

**EVALUATION OF THE INCIDENCE OF ENTERIC VIRUSES, *VIBRIO*  
SPECIES AND *ESCHERICHIA COLI* PATHOTYPES IN EFFLUENTS  
OF TWO WASTEWATER TREATMENT PLANTS LOCATED IN  
KEISKAMMAHOEK AND STUTTERHEIM**

**ADEFISOYE MARTINS AJIBADE**

**DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY  
FACULTY OF SCIENCE AND AGRICULTURE UNIVERSITY OF  
FORT HARE**



**University of Fort Hare**  
*Together in Excellence*

**Evaluation of the Incidence of Enteric Viruses, *Vibrio* species and *Escherichia coli*  
Pathotypes in effluents of two Wastewater Treatment Plants Located in  
Keiskammahoek and Stutterheim in the Eastern Cape Province of South Africa**

By

Martins Ajibade Adefisoye

*A thesis submitted in fulfilment of the requirements for the degree of*

Doctor of Philosophy

(Microbiology)

Department of Biochemistry and Microbiology

Faculty of Science and Agriculture

University of Fort Hare

Supervisor: Prof Anthony I. Okoh

September 2016

## **Declaration**

I hereby declare that the research work documented in this thesis was carried out by **Martins Ajibade Adefisoye** under the supervision of Professor Anthony I. Okoh in the Department of Biochemistry and Microbiology, University of Fort Hare, Alice South Africa in accordance with the requirements for the award of Doctor of Philosophy (Microbiology) degree and the work herein is original and has not been submitted in part or as a whole at any other university for the award of any degree.

**Candidate Name:** Martins A. Adefisoye

**Signature:** .....

**Date:** .....

**Supervisor:** Prof. Anthony I. Okoh

**Signature:** .....

**Date:** .....

## **Acknowledgements**

*The journey of a thousand miles begins with a single step. More than two years later, here I am ready for the next phase in my scientific adventures. There are quite a lot of people whom one way or the other contributed in this journey thus far. I am highly grateful to all of them!*

I would never have been able to complete this work without the special **GRACE** of God over my life. You are the one and Holy God, to whom all praise is due; **I stand in awe of you!**

I am highly indebted to my professor and mentor, Prof Anthony I Okoh, under whose excellent supervision this research was carried out. Your fatherly love, professorial guidance, never-ending patience, unquantifiable support and encouragement at all times have made this possible. Thank you Prof for the privilege to work with you and, for providing me an enabling atmosphere throughout my study/training period under your tutelage. Working with you was really an enjoyment, and if I was asked to choose a supervisor, I will choose you again and again Prof. You are more than just a supervisor to me, you are a father and a mentor. Also to Prof. (Pastor) Omobola Okoh, thank you for your support and prayers at all times.

I cannot but appreciate my late dad, Overseer (Engr.) Martins Ajibade Adefisoye (Snr.), who on his sick bed, and at the point of death emphasised the need for all his children to ensure by all means attain the peak in our academic pursuit. The power of death will never erase the best legacies you have bequeathed to us. We are forever grateful for coming to this world through you. Sleep on dad till we meet again!

To the best mum in the world, you remain my pillar of strength and comfort. Who in the world would ever ask for a better mum? You stood in the gap for us, when all hope was lost. May you live long to eat the fruit of you labour mother.

To all my siblings: Oluyomi, Adejumoke, Aderogba, Temilade, Modupe, Olusola and Olusegun for your prayers, support and encouragement. You are the best anyone could ever ask for. Our best is yet to come by His grace.

Special thanks to all our founding organisations: the Water Research Commission (WRC), the National Research Foundation (NRF) (Grant UID: 89040) and the South African Medical Research Council (SAMRC). Also, the Govan Mbeki Research and Development Centre (GMRDC) and the University of Fort Hare for providing us with enabling platform to carry out this project.

To all staff and student of the Department of Biochemistry and Microbiology, University of Fort Hare, and all members of the Applied Environmental and Microbiology Research Group (AEMREG) and the South African Medical Research Council (SAMRC) Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice, South Africa, you guys are the best team. Special thanks to the AEMREG/SAMRC leadership team: Prof AI Okoh, Prof. CL Obi, Prof. LV Mabinya, Prof. E Green, Prof. U Nwodo and Dr. BC Iweriebor whose advices and inputs made this possible.

And to my wonderful friends in Nigeria, South Africa and other parts of the globe thank you all. Dr Ýinka Titilawo, Mr Taiwo Fadare, Mr Emmanuel Abioye and Mr Charles Osunla thank you guys for your support at all times. We started this journey together years back and by His grace we shall reach the peak.

Finally to my ‘Wuraola’ Temitope K. Lawal, not enough words to appreciate you for being there for me at all times, I love you more than words can express.

## **Dedication**

“How great is our God – sing with me,  
How great is our God – and all will see,  
How great, how great is our God”.

To the memory of my late dad

**Late Overseer (Engr.) MA Adefisoye & my lovely mum Deaconess AM Adefisoye,**

and my siblings.

I love you all!

# Table of Contents

Declaration .....	ii
Acknowledgements .....	iii
Dedication .....	v
Table of Contents .....	vi
List of Figures .....	xiii
List of Tables.....	xvi
List of Abbreviations.....	xviii
General Abstract.....	xxii
CHAPTER ONE .....	1
General Introduction.....	1
1.1 Background to the study .....	2
1.2 Aim and Objectives of the study.....	14
References.....	16
CHAPTER TWO.....	30
Literature Review .....	30
2.1 Introduction.....	31
2.2 Wastewater treatment plants as important sources of pollution .....	32
2.3 Water pollution and human diseases.....	36
2.4 Mechanisms and pathogenesis of infectious diarrhoea.....	38
2.5 <i>Escherichia coli</i> as aetiological agent of acute diarrhoea and other infectious diseases .....	43

2.5.1	Enterotoxigenic <i>E. coli</i> (ETEC).....	49
2.5.2	Enterohemorrhagic <i>E. coli</i> (EHEC).....	54
2.5.3	Enteropathogenic <i>E. coli</i> (EPEC).....	58
2.5.4	Enter aggregative <i>E. coli</i> (EAEC).....	62
2.5.5	Enteroinvasive <i>E. coli</i> (EIEC).....	65
2.5.6	Diffusely adhering <i>E. coli</i> (DAEC).....	67
2.5.7	Extraintestinal pathogenic <i>E. coli</i> (ExPEC).....	69
2.6	Contaminated water as a vehicle for cholera disease.....	73
2.7	Water pollution and enteric viruses.....	77
2.8	Antimicrobial resistance in the aquatic environment.....	79
2.9	Good practices and policies for controlling water pollution.....	81
2.10	Conclusions and closing remarks.....	82
	References.....	84
CHAPTER THREE.....		130
Seasonal variation in the physicochemical quality indices of the final effluents of two wastewater treatment plants in Amathole District Municipality Area of South Africa.		130
3.1	Abstract.....	131
3.2	Introduction.....	132
3.3	Materials and Methods.....	134
3.3.1	Description of study sites.....	134
3.3.2	Sample Collection and Analytical Procedures.....	136
3.3.3	Analytical Quality Control.....	136



3.3.4	Statistical Analysis .....	137
3.4	Results and Discussion .....	137
3.5	Conclusions.....	154
	References.....	156
CHAPTER FOUR.....		167
	Assessment of the microbiological qualities of discharged final effluents of two wastewater treatment facilities located in Amathole District Municipality of the Eastern Cape Province, South Africa. ....	167
4.1	Abstract.....	168
4.2	Introduction.....	170
4.3	Materials and Methods.....	174
4.3.1	Description of study sites .....	174
4.3.2	Sample collection and treatment.....	174
4.3.3	Detection and enumeration of <i>E. coli</i> and <i>Vibrio</i> spp.....	175
4.3.4	Molecular confirmation of presumptive <i>E. coli</i> and <i>Vibrio</i> isolates.....	176
4.3.5	Antibiogram of PCR-confirmed <i>Vibrio</i> spp. isolates .....	179
4.3.6	Analysis of selected physicochemical variables.....	180
4.3.7	Statistical analysis.....	180
4.4	Results.....	180
4.4.1	Prevalence and distribution of <i>E. coli</i> and <i>Vibrio</i> species in the final effluents .....	180
4.4.2	Molecular identification of <i>E. coli</i> and <i>Vibrio</i> isolates.....	186

4.4.3	Antibiotic susceptibility pattern of <i>Vibrio</i> spp. ....	189
4.4.4	Free chlorine and turbidity profiles of the discharged effluent samples. .	189
4.5	Discussion .....	194
4.6	Conclusion .....	197
	References.....	1981
CHAPTER FIVE.....		211
	Identification and antimicrobial resistance prevalence of pathogenic <i>Escherichia coli</i> strains from treated wastewater effluents in Eastern Cape, South Africa .....	211
5.1	Abstract.....	212
5.2	Introduction.....	213
5.3	Materials and Methods.....	217
5.3.1	Study design and source of samples .....	217
5.3.2	Molecular confirmation and characterisation of <i>E. coli</i> pathotypes.....	217
5.3.3	Antibiotic resistance profiling of confirmed <i>E. coli</i> isolates .....	219
5.3.4	Multiple Antibiotic Resistance Phenotypes and Multiple Antibiotic Resistance Indexing.....	219
5.3.5	Detection of Antibiotic Resistant Determinants Genes (ARDs) .....	220
5.4	Results.....	222
5.4.1	Molecular Confirmation and Characterisation of <i>E. coli</i> Isolates into various Pathotypes.....	222
5.4.2	Antibiogram profiling of confirmed <i>E. coli</i> isolates.....	222

5.4.3	Multiple antibiotic resistance phenotypes and multiple antibiotic resistance indexing .....	223
5.4.4	Detection of antibiotic resistant determinants (ARDs).....	227
5.5	Discussion .....	230
5.6	Conclusions.....	236
	References.....	237
CHAPTER SIX .....		249
	Faecal coliform abundance and qPCR detection of human adenovirus, hepatitis A and rotavirus in discharged effluents of two wastewater treatment facilities in the Eastern Cape Province, South Africa .....	249
6.1	Abstract.....	250
6.2	Introduction.....	251
6.3	Materials and Methods.....	255
6.3.1	Study site description and collection of samples.....	255
6.3.2	Detection and enumeration of faecal coliforms.....	256
6.3.3	Concentration of viruses in wastewater samples and extraction of viral nucleic acids .....	256
6.3.4	Reverse transcription of HAV and RoV genomes.....	257
6.3.5	Construction of standard curves and qPCR sensitivity studies .....	258
6.3.6	TaqMan probe-based qPCR assays for the detection and quantification of HAdVs, HAV and RoV genomes .....	259
6.3.7	Characterisation of human adenovirus species and serotypes.....	261

6.3.8	Quality Control .....	261
6.3.9	Statistical Analysis .....	262
6.4	Result .....	264
6.4.1	Detection and enumeration of faecal coliforms.....	264
6.4.2	Real-time PCR sensitivity and detection limits.....	265
6.4.3	Detection and quantification of human adenovirus genome .....	268
6.4.4	Characterisation of human adenovirus virus species and serotypes.....	268
6.4.5	Detection and quantification of hepatitis A virus and rotavirus.....	274
6.5	Discussion .....	274
6.6	Conclusion .....	280
	References.....	281
CHAPTER SEVEN.....		295
	General Discussion and Conclusion .....	295
7.1	Discussion .....	296
7.2	Conclusions.....	306
7.3	Recommendations.....	307
	References.....	309
Appendix .....		317
Appendix I: Photographs showing some of the sampling points .....		317
Appendix II: Some Photographs of membrane filtration plates .....		318
Appendix III: Representative agarose gel electrophoresis images of some of the characterisation of <i>E. coli</i> isolates. ....		320

Appendix IV: Publications and conference presentations .....	326
Publications:.....	326
Adefisoye, Martins Ajibade Adefisoye, Uchechukwu U. Nwodo, Ezekiel Green and Anthony Ifeanyin Okoh (2016). Quantitative PCR detection and characterisation of human adenovirus, rotavirus and hepatitis A virus in discharged effluents of two wastewater treatment facilities in the Eastern Cape, South Africa. <i>Food and Environmental Virology</i> . 2016 May 28. DOI 10.1007/s12560-016-9246-4 [Epub ahead of print].....	326
Adefisoye, M.A. and Okoh, A.I. (2016). Identification and antimicrobial resistance prevalence of pathogenic <i>Escherichia coli</i> strains from treated wastewater effluents in Eastern Cape, South Africa. <i>Microbiologyopen</i> . 2016 Jan 13. doi: 10.1002/mbo3.319. [Epub ahead of print].....	326
Martins Ajibade Adefisoye and Anthony Ifeanyi Okoh (2015). Real-time detection of adenovirus and faecal indicator bacteria in the discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. <i>J Microb Biochem Technol</i> 2015, 5:4 <a href="http://dx.doi.org/10.4172/1948-5948.S1.014">http://dx.doi.org/10.4172/1948-5948.S1.014</a>	326
Okoh, A.I., Sibanda, T., Nongogo, V., Adefisoye, M.A., Olayemi, O.O. and Nontongana, N. (2015). Prevalence and characterisation of non-cholerae <i>Vibrio</i> spp. in the final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. <i>Environ Sci Pollut Res Int</i> . 22(3): 2008-2017.....	326
Conference Presentations:.....	327

## List of Figures

Figure 1.1:	Stages of conventional wastewater treatment operation.....	6
Figure 2.1:	A schematic diagram showing the general molecular mechanisms triggering diarrhoea. Diarrhoea results basically from impaired absorption or increased secretion of fluid and electrolytes. ....	40
Figure 2.2:	The pathogenic pathways of the various diarrhoeagenic (DAEC) <i>Escherichia coli</i> pathotype. ....	48
Figure 2.3:	Distribution of reported cases of cholera cases by continent by year (1989 – 2013). ....	74
Figure 2.4:	Model summarising the cellular processes of <i>V. cholera</i> -induced diarrhoea. .	76
Figure 4.1:	Prevalence and distribution of bacterial groups at SWWTP final effluent (SFE). ....	182
Figure 4.2:	Prevalence and distribution of bacterial groups at SWWTP discharge point (SDP). ....	183
Figure 4.3:	Prevalence and distribution of bacterial groups at KWWTP final effluents (KFE). . ....	184
Figure 4.4:	Prevalence and distribution of bacterial groups at KWWTP discharge point (KDP). ....	185
Figure 4.5:	Molecular confirmation of <i>E. coli</i> isolates. Legend: Lane 1: 100 bp Molecular weight marker; lane 2: Positive control ( <i>E. coli</i> ATCC 25922 strain); lane 3: Negative control; lanes 4 to 13 <i>E. coli</i> isolates. ....	187

Figure 4.6:	Molecular confirmation of <i>Vibrio</i> spp. isolates. Legend: Lane 1: 100 bp Molecular weight marker; lane 2: Positive control (DSM 11058 strain); lane 3: Negative control; lanes 4 to 13 <i>Vibrio</i> spp. isolates. ....	188
Figure 4.7:	Concentration of free chlorine in the discharged effluent at SWWTP over the 12 month sampling period. ....	191
Figure 4.8:	Concentration of free chlorine in the discharged effluent at KWWTP over the 12 month sampling period. ....	192
Figure 4.9:	Turbidity profiles of the discharged final effluents over the sampling period. ....	193
Figure 5.1:	Antibiotic resistant determinants. Lane M: 100 bp molecular weight marker; lane N: negative control; lane A: <i>strA</i> (546 bp); lane B: <i>aadA</i> (525 bp); lane C: <i>cat I</i> (547 bp); lane D: <i>cmlA1</i> (115 bp); lane E: <i>blaTEM</i> (690 bp); lane H: <i>tetC</i> (385 bp); lane I: <i>tetD</i> (787 bp); lane J: <i>tetK</i> (460 bp); lane K: <i>tetM</i> (125 bp). ....	228
Figure 6.1:	Monthly variation in FC counts in wastewater samples from SWTW (SFE: SWTW final effluent sample; SDP: SWTW discharge point sample). ....	266
Figure 6.2:	Monthly variation in FC counts in wastewater samples from KWTW (KFE: KWTW final effluent sample; KDP: KWTW discharge point sample). ....	267
Figure 6.3:	Amplification plot for HAdV detection and quantification from the discharged effluents samples. ....	269

Figure 6.4:	Monthly variation in HAdV concentrations in wastewater samples from SWTP (SFE: SWTP final effluent sample; SDP: SWTP discharge point sample). .....	270
Figure 6.5:	Monthly variation in HAdV concentrations in wastewater samples from KWTP (KFE: KWTP final effluent sample; KDP: KWTP discharge point sample). .....	271
Figure 6.6:	Seasonal distribution of HAdV concentration at all sampling points. ....	272



## List of Tables

Table 1.1:	Some microbial indicators and common pathogens found in raw sewage. ....	10
Table 2.1:	Wastewater treatment advancement levels and the various technologies employed.....	34
Table 2.2:	Mechanisms of pathogenesis and some clinical presentations of various diarrhoeagenic <i>E. coli</i> (DEC) infections.....	47
Table 2.3:	Prevalence of enteropathogenic <i>E. coli</i> strains among children with diarrhoea in developing countries. ....	61
Table 3.1:	Description of the study sites.....	135
Table 3.2:	Seasonal variations in physicochemical characteristics of the two selected wastewater treatment plants.....	139
Table 3.3:	Seasonal ranges of the physicochemical qualities of WWTP1 and WWTP2 plants.....	141
Table 3.4:	Correlation half-matrix of physicochemical wastewater quality parameters.	152
Table 4.1:	Primer sequences and sizes of PCR-amplified target genes of the <i>E. coli</i> and <i>Vibrio</i> spp. isolates.....	178
Table 4.2:	Antibiogram of randomly selected PCR-confirmed <i>Vibrio</i> species isolates.	190
Table 5.1:	Primer sequences and sizes of PCR-amplified target genes of <i>E. coli</i> pathotypes. ....	217
Table 5.2:	PCR conditions for antibiotic resistant determinants assayed. ....	220

Table 5.3:	Antibiotic resistance profiles of <i>E. coli</i> pathotypes showing percentages of resistance to the test antibiotics.....	223
Table 5.4:	The various multiple antibiotic resistant (MARP) phenotypes of <i>E. coli</i> pathotypes. ....	224
Table 5.5:	Distribution of antibiotic resistant genes <i>E. coli</i> isolates.....	227
Table 6.1:	Some commonly studied enteric viruses and the diseases caused. ....	253
Table 6.2:	Specific oligonucleotide primers and probes for the qPCR detection and quantification viral genomes.....	260
Table 6.3:	Primer sets and amplicon sizes for the detection of HAdV species and serotypes .....	263
Table 6.4:	Viral control strains used for HAdV species and serotype detection. ....	264
Table 6.5:	Characterisation of HAdV in the effluent samples into species and serotypes. .....	273

## List of Abbreviations

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
APEC	Avian pathogenic <i>Escherichia coli</i>
APHA	American Public Health Association
ARD	Antibiotic resistance determinants
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BOD	Biological oxygen demand
cAMP	Cyclic adenosine monophosphate
CDC	Centers for Disease Control and Prevention
cDNA	Complementary deoxyribonucleic acid
CFs	Colonisation factors
CFU	Colony forming units
cGMP	Cyclic guanine monophosphate
CLSI	Clinical and Laboratory Standards Institute
COD	Chemical oxygen demand
DAEC	Diffusely adherent <i>Escherichia coli</i>
DEC	Diarrhoeagenic <i>Escherichia coli</i>

DNA	Deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DO	Dissolved oxygen
DWAF	Department of Water Affairs and Forestry
EAggEC	Enterohaemorrhagic <i>Escherichia coli</i>
EC	Electrical conductivity
EHEC	Enterohaemorrhagic <i>Escherichia coli</i>
EIEC	Enteroinvasive <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>
ETEC	Enterotoxigenic <i>Escherichia coli</i>
ExPEC	Extraintestinal pathogenic <i>Escherichia coli</i>
FC	Faecal coliforms
FDA	Food and Drug Administration
GBS	Group B <i>streptococcus</i>
GTP	Guanine triphosphate
HAdV	Human adenovirus
HAV	Hepatitis A virus
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus

HUS	Heamolytic ureamic syndrome
InPEC	Intestinal pathogenic <i>Escherichia coli</i>
KHD	Keiskammahoek discharge point
KHF	Keiskammahoek final effluent tank
KWWTP	Keiskammahoek wastewater treatment plants
LPS	Lipopolysaccharide
LT	Heat-labile toxin
MARI	Multiple antibiotic resistance index
MARP	Multiple antibiotic resistance phenotype
MDR	Multidrug resistance
MF	Membrane filtration
MLTS	Multilocus sequence typing
NAD	Nicotinamide adenine dinucleotide
NMEC	Noenatal meningitis <i>Escherichia coli</i>
NTU	Nephelometric unit
PAIs	Pathogenic islands
PCR	Polymerase chain reaction
qPCR	Quantitative (real-time) polymerase chain reaction
RoV	Rotavirus

ST	Heat-stable toxin
STD	Stutterheim discharge point
STEC	Shiga-toxin-producing <i>Escherichia coli</i>
STF	Stutterheim final effluent tank
SWWTP	Stutterheim wastewater treatment plants
TBE	Tris-Borate-EDTA
TCBS	Thiosulfate-Citrate-Bile-Sucrose agar
TDS	Total dissolved solid
THM	Trihalomethane
TTP	Thymine triphosphate
UNESCO	United Nations Educational, Scientific and Cultural Organisation
UNICEF	United Nations International Children's Emergence Fund
UPEC	Uropathogenic <i>Escherichia coli</i>
US EPA	United States Environmental Protection Agency
UTI	Urinary tract infection
WaSH	Water, Sanitation and Hygiene
WHO	World Health Organisation
WWTP	Wastewater treatment plants

## General Abstract

South Africa is currently experiencing water shortage crisis, a challenge that has been attributed not only to the scarcity of freshwater, but also to fast degrading water quality. Factors such as rapid urbanisation, population and economic growth, climate change as well as poor operational and maintenance of many of the existing water/wastewater treatment facilities have been acknowledged as important contributors to degrading water quality in the country. Untreated or inadequately treated discharged wastewater effluents constitute point source pollution to many freshwater environments in South Africa. Hence, it becomes imperative to evaluate wastewater discharges in order to protect the scarce freshwater resource, the environment and public health. Over a twelve-month sampling period (September 2012 to August 2013), we assessed the bacteriological, virological and physicochemical qualities of the discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. For the physicochemical assessment, a total of 144 final effluent samples were collected from both the final effluent tanks (FE) and the discharge points (DP) of the treatment facilities. Physicochemical parameters including pH, temperature, turbidity, total dissolved solids (TDS), dissolved oxygen (DO), electrical conductivity (EC) and free chlorine concentration were determined *on site* while biological oxygen demand (BOD), nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), phosphate ( $\text{PO}_4^-$ ) and chemical oxygen demand (COD) were determined in the laboratory. The bacteriological analysis of the samples was done using standard membrane filtration (MF) technique. Bacterial groups assessed included: faecal indicator bacteria (faecal coliforms and *E. coli*) and *Vibrio* species, while the antibiotic susceptibility profiles of selected *E. coli* and *Vibrio* species isolates against some selected antibiotics commonly used in human therapy and veterinary medicine were determined using the standard agar-disc diffusion method. The occurrence and concentrations of human enteric viruses including: human adenovirus (HAdV), hepatitis A

virus (HAV) and rotavirus (RoV) in the samples were determined by TaqMan-based real-time polymerase chain reaction (qPCR) following concentration by adsorption-elution method. The physicochemical characteristics of the samples ranged as follows: pH (6.5 – 7.6), TDS (95 – 171 mg/L), EC (134 – 267  $\mu$ S/cm), temperature (12 – 27 °C), turbidity (1.5 – 65.7 mg/L), free chlorine (0.08 – 0.72 mg/L), DO (2.06 – 9.81 mg/L), BOD (0.13 – 9.81 mg/L),  $\text{NO}_3^-$  (0 – 21.5 mg/L),  $\text{NO}_2^-$  (0 – 0.72 mg/L),  $\text{PO}_4^-$  (0 – 18.3 mg/L) and COD (27 – 680 mg/L). Some of the characteristic such as pH, TDS, EC, temperature, nitrite and DO (on most instances) complied with recommended guidelines. Other characteristics, however, including turbidity, BOD, nitrate, phosphate and COD fell short of the recommended guidelines. All the 48 samples analysed for bacteriological qualities tested positive for the presence of the bacterial groups with significant ( $P \leq 0.05$ ) seasonal variation in their densities. Faecal coliforms were detected in counts ranging from 1 CFU/100ml to  $2.7 \times 10^4$  CFU/100ml. Presumptive *E. coli* counts ranged generally between 1 CFU/100ml –  $1.4 \times 10^5$  CFU/100ml while counts of presumptive *Vibrio* species ranged between 4 CFU/100ml –  $1.4 \times 10^4$  CFU/100ml. Molecular identification of the presumptive isolates by polymerase chain reactions PCR gave positive reaction rates of 76.2% (381/500) and 69.8% (279/400) for *E.coli* and *Vibrio* species respectively. The antibiotic susceptibility profiling of 205 PCR-confirmed *Vibrio* isolates against 18 commonly used antibiotics showed resistance frequencies ranging from 0.5% (imipenem) to 96.1% (penicillin G) at recommended breakpoint concentrations. Eighty one percent (166/205) of the *Vibrio* isolates showed multidrug resistance (resistance to 3 or more antibiotics) with the most common multiple antibiotic resistance phenotype (MARP) being AP-T-TM-SMX-PG-NI-PB, occurring in 8 isolates. Multiple antibiotic resistance indices (MARI) estimated for the *Vibrio* isolates at both study sites were 0.35 and 0.33 at SWWTP and KWWTP respectively. Further molecular characterisation of 223 PCR-confirmed *E. coli* isolates revealed five different pathotypes in



the following proportions: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%), UPEC (41.7%) and others (26.9%). The *E. coli* isolates showed marked susceptibility to meropenem and imipenem while exhibiting varying (1.7% – 70.6%) degrees of resistance to 15 out of the 17 test antibiotics. Multidrug resistance was exhibited by 32.7% of the *E. coli* isolates with the commonest MARP being AP-T-CFX (12 isolates), while MARI estimates are 0.23 (SWWTP) and 0.24 (KWWTP). The associated antibiotic resistance determinants (ARDs) detected in the *E. coli* isolates include: *strA* (88.2%), *aadA* (52.9%), *cat I* (15%), *cmlA1* (4.6%), *blaTEM* (56.4%), *tetA* (30.4%), *tetB* (28.4%), *tetC* (42.2%), *tetD* (50%), *tetK* (11.8%) and *tetM* (68.6%). Human adenovirus was detected by qPCR in 62.5% (30/48) of the effluent samples with concentration ranging from  $8.4 \times 10^1$  genome copies/L to  $1.0 \times 10^5$  genome copies/L. HAV and RoV were not detected in the samples analysed over the sampling period. The molecular characterisation of the adenovirus-positive samples by conventional PCR showed adenovirus species HAdV-B (serotype 2) and HAdV-F (serotype 41) in 86.7 % and 6.7% of the samples respectively. No consistent seasonal trend was observed in HAdV concentration, however, increased concentrations of HAdV was generally observed in the winter months. Also, there was no correlation between faecal coliforms counts and HAdV prevalence at both study sites. Our findings highlight the detrimental impacts that discharged effluents may have on the physicochemical characteristics of the receiving watersheds overtime, it also shows the ability of potentially pathogenic microorganisms to circumvent the various wastewater treatment processes and be released directly into surface water with the attendant risks associated upon human exposure. We therefore conclude as follows: i) inadequately treated municipal effluents are potential contributors to water pollution problems in the Eastern Cape Province; ii) municipal wastewater effluents are important reservoirs for the dissemination of potentially pathogenic *E. coli* (and possibly other pathogens) and antibiotic resistance determinants (ARDs) in the

aquatic milieu of the Eastern Cape Province and a risk to public health; iii) the persistent occurrence of HAdV in the discharged treated effluents points to the potential public health risk through the release of HAdV genomes into the receiving watersheds and the possibility of their transmission to human population. Hence, there is need for consistent monitoring of the treatment processes and facilities by the relevant authorities, while well-coordinated efforts and approaches should be deployed towards curbing indiscriminate discharge of poor quality effluents into the aquatic milieu of the Eastern Cape in the interest of public health and freshwater ecosystem conservation.

# **CHAPTER ONE**

## **General Introduction**

## **1.1 Background to the study**

Air, water and food are vital for survival of all life forms. Human beings can survive for about 3 to 4 min without air, about 3 to 4 days without water, and up to 4 weeks without food (Falvo, 2010). Therefore, potable water must be considered a human right (WHO, 2003a). The concomitant hygienic removal of anthropogenic wastes as well as chemical and heavy metals from municipal and industrial wastewaters are essential for consistent availability of clean and safe water (Falvo, 2010). Although, the ranges of water consumption often varies across the world; the World Health Organisation (WHO) recommended a minimum of 20 litres of freshwater per person per day (WHO/UNICEF, 2000). Whereas affluent urban dwellers have access to hundreds of litres of clean and safe water per day, most poor rural dwellers in many Asian, Latin American, and African countries have access to about six or less litres of water per person per day (Falvo, 2010).

The productions of wastewater final effluents that meet regulatory requirements remain a major challenge in developing countries with the consequential problem of surface water pollution. Point source pollution of freshwater sources from inadequately treated wastewater effluents constitutes a major burden of pollution load to the limited available freshwater resource, and remains a threat to public health as well as conservation of the natural environment (Kraemer *et al.*, 2001; Igbinosa and Okoh, 2009). Polluted water sources endanger both the physical and social health of all people and it is an affront to human dignity (WHO, 2003b) and a number of water-related epidemics have reportedly been linked to the ingestion of contaminated water and food items with reference to risk factors exposure (CDC 1998; WHO, 2004; Fong and Lipp, 2005).

Water is a critical resource that underpins economic growth and development as well as human well-being, and environmental protection contemporarily, and in the future (Love and Luchsinger, 2014). The quality of water in developed and developing countries varies and often reflects the level of socioeconomic and physical development (Abbaspour, 2011). One of the most pressing tasks facing our world presently is guaranteeing adequate supply of good quality water in spite of rapidly increasing human demand and climate changes (NSF, 2011; Chandra, 2014). The World Bank in 2007 suggested that water-related environmental problems will cost countries between 0.5 and 2.5 of their Gross Domestic Product (GDP) (World Bank, 2007). Also, more than three quarter of mortality cases from infections have been attributed to water-borne disease in developing countries (Love and Luchsinger, 2014).

Lack of access to safe water or adequate hygiene largely remains important risk factors for increasing morbidity and mortality, particularly in developing countries (Pruss-Ustun *et al.*, 2008; WHO, 2011). Although about 87% of human population now have access to better-quality water sources, an estimated 39% still lack access to better-quality water. Additionally, more than 1.1 billion human populations still excrete in the open, while only about 17% washing their hands with soap after toilet use in developing countries (Pruss-Ustun *et al.*, 2008; WHO/UNICEF, 2010). In low- and middle-income countries such as South Africa, health services continue to struggle under huge burden of diseases resulting from environmental degradation, and more importantly water pollution (Nugent, 2008; Govender *et al.*, 2011). The interrelated impacts of water quality, anthropogenic activities, waste disposal, and health status, particularly in terms of diseases transmitted through the faecal-oral route, are well recognised (Curti *et al.*, 2000; Fewtrell *et al.*, 2005). Enteric bacterial and viral pathogens are transmitted at household and community levels through a complex network of interdependent routes, such as contaminated food and water, poor waste disposal,

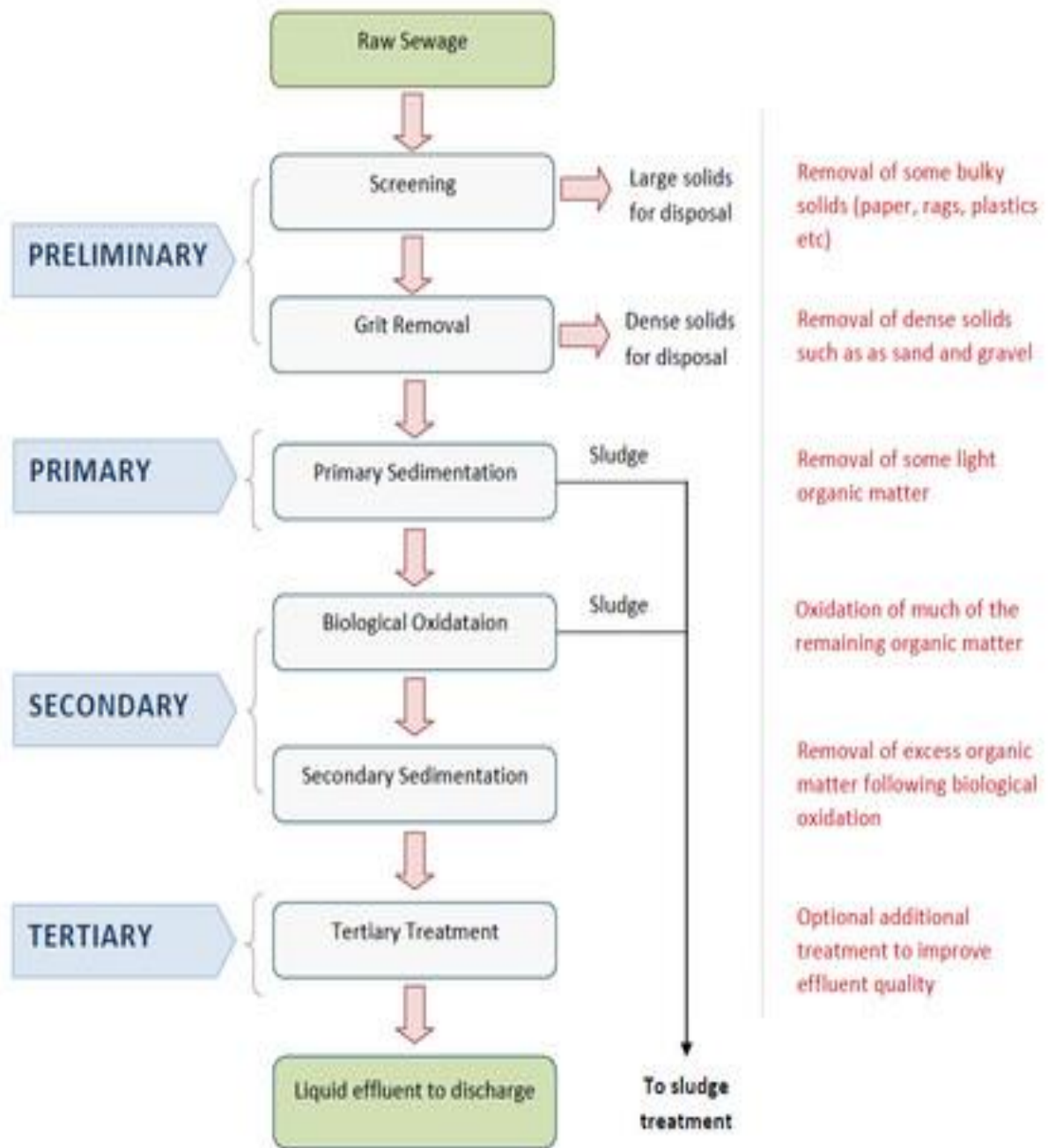
and intricate household and community human-to-human interactions (Eisenberg *et al.*, 2007).

The composition of wastewater is often a reflection of the habitual and technological practices of the producing community, and usually it is a complex mixture of organic, inorganic and man-made compounds (Abdel-Raouf *et al.*, 2012). Historically, wastewater collection can be traced back to ancient times. Its treatment is however a relatively recent development dating from the late 1800s and early 1900s (Chow *et al.*, 1972, Okoh *et al.*, 2007). Wastewater treatment practises basically involves a combination of physical, chemical and biological processes toward removing contaminants from wastewater. The basic steps involved in treating wastewater are usually divided into; preliminary, primary, secondary, tertiary, quaternary (usually optional and expensive) and disinfection stages (Figure 1.1) (Abdel-Raouf *et al.*, 2012). The methods and techniques used for treating wastewater differs among various nations and from plant to plant although, the main objectives of wastewater treatment remain the same, which apparently is to produce clean discharged effluents that are safe in terms of environmental conservation and public health protection.

South Africa has been described as a water scarce country (Momba *et al.*, 2006; Mema, 2009), a situation worsened by climate change, proliferation of alien plant species (Blignaut and van Heerden, 2009), and discharges of untreated or poorly treated effluents (DEAT, 2006). Improper operational procedures as well as inadequate maintenance of wastewater treatment facilities call for concern in the country. Health risks associated with inadequately treated wastewater may arise from pathogenic microorganisms (bacteria, viruses, protozoans and helminths), toxic organic and inorganic chemicals, and trace and heavy metals

(Odjadjare, 2010). The occurrence of these contaminants in high concentrations above critical values recommended by national and international regulatory organisations is considered unacceptable in receiving watersheds and the environment (Akpor and Muchie, 2011).

Heavy metals, also referred to as trace metals, are one of the most persistent pollutants contained in municipal and industrial wastewater discharges. These metals have atomic densities greater than  $6 \text{ g/cm}^3$ ; they are non-biodegradable and, are toxic in nature (Jern, 2006). Some heavy metals of public health and environmental concerns in municipal and industrial wastewater are zinc, nickel, mercury, chromium, cadmium, lead, copper, silver and arsenic (Akpor *et al.*, 2014). High concentrations of heavy metals in municipal wastewater can lead to increased cost in wastewater treatment, retarded growth in aquatic plants and animals, increased incidences of cancer in human, nervous system breakdown, organ failure and damage, reduced enzyme activity, reduced lipid content of plant by cadmium, photosynthesis inhibition by copper and mercury, and reduction in the level of chlorophyll production by lead (Gardea-Torresdey *et al.*, 2005; Akpor *et al.*, 2014).



**Figure 1.1:** Stages of conventional wastewater treatment operation. *Source:*

<http://www.oilgae.com/algae/cult/sew/new/new.html>



Monitoring of physicochemical variables in water resources helps to evaluate the suitability of such water for various uses as well as the identification of impairment in water quality in order to ensure the protection of public health and the environment (Okoh *et al.*, 2007). Although, some physicochemical variables may have limited health significance, domestic and international guidelines often require their evaluation in monitoring water quality from various sources (Tebbut, 1992; WHO, 2008). Important physicochemical variables that are monitored in water include: pH, turbidity, odour, temperature, odour, radioactivity, total dissolve solids, total suspended solids, nitrate, nitrite, orthophosphate, total and unionised ammonia, electrical conductivity, dissolved oxygen (DO), biochemical oxygen demand, total hardness, salinity and total alkalinity among others (Tebbut, 1992; Mara, 2003; Joshi *et al.*, 2009). Many studies across the world have reported on the importance of physicochemical quality monitoring of different water resources including drinking water, river water and reclaimed water (Joshi *et al.*, 2009; Odjadjare and Okoh, 2010; Sulieman *et al.*, 2010; Tabor *et al.*, 2011; O'Hogain *et al.*, 2011; Fadaeifard *et al.*, 2012; Al-Bayatti *et al.*, 2012; Popa *et al.*, 2012; Bayram *et al.*, 2013; Chigor *et al.*, 2013; Awoyemi *et al.*, 2014; Platikanov *et al.*, 2014; Serpa *et al.*, 2014; Sheshe and Magashi, 2014; Aakame *et al.*, 2015).

Water contaminated with faecal matter are largely regarded as hazardous to public health, as they may contain human specific enteric pathogens, such as pathogenic strains of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., hepatitis A virus and Norwalk group viruses among others (Scott *et al.*, 2002). Indicator microorganisms including total coliforms (TC), faecal coliform (FC), enterococci, faecal streptococci (FS) and *E. coli* groups have conventionally been used to suggest the possible presence of pathogens for the purpose of water quality assessment (Berg 1978; Myers and Sylvester, 1997; Ashbolt *et al.*, 2001). *E. coli* is included in the faecal coliform group and is a more specific indicator of faecal pollution than other

faecal coliform bacteria. Two important factors that have led to the use of *E. coli* as the preferred indicator for the detection of faecal contamination are, firstly, some of the other faecal coliforms are non-faecal in origin, and secondly, the development of improved rapid testing methods for *E. coli* (Odonkor and Ampofo, 2013). *E. coli* is still mostly used as the best bacterial indicator of faecal contamination in water, based on the prevalence of thermotolerant coliforms in temperate environments as compared to the rare incidence of *E. coli*, its predominance in human and animal faecal materials as compared to other thermotolerant coliforms, and the availability of inexpensive, fast, sensitive, specific and easier to perform methods for its detection (Odonkor and Ampofo, 2013).

Ideally, indicator organisms are non-pathogenic, easily detected by simple and rapid methods, have survival characteristics that are similar to the pathogens of interest, and help circumvent the need to assay every pathogen that may be present in water system (Scott *et al.*, 2002). However, reports have underlined the differences in the ecology, prevalence, and stress resistance of some bacterial indicators such as coliforms, from those of the many pathogens they are proxy for (Desmarais *et al.*, 2002). These differences limit the usefulness of such indicator for faecal pollution in water. Additionally, faecal bacteria densities may provide some information about enteric viruses originating from human sources, such information may not exist for faecal contaminations of animal origin (Payment *et al.*, 1997), and studies have revealed that the detection of indicator bacteria do not always correlate with the presence or absence of enteric viruses in water (Miagostovich *et al.*, 2004; Espinosa *et al.*, 2009; Jurzik *et al.*, 2010). Moreover, enteric viruses have been detected in surface, ground and treated drinking water despite meeting quality standard for bacterial indicators (Cho *et al.*, 2000; Pusch *et al.*, 2005; Lin and Ganesh, 2013). Therefore, other microorganisms such as bacteriophages, somatic coliphages, and human adenoviruses have been suggested as

alternative indicators of faecal contamination in water systems (Lin and Ganesh, 2013). Table 1.1 shows some microbial indicators and common pathogens found in raw sewage.

While certain pathogens including *Vibrio* species occur naturally in marine and estuarine water environment, anthropogenic contaminations may be introduced into freshwater environment through sewage or wastewater pollution (Scott *et al.*, 2004). *Vibrios* can cause human infections including gastroenteritis, wound infections, and septicaemia (Morris and Black, 1985). Infection caused by *Vibrio* is usually contracted via ingestion of contaminated food or water or by direct colonization through wounds. The genus *Vibrio* comprises more than thirty species, of which about fourteen have been recognised as human pathogen (Iwamoto *et al.*, 2010). *Vibrio cholerae* is one of the waterborne bacterial agents of public health concern and the most studied member of the genus, and comprises numerous strains classified according to O group (Hlady and Klontz, 1996). Other pathogenic species include *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus* etc. while non-pathogenic species include *V. aestuarianus*, *V. campbelli*, *V. fischeri*, *V. nereis* (Thompson *et al.*, 2005).

The two other species of *Vibrio* that have been linked with reported infection cases are *V. vulnificus* and *V. parahaemolyticus*. The clinical manifestations often associated with *V. parahaemolyticus* infections may include diarrhoea, abdominal cramps, nausea and vomiting, while septicaemia and wound infection are less frequent (Levine and Griffin, 1993; Daniels *et al.*, 2000). *V. vulnificus* infections can result in sepsis and severe wound infections. The fatality rate of bloodstream infection caused by *V. vulnificus* is about 50% while *V. vulnificus* associated wound infections has about 25% fatality rate (Klontz *et al.*, 1988; Shapiro *et al.*, 1998; Dechet *et al.*, 2008).

**Table 1.1:** Some microbial indicators and common pathogens found in raw sewage

	<b>Microorganisms</b>	<b>Number (CFU/100 ml)</b>	<b>Source and pathogenicity</b>
Bacteria	<i>E. coli</i> ( <i>E. coli</i> O:148; O:157; O:124)	$10^6 - 10^7$	Human faeces; gastroenteritis, diarrhoea
	Total coliforms	$10^7 - 10^9$	
	Faecal coliforms	$10^6 - 10^7$	Human/animal
	Faecal streptococci	$10^5 - 10^6$	Human/animal
	Enterococci	$10^4 - 10^5$	Human/animal
	<i>Campylobacter</i> sp.	$10^4 - 10^5$	Human/animal; gastroenteritis
	<i>Staphylococcus</i> (coagulase positive)	$10^3$	
	<i>Pseudomonas aeruginosa</i>	$10^5$	
	<i>Shigella</i> spp.	$0 - 10^7$	Human faeces; dysentery
	<i>Salmonella</i> spp.	$0 - 10^7$	Human faeces; salmonellosis, gastroenteritis, typhoid fever, paratyphoid fever
	<i>Vibrio cholerae</i>	$0 - 10^7$	Human faeces; cholera, diarrhoea, dehydration
	Bacteroides	$10^7 - 10^{10}$	
	Acid-fast bacteria	$10^2$	
Protozoa	<i>Entamoeba histolytica</i>	$0 - 10^5$	Amoeba dysentery
	<i>Cryptosporidium parvum</i>	$10^1 - 10^3$	Diarrhoea, low-grade fever
	<i>Giardia</i> cysts	$10^3 - 10^4$	Diarrhoea, nausea, indigestion
	<i>Balantidium coli</i>		Diarrhoea, dysentery, intestinal ulcers
	Cyclospora		Severe diarrhea, nausea, vomiting, severe stomach cramps
Enteric virus	Coliphages	$10^2 - 10^3$	
	Norwalk virus		Human faeces; diarrhoea, vomiting
	Rotavirus	400 – 85000	Human faeces; diarrhoea, vomiting
	Adenovirus		Human faeces; gastroenteritis
	HepatitisA virus		Human faeces; hepatitis
	Poliovirus	180 – 500000	Human faeces; poliomyelitis

Adapted from: Gerba, 2008; Lusk *et al.*, 2014; Naidoo and Olaniran, 2014.

*Vibrio* infections cases have marked seasonal distribution, with high frequency reported in summer and early fall, this often correspond to warmer temperature periods (Iwamoto *et al.*, 2010). *Vibrio* is best identified and isolated on selective media such as thiosulfate-citrate-bile-salts-sucrose (TCBS) agar (Janda *et al.*, 1988; Abbott *et al.*, 2007).

Human enteric viruses are known to primarily infect and reproduce in the intestinal tract, and have been associated with waterborne transmission; however public health influence of viral infection associated with waterborne outbreaks is often underestimated (Carter 2005; Grabow 2007; Mena 2007; Kiulial *et al.*, 2010). Enteric viruses are shed in large numbers by infected individuals and can persist in the environment including surface water and ground water as well as circumvent wastewater treatment processes (Baggi and Peduzzi 2000; Carter 2005). Over one hundred types of enteric viruses may exist in faecally contaminated water (Fong and Lipp 2005), and their presence in water sources is of public health concern because of their low infectious dose (Wyn-Jones and Sellwood 2001; Fong and Lipp 2005; Teunis *et al.* 2008; Kiulial *et al.*, 2010).

In recent times, about two hundred and thirty four viruses have been identified in environmental matrices (such as surface water, human faeces, plants materials and sewage) (Prado *et al.*, 2014). Human enteric viruses may exist in water by adsorption onto solid matters or as free-floating, wastewater treatment process are such as flocculation, sedimentation, filtration and disinfection are designed to reduce their concentrations or inactivate them (Templeton *et al.*, 2008). Although, some of the viruses associated with large particle are removed during the treatment processes, viral particles attached to smaller colloidal materials may evade the process; some of these may persist in the environment, and

are capable of causing diseases upon ingestion, even at low doses (Li *et al*, 1998; Okoh *et al*, 2010). Some genera of enteric viruses which may be associated with waterborne transmission include noroviruses, sapovirus, rotavirus, orthoreovirus (Reovirus), astroviruses (Human astrovirus), Mastadenovirus (human adenovirus A- F), polyomavirus (JC; BK; KI; WU; MC), bocavirus (human bocavirus), hepevirus (hepatitis E virus), hepatovirus (hepatitis A virus), enterovirus (human enterovirus A-D; human rhinovirus A-C); kobovirus (Aichi virus), torque teno virus, picobirnavirus (human picobirnavirus), influenza virus and coronavirus (Carter 2005; Hamza *et al.*, 2011). Diseases caused by these include gastroenteritis, respiratory tract disease, pneumonia, keratoconjunctivitis, cystitis, progressive multifocal leukoencephalopathy, kidney nephritis, hepatitis, paralysis, meningitis, myocarditis, diabetes and influenza among others (Hamza *et al.*, 2011).

A few countries including South Africa incorporated in their constitution the right to good and safe water supply as a basic right for everyone. This is stated under the Bill of Rights in the South African constitution: “*everyone has the rights to have access to sufficient food and water*” (Constitution of South Africa, 1996 s27b). Every South African deserves clean, safe and affordable water (Odjadjare, 2010). In its attempt to ensure this right, the governments of South Africa have made several efforts at protecting and conserving the limited available freshwater resources in the country. For instance, in 1956, the government of South Africa made it mandatory through the South African Water Act (Act 54 of 1956) that effluents be treated to acceptable standards and returned to the water course from where water was originally obtained (Morrison *et al.*, 2001). Nevertheless, as the quest for water usage increased due to economic growth and human population growth, wastewater and sewage treatment facilities increasingly worked under increased pressure and this situation in turn put

burden on water and sanitation authorities to find ways to sustain the quality of water resources (Mema, 2009).

Remarkably, South Africa boasts one of the cleanest water systems in the world; however, lack of access to proper hygiene or sanitation facilities most especially in the country's rural communities has significantly led to steady increase in waterborne diseases (AgriPortal, 2015). For instance, the Vaal River which is the largest river in South Africa is increasingly being polluted by faecal materials due to lack of adequate sanitation facilities. The situation is so worrisome that a local water agency issued a directive that human contacts with the river water may cause severe infection (Groenewald, 2000; AgriPortal, 2015; The Water Project, 2015). A court injunction was issued to remove about 20 tonnes of dead fish from the river after a non-governmental organisation took the municipality authority to court for discharging raw sewage/wastewater into the river (The Water Project, 2015). The continuous pollution of surface waterbodies by the frequent discharge of wastewater and the use of water from these sources for domestic and agricultural purposes as observed in South Africa and other developing countries may present serious public health risk (Gemmell and Schmidt, 2012; Chigor *et al.*, 2013).

The Eastern Cape Province of South Africa is the second poorest province with 35.7% of households living below poverty level, this combined with the highest provincial unemployment rate in the country (Statistics South Africa, 2012). The Province largely comprises of rural settlements which mostly lack pipe-borne water supply and adequate sanitary infrastructures with the resultant reliance of the people on water from open sources (Okoh *et al.*, 2007) for domestic, recreational and agricultural purposes. River water and

beaches equally constitute vital economic resources to the Province. However, water from these sources are often polluted by discharges from municipal wastewater treatment facilities with it attendant public and environmental health implications (Fatoki *et al.*, 2003).

Although, the effect of water and sanitation is frequently studied in many developing countries across the globe, yet studies in this area do not usually lead to robust conclusions as to which variable is associated with the most health benefits and under which circumstances. Limited information is available in the literature on the detrimental impacts of incessant discharged of poorly treated effluents into the aquatic milieu of the Eastern Cape Province (Igbiosa and Okoh, 2009; Osode and Okoh, 2009; Odjadjare and Okoh, 2010). Reports from those studies have also been limited to physicochemical and bacteriological assessment, while there is no information on the virological assessments of the discharged effluents and the possible impacts on the limited available freshwater resources of the Province. In view of this, the current study is therefore designed to evaluate the incidence of enteric viruses, *Vibrio* spp. and *Escherichia coli* pathotypes in two wastewater treatment plants located in Keiskammahoek and Stutterheim in the Eastern Cape Province.

## **1.2 Aim and Objectives of the study**

The main **aim** of this study was to assess the incidences of some key microbial pathogens (including enteric virus, *Vibrio* species and *Escherichia coli* pathotypes) in the final effluents of two wastewater treatment plants (WWTPs) located in Keiskammahoek and Stutterheim in the Eastern Cape Province of South Africa. Specific objectives of this study are therefore:



1. To determine the physicochemical characteristics of the final effluents of the two WWTPs over a 12 month sampling period.
2. To evaluate the incidence of *Vibrio* spp. and *E. coli* pathotypes in final effluents.
3. To elucidate the antibiogram profiles and the presence of antibiotic resistance determinants in the confirmed isolated *E.coli* and *Vibrio* pathotypes.
4. To assess the occurrence and distribution of human viral pathogens in the final effluents.
5. To determine the correlation relationship between the assessed microbiological and the physicochemical qualities of the discharged effluents.

## References

- Aakame, R.B., Fekhaoui, M., Bellaouchou, A., El Abidi, A., El Abbassi, M. and Saoiabi, A. (2015).** Assessment of physicochemical quality of water from groundwater in the areas of Northwest on Morocco and health hazard. *J. Mater. Environ. Sci.* 6(5), 1228-1233.
- Abbaspour, S. (2011).** Water quality in developing countries, South Asia, South Africa, water quality management and activities that cause water pollution. 2011 International Conference on Environmental and Agriculture Engineering 2011, 15, 94-102.
- Abbott, S.L., Janda, J.M., Johnson, J.A. and Farmer, J.J. (2007).** *Vibrio* and related organisms, 9th ed. ASM Press, Washington, DC.
- Abdel-Raouf, N., Al-Homaidan, A.A and Ibraheem, I.B.M (2012).** Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences*, 19, 257-275.
- AgriPortal (2015).** Water in crisis – South Africa. Available on: [http://www.farmingportal.co.za/index.php?option=com\\_k2&view=item&id=4969:water-in-crisis-south-africa&tmpl=component&print=1](http://www.farmingportal.co.za/index.php?option=com_k2&view=item&id=4969:water-in-crisis-south-africa&tmpl=component&print=1). (Accessed 11 August 2015).
- Akpor, O.B and Muchie, M. (2011).** Environmental and public health implications of wastewater quality. *African Journal of Biotechnology*, 10(13), 2379–2387.
- Akpor, O.B, Ohiobor, G.O. and Olaolu, T.D. (2014).** Heavy metal pollutants in wastewater effluents: source, effects and remediation. *Advances in Bioscience and Bioengineering*. 2(4), 37–43.
- Al-Bayatti, K.K., Al-Arajy, K.H and Al-Nuaemy, S. (2012).** Bacteriological and physicochemical studies on Tigris River near the water purification stations within

Baghdad Province. *Journal of Environmental and Public Health*, Article ID 695253, 8 pages, 2012. doi:10.1155/2012/695253.

**Ashbolt, N.J., Grabow, W.O.K. and Snozzi, M. (2001).** Water Quality: Guidelines, Standards and Health. World Health Organization (WHO). Chapter 13: Indicators of Microbial Water Quality. p. 289-316.

**Awoyemi, M.O., Achudume, A.C. and Okoya, A.A. (2014).** The physicochemical quality of groundwater in relation to surface water pollution in Majidun Area of Ikorodu, Lagos State, Nigeria. *American Journal of Water Resources*, 2, 126-133.

**Baggi, F. and Peduzzi, R. (2000).** Genotyping of rotaviruses in environmental water and stool samples in southern Switzerland by nucleotide sequence analysis of 189 base pairs at the 5' end of the VP7 gene. *J Clin Microbiol*, 38, 3681–3685.

**Bayram, A., Onsoy, H., Bulut, V.N. and Akinci, G. (2013).** Influences of urban wastewater on the stream water quality: a case study from Gumushane Province, Turkey. *Environmental Monitoring and Assessment*, 185, 1285-1303.

**Berg, G. (1978).** The indicator system. In *Indicators of Viruses in Water and Food* (ed. G. Berg), pp.1-13, Ann Arbor Science Publishers, Ann Arbor, MI.

**Blignaut, J. and van Heerden, J. (2009).** The impact of water scarcity on economic development. *Water SA*, 35, 415-420.

**Carter, M.J. (2005).** Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *J Appl Microbiol*, 98, 1354–1380.

- Centers for Disease Control and prevention (CDC). (1998).** Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters – Pacific Northwest, 1997. *Morbidity and Mortality Weekly Report*, 47, 457–462.
- Chandra, G. (2014).** Water climate and energy. *International journal of Technical Research and Applications*, 2(2), 73-77.
- Chigor, V.N., Sibanda, T. and Okoh, A.I. (2013).** Variations in the physicochemical characteristics of the Buffalo Rivers in the Eastern Cape Province of South Africa. *Environmental Monitoring and Assessment*, 185, 8733-8747.
- Cho, H.B., Lee, S.H., Cho, J.C. and Kim, S.J. (2000).** Detection of adenoviruses and enteroviruses in tap water and river water by reverse transcription multiplex PCR. *Canadian Journal of Microbiology*, 46, 417-424.
- Chow, V.T., Eliason, R., and Linsley, R.K. (1972).** Development and trends in wastewater Engineering. In *Wastewater Engineering*. McGraw-Hill Book Company. New York, St. Louis, Dusseldorf, Johannesburg, Kuala Lumpur, London, Mexico, Montreal, New Delhi, Panama, Rio de Janeiro, Singapore, Sydney, Toronto. pp. 1-11.
- Constitution of Republic of South Africa (1996)**, (Chapter two, Section Seven Bill of Rights), As adopted on 8 May 1996 and amended on the 11 October 1996 by Constitutional Assembly, Act No 108 of 1996. Available on: <http://www.justice.gov.za/legislation/constitution/SACConstitution-web-eng.pdf> (Accessed 07 September 2015).
- Curtis, V., Cairncross, S. and Yonli, R. (2000).** Domestic hygiene and diarrhoea-pinpointing the problem. *Trop Med Int Health*, 5(1), 22-32.

- Daniels, N.A., MacKinnon, L.C., Bishop, R., Altekruise, S., Ray, B., Hammond, R.M., Thompson, S., Wilson, S., Bean, N.H., Griffin, P.M. and Slutsker, L. (2000).** *Vibrio parahaemolyticus* infections in the United States, 1973– 1998. *Journal of Infectious Diseases*, 181, 1661–1666.
- Dechet, A.M., Yu, P.A., Koram, N. and Painter, J. (2008).** Non-foodborne *Vibrio* infections: an important cause of morbidity and mortality in the United States, 1997– 2006. *Clin. Infect. Dis.*, 46, 970–976.
- Department of Environment Affairs and Tourism (DEAT) (2006).** South Africa environment outlook. A report on the state of the environment. Department of Environmental Affairs and Tourism, Pretoria. Available on: [https://www.environment.gov.za/sites/default/files/docs/message\\_synthesis.pdf](https://www.environment.gov.za/sites/default/files/docs/message_synthesis.pdf) (Accessed 22 April, 2015).
- Desmarais, T.R., Solo-Gabriele, H.M. and Palmer, C.J. (2002).** Influence of soil on faecal indicator organisms in tidally influenced subtropical environment. *Appl. Environ. Microbiol.* 68, 1165-1172.
- Eisenberg, J.N.S., Scott, J.C. and Porco, T. (2007).** Integrating disease control strategies: balancing water sanitation and hygiene interventions to reduce diarrheal burden. *American Journal Public Health*, 97(5), 846-852.
- Espinosa, A.C., Arias, C.F., Sanchez-Colon, S. and Mazari-Hiriart, M. (2009).** Comparative study of enteric viruses, coliphages and indicator bacteria for evaluating water quality in a tropical high-altitude system. *Environmental Health*, 8, 49. doi:10.1186/1476-069X-8-49.

- Fadaeifard, F., Raissy, M., Faghani, M., Majilesi, A. and Farahani G.N. (2012).** Evaluation of physicochemical parameters of waste water from rainbow trout fish farm and their impacts on water quality of Koohrang stream – Iran. *International Journal of Fisheries and Aquaculture*, 4, 170-177.
- Falvo, C.E. (2010).** In, Women's global health and human rights. Murthy, P. and Smith, C.L. (eds). Jones and Bartlett Publishers Sudbury, MA, United States.
- Fatoki, S.O. Gogwana, P. and Ogunfowokan, A.O. (2003).** Pollution assessment in the Keiskamma River and in the impoundment downstream. *Water South Africa*, 29(3), 183-189.
- Fewtrell, L., Kaufmann, R.B., Kay, D, Enanoria, W., Haller, N. and Colford, J.M., Jr. (2005).** Water, sanitation, and hygiene intervention to reduce diarrhoea in less developed countries: a systemic review and meta-analysis. *Lancet Infect Dis*. 5(1), 42-52.
- Fong, T.T. and Lipp, E.K. (2005).** Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews*, 69, 357–371.
- Gardea-Torresdey, J.I., Peralta-Videa, J.R., Rosa, G.D. and Parsons, J.G (2005).** Phytoremediation of heavy metals and study on the metal coordination by X-ray absorption spectroscopy. *Coordination Chemistry Review*. 249 (17-18), 1797–1810.
- Gemmell, M.E. and Schmidt, S. (2012).** Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa. *Food Res. Int.*, 47, 300–305.

- Gerba, C.P (2008).** Pathogen removal. In *Biological wastewater treatment: principles, modelling and design*. Henza, M., Loosdrecht, M., Ekama, G. and Brdjanovic, D. IWA Publishing, Cambridge University Press, London UK.
- Govender, T., Barnes, J.M. and Pieper, C.H. (2011).** Contribution of water pollution from inadequate sanitation and housing quality to diarrheal disease in low-cost housing settlements of Cape Town, South Africa. *American Journal of Public Health, 101(7)*, e4-e9.
- Grabow, W.O.K. (2007).** Overview of health-related water virology. In, *Human Viruses in Water*. Bosch, A. (ed.). 1–25. Amsterdam: Elsevier B.V.
- Groenewald, M. (2000).** Bioaccumulation of metals and the general health of fish from the Vaal Dam and Vaal River Barrage. Masters Dissertation, Rand Afrikaans University South Africa.
- Hamza, I.A., Jurzik, L., Uberla, K. and Wilhelm, M. (2011).** Methods to detect infectious human enteric viruses in environmental water sample. *International Journal of Hygiene and Environmental Health, 214(6)*, 424-436.
- Hlady, W.G. and Klontz, K.C. (1996).** The epidemiology of *Vibrio* infections in Florida, 1981–1993. *J. Infect. Dis.* 173, 1176–1183
- Igbinosa, E.O. and Okoh, A.I. (2009).** Impact of discharged wastewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community. *International Journal of Environmental Science and Technology, 6*, 175-182.
- Iwamoto, M., Ayers, T., Mahon, B.E. and Swerdlow, D.L. (2010).** Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews, 23(2)*, 399–411.

- Janda, J.M., Powers, C., Bryant, R.G. and Abbott, S.L. (1988).** Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin. Microbiol. Rev.*, 1, 245–267.
- Jern, W.N.G. (2006).** Industrial wastewater treatment. Singapore: Imperial College Press.
- Joshi, D.M., Bhandari, N.S., Kumar, A. and Agrawal, N. (2009).** Statistical analysis of physicochemical parameters of water of River Ganga in Haridwar District. *Rasayan J. Chem.*, 2, 579-587.
- Jurzik, L., Hamzaa, I.A., Puchert, W., Uberla, K. and Wilhelm, M. (2010).** Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. *International Journal Hygiene and Environmental Health*, 213, 210-216.
- Kiulia, N.M., Netshikweta, R., Page, N.A., van Zyl, W.B., Kiraithe, M.M., Nyachio, A., Mwenda, J.M. and Taylor, M.B. (2010).** The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special reference to rotaviruses. *Journal of Applied Microbiology*, 109, 818-828.
- Klontz, K.C., Lieb, S., Schreiber, M., Janowski, H.T., Baldy, L.M. and Gunn, R.A. (1988).** Syndromes of *Vibrio vulnificus* infections. Clinical and epidemiologic features in Florida cases, 1981–87. *Ann. Intern. Med.*, 109, 318–323.
- Kraemer RA, Choudhury K, Kampa E (2001)** Protecting water resources: pollution prevention. Thematic Background Paper .International Conference on Freshwater, Bonn 2001.
- Levine, W.C., and Griffin, P.M. (1993).** *Vibrio* infections on the Gulf coast: results of first year of regional surveillance. *Journal of Infectious Diseases*, 167, 479–483.



- Li, W.J., Xin, W.W., Rui, Q.Y., Song, N., Zhang, F.G., Ou, Y.C. and Chao, F.H. (1998).** A new and simple method for concentration of enteric viruses from water. *J. Virol. Meth.*, 74, 99-108.
- Lin, J. and Ganesh, A. (2013).** Water quality indicators: bacteria, colphages, enteric viruses. *International Journal of Environmental Health Research* 23(6), 484-506.  
DOI:10.1080/09603123.2013.769201.
- Love, J. and Luchsinger, V. (2014).** Sustainability and water resources. *Journal of Sustainability and Green business*, 2(1), 1-12.
- Lusk, M., Toor, G.S. and Obreza, T. (2014).** Onsite sewage treatment and disposal systems: bacteria and protozoa. Available on: <http://edis.ifas.ufl.edu/ss552> (Accessed 02 September 2015).
- Mara, D. (2003).** Domestic wastewater treatment in developing countries. Published by Earthscan UK.
- Mema, V. (2009).** Impact of poorly maintained wastewater and sewage treatment plants: lessons from South Africa. Council for Scientific and Industrial Research (CSIR) Pretoria, South Africa, 2009.
- Mena, K.D. (2007).** Waterborne viruses: assessing the risks. In, *Human Viruses in Water* ed. Bosch, A. pp. 163–175. Amsterdam: Elsevier B.V.
- Miagostovich, M.P., Ferreira, F.F., Guimraes, F.R., Fumian, T.M., Diniz-Mendes, L., Luz, S.L., Morens, D.M., Folkers, G.K. and Fauci, A.S. (2004).** The challenge of emerging and re-emerging infectious diseases. *Nature* 430, 242-249.

- Momba, M.N.B., Osode, A.N. and Sibewu, M. (2006).** The impact of inadequate wastewater treatment on the receiving water bodies – Case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *Water SA*, 32, 687-692.
- Morris, J.G. and Black, R.E. (1985).** Cholera and other vibrios in the United States. *N Engl J Med* 312, 343-50.
- Morrison, G., Fatoki, O.S., Persson, L. and Ekberg, A. (2001).** Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River - pH, electrical conductivity, oxygen-demanding substrate (COD) and nutrients. *Water SA*, (27)4, 475-480.
- Myers, D.M. and Sylvester, M.A. (1997).** Biological indicators, faecal indicator bacteria in National field manual for the collection of water-quality data: U.S. Geological Survey Techniques of Water-Resources Investigations book 9, chapter A7.1, variously paginated.
- Naidoo, S. and Olaniran, A.O. (2014).** Treated wastewater effluents as a source of microbial pollution of surface water resources. *International Journal of Environmental Research and Public Health*, 11, 249-270.
- National Science Foundation (NSF) (2011).** Water sustainability and climate. Available on: [www.nsf.gov/pubs/2010/nsf10524/nsf10524.htm](http://www.nsf.gov/pubs/2010/nsf10524/nsf10524.htm). (Accessed 10 August 2015).
- Nugent, R. (2008).** Chronic diseases in developing countries health and economic burdens. *Annals of the New York Academy of Sciences*, 1136, 70-79.
- O'Hogain, S., McCarton, L., McIntyre, N., Pender, J. and Redi, A. (2011).** Physicochemical and microbiological quality of water from a pilot domestic rainwater harvesting facility in Ireland. *Water and Environmental Journal*, 25, 498-494.

- Odjadjare, E.E. (2010)** Prevalence of *Listeria* pathogens in effluents of some wastewater treatment facilities in the Eastern Cape Province of South Africa. PhD. Thesis University of Fort Hare.
- Odjadjare, E.E. and Okoh, A.I. (2010).** Physicochemical quality of and urban municipal wastewater effluent and its impact on the receiving environment. *Environmental Monitoring and Assessment*, 170, 383-394.
- Odonkor, S.T. and Ampofo, J.K. (2013).** *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, 4, e2.
- Oilgae (2015).** New Technologies in Wastewater Treatment. Available on: <http://www.oilgae.com/algae/cult/sew/new/new.html>. (Accessed 10 August 2015).
- Okoh, A.I., Odjadjare, E.E., Igbinsosa, E.O. and Osode, A.N. (2007).** Wastewater treatment plants as a source of microbial pathogens in the receiving watershed. *Afri. J. Biotechnol.*, 6, 2932-2944.
- Okoh, A.I., Sibanda, T. and Gusha, S.S. (2010).** Inadequately Treated Wastewater as a Source of Human Enteric Viruses in the Environment. *Int. J. Environ. Res. Public Health* 7, 2620-2637.
- Osode, A.N. and Okoh, A.I. (2009).** Impact of discharged wastewater final effluent on the physicochemical quality of a receiving watershed in a suburban community of the Eastern Cape Province. *Clean*, 37, 938-944.
- Payment, P., Siemiatycki, J., Richardson, L., Renaud, G., Franco, E. and Prevost, M. (1997).** A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. *International Journal of Environmental Health Research*, 7, 5-31.

- Platikanov, S., Rodriguez-Mozaz, S., Huerta, B., Barcelo, D. Cros, J., Batle, M., Poch, G. and Tauler, R. (2014).** Chemometrics quality assessment of wastewater treatment plants effluents using physicochemical parameters and UV absorption measurements. *Journal of Environmental Management*. 140, 33-44.
- Popa, P., Timofti, M., Voiculescu, M., Dragan, S., Trif, C and Georgescu, L.P. (2012).** Study of physicochemical characteristics of wastewater in an urban agglomeration in Romania. *The Scientific World Journal*. Volume 2012, Article ID 549028, 10 pages.
- Prado, T., Gaspar, A.M.C. and Miagostovich, M.P. (2014).** Detection of enteric viruses in activated sludge by feasible concentration methods. *Brazilian Journal of Microbiology*, 45(1), 343–349.
- Pruss-Ustun, A. Bos, R., Gore, F. and Bartram, J. (2008).** Safer water, better health: costs, benefits and sustainability of interventions to protect and promote health. Geneva: World Health Organisation.
- Pusch, D., Oh, D.Y., Wolf, S., Dumke, R., Schroter-Bobsin, U., Hohne, M., Roske, I. and Schreier, E. (2005).** Detection of enteric viruses and bacterial indicators in German environmental waters. *Archives of Virology*, 150, 929-947.
- Scott, T., Lipp, E., Rose, J. (2004).** The effect of climate change on waterborne disease. In, *Microbial Waterborne Pathogens*. Cloete, T.E., Rose, J., Nel, L.H. and Ford, T. (Eds.). IWA Publishing, London, UK; pp. 143-154.
- Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R. and Lukasik, J. (2002).** Microbial source tracking: current methodology and future directions. *Appl. Environ. Microbiol.* 68, 5796-5803.

- Serpa, D., Keizer, J.J., Cassidy, J., Cuco, A., Silva, V., Goncalves, F., Cerqueira, M. and Abrantes, N. (2014).** Assessment of river water quality using an integrated physicochemical, biological and ecotoxicological approach. *Environmental Science: Processes & Impacts*, 16, 1434-1444.
- Shapiro, R.L., Altekruze, S., Hutwagner, L., Bishop, R., Hammond, R., Wilson, S., Ray, B., Thompson, S., Tauxe, R. and Griffin, P.M. (1998).** The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996. *J. Infect. Dis.*, 178, 752–759.
- Sheshe, M.U. and Magashi, A. M. (2014).** Assessment of physicochemical quality of sachet water produced in selected local government areas of Kano metropolis, Kano State-Nigeria. *Bayero Journal of Pure and Applied Science*, 7, 31-35.
- Statistics South Africa (2012).** Poverty profile of South Africa: application of the poverty lines on the LCS 2008/2009/Statistics South Africa. Pretoria.
- Sulieman, A.M.E.H., Yousif, A.W.M. and Mustafa, A.M. (2010).** Chemical, physicochemical and physical properties of wastewater from the Sudanese fermentation industry (SFI). In, Fourteenth International Water Technology Conference. Cairo, Egypt, pp 305-315.
- Tabor, M., Kibret, M. and Abera, B. (2011).** Bacteriological and physicochemical quality of drinking water and hygiene-sanitation practices of the consumers in Bahir Dar city, Ethiopia. *Ethiop. J. Sci*, 21, 19-26.
- Tebbut, T.H.Y. (1992).** *Principles of Water Quality*. Pergamon Press, Oxford, England; pp. 1 – 251.

- Templeton, M.R., Andrews, R.C., Hofmann, R. (2008).** Particle-associated viruses in water: impacts on disinfection processes. *Critical Revs. Environ. Sci. Tech.*, 38, 137–164.
- Teunis, P.F.M., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J. and Calderon, R.L. (2008).** Norwalk virus: how infectious is it. *J Med Virol*, 80, 1468–1476.
- The Water Project (2015).** Water-crisis in South Africa. Available on: <http://thewaterproject.org/water-in-crisis-south-africa> (Accessed 08 August 2015).
- Thompson, F.L., Gevers, D., Thompson, C.C., Dawyndt, P., Naser, S., Hoste, B., Munn, C.B. and Swings, J. (2005).** Phylogeny and Molecular Identification of *Vibrios* on the basis of multilocus sequence analysis. *Applied and Environmental Microbiology* 71(9), 5107-5115.
- World Bank (2007).** Coping with Scarce Water in the Middle East and North Africa. Available on: <http://web.worldbank.org/WBSITE/EXTERNAL/NEWS/0,,contentMDK:21253203> (Accessed 10 August 2015)
- World Health Organisation (WHO) (2003a).** Water, sanitation and health programme: Health and human rights publications series; no 3. Available on: [www.who.int/hhr](http://www.who.int/hhr). (Accessed 14 June 2015).
- World Health Organisation (WHO) (2003b).** Emerging issues in water and Infectious Disease. Geneva: World Health Organization. Available on: [https://www.who.int/water\\_sanitation\\_health/emerging/emerging.pdf](https://www.who.int/water_sanitation_health/emerging/emerging.pdf) (Accessed 22 April, 2015).

**World Health Organisation (WHO) (2004).** Guidelines for drinking water quality 3rd edition. Volume 1 Recommendations. World Health Organization, Geneva. Available on: <http://www.who.int/> (Accessed 22 April, 2105).

**World Health Organisation (WHO) (2008).** Guidelines for drinking water quality 3rd edition. Volume 1 Recommendations. World Health Organization, Geneva.

**World Health Organisation (WHO) (2011).** Water, sanitation and hygiene interventions and the prevention of diarrhoea. Available on: [www.who.int/elena/title/bbc/wsh\\_diarrhoea/en/](http://www.who.int/elena/title/bbc/wsh_diarrhoea/en/) (Accessed 06 August 2015).

**World Health Organisation/United Nations International Children's Emergency Fund (WHO/UNICEF) (2010).** WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation. Progress on sanitation and drinking-water 2010 update. Geneva, World Health Organization, 2010. Available on: [http://www.who.int/water\\_sanitation\\_health/publications/9789241563956/en/](http://www.who.int/water_sanitation_health/publications/9789241563956/en/). (Accessed 05 August 2015).

**World Health Organisation/United Nations International Children's Emergency Fund (WHO/UNICEF) (2000).** WHO / UNICEF Joint Monitoring Programme for Water Supply and Sanitation, Global Water Supply and Sanitation Assessment 2000 Report. WHO / UNICEF, Washington, 2000.

**Wyn-Jones, A.P. and Sellwood, J. (2001).** A review: enteric viruses in the aquatic environment. *Journal of Applied Microbiology*, 91, 945–962.

## **CHAPTER TWO**

### **Literature Review**



## 2.1 Introduction

Water is a unique and finite resource vital in all human activities. It is pivotal for maintaining an adequate supply of food and the sustenance of a productive environment to support the teeming human and animal populations (Pimentel *et al.*, 2004). Global demand for freshwater is growing rapidly in accordance with increasing human population, rapid urbanisation and economic expansion. The ever-increasing world population, changes in consumption habits and the urbanisation growth have raised concerns about the capacity of many nations sustainability capacity; most importantly with regards to freshwater availability (Fernandez-Jauregui, 2010). The present situation about requirements for water, and forecast for water demand in the nearest future, coupled with the fact that more than one third of the world population currently suffers from some degree of water shortage has led to the perception of a critical situation which may turn into a global water crisis (Fernandez-Jauregui, 2010).

The debilitating effects of global water shortages include: threats to human food supply, severe reduction in aquatic and terrestrial ecosystems biodiversity, spread of serious human diseases and acute water quality degradation (Postel *et al.*, 1996, Pimentel *et al.*, 2004). Water quality issue is one of the major challenges facing humanity presently. Pollution has no bounds: approximately 90% of wastewater in developing countries is documented as untreated and more often than not flows into surface waters such as river, lakes and highly productive coastal zones (UNESCO, 2013). Socio-economic, as well as environmental factors such as hydrological variability and climate change compound this problem; more so in developing countries, where water, sanitation and hygiene (WaSH) related diseases still pose a great risk to billions of people (Naidoo and Olaniran, 2014).

## **2.2 Wastewater treatment plants as important sources of pollution**

Water pollution sources can be categorised as either point or non-point. Runoff of polluting substances into waterways constitute a nonpoint source of pollution, while point source pollution occurs when polluting substances are released directly in high concentrations into waterways (Alemad *et al.*, 2013). Wastewater or sewage can be described as used water discharges from home, businesses, industries, cities and agriculture facilities (Asano *et al.*, 2007). Municipal wastewater usually comprises a mixture of one or more of domestic effluents (consisting of blackwater from toilets and greywater from kitchen and bathing); water discharges from commercial centres (including hospitals); industrial effluents, stormwater and other urban runoff (Mateo-Sagasta *et al.*, 2015). There is limited available data/information in literature on the generation, treatment quality and usage of wastewater effluents in many countries. Of the limited information available, only about 37% of the data could be regarded as recent (reported between, 2008 and 2012) (Sato *et al.*, 2013). The available figures show that 70% of wastewater generated in high-income countries are treated, while upper-middle-income, lower-middle-income, and low-income countries have 38%, 28% and 8% of their generated wastewater treated respectively (Sato *et al.*, 2013). The United Nations, however, estimates that over 1,500 km<sup>3</sup> of wastewater is produced annually, a figure representing six times more water than exists in all the world's rivers combined (UN WWAP, 2003; Qureshi *et al.*, 2015).

The volumes of municipal wastewater generated have drastically increased over the past decades as a result of rapid population growth and overreliance on shrinking freshwater resources. Efficient wastewater treatment systems are indicative of the level of development within a municipality and reflect on public health, with the degree and quality of discharged wastewater effluents defining the impact of treatment facilities on environmental water

sources which often serve as receiving watersheds (DWA, 2011). Consequentially, consistent monitoring of the working conditions of existing wastewater treatment facilities and emphasis on environmental water and community health has become important factors in determining the quantity and quality of wastewater generated by respective municipalities (Naidoo and Olaniran, 2014).

Wastewater treatment or water reclamation involves the use of several combinations of chemical, biological and mechanical/electrical processes to treat municipal and industrial effluent discharges, in order to remove, reduce or neutralise pollutants before the effluents are released back to the environment or reused. A typical water reclamation installation combines mechanical and electrical technologies to remove solids, dissolved and suspended materials, as well as nutrients such as nitrogen and phosphorus compounds from wastewater. Numerous factors are often considered when selecting water reclamation technology. Some of the important factors include: the characteristics and source of the effluent; reclaimed water objectives; water reuse applications; process flexibility and compatibility with existing conditions; operational and maintenance requirements; residual disposal options and environmental restrictions (Asano *et al.*, 2007; NRC, 2012). Table 2.1 below shows different levels of wastewater treatment and the various technologies often employed.

Established water reclamation technologies such as activated sludge and trickling filters have aided in the management of municipal and industrial wastewater effluents hitherto. However, the need for better management of millions of gallons of wastewater generated on daily basis, and rising global standards of living motivate the need for improved technology development

for enhanced wastewater management (Daigger *et al.*, 2008). In addition to these, pollution from large amounts of residual sludge production, high energy requirements

**Table 2.1** Wastewater treatment advancement levels and the various technologies employed.

<b>Treatment Level</b>	<b>Examples of Technologies Employed</b>
Primary treatment	Bar or bow screen, grit removal, primary sedimentation, comminution, oil/fat removal, flow equalisation, pH neutralisation and Imhoff tank
Secondary treatment	Activated sludge, extended aeration, aerated lagoon, trickling filter, rotating bio-discs, anaerobic treatment/UASB, anaerobic filter, stabilisation ponds, constructed wetlands, aquaculture wetlands and aquaculture
Tertiary treatment	Nitrification, denitrification, chemical precipitation, disinfection, direct filtration, chemical oxidation, biological P removal, constructed wetlands and aquaculture
Advance treatment	Chemical treatment, reverse osmosis, electrodialysis, carbon adsorption, selective ion exchange, hyperfiltration and detoxification

*Source: Veenstra et al. (1997).*

(increasing carbon footprint), emission of greenhouse gases (GHG; including CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>) and lack of effective response to diurnal seasonal or long-term variations are some of the shortcomings of the current available treatment technologies (Cao *et al.*, 2011).

Innovative combinations of established water reclamation technologies and more recent advances in treatment technologies/researches (mostly incorporating anaerobic carbon oxidation and phototrophic technologies) (Shoener *et al.*, 2014), have led to the emergence of some less energy-consuming, less expensive and more environmental friendly systems of wastewater treatment. One of such system is the Aquanos Energy's Algae Based System; a system which harnesses the natural symbiotic association between bacteria and algae to achieve approximately 90% reduction in the amount of energy required to operate a wastewater treatment plant, reduces treatment systems' operational cost by about 40-60%, and eliminates chemical-dependent system, thereby reducing overall capital expenditure (Fosshage, 2014).

Some of the emerging technologies (many at different developmental stages) are described below.

- ❖ Research stage – these are technologies at the developmental stage and/or have been tested in the laboratory or bench scale only e.g. Anaerobic Membrane BioReactor (An-MBR), Microbial Fuel Cell (MFC) and Anaerobic Migrating Blanket Reactor (AMBR);
- ❖ Emerging stage – these are technologies that have been tested at a pilot or demonstrations scale, or have been implemented at full scale in three or fewer

installations or for less than one year e.g. Multi-Stage Activated Biological Process (MSABP™), Aerobic Granular Sludge Process (AGSP), Critical Oxygen Point Control;

- ❖ Innovative stage – these are technologies that have been implemented at full scale for less than five years, or have some degree of initial use e.g. Multi-stage Filtration, Treatment Shaft, Bioaugmentation, Cyclic Metabolic Environment etc; and
- ❖ Adaptive use stage – these are established technologies that are being modified or adapted, thereby resulting in emerging technology e.g. Modified University of Cape Town (MUCT) Process, Westbank Process, Actiflo® Process among others (US EPA, 2013).

### **2.3 Water pollution and human diseases**

Water pollution is caused by a variety of natural and human activities and involves the alteration of the physical and chemical nature of water. Water pollution destroys the natural ecosystems that support human and environmental health and biodiversity. Biodiversity of freshwater can be altered by excessive accumulation of nutrients such as nitrogen and phosphorus which are often added to the soil in the form of fertilizers or animal manure. High concentration of these minerals in water leads to eutrophication, usually causing algal blooms. This in turn may lead to the release of harmful toxins during the decay of the cyanobacteria (Chakraborty and Mukhopadhyay, 2014). High concentrations of nitrates and nitrites in fresh or underground drinking water environments may also result from the excessive use of nitrogenous fertilizers; this may result in a life-threatening disease called methemoglobineamia or ‘blue baby’ syndrome in infants (Navarro and Zagmutt, 2009). The release of heated water from industries such as thermal stations or municipal wastewater into

surface waters causes thermal pollution, damaging aquatic organisms, and leading to disruption of ecological balance of water (Cheppi, 2012).

Microbial water quality assessment deals with the microorganisms that may be present in water. Although, microbial water quality has been measured by endpoint monitoring for faecal indicators, innumerable evidences have shown lack of correlation between pathogens and indicator organisms in water systems (Jofre and Blanch, 2010; Odonkor and Ampofo, 2013). Acute disease hazards associated with pathogenic microorganisms originates from the presence of bacteria, viruses, fungi, protozoa and algae which can find their way into surface, recreational and groundwater intended for drinking and spread via the faecal oral route (Cabral, 2010; Schwarzenbach *et al.*, 2010; Shannon *et al.*, 2011). Other parasites such as zoonotic worms (e.g. helminths) may also be transmitted to humans through contamination of water sources or by zoonotic transmission (Otranto and Eberhard, 2011).

The adverse health effects associated with the presence of disease-causing contaminants in water are numerous, and may include: gastrointestinal illnesses, acute respiratory diseases, neurological disorders, skin diseases, reproductive problems and more importantly diverse forms of diarrhoea. For instance, waterborne disease outbreaks were at the top of the list of outbreaks of infectious diseases between 1998 and 2001 in 132 countries, cholera being the most common disease, followed by acute infectious diarrhoea, legionellosis and typhoid fever (WHO, 2002; Schwarzenbach *et al.*, 2010). The reappearance of cholera in Africa (accounting for about 94% of the total global cases during that period) was alarming, considering its absence for almost about a century. Typhoid and paratyphoid fever were the next common waterborne diseases outbreak reported. Similarly, waterborne outbreaks

associated with viral agents such as hepatitis A, hepatitis E, and rotaviruses, as well as parasitic protozoa have been associated with lack of access to improved water sources and inadequate hygiene (Ashbolt, 2004; Schwarzenbach *et al.*, 2010).

#### **2.4 Mechanisms and pathogenesis of infectious diarrhoea**

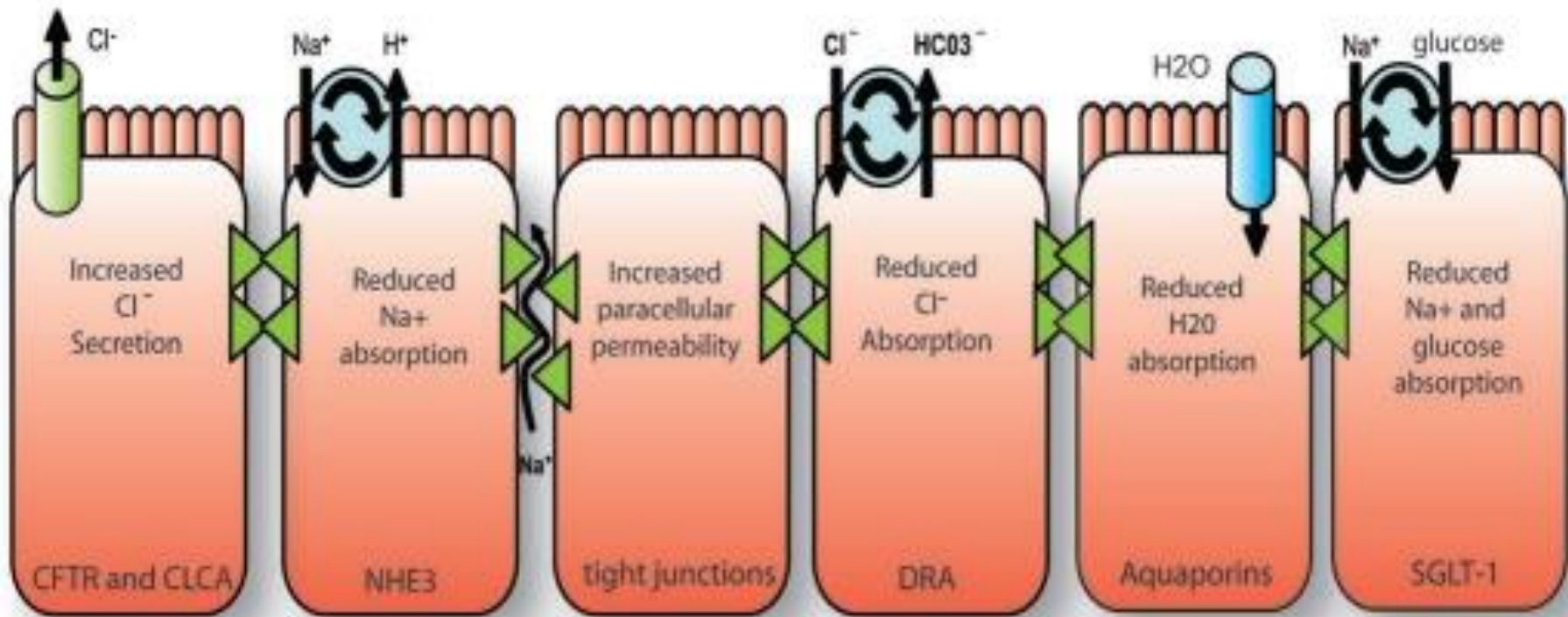
Diarrhoea is described as the passing of three or more watery stools per day (Grandy *et al.*, 2010). It is usually a symptom of a variety of bacterial, viral and parasitic infection in the gastrointestinal tract. Even though, diarrhoea is preventable and treatable, the World Health Organisation (WHO) reported this scourge as still the second leading cause of mortality; particularly in children and infants, with about 760 000 cases of diarrhoea associated death in children under 5 years old annually (WHO, 2015a). Globally, about 1.7 billion diarrhoeal disease cases are reported every year, with half of the diarrhoeal death cases occurring in five countries, namely: India, Pakistan, Nigeria, Afghanistan and Ethiopia (UNICEF, 2012; Ramanaiah *et al.*, 2015).

Polluted water and lack of adequate sanitation or poor hygiene have been the major cause of about 90% cases of child deaths from diarrhoea diseases (Schlipkoter and Flahault, 2010). According to UNICEF (2013), deaths of under-five aged children occur mainly in five countries including: India, Nigeria, Pakistan, China and the Democratic Republic of Congo (DRC). Two of these countries, namely, India and Nigeria accounted for more than 1/3 of all under-five's deaths, while these two countries also have substantial fraction of their human populations lacking access to improved water sources and adequate sanitation. UNICEF (2013) statistics paint a drastic global picture of population (out of about 783 million) living without improved drinking water thus: China - 119 million; India 97 million; Nigeria 66



million; DRC 36 million and Pakistan 15 million. Similarly, statistics for populations without improved sanitation are: India - about 814 million; China 477 million; Nigeria 109 million; Pakistan 91 million and DRC 50 million.

Death from diarrhoea often results from malnutrition and dehydration, while children with impaired immunity and immunocompromised adults are vulnerable to life-threatening diarrhoea (WHO, 2015a). This disease manifests when the gastro-intestinal tract loses its ability to absorb fluids and electrolytes, thus leading to altered balance towards net secretion (Hodges and Gill, 2010). Different cellular and molecular mechanisms have been proposed for pathogen-induced diarrhoea; however, diarrhoea basically involves the alteration of many cellular functions such as ion absorption and secretion, barrier function, and membrane trafficking pathways, thus leading to accumulation of fluid in the intestine (Buccigrossi and Spagnuolo, 2015). The net shift in the movement of ions may occur either through molecule transporters or the lateral spaces between cells, which are controlled by tight junctions as shown in Figure 2.1. Some ion transport processes are principally associated with diarrhoea and include cystic fibrosis transmembrane conductance regulator (CFTR) and  $\text{Ca}^{2+}$ -activated chloride channel (CLCA), which are chloride channels, and the  $\text{Na}^+/\text{H}^+$  exchange isoform (NHE3), which is involved in  $\text{Na}^+$  absorption (Hodges and Gill, 2010). The afore-mentioned authors further explain that changes in tight junctions create a pathway for the migration of both ions and water. Downregulated in adenoma (DRA) is responsible for chloride absorption and is associated with congenital chloride diarrhoea. The actual role of aquaporins is not clear; however, they are thought to contribute to diarrhoea when absorption is reduced; on the other hand, sodium and glucose transporter (SGLT-1) is tightly coupled with the movement of water, and is the reason for oral rehydration using glucose to enhance sodium absorption (Hodges and Gill, 2010).



**Figure 2.1:** A schematic diagram showing the general molecular mechanisms triggering diarrhoea. *Sources:* Hodges and Gill (2010); Buccigrossi and Spagnuolo (2015).

Diarrhoea manifestation generally follows one or more of the four mechanisms listed below:

1. Inflammatory and infectious diarrhoea: it is caused by two groups of pathogen-cytotoxin producing, non-invasive organisms (e.g. enterohaemorrhagic *E. coli*, enteroaggregative *E. coli*, and *Clostridium difficile*), or by invasive microorganisms (e.g. *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Entamoeba histolytica*). The cytotoxin-producing organisms adhere to the mucosa, activate cytokines and stimulate the intestinal mucosa to release inflammatory mediators. Invasive organisms, which may also produce cytotoxin, invade the intestinal mucosa to induce an acute inflammatory reaction, consequently involving the activation of cytokines and inflammatory mediators (Navaneethan and Giannella, 2008). Viral causes of infectious diarrhoea include coronaviruses, norovirus, rotaviruses and parvoviruses, while protozoa such as coccidian species and *Giardia* have also been implicated in infectious diarrhoea (Kiser *et al.*, 2008; Navaneethan and Giannella, 2008; Greenberg and Estes, 2009; Patel *et al.*, 2009);
2. Osmotic diarrhoea: it occurs due to ingestion of poorly absorbed substrates or malabsorption of osmotically-active substances (salt, sugar or water) in the small intestine. It is common in adult humans who are lactose intolerant resulting from lack of lactase enzyme to break down lactose into glucose and galactose for absorption. The osmotically-active lactose remains in the lumen where it absorbs water. Osmotic diarrhoea usually stops when the patient is fasted or stops the consumption of the poorly absorbed substrates (Bowen, 2006; Rehydration Project, 2014);
3. Secretory diarrhoea: This occurs when the rate of secretion in the mucosa exceeds the absorption rate (Castro-Rodriguez *et al.*, 1997; Bowen, 2006). Secretory diarrhoea mechanisms often follow strong activation of adenylyl cyclase by bacterial toxins

such as the cholera toxin, thereby leading to prolonged increase of cAMP concentration within crypt enterocytes.

The A<sub>1</sub> subunit of the cholera toxin is a NAD (nicotinamide adenine dinucleotide) dependent ribosyl transferase which covalently bonds ADP (adenosine diphosphate) ribose to G protein (guanine nucleotide binding protein), leading to activation of G, the catalytic unit of the adenylate cyclase enzyme. These changes result in the opening of the chloride channels for extended periods of time, thereby leading to uncontrolled secretion of water from the crypts (Farthing, 1993, 2002; Navaneethan and Giannella, 2010). Moreover, the cholera toxin may also stimulate the enteric nervous system, thereby causing independent secretion (Bowen, 2006). Other microbial causes of secretory diarrhoea include *E. coli* heat labile toxins (LT1 and LT2), Giardia, Cryptosporidium, rotaviruses, noroviruses etc. (Schiller, 1999; Bowen, 2006; Navaneethan and Giannella, 2010);

4. Diarrhoea associated with deranged motility: For proper nutrient and water absorption to occur, there is need for adequate exposure to mucosal epithelium and enough retention time. Increase in bowel motion (such as irritable bowel syndrome and thyrotoxicosis) leads to rapid transit and prevents sufficient absorption time resulting in diarrhoea even though mucosal absorptive capacity is not altered (Sung, 2002; Shah, 2004;). On the other hand, diseased conditions such as, diabetes mellitus, systemic sclerosis and intestinal pseudoobstruction can impair bowel mobility thus encouraging bacterial growth in the small intestine leading to disruption of digestion and altered electrolyte transport (Sung, 2002; Shah, 2004).

## 2.5 *Escherichia coli* as aetiological agent of acute diarrhoea and other infectious diseases

*Escherichia coli*, also known as *E. coli*, remain one of the most recognised and intensively studied of all bacteria forms. *E. coli* is a Gram–negative, facultative anaerobic, rod bacterium known for its close association with the human digestive tract (Nataro and Kaper, 1998). It establishes a beneficial association with the host by preventing the colonisation of the colon by pathogenic bacteria, and also producing some useful vitamins (e.g., vitamin K) which are useful to the host (Vogt and Dippold, 2005; CDC, 2012). *E. coli* was first discovered by Theodor Escherich (a German paediatrician) who called it *Bacterium coli commune* because it is was found in the colon and faeces of humans. It was subsequently renamed *Escherichia coli* after the original discoverer (Castellani and Chalmers, 1919).

Although it has been a subject of scientific investigations for over a century, occupying the main stage of genetic and molecular manipulation in the laboratory, *E. coli* continues to startle scientists' understanding of how bacteria infect, adapt, reproduce, colonise new niches and cause pathogenesis in their host organisms (Souza *et al.*, 2002). *E. coli* is classified in the genus *Escherichia* and belongs to the family *Enterobacteriaceae*. The genus *Escherichia* contains four other species besides *E. coli* these including *E. hermannii*, *E. fergusonii*, *E. vulneris*, and *E. blattae* (Osode and Okoh, 2010). *Escherichia blattae* were isolated from cockroaches, whereas *E. hermannii*, *E. fergusonii*, and *E. vulneris* have been isolated from both intestinal and extra-intestinal human sources (Wilshaw *et al.*, 2000).

Though most *E. coli* strains are non-pathogenic members of the normal intestinal flora, some highly adapted strains of the bacteria cause diarrhoeal and other gastrointestinal illness along

with other, more serious health problems (Vidal *et al.*, 2005; Health Canada, 2006b; Prescott *et al.*, 2008). The *E. coli* groups that cause diarrhoea are often transmitted via the faecal-oral route through the ingestion of contaminated water or food, or through contact with animals or persons. This diarrhoea-causing group is designated diarrhoeagenic *E. coli* (DEC) and consists of a diverse group of *E. coli* pathotypes (Moyo *et al.*, 2007; Jafari *et al.*, 2012; Dutta *et al.*, 2013); a group that includes emerging pathogens of public health importance worldwide (Nataro and Kaper, 1998; Vidal *et al.*, 2005). *E. coli* has long been known as the etiological agent of endemic childhood diarrhoea worldwide, and particularly in the developing world (Okeke *et al.*, 2000; Guerrant *et al.*, 2002; Gomez-Duarte *et al.*, 2013; Pourakbari *et al.*, 2013). Several diarrhoea outbreaks have been linked to *E. coli* through consumption of food material or water contaminated with faecal materials (Hedberg *et al.*, 1997; Itoh *et al.*, 1997; Koo *et al.*, 2008; Vigil *et al.*, 2009; Okhuysen and DuPont, 2010). Enteropathogenic *E. coli* and other diarrhoeagenic *E. coli* (DEC) pathotypes have also been implicated as etiological agents of diarrhoea outbreak in South Africa and other parts of the world (Robins-Browne *et al.*, 1980; Levine and Edelman, 1984; Isaacson *et al.*, 1993; Germani *et al.*, 1997; Cunin *et al.*, 1999; Effler *et al.*, 2001; Okeke *et al.*, 2003; Kelly *et al.*, 2004; Raji *et al.*, 2006). Although, statistical data are largely scarce on *E. coli* pathotypes and serotypes in South Africa, Tau *et al.*, (2012) identified strains of *E. coli* O104 from South Africa which were mostly associated with enteroaggregative *E. coli* (EAaggEC) pathotypes as causative agents of diarrhoea, particularly in infants.

Biologically significant strains of *E. coli* have broadly been classified into 3 major classes on the basis of both genetic and clinical criteria. Commensal *E. coli* strains make up a major part of the facultative faecal flora in most healthy humans and other warm-blooded animals where they are adapted for innocuous coexistence within the colon of the host organism and do not

appear to cause diseases in their host (Russo and Johnson, 2000; Croxen *et al.*, 2013). The greater part of human commensal strains of *E. coli* are known to come from phylogenetic group A, as defined by multilocus enzyme electrophoresis, as well as the familiar laboratory strains of *E. coli* K-12 and its other derivatives (Selander *et al.*, 1987; Herzer *et al.*, 1990). Commensal *E. coli* strains are typically devoid of the specialized virulence characteristic often present in intestinal and extra-intestinal pathogenic strains; and as commensals, they often contribute to the maintenance of their host's health by resisting colonisation by other pathogenic microorganisms and also by producing certain beneficial products (e.g. vitamins and fatty acids) useful to the host organism (Russo and Johnson, 2000; Donnenberg, 2002; Vogt and Dippold, 2005; Alhetar *et al.*, 2011; CDC, 2012; Maltby *et al.*, 2013). Furthermore, commensal *E. coli* strains usually do not cause disease outside the intestinal tract, except when precipitating factors such as occurrence of an indwelling foreign body or disorder in host defences occur (Russo and Johnson, 2000).

Enteric or diarrhoeagenic *E. coli* (DEC), are the pathogenic strains of *E. coli* often associated with diarrhoea. This group is divided into different pathogenic types or variants (pathotypes/pathovars) based on their specific virulence factors, specific gene patterns, phenotypic profile and clinical disease symptoms (Vidal *et al.*, 2005; Palaniappan *et al.*, 2006; Rasko *et al.*, 2008; CFSPH, 2009; Jafari *et al.*, 2012; Clements *et al.*, 2012; Croxen *et al.*, 2013). Conventionally, six categories of the diarrhoeagenic *E. coli* that possess different virulence factors such as exotoxins have been described (Figure 2.2). These include enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAaggEC) and diffusely adhering *E. coli* (DAEC) (Vidal *et al.*, 2005; Prescott *et al.*, 2008). Although other pathovars have been proposed, these six groups are, however, unique and have been agreed upon in the

scientist community in general (Kaper *et al.*, 2004). The different mechanisms of pathogenesis and typical clinical presentations of DEC infections are summarised in Table 2.2 below.

Enteric *E. coli* strains can also be classified by their serogroup, for instance, *E. coli* O157, where O refers to the LPS (lipopolysaccharide) O-antigen or by their serotype e.g., enterohemorrhagic *E. coli* O157:H7, where H refers to the flagellar antigen (Clements *et al.*, 2012). Conversely, since each pathotype contains many serotypes, and some serotypes may be found in more than one pathotype, serotyping may not give a definitive identification of pathotypes (Wolf, 1997; Clements *et al.*, 2012). The O and K antigens shield *E. coli* from antimicrobial effects of complement and help it to escape phagocytosis in the absence of specific antibodies (Baldwin *et al.*, 1992; Blanco *et al.*, 1992).

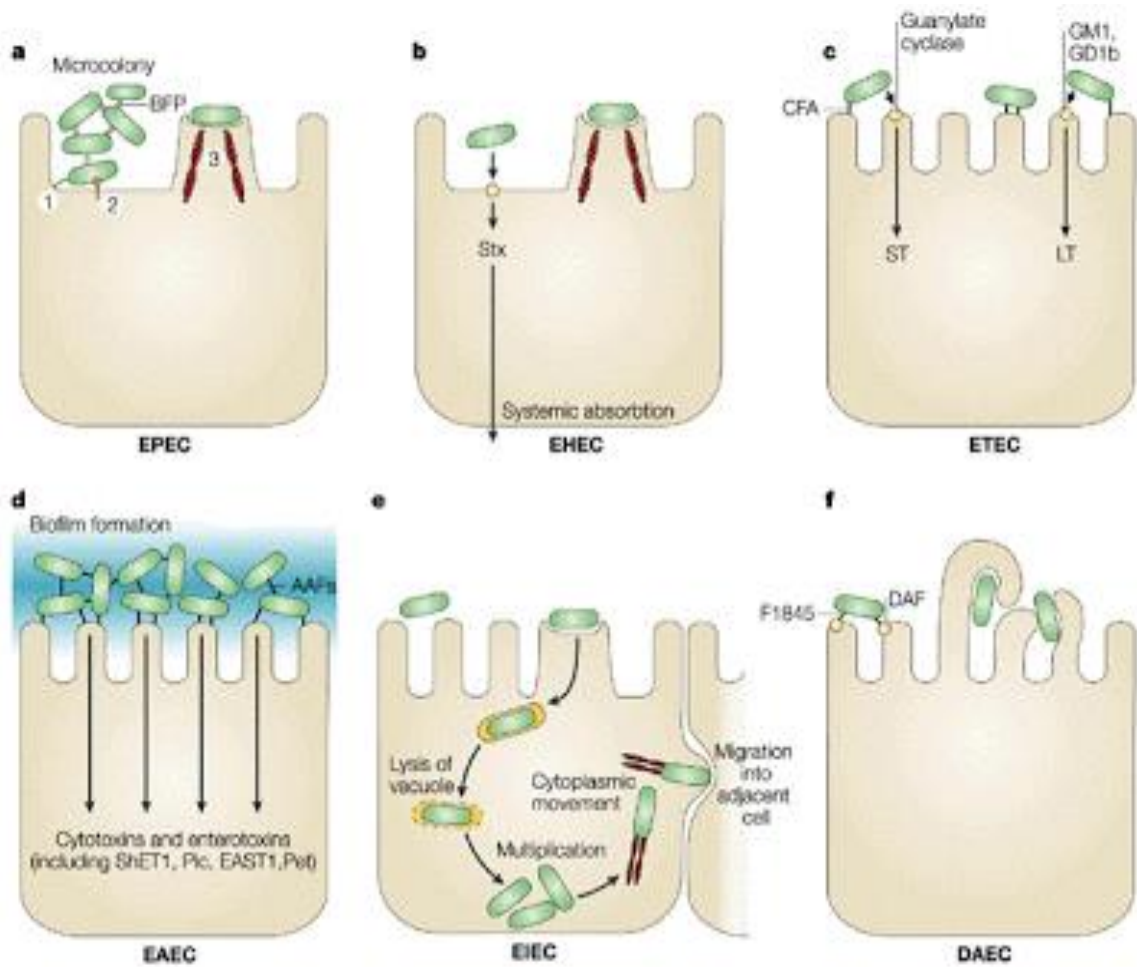
While pathogenicity in *E. coli* has been more commonly associated with DEC, extraintestinal *E. coli* (ExPEC) infections are also a major source of morbidity, mortality, and increased health costs (Russo and Johnson, 2003; Pitout, 2012; Lamprecht *et al.*, 2014). Extraintestinal *E. coli* strains become pathogenic when they exit their usual reservoir in the host's intestinal tract to other anatomical sites such as urinary tract, bloodstream, cerebral spinal fluid, respiratory tract, and peritoneum (spontaneous bacterial peritonitis), resulting in infections (Diard *et al.*, 2007; Pastorello *et al.*, 2013). Urinary tract infections have been commonly linked to ExPEC and are also a leading cause of meningitis and other forms of sepsis in human neonates, which can lead to serious complications, and even deaths, in some cases (de Louvois, 1994; Hamelin *et al.*, 2007). Figure 2.2 below shows the pathogenic pathways of the various diarrhoeagenic (DAEC) *Escherichia coli* pathotype.



**Table 2.2:** Mechanisms of pathogenesis and some clinical presentations of various diarrhoeagenic *E. coli* (DEC) infections.

<b>DEC pathotype</b>	<b>Mechanism of Pathogenesis</b>	<b>Typical Clinical Presentation</b>
ETEC	Heat labile/ heat stable enterotoxin production	Acute watery diarrhoea, afebrile, sometimes severe
EAEC	Adherence to small and large intestine; production of enterotoxin and cytotoxin	Watery diarrhoea, bloody diarrhoea; may cause persistent diarrhoea in children
EPEC	Small intestine adherence and epithelial cell effacement by intimin	Severe acute watery diarrhoea, bloody diarrhoea; may be persistent; common aetiological agent of infantile diarrhoea in developing countries
EIEC	Adherence, mucosal invasion and inflammation of large intestine	Watery diarrhoea, dysentery-like diarrhoea and fever
DAEC	Diffuse adherence to epithelial cell	Watery diarrhoea, but pathogenicity not conclusively demonstrated
EHEC (STEC)	Large bowel adherence (intimin-mediated); Shiga toxin 1, Shiga toxin 2 production	Watery diarrhoea which often progresses to bloody diarrhoea within 1 to 3 days; painful defaecation; abdominal tenderness; patient may report a history of fever but are often afebrile on presentation, often >5 stools in 24 h

Source: CDC, 2015



Nature Reviews | Microbiology

**Figure 2.2:** The pathogenic pathways of the various diarrhoeagenic (DAEC) *Escherichia coli* pathotype. Source: Kaper *et al.*, (2004).

Osteomyelitis, pulmonary, intra-abdominal, soft tissue, and intravascular device-associated infections, among others, have also been associated with ExPEC (Russo and Johnson, 2000). Some of the well-characterised virulence factors which help ExPEC evade host defence mechanisms include: P-fimbriae, type 1 fimbriae, haemolysin, aerobactin, and serum resistance. Several decades of microbiology research has revealed different strains of pathogenic *E. coli* associated with diarrhoea and other human diseases (Nataro and Kaper, 1998; Okeke, 2009; Yang and Wang, 2014). An overview of some of the important pathogenic *E. coli* strains and their public health concerns are discussed in the sections below:

### **2.5.1 Enterotoxigenic *E. coli* (ETEC)**

Enterotoxigenic *E. coli* (ETEC) is the most common pathotype of *E. coli* and a leading cause of diarrhoea mainly in developing countries, as well as the most common etiological agent of infantile and traveller's diarrhoea (mostly during the warm and wet months and among first-time travellers to the developing world) (Qadri *et al.*, 2005; Osode and Okoh, 2008; Osode and Okoh, 2010; WHO, 2010). ETEC strains of *E. coli* were first recognised as a cause of human diarrhoeal illness in the 1960s but have since emerged as a major bacterial cause of diarrhoea among travellers and children in the developing world (CDC, 2012). Although, there is insufficient data on the extent of the severity of the diseases caused by ETEC, some studies have suggested that ETEC is responsible for about 210 million cases of infection and a mortality rate of approximately 380, 000, mostly in children (Gupta *et al.*, 2008; Okeke, 2009; WHO 2010). While other endemic pathogens such as *Shigella* spp., *Vibrio cholerae*, and rotavirus can be easily detected by conventional standard assays, ETEC is more difficult to recognise and therefore, often, overlooked as a major etiological agent of diarrhoea in infants or cholera-like illness across all age groups (Kosek, 2003; Qadri, 2005). ETEC causes

watery diarrhoea which often lasts up to a week, but can be protracted. Infections are known to be common in areas where there are high levels of faecal contamination of water and food supplies.

The chronology of ETEC originated about six decades ago in Calcutta, India. In their study, De *et al.*, (1956) isolated live strains of *E. coli* from infants and adults who had cholera-like disease. They injected these into ileal loops isolated from rabbits and discovered that large amounts of fluid accumulated in the loops, close to that seen with *Vibrio cholerae*. Nonetheless, they did not test the filtrates for the presence of toxins (Qadri, 2005). Sack (1968) followed up these findings and reported, almost twelve years later, that adults and children with a cholera-like disease had almost pure growth of *E. coli* in both stool and small intestine. Further studies showed that these isolates produce cholera-like secretory response in rabbit ileal loops, both as live and culture filtrates (Gorbach *et al.*, 1971). The patients also produced antitoxin responses to the heat-labile enterotoxin produced by these organisms (Quiroga *et al.*, 2000). Similar studies on animals also revealed strains of *E. coli* to be the causative agents of diarrhoeal disease in several other animals (Gyles and Barnum, 1969; Smith and Linggood, 1971). The *E. coli* strains in those studies were found to produce enterotoxins and specific colonisation factors. Several other studies in other developing countries also support these findings (DuPont *et al.*, 1971; Black *et al.*, 1981; Black *et al.*, 1989).

Travellers' diarrhoea (TD), caused by ETEC, is a self-limited disease which often resolves naturally within a few days. The incubation period usually is 2–3 days, and the major symptom is diarrhoea (4–6 loose, watery or bloody bowel movements per /day) often

associated with abdominal pain, cramps, nausea, vomiting, muscle aches, weakness and sometimes low-grade fever. Sometimes high-grade fevers and bloody stool occur in patients with TD. When these symptoms occur, the illness is described as dysentery and not “ordinary” travellers’ diarrhoea (Sack *et al.*, 2007). The duration of TD usually is 2–6 days, if untreated. Occurrence of TD varies widely from high areas of incidence such as northern Africa, Latin America, the Middle East and Southern Asia to low areas of incidence such as northern America, northern Europe, Australia, New Zealand and the United Kingdom (Mc Farland, 2007). TD is usually acquired by ingesting enterotoxins-producing ETEC faecal contaminated water and food materials. Foods with high risk of infection include raw or undercooked meats and seafood, raw fruits and vegetables, unpasteurised milk and other dairy products among others (Mc Farland, 2007).

The utmost important virulence determinants of ETEC bacteria are classically pronounced to be the colonisation of the host small intestine epithelium with the aid of plasmid-encoded colonisation factors (CFs) and the subsequent release of plasmid-encoded enterotoxins that induce a net secretory condition causing copious watery diarrhoea (Viboud *et al.*, 1999; O’Sullivan *et al.*, 2006; Turner *et al.*, 2006; Crossman *et al.*, 2010; Fleckenstein *et al.*, 2010; CDC, 2012). CFs are known to be genetically and structurally diverse and to date, more than 25 CFs have been identified (Gaastra and Svennerholm, 1996). Two different enterotoxins have been described; a heat labile toxin (LT) and a heat stable toxin (ST) (Erume *et al.*, 2008; Rajendran *et al.*, 2010). Strains of ETEC may produce either one or both of these toxins; two-thirds of ETEC produce a heat-labile toxin which is structurally and functionally similar to cholera toxin, and which induces secretory diarrhoea (Benenson, 1995; Al-Abri *et al.*, 2005; Qadri, *et al.*, 2005; Chowdhury *et al.*, 2010). The release of the ST and /or the LT in the epithelium increases intracellular cyclic AMP (cAMP) which, in turn, activates the cystic

fibrosis transmembrane regulator (CFTR) chloride channel (Sahl and Rasko, 2012). This activation causes production of electrolytes and, eventually release of water as diarrhoea (Sack *et al.*, 1971).

In addition to the ST and LT enterotoxins, the enteroaggregative *E. coli* (EAEC) heat-stable enterotoxin 1 (EAST1) protein, which was firstly recognised in enteroaggregative *E. coli*, has also been found in many ETEC isolates with established colonisation factor profiles; however, the benefaction of EAST1 to ETEC diarrhoeal disease is currently unknown (Savarino *et al.*, 1991; Yamamoto and Echeverria, 1996; Fleckenstein *et al.*, 2010). Roy *et al.*, (2009), discovered the ETEC glycoprotein EtpA can act as a link between the bacterial flagella and the host epithelial cells. The glycoprotein has also been recognised as a promising target for vaccines development (Roy *et al.*, 2008; Roy *et al.*, 2009). Besides, other virulence factors have also been implicated in ETEC's pathogenesis, including: EatA serine protease autotransporter of the *Enterobacteriaceae* (SPATE) family and EtpA protein, which acts as an intermediate in the adhesion between bacterial flagella and host cells (Henderson *et al.*, 1998; Patel, 2004; Fleckenstein *et al.*, 2006; Roy *et al.*, 2009a).

While the actual role of the *eataA* gene product in ETEC pathogenesis has not been established, a study used a mouse model to show that EatA is immunogenic (Roy *et al.*, 2010). Moreover, a number of other chromosomal factors are also thought to be involved in ETEC virulence, e.g., the invasion Tia; the TibA adhesion/invasion; and LeoA, a GTPase with unknown function (Elsinghorst and Weitz, 1994; Fleckenstein *et al.*, 1996; Fleckenstein *et al.*, 2000). Many of the recognised pathogenic factors in ETEC are distributed among its genomes, without a universally conserved core virulence gene sets; however, results of gene

independent comparative analyses of the genome indicate that ETEC isolates share a conserved backbone that, while not outstanding to ETEC, shows that regardless of the phylogeny, ETEC isolates share a common core genomic structure (Rasko *et al.*, 2008; Sahl *et al.*, 2011).

Although, the use of a cultural-based diagnostic method remains the gold standard for disease-testing in clinical settings, ETEC disease diagnosis is more difficult to recognise using cultural-based techniques; an efficient diagnostic method for the detection of ETEC during outbreaks of diarrhoea requires a sensitive and specific protocol which detects the bacterial ST or LT encoding genes (Osode and Okoh, 2010). In the early stages, ETEC strain diagnosis and detection was done with animal assays and cell culture techniques which often require specific antibodies to effectively identify the presence of the target toxins (Zuber, 1999).

Enzyme-linked immunosorbent assays and membrane-based DNA hybridisation assays were later developed, and this increased the speed and ease of pathogen detection (Reischl *et al.*, 2004). Further improvement in the technique for the detection of ETEC bacteria has led to the development of polymerase chain reaction (PCR) protocols which specifically amplify the enterotoxin encoding genes of the bacterial isolates, with the advantage of the possibility of rapid diagnosis of the bacterial infection, particularly during outbreaks of diarrhoea (Olive, 1989; Candrain *et al.*, 1991; Lin *et al.*, 1993; Schultsz, 1994; Saulnier, 1997; Yavzori *et al.*, 1998; Tsen and Jian, 1998; Zuber, 1999; Pass *et al.*, 2000). The use of PCR gives high levels of sensitivity and specificity as well as speed, and more recent advances in PCR amplification and fluorescence-based detection technologies have led to improvements in conventional

block cyler PCR assays, thus enabling the simultaneous, sequence-specific detection of multiple PCR products in real time (Reischl *et al.*, 2004). The development of PCR cycling conditions with the capability to rapidly amplify genes and the ability to constantly and continuously monitor the cumulating specific PCR products labelled with fluorescent probes eliminates the need for labour-intensive agarose gel procedures and made possible a real time, more reliable and more efficient identification of target genes (Bellin *et al.*, 2001; Maguire *et al.*, 2001; Reischl *et al.*, 2000; Reischl *et al.*, 2002; Reischl *et al.*, 2004).

### **2.5.2 Enterohemorrhagic *E. coli* (EHEC)**

Enterohemorrhagic *E. coli* (EHEC) strains are Shiga-toxin-producing (due to the similarity of toxins to that produced by *Shigella dysenteriae*) pathogenic variants (pathovars) of *E. coli* that are capable of causing haemolytic colitis, and bloody diarrhoea which has been associated with haemolytic ureamic syndrome (HUS); an important cause of hemolytic anaemia, thrombocytopenia, and acute renal failure mainly in infants and young children (Tarr *et al.*, 2005; Mariani-Kurkdjian *et al.*, 2014). A highly pathogenic strain belonging to this serotype, (Enterohemorrhagic *E. coli* O157:H7) was first identified as an etiological agent of disease in the United State in 1982, following an outbreak of gastrointestinal illness (Riley *et al.*, 1983).

*E. coli* O157:H7 strain has been the most notorious strain responsible for most infection in this group; however, non-H7 strains have also been implicated in illnesses (Lawson, 2004; Panos *et al.*, 2006; Friesema *et al.*, 2015). Contrary to *E. coli* O157:H7 strains, the non-H7 strains are sorbitol-positive, non-motile and have mainly been limited to Continental Europe; about 86 cases from 37 outbreaks were investigated in Europe between 1993 and 1998



(Lienemann *et al.*, 2012; Mariani-Kurkdjian, 2014; Doulgere *et al.*, 2015; Berger, 2015). High incidences of non-H7 infections are usually recorded during September to April, mostly among infants; conversely, infections due to H7 strains peak during June to August, and are more common among children usually above 3 years (Berger, 2015). Approximately 30 to 60% of human EHEC infections are caused by non-O157 strains in the United States (EFSA, 2013; Delannoy *et al.*, 2013), while outbreaks of O111 infections have also been reported in the United State, Japan, Australia and European countries (Vally *et al.*, 2012; Berger, 2015).

The pathogenesis of *E. coli* O157:H7 is due mainly to the production of Shiga-like toxins or verotoxins, which are lethal to Vero cells. The Shiga toxins act by inhibiting protein synthesis in infected cells, thus leading to cellular death (Ogasawara *et al.*, 1988; Rahal *et al.*, 2011). Although the relevant nomenclature remains unsettled; however, two major classes of Shiga-like toxins (Shiga-toxins or verotoxins), SLT1 (Stx1, VT1) and SLT2 (Stx2, VT2) have been identified (Acheson *et al.*, 1998; Panos *et al.*, 2006; Rahal *et al.*, 2011). The Shiga toxins are thought to be stored in the periplasmic space of the bacteria cells after production and released later (Rahal *et al.*, 2011).

Transmission of EHEC is, largely foodborne through the consumption of contaminated food such as raw or undercooked ground-meat products and raw milk. Faecal contamination of water and food items, cross contamination of food during preparation as well as person-to-person contacts are also important in the oral-faecal route transmission of EHEC infections (WHO, 2015e). A number of outbreaks have also been linked to the consumption of fruits and raw vegetables such as sprout, lettuce, coleslaw, salad that are irrigated or processed with sewage-contaminated water. EHEC strains have been recovered from fresh, drinking and

recreational water. An outbreak of O157:H7 involving 243 cases and 4 deaths was linked to contaminated municipal water in Cabool, Mo between December 1989 and January 1990 (Swerdlow *et al.*, 1992). Also in 1991, swimmers, including many toddlers were noticed to have ingested faecally contaminated water while swimming at a lakeside park in Portland, Ore, and this led to an outbreak of *E. coli* O157:H7 infection involving 21 reported cases (Keene *et al.*, 1994). Water has also been suggested as an important means of transmitting *E. coli* O157:H7 among cattle, and the pathogen has been noted to survive for a prolonged period of time in the environment (Faith *et al.*, 1996; Kim and Harrison, 2007; WHO, 2015e).

Cattle have been identified as the major reservoir of EHEC, while other animals such as deer, sheep and goats are also considered significant hosts; however, unlike humans, cattle are asymptomatic carriers of EHEC (Wray *et al.*, 2000; Nguyen and Sperandio, 2012; Fernandez *et al.*, 2013). Even though the molecular basis and implications of Shiga-toxins have been extensively studied, the mechanisms behind the site-specific colonization of the bacteria remain unclear. Successful colonisation is crucial for EHEC and EPEC infections because both are non-invasive pathogens believed to have evolved from a common ancestor (Karch, 2001; Vallance *et al.*, 2002; Yang *et al.*, 2015).

Much of the pathophysiology associated with EHEC Shiga toxin is promoted by Gb3 receptors which are expressed on human vascular endothelium; even though Gb3 receptors have also been detected in the kidney and brain of cattle, Shiga toxin, however, was not able to bind unto the gastrointestinal tract blood vessels of cattle (Pruimboom-Brees *et al.*, 2000). Due to this, it is impossible to endocytose nor transport Shiga toxin to other organs for vascular damage induction in cattle (Nguyen and Sperandio, 2012). Additionally, EHEC has

developed several acid resistance mechanisms such as the alternative sigma factor RpoS which allows it to survive the harsh pH conditions of the cattle gastrointestinal tract (Ramamurthy and Albert, 2012). Unlike human whose colonisation of the colon by EHEC causes electrolyte imbalances, EHEC colonisation in cattle occurs at the rectoanal junction where it is insusceptible to Shiga toxin effects (Naylor *et al.*, 2003). This combination (that is lack of sensitivity to Shiga toxin and differential preference of colonisation sites) allows cattle to be tolerant hosts of EHEC, and this may contribute to its persistence and transmission to humans (Nguyen and Sperandio, 2012).

Enterohaemorrhagic *E. coli* have evolved and acquired specific virulence factors which permit them to colonise and infect the human gastrointestinal tract, often without invading the bloodstream (Goldwater and Bettelheim, 2012). They have an incubation period of about 3 to 4 day after ingestion. In addition to bloody diarrhoea, EHEC infection clinical manifestation range from asymptomatic carriage to haemorrhagic colitis to HUS characterised by the triad of acute renal failure, microangiopathic haemolytic anaemia and thrombocytopenia (Boyce *et al.*, 1995; Kehl, 2002).

Clinical diagnosis of EHEC infection includes culturing of stool samples testing specifically for O157:H7 strains on Sorbitol-MacConkey agar. Sorbitol negative *E. coli* colonies are further tested for reaction with antiserum to O157 antigen (Boyce *et al.*, 1995). Serotype-specific enzyme immunoassays with about 73 to 100% sensitivity when compared to Sorbitol-MacConkey testing can also be used to detect O157 directly from stool (Kehl, 2002). A more efficient way of identifying all types of EHEC is the detection of Shiga toxin/Verotoxin or its associated genes, involving DNA-based methods. PCR and multiplex

PCR (mPCR) with a specific oligonucleotide primer have become accepted sensitive techniques for the detection of short selected segment of the Shiga toxin-producing genes in EHEC (Kehl, 2002; Welinder-Olsson and Kaijser, 2005). There is no appropriate treatment for EHEC infection currently, and only supportive care with monitoring for complications of haemolytic uremic syndrome have been used in cases of infection. The use of antibiotics is also not recommended because it can exacerbate Shiga toxin-mediated cytotoxicity (Kehl, 2002; Goldwater and Bettelheim, 2012).

### **2.5.3 Enteropathogenic *E. coli* (EPEC)**

Enteropathogenic *E. coli* (EPEC) strains which are characterised partly by their ability to induce attaching-effacing (A/E) lesions permitting intimate adherence of the pathogen to colon epithelial cells are well-acknowledged aetiological agents of childhood diarrhoea disease in under-developed countries (Tennant *et al.*, 2009). Even though, the occurrence of the bacteria has reduced in developed countries, they however, continue to be a significant cause of diarrhoea (Trabulsi *et al.*, 2002; Sousa, 2006). EPEC strains possess locus for enterocyte effacement (LEE) pathogenicity island, carrying multiple virulence factors, such as *eae* and *tir* genes (encoding intimin and intimin receptor respectively) (FDA, 2012). EPEC lesion involves the intimate attachment of the bacteria to intestinal mucosa at the point of cytoskeletal rearrangements thus producing morphological changes called cupping and pedestal formation, accompanied by the effacement of microvilli (Tennant *et al.*, 2009).

Enteropathogenic *E. coli* strains have been classified into two main groups based on the presence of an *E. coli* adherence factor (EAF) plasmid. The *eaeA* gene, located in the locus of enterocyte effacement pathogenicity Island, and the *bfpA* gene, located on the EAF

plasmid, have both been used to categorise the bacteria as either typical or atypical strains. *E. coli* strains that are *eaeA*<sup>+</sup>/*bfpA*<sup>+</sup> bearing genes involved in localised adherence pattern which produce attaching and effacing lesions are categorised as typical EPEC; most strains in this category fall under the classic O:H serotypes, while *E. coli* strains that are *eaeA*<sup>+</sup>/*bfpA*<sup>-</sup> displaying localised adherence-like, diffuse adherence or aggregate adherence patterns are categorised as atypical EPEC (Kaper, 1996; Trabulsi, 2002; Alikhani *et al.*, 2006; Ochoa and Contreras, 2011; Duda-Madej *et al.*, 2013; Ochoa and Cleary, 2015). Based on this classifications, the basic difference between strains of typical and atypical EPEC is the possession of EAF plasmid by the former group while it is absent in the latter group (Trabulsi, 2002).

Enteropathogenic *E. coli* are responsible for a significant number of infant deaths and young children yearly, a situation exacerbated by seasonal outbreaks. Certain serotypes of the pathogen such as O55:H12/45, O86:H48, O127:H21, O142:H48, O126:H48, and O126:H19 are more frequently encountered and have been more significantly associated with diarrhea in children (Ochoa *et al.*, 2008). Other serogroups including O55:H6, O111:H2 and O111: H have reportedly been isolated frequently in different geographical areas around the world (Elias *et al.*, 2002; Botelho *et al.*, 2003). Typical EPEC were considered in developing countries for many years as the leading agent of diarrhoea in infant and were thought to be rare in developed countries where atypical EPEC seemed to be more important agent of diarrhoea. Contrarily, relatively recent data reveal that atypical strains are more frequently encountered than typical EPEC strains in both developing and industrialised countries Table 2.3 (Ochoa *et al.*, 2008). Interestingly, atypical EPEC were responsible for a larger percentage ( $\approx 78\%$ ) of all EPEC cases in children under 5 years of age with diarrhoea (according to studies reporting data for children under 5 years old as shown in Table 2.3).

Likewise, atypical strains of EPEC were also reported to have accounted for more than half of the episodes of diarrhoea in Australia and Norway (Afset *et al.*, 2004; Nguyen *et al.*, 2006).

Enteropathogenic *E. coli* is a non-invasive bacterium which depends on a type III secretion system (T3SS) deliver effector proteins into infected host cells to disrupt the various host cell functions eventually leading to disease. The first effectors discovered were all encoded on the LEE pathogenicity island. Recently, effector proteins encoded outside the LEE area have been discovered in all A/E pathogens (Ochoa and Contreras, 2011). The complete genome sequence analysis of over 400 known and/or predicted effector sequences and identified 21 putative effectors of EPEC strains E2348/69 affords a clear representation of the core LEE and non-LEE effector genes (Iguchi *et al.*, 2009).

The hallmark of EPEC pathogenicity involves a histological change called the attaching and effacing (A/E) lesion. The pathogen adheres intimately to the enterocyte and prompts assemblage of cytoskeleton intracellular actin on the surface of the infected host cell. The actin cytoskeleton rearrangements form a pedestal-like assembly where bacterium firmly cups the cells, causing the disappearance or degeneration of brush border microvilli (Vidal *et al.*, 2007). Although the mechanism of pedestal formation caused by EPEC has been well studied, the complete mechanism of EPEC induced diarrhoea remains largely unclear. It has been suggested that EPEC-mediated diarrhoea is related to: i) effacement of intestinal microvilli, ii) massive loss of intracellular electrolyte into the intestinal milieu and iii) secretion of enterotoxin (Vidal *et al.*, 2007; Ochoa *et al.*, 2008).

**Table 2.3:** Prevalence of enteropathogenic *E. coli* strains among children with diarrhoea in developing countries

Country	Sampling period	Children's age (years)	No. of samples	EPEC <i>n</i> (%)	EPEC type	
					Typical <i>n</i> (%)	Atypical <i>n</i> (%)
Tanzania	1996 – 1997	<5	451	24 (5.3)	9 (37.5)	15 (62.5)
Thailand	1996 – 2000	<12	2629	85 (3.2)	24 (28.2)	61 (71.8)
Mozambique	1998 – 1999	<7	548	13 (2.4)	4 (30.8)	9 (69.2)
Brazil	1998 – 1999	<2	237	34 (14.3)	21 (61.8)	13 (38.2)
Mongolia	2001 – 2002	<16	238	9 (3.9)	5 (55.6)	4 (44.4)
Brazil	2001 – 2002	<5	175	13 (7.4)	1 (7.7)	12 (92.3)
Vietnam	2001 – 2002	<5	587	39 (6.6)	0 (0)	39 (100)
Mexico	2000 – 2004	<5	430	16 (3.7)	3 (18.7)	13 (81.3)
Tunisia	2001 – 2004	<15	115	6 (5.2)	2 (33.3)	4 (66.7)
Brazil	2002 – 2003	<5	446	25 (5.6)	2 (8.0)	23 (92.0)
Chile	2004 – 2005	<9	509	54 (10.6)	14 (25.9)	40 (74.1)
Peru	2006 – 2007	<1	461	18 (3.9)	2 (11.1)	16 (88.9)
Iran	-	<10	247	45 (18.2)	29 (64.4)	16 (35.6)

Source: Ochoa *et al.*, 2008.

Some of the most relevant specific diagnoses of diseases caused by EPEC include:

1. Serotyping;
2. FAS test;
3. The adherence assay; and
4. The specific detection of virulence factors including *eaeA* and *bfpA* genes using molecular techniques (Vidal *et al.*, 2007).

From the clinical perspective, fast, simple and cheap diagnostic techniques are required in order to define appropriate treatments and adequate prevention of EPEC-induced diseases for children in endemic regions (Ochoa and Contreras, 2011).

#### **2.5.4 Enteroaggregative *E. coli* (EAEC)**

Enteroaggregative *E. coli* (EAEC) is a heterogeneous subgroup of diarrhoeagenic *E. coli* that have increasingly been regarded as an important aetiological agent of persistent watery diarrhoea in infants, children and adults worldwide (Weintraub, 2007), as well as an important cause of travellers' diarrhoea, and infections in HIV patients. Strains of EAEC have been linked to sporadic outbreaks of food-poisoning most importantly in industrialised countries (Huang *et al.*, 2006). A highly virulent strain, EAEC O104:H4 (which had acquired Shiga toxin producing capability), raised awareness about the importance of EAEC as important emerging pathogen; causing a severe outbreak in Germany in 2011, resulting in more than 400 morbidity and at least 50 fatality cases (Boisen *et al.*, 2015). The major defining characteristic of EAEC strains compared to others is their ability to form a “stacked-brick” pattern of bacterial network adhesion with human epithelial cells line such as Hep-2 or HeLa (Jafari *et al.*, 2013). This adhesion pattern was described and termed “aggregative adherence” (AA) by Nataro *et al.* (2006).



EAEC strains of *E. coli* have caused a number of significant outbreaks around the world. However, historical data concerning outbreaks of EAEC has mainly been limited to Europe and, North and South America, while efforts are being made to fully understand the global impact of EAEC incidences and outbreaks (Croxen *et al.*, 2013). In a large scale study carried out in the emergency and outpatient departments of two large academic health care facilities in Maryland and Connecticut, Nataro *et al.* (2006) reported EAEC has the most encountered bacterial cause of diarrhoea. In 1997, EAEC was responsible for a school lunch outbreak involving 2,696 children in Japan, and an epidemic involving about 15% of a village population in India (Pai *et al.* 1997). More recent surveillance studies have also established the presence of EAEC strains in some sub-Saharan Africa countries including Nigeria and Libya (Dow *et al.*, 2006; Okeke *et al.*, 2010; Boisen *et al.* 2012; Kotloff *et al.*, 2013).

Transmission of EAEC-induced travellers' diarrhoea has mostly been linked to contaminated water, food and vegetables. An important outbreak occurred in Germany in 2011 where 4321 previously healthy persons from sixteen countries were infected with hybrid strains of EAEC and STEC, with more than 900 patients developing haemolytic uraemia syndrome and over 50 death cases (Grad *et al.*, 2012). The Shiga toxin-encoding hybrid strains of EAEC, identified as *E. coli* O104:H4 pathogens, were found to carry both the virulence genes for EAEC (including *aggA*, *aggR*, *set1*, *pic*, and *aap*) and STEC (*stx 2*) (Bielaszewska *et al.*, 2011; Brzuszkiewicz *et al.*, 2011; Estrada-Garcia and Navarro-Garcia, 2012). The ability of EAEC strains to acquire Shiga toxin-producing capabilities, as reported by various studies, indicate the possibility of outbreaks for this pathogen in future (Morabito *et al.*, 1998; Iyoda *et al.*, 2000; Mossoro *et al.*, 2002; Dallman *et al.*, 2012).

The primary features of EAEC pathogenesis include i) adherence to the intestinal mucosa by aggregative adherence fimbriae (AAF) and adherence factors, ii) increased formation of mucoid biofilm which encrust EAEC bacteria on the surface of enterocytes; and iii) elaboration of enterotoxins (plasmid-encoded toxin (Pet) and plasmid-encoded EAEC heat-stable toxin 1(EAST1)) and elicitation of mucosal inflammation response, toxicity, and intestinal secretion (Kaur *et al.*, 2010). Plasmid (pAA)-encoded AAF (aggregative adherence fimbriae) and AggR (regulator) are required for colonisation; however, not all EAEC strains carry AAF, therefore, other adherence factors might be involved in EAEC colonisation. EAST 1 is a small polypeptide enterotoxin which induces diarrhoea by secreting cyclic GMP while *pet* is an enterotoxin which induces cytoskeletal alterations and rounding of epithelial cells. EAEC bacteria strains showing the stacked brick phenotype and pAA are designated typical EAEC while those having the stack phenotype but lacking pAA are referred to as atypical EAEC (Smith and Fratamico, 2015).

Even though, most EAEC infections are asymptomatic, some clinical manifestations associated with EAEC colonisation may include watery diarrhoea and intermittently very mucoid diarrhoea, low-grade fever, anorexia, nausea, tenesmus and borborygmi (Mendez-Aracibia *et al.*, 2008; Okeke *et al.*, 2011). The incubation period range between 8 to 52 h. The Shiga toxin-producing EAEC O104:H4 responsible of the 2011 German outbreak caused several cases of haemorrhagic colitis and HUS, resulting in a substantial number of morbidity and causality cases (Jensen *et al.*, 2014).

The identification of the characteristic “stacked-brick” aggregative adherence when grown in static Luria-Bertani borth at 37°C for 3 h in Hep2 cells remains the gold standard of diagnosis

for EAEC (Tokuda *et al.*, 2010). This test is, however, only carried out in reference laboratories with cell culture facilities and it is time consuming and laborious (Cennimo *et al.*, 2007). Polymerase chain reactions (PCR) have been developed to detect specific EAEC virulence-associated genes, however, high heterogeneity of EAEC strains and the notation that the virulence genes of EAEC, possibly, result from the combination of multiple factors poses a challenge in differentiating between pathogenic and non-pathogenic strains by molecular techniques (Jensen *et al.*, 2014). Biofilm assay has also been useful for screening large numbers of strains in clinical and epidemiological studies (Kaur *et al.*, 2010).

The effectiveness of oral rehydration therapy alone in treating and managing EAEC disease is limited due to the persistent nature of the pathogen; therefore, the use of preventive interventions include vaccination, which is a high priority endemic area (Kaur *et al.*, 2010). The use of antibiotics is also generally recommended for treating travellers diarrhoea; however, due to rising resistance to some of the established antibiotics, selection of antimicrobials for treating EAEC diseases should take into cognisance the location or region of outbreak/infection as the different strains exhibit different antimicrobial susceptibility/resistance patterns based on geographical region (Cennimo *et al.*, 2007; Croxen *et al.*, 2013).

### **2.5.5 Enteroinvasive *E. coli* (EIEC)**

Enteroinvasive *E. coli* (EIEC) bacteria possess biochemical and genetic features similar to both *E. coli* and *Shigella* species. This pathogen was first recognised in 1944 and at first referred to as paracolony bacillus but was later called *E. coli* O124. Further isolation of similar strains of bacteria led to their grouping as *Shigella* species; however, the strains were

reclassified to specific serotype of Enteroinvasive *E. coli*. EIEC and 4 other species of *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*) are known to cause bacillary dysentery, which usually occurs after 8 to 24 h of consumption of contaminated water or food (Lampel, 2014). In contrast to other *E.coli* strains, most EIEC strains (about 70%) are not motile, lack lysine decarboxylase and mostly do not ferment, or have delayed reaction with lactose (attribute similar to the four species of *Shigella*) (Lampel, 2014).

EIEC incidences cannot be associated with any specific geographical pattern due to their close resemblance to *Shigella*. More recent health-related surveillance programs in the US, Canada, and Europe have not been able to link EIEC to any outbreaks (NESP, 2010; ECDC, 2011; CDC, 2012). Some of the initial outbreaks and sporadic cases of EIEC were reported in Australia and Israel in 1968 and 1974 respectively (Riley, 1968; Shmilovitz *et al.*, 1974). EIEC strains have been known to cause diarrhoeal and dysentery in children in underdeveloped countries and have also been associated mostly with cruise ships or water and foodborne outbreaks in industrialised countries (Ramamurthy and Albert, 2012). In a study conducted in Taiwan by Yang *et al.*, (2007), EIEC strains were reported to be responsible for a proportion as high as 20% of patients, which was next only to ETEC with 67% prevalence. Vieira *et al.*, (2007) also reported while investigating a case-control study in some 22 rural communities in north-western Ecuador that EIEC incidence of (3.2 cases/100 persons) was higher than those of other diarrhoea-causing pathogens. EIEC-induced diarrhoea has also been reported among Costa Rica children with infection rates as high as 19% (Perez *et al.*, 2010). Meanwhile, EIEC bacteria have been recovered from healthy and diseased individuals in parts of Africa including, Nigeria and South Africa (Okeke *et al.*, 2000; Nweze, 2009; Tau *et al.*, 2012; Onanuga *et al.*, 2014).

Watery diarrhoea is the most common typical clinical presentation of EIEC infection which may be accompanied by cramps in the abdomen and fever (may proceed to bloody diarrhoea with mucous and leukocytes on some occasions). Patients may, however, show characteristic dysentery syndrome in some instances, with frequent passing of stool, but of low volume contrary to watery stools observed in other DEC infections (Tau *et al.*, 2012; Lampel, 2014). Microbiological culturing and isolation of EIEC bacteria from stool of infected patients, coupled with the demonstration of invasiveness of isolates in tissue culture is useful in diagnosing EIEC-induced dysentery. Genetic probe using DNA hybridisation technique as well as the detection of genes mediating invasiveness e.g. *ial* and *ipaH* are also important diagnostic tool for EIEC infection.

As with other bacterial-induced diarrhoea, effective formulation of oral rehydration therapy plays an important part in treating EIEC patients, while use of antimicrobials has been effective in reducing the duration of the symptoms and may lessen risk associated with complications and fatalities (Niyogi, 2005; WHO, 2005). It should be noted that no vaccine is currently available for the prevention of EIEC or *Shigella*.

### **2.5.6 Diffusely adhering *E. coli* (DAEC)**

Diffusely adherent *E. coli* (DAEC) are important agents of diarrhoea characterised by the formation of diffuse adherence patterns on cultured epithelial cells HeL or Hep-2. Approximately 75% of DAEC strains carry adhesins genes from the family *afa/dra/daa*-related operon, which accounts for the observed phenotype seen in this pathotype. This group of pathogens has emerged as a unique class different from other pathotypes; however, the group requires further epidemiological studies because of difficulties in their classification

and identification (Snelling *et al.*, 2009). DAEC has been isolated from children having diarrhoea in many regions of the world including: Australia, Mexico, Brazil, Columbia, Chile, Peru, UK and the United States (Giron *et al.*, 1991; Gunzburg, 1993; Levine *et al.*, 1993; Gomes, 1998; Knutton *et al.*, 2001; Lopes *et al.*, 2005; Gomez-Duarte *et al.*, 2010; Ochoa *et al.* 2010). DAEC has also been identified from healthy individuals who did not present any diarrhoea symptoms, emphasising the importance of developing a suitable and rapid technique for detecting diarrhoea associated with the bacteria. The reservoir and transmission routes of DAEC also remain unclear.

Although similar virulence factors identified in other pathogenic *E. coli* have also been observed in DAEC strains from patients with intestinal and extraintestinal infections and healthy individuals, secretion system-encoding genes, however, have not been found associated with pathogenic *afa/dra*-DAEC isolates. Cellular lesions found in *afa/dra*-DAEC infected intestinal cells were obviously adhesion-independent suggesting the possible presence of additional virulence factors. Furthermore, the *sat* gene has been detected in *afa/dra* DAEC isolated from a child with diarrhoea, expressing the *afaE-V* adhesion (Taddei *et al.*, 2005; Le Bouguenec and Servin, 2006).

The *afa-dr* adhesins interact with brush border associated complement decay-accelerating factor (DAF), usually found on the surface of colon and urinary epithelia cells. Aggregation of the DAF molecules underneath the adherent bacteria occurs due to the binding of the DAF. Additionally, it triggers a  $\text{Ca}^{2+}$ -dependent signalling cascade, resulting in the elongation and destruction of the brush border microvilli through the disorganisation of the vital components of the cytoskeleton. Moreover, in addition to the flagella interaction between *afa-dr* adhesins

and DAF induces IL-8, secretion from enterocytes promotes transmigration of polymorpho nuclear neutrophils (PMNs) across the mucosal epithelial layer. This activates the upregulation of DAF on the apical surface of epithelial cells, providing DAEC with more receptors for tighter adherence. DAEC interaction with PMNs, mediated by *afa-dr*, lead to an accelerated rate of PMN apoptosis and a decreased rate of PMN-mediated phagocytosis (Croxen and Finlay, 2010).

Presentations of DAEC infections include watery diarrhoea which may become persistent in children, with rise in severity of disease from 18 month to 5 years of age. It is also thought that adults are healthy asymptomatic carriers of DAEC, and there have been speculations that carriage of DAEC may lead to chronic inflammatory intestinal diseases (Le Bouguenec and Servin, 2006). DNA hybridisation probes and PCR-based screening have been developed for detecting the common genes associated with DAEC, including *daaC*, *daaE*, and *afaB* and –C (Le Bouguenec *et al.*, 2001). Oral rehydration is the only therapy currently recommended for treating watery diarrhoea associated with DAEC. No vaccines have been developed for the prevention of DAEC to date.

### **2.5.7 Extraintestinal pathogenic *E. coli* (ExPEC)**

Extraintestinal pathogenic *E. coli* are facultative bacteria that form part of the normal intestinal microflora of a few healthy individuals, thereby colonising the human colon asymptotically. However, they produce disease once they gain access and effectively colonise niches outside the colon. Uropathogenic *E. coli* (UPEC) and Neonatal Meningitis *E. coli* (NMEC) are two prominent ExPEC strains that have been associated with diseases at anatomical sites outside the gastrointestinal tract.

Human diseases associated with ExPEC include urinary tract infections (UTIs), sepsis, pneumonia, neonatal meningitis and surgical site infections (Smith *et al.*, 2007). Diseases induced by ExPEC represent a huge burden in terms of medical cost and loss of productivity (Smith *et al.*, 2007), and ExPEC are known to cause diseases in animals and pets. ExPEC are a major cause of community-acquired UTI responsible for more than 85% of infection cases. In the United States, approximately six to eight million ExPEC-related cases are diagnosed, and about 130 to 175 million cases diagnosed globally every year. The estimated direct health care-related cost of uncomplicated UTIs has been put at about 1 to 2 billion US dollars annually in the United States (Zhang and Foxman, 2003; Bergeron *et al.*, 2012).

ExPEC strains have been linked to some possible outbreaks in different geographical regions in the world. Community-related outbreaks have been described in places such as the United State, the United Kingdom, and Canada; suggesting that ExPEC can be disseminated to the intestinal tracts of individuals in the community by a common source or route (Phillips *et al.*, 1988; Olsen *et al.*, 1994; Manges *et al.*, 2001; Pitout *et al.*, 2005; George and Manges, 2010; Bergeron *et al.*, 2012). Molecular studies of the epidemiology of ExPEC have revealed the unique pathogenic versatility of this group of *E. coli*, and have helped in understanding the ecological, evolutionary, mode of transmission, host-pathogen interactions, and the virulence mechanisms of this important group of pathogen (Johnson and Russo, 2005).

Some of the important virulence-associated factors linked to severe UTIs in UPEC strains include: P fimbriae (*pap*), S fimbriae (*saf*), afimbrial adhesion I (*afaI*), type 1 fimbriae, hemolysin (*hly*), aerobatic (*aer*), cytotoxic and adhesins. Other virulence-associated factors that have also been identified in relation to pathogenicity of UPEC include *astA*, *set 1*, *ompT*,



*kpsMT*, *sfa/foc*, *traT*, *iutA*, *fimH*, S and *F1C* fimbriae (*sfa*), group II capsule synthesis and serum resistance (Momtaz *et al.*, 2013).

Noenatal Meningitis *E. coli* (MNEC) strains are the second largest cause of neonatal bacterial meningitis after Group B *streptococcus* (GBS), with high mortality rate of over 10% and may induce neurological sequelae in about 20 to 50% of infection cases (Gaschignard *et al.*, 2012). Some of the specific virulence-associated genes that have been identified and characterised in NMEC include factors such as the K1 capsular polysacchide, the S fimbriae, and the Ibe10 protein. However, it is believed that other unidentified/uncharacterised genes are likely to be involved in NMEC pathogenesis (Bonacorsi *et al.*, 2000). An important clone of MNEC, the O18:K1:H7 clone is known to be distributed worldwide while other clones such as O83:K1 and O45:K1 are restricted to certain countries or regions.

In addition to the virulence-associated factors mentioned above, genomic island including, pathogenicity islands (PAIs) have been recognised in ExPEC, which carry blocks of genes coding numerous virulence associated genes. The PAIs acquisition might have occurred by means of plasmids and bacteriophages, and can encode biochemical factors which enable these strains to colonise and survive in the UTI and other anatomical sites outside the colon (Cyويا *et al.*, 2015). Some of the most studied PAIs in UPEC include, PAI I, PAI II and PAI IV<sub>536</sub> recognised in *E. coli* J96 and CFT073. PAIs have been associated with severe clinical conditions in UPEC (Cyويا *et al.*, 2015).

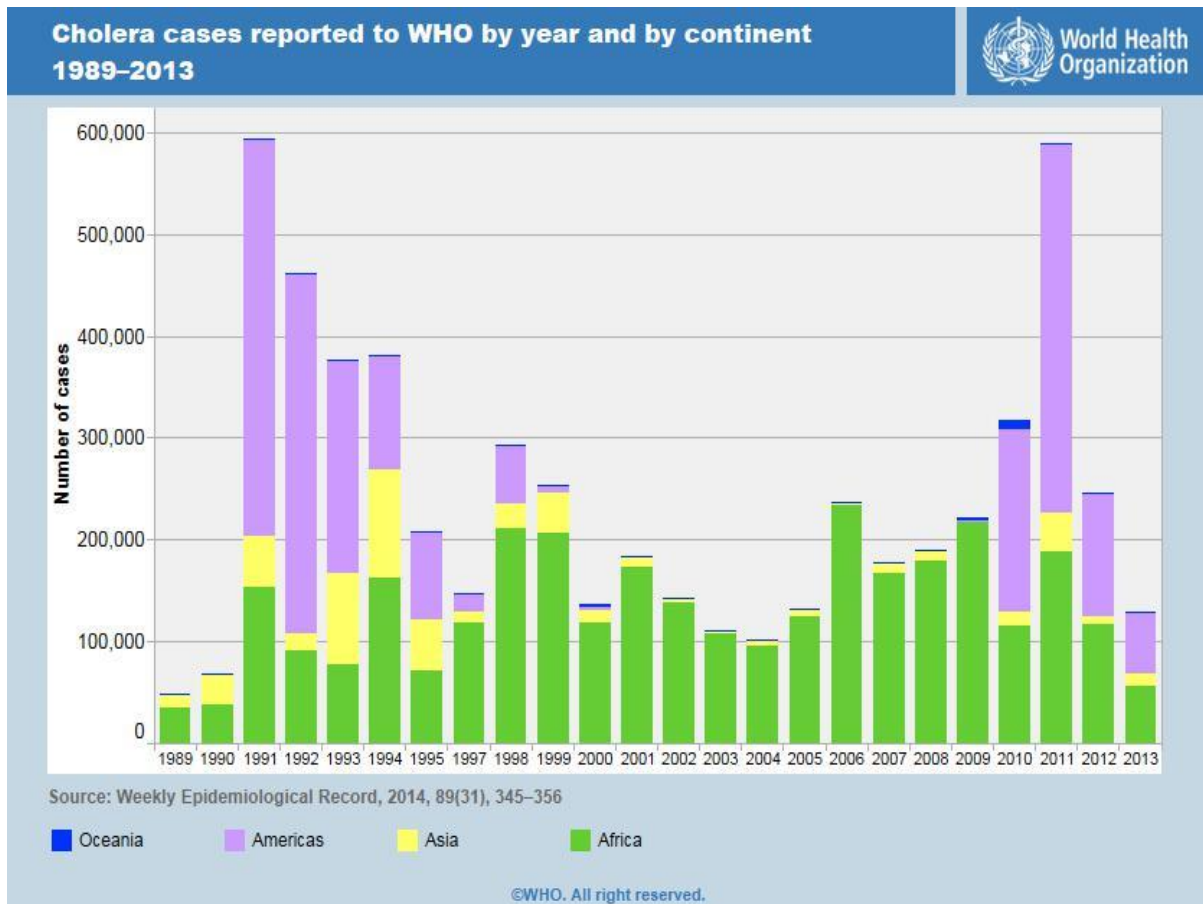
A reliable discrimination between commensal *E. coli* and the various pathotypes is essential for risk evaluation, epidemiological and ecological assessment as well as population genetics. Although various important virulence-associated factors and their roles in the pathogenesis of ExPEC have been described, the use of multiple virulence factors in a mix-and-match fashion makes it difficult to clearly distinguish many ExPEC strains from commensal *E. coli* using a set of discriminatory virulence factors. While the search for the proper typing system that allows a fast and precise determination of ExPEC pathogens, its evolutionary history and pathogenic potentials is still ongoing; a classical typing method uses analysis of the somatic, capsular and flagellar (O:K:H) antigen to differentiate among serogroups (Kohler and Dobrindt, 2011). Currently, the accepted gold standard for ease of use and global comparability is multilocus sequence typing (MLST) which was introduced by Maiden *et al.*, (1998). Even though, many types of *E. coli* MLST schemes are available, nonetheless, they all result in a similar delimitation of the phylogenetic diversity of *E. coli* strains (Kohler and Dobrindt, 2011).

ExPEC strains are implicated in many human and animal infections including UTIs, meningitis, intra-abdominal infections, pneumonia, bacteremia, osteomyelitis and soft-tissue infections among others. Even though the global morbidity and mortality rates are considerable and rising, the increasing emergence of multidrug resistant strains worldwide presents a major challenge to the prevention and management of infections (Poolmani and Wacker, 2015). No prophylactic vaccine exists against ExPEC infection presently.

## 2.6 Contaminated water as a vehicle for cholera disease

Cholera is an acute infectious disease of the gastrointestinal tract with painless watery diarrhoea, nausea and vomiting at the onset, and can kill within hours if left untreated. It is one of the most frequently encountered water-borne diseases caused by certain strains of *Vibrio cholerae* (O1 and O139) and can occur sporadically in many countries where water supplies, sanitation, food safety and hygiene practices are inadequate (Ranjbar *et al.*, 2011; WHO, 2015b). Although the WHO reported a decrease in cholera cases for the year 2013, cholera outbreaks still affect many regions of the world and continue to pose a severe public health challenge in developing countries where access to improved drinking water and adequate sanitation resources are still lacking (WHO, 2015c). The World Health Organisation estimates that documented or reported cases represent only 5 – 10% of the actual number of cases that occur yearly worldwide. Figure 2.3 below shows the distribution of reported cholera cases at different continents between 1989 and 2013 according to the WHO (2015c).

Although, *V. cholerae* is the most virulent and most studied member of the *Vibrio* genus, other species such as *V. fluvialis*, *Grimontia hollisae* (*V. hollisae*), *V. mimicus*, *V. vulnificus* and *V. parahaemolyticus* can also cause diarrhoea or other gastrointestinal infections. *V. cholerae* consists over 200 serogroups based on O-antigen; however, only two serogroups, that is, O1 (causing majority of outbreaks) and O139 (initially identified in Bangladesh in 1992-and confined to South-East Asia) have been responsible for epidemics and pandemics of cholera disease worldwide (Goel *et al.*, 2007; Islam *et al.*, 2013; Menezes *et al.*, 2014; Runft *et al.*, 2014; Unterweger *et al.*, 2014; Rapa *et al.*, 2015). The distribution of the various *Vibrio* species in the environment often depends on salinity and water temperature (Farmer *et al.*, 2005).

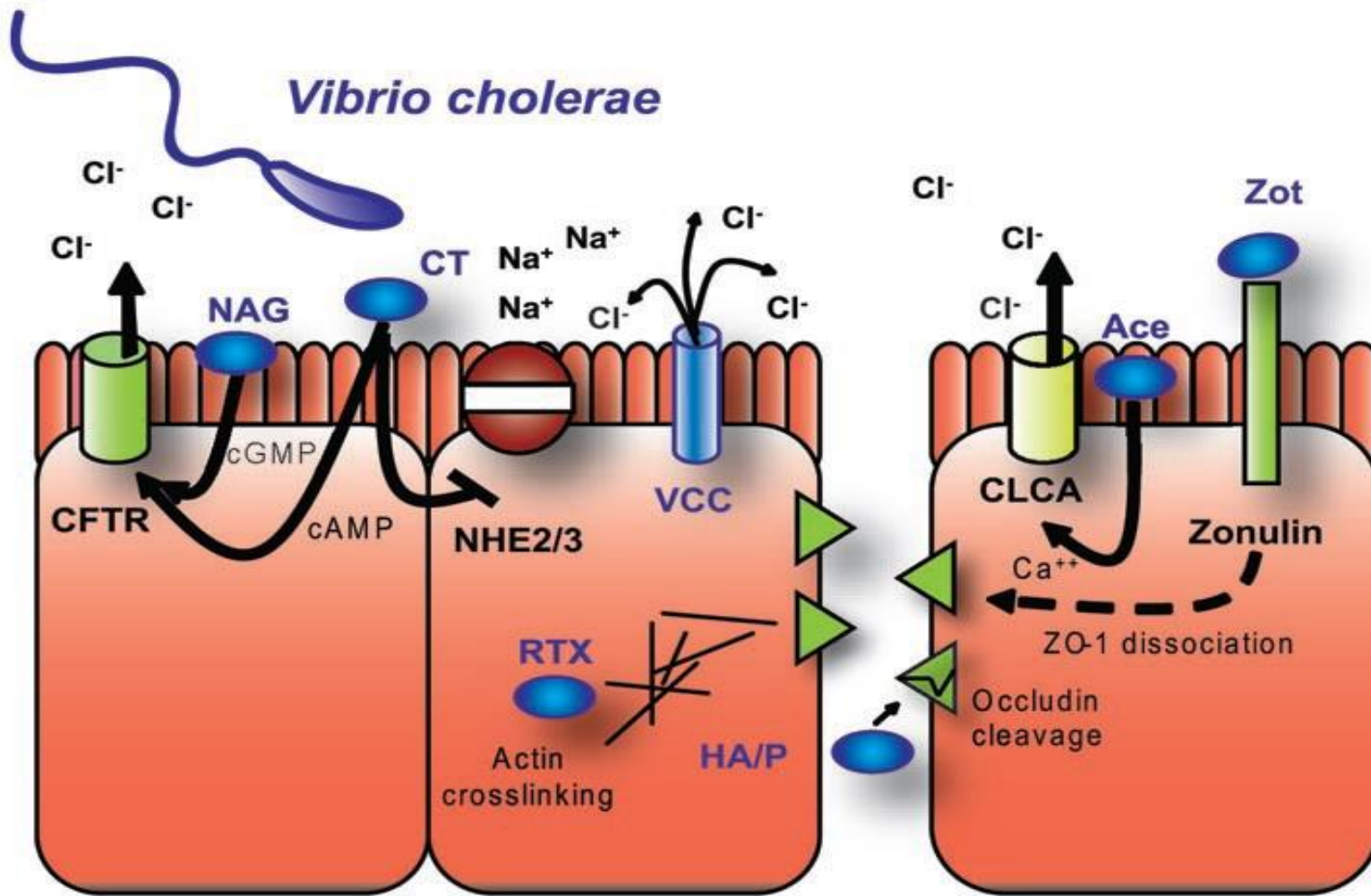


**Figure 2.3:** Distribution of reported cases of cholera cases by continent by year (1989 – 2013). *Source:* WHO (2015c).

Vibrios are commonly found in marine and estuarine habitats while species with low salinity requirements can be found in freshwater environments (Wright *et al.*, 1996; Farmer *et al.*, 2005; Blackwell and Oliver, 2008). The prevention and control of cholera outbreaks require multi-disciplinary approaches such as water and sanitation interventions, social mobilisation, good surveillance programmes, administration of oral cholera vaccines and regulated travel and trade (WHO, 2015d).

The onset of *V. cholerae*-induced diarrhoea is abrupt, with massive loss of fluid which may be up to 1 L/h, thus resulting in acute dehydration and can be lethal within a few hours. The disease is characterised by watery diarrhoea with typical rice-water stools as a result of change in ion secretion and absorption. The release of virulence factors including Ace, cholera toxin and NAG (*N*-acetylglucosamine) heat-stable toxin leads to the activation of both CLCA (Ca<sup>2+</sup>-activated chloride channel) and CFTR (cystic fibrosis transmembrane conductance regulator)-dependent Cl<sup>-</sup> secretion. Increased levels of cyclic adenosine monophosphate (cAMP) also cause the blockage of sodium absorption through NHE2 (Na<sup>+</sup>/H<sup>+</sup> exchanger 2) and NHE3 (Na<sup>+</sup>/H<sup>+</sup> exchanger 3) (Figure 2.4). Additionally, *V. cholerae* creates anion-permeable pores by the insertion of VCC; paracellular permeability also decreases in combination with changes in ion transport/absorption. Interaction between Zot and zonulin leads to its dissociation from tight junctions while HA/P cleaves occluding and rTX interfere with the contractile actin ring (Hodges and Gill, 2010).

Aggressive oral or intravenous rehydration is the mainstay of cholera-induced diarrhoea treatment. The use of antibiotics is also recommended for severely ill and hospitalised patients. However, the choice of antibiotic(s) should be based on the local antibiotic susceptibility pattern or the region; while in most countries, doxycycline is usually recommended as first line of therapy for adults, and azithromycin as the first line of treatment for pregnant women and children. The use of antibiotic as prophylaxis is not recommended for cholera prevention (CDC. 2015a).



**Figure 2.4:** Model summarising the cellular processes of *V. cholera*-induced diarrhoea. Source: Hodges and Gill, 2010.

## 2.7 Water pollution and enteric viruses

Waterborne enteric viral agents constitute a major threat to human and animal health. Enteric viruses may be found in the natural aquatic habitats or be introduced to aquatic environments via anthropogenic activities including: municipal wastewater discharges, urban and agricultural runoffs, leaking sewage and septic networks, among others. Numerous types of disease-causing enteric viruses are shed in the faeces of infected patients and animals (Melnick 1984; Bofill-Mas, 2013; Petrovic, 2013). Viruses can be disseminated in the environment through inadequately treated wastewater and drinking water, surface water that receive treated and untreated wastewater either directly or indirectly, ground water, seawater, estuarine water, and via aerosols emitted from wastewater treatment facilities.

Viruses are host-specific and can cause a variety of diseases and symptoms in humans and animals. Although some enteric viruses have been detected in aquatic environments, the lack of a comprehensive indicator system for monitoring viruses is still a major challenge (Wyn Jones *et al.*, 2011). Varieties of analytical methods are applied to the analysis of environmental water samples for the detection and monitoring of pathogenic microorganisms. Still, the use of bacterial indicator-based techniques for the assessment of microbiological quality of water is insufficient as proxies for pathogenic enteric viruses (Fong and Lipp, 2005).

Enteric viruses are continuously released to the aquatic environment from their host and have the ability to survive in the environment for prolonged periods (about 130 days while type 1 poliovirus have been reportedly survived for about 296 days in sterile pH 7 water at a temperature between +18 °C and +23 °C (Stenstrom *et al.*, 1994)), and remain infectious even

at low concentrations (Eifan, 2013). Therefore, monitoring of the presence and concentration of enteric viruses in various aquatic habitats will be helpful in assessing the public health risks associated with human exposure to such environments.

Diverse environmental factors may contribute to the persistence and survival of enteric viruses in the aquatic habitats. Factors such as pH, temperature, salinity and UV have huge influence on the survival of organisms in the aquatic environments. Low temperature observed in winter months may favour the survival of viruses for longer times while higher temperature may cause inactivation of viral particles due to the denaturation of the viral proteins and nucleic acid (Daczowska-Kozon *et al.*, 2010; Eifan, 2013). Ultraviolet radiation from the sun can inactivate viruses; however, the presence of suspended particles can reduce this effect. Extreme pH such as lime treatment or acidic conditions can also lead to virus inactivation due to direct toxic effect by fragmenting the nucleic acids (Redwan *et al.*, 2008).

More than 140 different types of pathogenic viruses can be transmitted from the aquatic environment to humans. The resultant effect of exposure to pathogenic viruses is usually not the same with different individuals and consequently for all populations. Exposure to waterborne viruses may lead to severe and, sometimes life-threatening illnesses such as infectious hepatitis caused by hepatitis A (HAV) or E (HEV) viruses while less severe diseases like self-limiting diarrhoeal may result from exposure to rotavirus. Even though diarrhoeal may be described as not severe, it should be noted that the disease has been associated with significant infant morbidity and mortality, most especially in some regions and among immune-compromised patients (Redwan *et al.*, 2008).



Enteric viruses are among the most common and most hazardous waterborne pathogenic agents, responsible for some sporadic outbreak of diseases around the world. Virus-associated waterborne outbreaks mainly due to hepatitis A virus and noroviruses have been reported in the USA, and European countries like Denmark, England, Wales, Finland, France, Sweden and Netherlands, while cases of waterborne disease outbreaks have also been documented in Angola, Egypt, Central Africa Republican and some other African countries (WHO, 2001; La Rosa *et al.*, 2012). The global effects of water-related viral diseases, however, remain difficult to evaluate, due to wide range of clinical manifestations associated with waterborne viral infections. Moreover, water contamination evidences are always not available by the time of identification of a disease outbreak mainly due to challenges associated with viral detection in water (La Rosa *et al.*, 2012). There is need for future studies to provide valid, reliable and reproducible techniques for the assessment of waterborne viral pathogens, to help in determining the magnitude of contamination of aquatic environments. More importantly, it is necessary to put in place preventive measures to check the pollution of water sources by viral-carrying pollutants such as inadequately treated sewage and municipal effluents.

## **2.8 Antimicrobial resistance in the aquatic environment**

The role of the aquatic environment as a medium for the spread and evolution, as well as a reservoir of clinically important resistance genes, has been acknowledged in aquatic microbiology. The incessant use of antimicrobial agents in poultry farming together with the practice of indiscriminate discharge of raw or inadequately treated sewage into receiving watershed, has led to significant increase in the number and varieties of antibiotic-resistant microorganisms encountered in the aquatic environment (Young, 1993).

Increasing antibiotic resistance among pathogenic microorganisms is a phenomenon of growing global concern. Presently, very little information is available on the fate and persistence of multidrug resistance organisms and their resistance genes in the natural aquatic environments. Even though the majority of investigations have focused more on drug resistance in the clinical settings, several other studies have, however, documented the contributions of discharged effluents to the spread of antibiotic-resistant bacteria and resistance genes in the aquatic environments (Garcia-Armisen *et al.*, 2011; Thevenon *et al.*, 2012; Rizzo *et al.*, 2013; Ramirez-Castillo *et al.*, 2014; Abia *et al.*, 2015). Antimicrobial resistance has been shown to be widespread among environmental microbial communities, while many resistance elements found in some pathogens are thought to have evolved from environmental bacterial communities (Martinez, 2009; Wright, 2010). Some resistance elements such as mobile genetic elements, transposable elements, plasmids, phages and metal resistance gene among others, have been detected and isolated from environmental matrices including soil, wastewater, marine and freshwater (Davies and Davies, 2010; Wright, 2010; Nesme and Simonet, 2015).

Antimicrobial resistance determinants are, ultimately, disseminated into receiving watershed and coastal line through sewage, agricultural and urban runoffs, and thus forming reservoirs of antimicrobial genes. Selective pressures such as antibiotic overuse and misuse in clinical settings, as well as agricultural and aquaculture application may also favour the emergence and dissemination of resistance factors in the environment (Davies and Davies, 2010; Port *et al.*, 2014). Humans can be exposed directly or indirectly to antibiotic resistance-carrying pathogens through food, such as livestock and seafood, consumption of contaminated drinking water and recreational activities (Wellington *et al.*, 2013; Port *et al.*, 2014).

Documented information remains scarce on the human health risk associated with the spread of multiple antibiotic resistance in the environment (Ashbolt *et al.*, 2013).

## **2.9 Good practices and policies for controlling water pollution**

Recently, there has been a growing awareness of, and concerns about, water pollution globally, and new interventions towards achieving sustainable usage and conservation of water resources have been developed internationally. Several regulatory agencies are involved with the formulation and supervision/enforcing of clean and safe water policies at international, regional, national and local levels in order to safeguard the limited available fresh water sources. These agencies also provide supports for municipal wastewater treatment plants, and also take part in prevention of pollution solely aimed at protecting watersheds, drinking water sources, the environment and human health, at large.

Control of water pollution is clearly one of the most critical challenges worldwide. The majority of industrialised nations have extensive experience of harm resulting from water pollution and have developed strategies and technologies to control such harm. This cannot be said of developing countries, however, due to rapid urbanisation and industrialisation as most of these developing countries have very limited knowledge and experience of pollution control measures. Even though public health problems associated with wastewater and drinking water supplies are intimately linked, issues related to sanitation are often treated politically, usually with lower priority than water supply problems, and larger funds are usually apportioned to water supply than sanitation (Schwarzenbach *et al.*, 2010).

Developing countries require urgent, proper and well coordinated actions to tackle the increasing problems of disease, environmental degradation and economic challenges arising from continuous contamination of the precious water resources. The majority of wastewater treatment technologies applied in many developing countries (where available) are only capable of generating discharged effluents which in most cases, do not meet most of the existing standards or effluent criteria. A stage-wise and well-planned improvement of discharged effluent quality will be a better alternative in developing countries (von Sperling and Chernicharo, 2000). The need for properly developed institutional and legislative policy framework for the protection of water resources from untreated or inadequately treated effluents remains a crucial element in water resource as well as environmental and public health management in developing countries (WHO/UNEP, 1997; von Sperling and Chernicharo, 2000).

## **2.10 Conclusions and closing remarks**

Water pollution may not be visible to the naked eye; however, its impact may for the most part, be far reaching. The presence of organic and inorganic, as well as biological contaminants, in waterbodies constitute major threat to the physicochemical, biological and the aesthetical qualities of such waterbodies in most cases, rendering the water unfit for human use. Impairment of water quality can have a direct bearing on human, animal, and environmental health. Even though there has been increased access to improved water sources over the years (especially in developed countries) due to water and wastewater treatment innovations and improved hygiene levels, nonetheless, it is not enough to give a clean bill of health, particularly in less developed countries where larger portion of sewage remain predominantly untreated or improperly treated before being discharged into the

environment. Although a significant number of disease burden caused by classical waterborne pathogens such as diarrhoea, typhoid and cholera have been well studied and controlled, newly-recognised pathogenic agents and new strains of established pathogens are being discovered concurrently; these present significant additional challenges to both water and public health sectors, worldwide. While the challenges faced in water management and conservation are enormous, evidence shows that they are not insurmountable. Addressing these challenges will require the political will, support and awareness of the general public to adopt stringent measures in controlling indiscriminate water pollution towards the sustenance and implementation of the clean and safe water strategies for all humanity.

## References

- Abia, A.L.K., Ubomba-Jaswa, E. and Momba, M.N.B. (2015).** High prevalence of multiple-antibiotic-resistant (MAR) *Escherichia coli* in river bed sediments of the Apies River, South Africa. *Environmental Monitoring and Assessment* 187, 652.
- Acheson, D.W., Reidi, J., Zhang, X., Keusch, G.T., Mekalanos, J.J. and Waldor, M.K. (1998).** In vivo transduction with Shiga toxin 1-encoding phage. *Infection and Immunity* 66, 4496-4498.
- Afset, J.E., Bevanger, L., Romundstad, P. and Bergh, K. (2004).** Association of atypical enteropathogenic *Escherichia coli* (EPEC) with prolonged diarrhoea. *Journal of Medical Microbiology* 53, 1137-1144.
- Al-Abri, S.S., Beeching, N.J. and Nye, F.J. (2005).** Traveller's diarrhoea. *The Lancet Infectious Diseases*. 5, 349-360.
- Alemad, A., Nagi, M., Ibeda, A. Naser, R., Alwathaf, Y., Elrhaouat, O., Elkharrim, K., Babaqi, A. and Belghyti, D. (2013).** The impacts of Sana'a solid waste on the quality of ground water in Yemen. In, *Water and Society II*, Brebbia, C.A. (ed.). WIT Press, Southampton, UK.
- Alhetar, K.Y.A., Sekawi, Z., Mariana, N.S. and Neela, V. (2011).** Molecular characterization of extended-spectrum beta-lactamase (ESBL) producing extra-intestinal pathogenic *Escherichia coli*. *African Journal of Microbiology Research* 5(31), 5662-5668.
- Alikhani, M.Y., Mirsalehian, A. and Aslani, M.M. (2006).** Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. *Journal of Medical Microbiology* 55, 1159-1163.

- Asano, T., Burton, F.L., Leverenz, H., Tsuchihashi, R. and Tchobanoglous, G. (2007).** Water Reuse: Issues, Technologies, and Applications, McGraw-Hill, New York.
- Ashbolt, N.J. (2004).** Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology* 198:229–38
- Ashbolt, N.J., Amézquita, A., Backhaus, T., Borriello, P., Brandt, K.K., Collignon, P., Coors, A., Finley, R., Gaze, W.H., Heberer, T., Lawrence, J.R., Larsson, D.G., McEwen, S.A., Ryan, J.J., Schönfeld, J., Silley, P., Snape, J.R., Van den Eede, C. and Topp, E. (2013).** Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. *Environmental Health Perspectives* 121, 993-1001.
- Baldwin, T.J., Knutton, S., Sellers, L., Hernandez, H.A., Aitken, A. and Williams, P.H. (1992).** Enteroaggregative *Escherichia coli* strains secrete a heat-labile toxin antigenically related to *Escherichia coli* haemolysin. *Infection and Immunity* 60, 2092-2095.
- Bellin, T., Pulz, M., Matusek, A., Hempen, H.G. and Gunzer, F. (2001).** Rapid detection of enterohemorrhagic *Escherichia coli* by real-time PCR with fluorescent hybridization probes. *Journal of Clinical Microbiology* 39, 370-374.
- Benenson, A.S. (ed.) (1995).** Control of communicable diseases Manual, sixteenth Edition. United Book press, Baltimore. 140-150.
- Berger, S. (2015).** *Escherichia coli* diarrhoea: Global status – 2015 edition. GIDEON Informatics, Inc, Los Angeles, California, USA.
- Bergeron, C.R., Prussing, C., Boerlin, P., Daignault, D., Dutil, L., Reid-Smith, R.J., Zhanel, G.G and Manges, R.A. (2012).** Chicken as Reservoir for Extraintestinal

Pathogenic *Escherichia coli* in Humans, Canada. *Emerging Infectious Diseases* 18, 415-421.

**Bielaszewska, M., Mellmann, A., Zhang, W., Kock, R., Fruth, A., Bauwens, A., Peters, G. and Karch, H. (2011).** Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiology study. *The Lancet Infectious Diseases* 11, 671-676.

**Black, R.E., Lopez de Romana, G., Brown, K.H., Bravo, N., Bazalar, O.G. and Kanashiro, H.C. (1989).** Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar. Peru. *American Journal of Epidemiology* 129, 785-799.

**Black, R.E., Merson, M.H., Huq, I., Alim, A.R. and Yunus, M. (1981).** Incidence and severity of rotavirus and *Escherichia coli* diarrhoea in rural Bangladesh. Implications for vaccine development. *Lancet* 1, 141-143.

**Blackwell, K.D. and Oliver, J.D. (2008).** The ecology of *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* in North Carolina estuaries. *Journal of Microbiology* 46, 146-153.

**Blanco, J., Gonzalez, E.A., Espinosa, P., Blanco, M., Garabal, J.I. and Alanso, M.P. (1992).** Enterotoxigenic and necrotizing *Escherichia coli* in human diarrhoea in Spain. *European Journal of Epidemiology* 8, 548-552.

**Bofill-Mas, S., Rusinol, M., Fernandez-Cassi, X., Carratala, A., Hundesa, A. and Girones, R. (2013).** Quantification of human and animal viruses to differentiate the origin of the fecal contamination present in environmental samples. *BioMed Research International*, Volume 2013, Article ID 192089, 11 pages.



- Boisen, N., Melto-Celsa, A.R., Scheutz, F., O'Brien, A.D. and Nataro, J.P. (2015).** Shiga toxin 2a and enteroaggregative *Escherichia coli* – a deadly combination. *Gut Microbes* 6, 272-278.
- Bonacorsi, S.P.P, Clermont, O., Tinsley, C., Le Gall, I., Beaudin, J-C., Elion, J., Nassif, X. and Bingen, E. (2000).** Identification of regions of the *Escherichia coli* chromosome specific for neonatal meningitis-associated strains. *Infection and Immunity* 68, 2096-2101.
- Botelho, B.A., Bando, S.Y., Trabulsi, L.R. and Moreira-Filho, C.A. (2003).** Identification of EPEC and non-EPEC serotypes in the EPEC O serotypes by PCR-RFLP analysis of the fliC gene. *Journal of Microbiology Methods* 54, 87-93.
- Bowen, R. (2006).** Pathophysiology of diarrhoea. Available on: [www.vivo.colostate.edu/hbooks/pathphys/digestion/smallgut/diarrhoea.html](http://www.vivo.colostate.edu/hbooks/pathphys/digestion/smallgut/diarrhoea.html)  
(Accessed 09 October 2015).
- Boyce, T.G., Swerdlow, D.L. and Griffin, P.M. (1995).** *Escherichia coli* O157:H7 and the haemolytic-uremic syndrome. *The New England Journal of Medicine* 333, 364-368.
- Buccigrossi, V. and Spagnuolo, M.I. (2015).** Bacterial infections of the small and large intestine. In, Textbook of paediatric gastroenterology, hepatology and nutrition: a comprehensive guide to practice. Guandalini, S., Dhawan, A. and Branski, D. (Eds.). Springer International Publishing, AG, Switzerland.
- Cabral, J.P.S. (2010).** Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health* 7, 3657-3703.
- Candrian, U., Furrer, B., Hofelein, C., Myer, R., Jermini, M. and Luthy, J. (1991).** Detection of *Escherichia coli* and identification of enterotoxigenic strains by primer-

directed enzymatic amplification of specific DNA sequences. *International Journal of Food Microbiology* 12, 339–352.

**Cao, Y.S. (2011).** Mass flow and energy efficiency of municipal wastewater treatment plants. IWA Publishing, London, UK.

**Castellani, A. and Chalmers, A.J. (1919).** Manual of tropical medicine, 3rd ed. Williams Wood & Co., New York. p. 959-960

**Castro-Rodriguez, J.A., Salazar-Lindo, E, and Leon-Barua, R. (1997).** Differentiation of osmotic and secretory diarrhoea by stool carbohydrate and osmolar gap measurements. *Archives of Disease in Childhood* 77, 201-205.

**Cennimo, D.J., Koo, H., Mohamed, J.A., Huang, D.B. and Chiang, T. (2007).** Enteroaggregative *Escherichia coli*: a review of trends, diagnosis, and treatment. *Infections in Medicine* 24, 100-110.

**Centers for Disease Control and Prevention (CDC National Centers for Emerging and Zoonotic Infectious Diseases) (2012).** *Escherichia coli*. URL: <http://www.cdc.gov/ecoli/index.html/>. (Accessed 20 December, 2014).

**Centers for Disease Control and Prevention (CDC) (2015).** Infectious diseases related to travel. URL: <http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/escherichia-coli>. (Accessed 01 November, 2015).

**Centers for Disease Control and Prevention (CDC) (2015a).** Cholera – *Vibrio cholera* infection. Available on: <http://wwwnc.cdc.gov/treatment/antibiotic-treatment.html> (Accessed 14 November, 2015).

**Centre for Food Security and Public Health (CFSPH) (2009).** Enterohemorrhagic *Escherichia coli* infections. Iowa State University. URL: [www.cfsph.iastate.edu/Factsheets/pdfs/e\\_coli.pdf](http://www.cfsph.iastate.edu/Factsheets/pdfs/e_coli.pdf)

**Chakraborty, D. and Mukhopadhyay, K. (2014).** Water pollution and abatement policy in India: a study from an economic perspective. Springer Dordrecht Heidelberg New York London.

**Cheppi, P. (2012).** Musi River water pollution its impact on cropping patterns. *Research Journal of Humanities and Social Sciences* 3, 514-518.

**Chowdhury, F., Begum, Y.A., Alam, M.M., Khan, A.I., Ahmed, T., Bhuiyan, Y.A., Harris, J.B., LaRocque, R.C. Faruque, A.S.G., Endtz, H., Ryan, E.T., Cravioto, A., Svennerholm, A.M., Calderwood, S.B. and Qadri, F. (2010).** Concomitant enterotoxigenic *Escherichia coli* infection induces increased immune response to *Vibrio cholera* O1 antigens in patients with cholera in Bangladesh. *Infection and Immunity* 78(5), 2117–2124.

**Clements, A., Young, J.C., Constantinou, N. and Frankel, G. (2012).** Infection strategies of enteric pathogenic *Escherichia coli*. *Gut Microbes* 3, 71-87.

**Crossmann, L.C., Chaudhuri, R.R., Beatson, S.A. Wells, T.J., Desvaux, M., Cunninham, A.F., Petty, N.K., Mahon, V., Brinkley, C., Hobman, J.L., Savarino, S.J., Turner, S.M., Pallen, M.J., Penn, C.W., Parkhill, J., Turner, A.K., Johnson, T.J., Thomson, N.R., Smith, S.G.J. and Henderson, I.R. (2010).** A Commensal Gone Bad: Complete Genome Sequence of the Prototypical Enterotoxigenic *Escherichia coli* Strain H10407. *Journal of Bacteriology* 192, 5822–5831.

- Croxen, M.A. and Finlay, B.B. (2010).** Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology* 8, 26-38.
- Croxen, M.A., Law, R.J., Schoz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B. (2013).** Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews* 26, 822-880.
- Cunin, P., Tedjouka, E., Germanii, Y., Ncharre, C., Bercion, R. and Morvan, J. (1999).** An epidemic of bloody diarrhoea: *Escherichia coli* O157 emerging in Cameroun? *Emerging Infectious Diseases* 5, 285- 290.
- Cyoia, P.S., Rodrigues, G.R., Nishio, E.K., Medeiros, L.P., Koga, V. L., Pereira, A.P.D., Vespero, E.C., Houle, S., Dozois, C.M., Nakazato, G. and Kobayashi, R.K.T. (2015).** Presence of virulence genes and Pathogenicity Island in extraintestinal pathogenic *Escherichia coli* from Brazil. *Journal of Infection in Developing Countries* 9, 1068-1075.
- Daczkowska-Kozon, E.G., Dabrowski, W., Bednarczyk-Drag, A and Szymczak, B. (2010).** Safety aspects of seafood. In, Environmental effects of seafood availability, safety, and quality. Daczkowska-Kozon, E.G and Pan, B.S. (eds.). CRC Press, Taylor & Francis Group, Boca Raton, FL.
- Daigger, G.T. (2008).** New approaches and technologies for wastewater management. In: Bugliarello, G. (Ed.). The Bridge: Linking Engineering and Society. The National Academy of Engineering, Washington, DC.
- Dallman, T., Smith, G.P., O'Brien, B., Chattaway, M.A., Finlay, D., Grant, K.A. and Jenkins, C. (2012).** Characterization of a verocytotoxin-producing enteroaggregative *Escherichia coli* serogroup O111:H21 strain associated with a

household outbreak in Northern Ireland. *Journal of Clinical Microbiology* 50, 4116-4119.

**Davies, J. and Davies, D. (2010).** Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews* 74, 417–433.

**de Louvois, J. (1994).** Acute bacterial meningitis in the newborn. *Journal of Antimicrobial Chemotherapy* 34 (Suppl. A):61-73.

**De, S.N., Bhattacharya, K. and Sarkar, J.K. (1956).** A study of the pathogenicity of strains of bacterium coli from acute and chronic enteritis. *The Journal of Pathology Bacteriology* 71, 201-209.

**Delannoy, S., Beutin, L. and Fach, P. (2013).** Discrimination of enterohemorrhagic *Escherichia coli* (EHEC) from non-EHEC strains based on detection of various combinations of type III effector genes. *Journal of Clinical Microbiology* 51(10), 3257-3262.

**Department of Water Affairs (DWA) (2011).** *Green Drop Handbook, Version 1*; Department of Water Affairs: Pretoria, South Africa.

**Diard, M., Baeriswyl, S., Clermont, O., Gouriou, S., Picard, B., Taddei, F., Denamur, E., Matic, I. (2007).** *Caenorhabditis elegans* as a simple model to study phenotypic and genetic virulence determinants of extraintestinal pathogenic *Escherichia coli*. *Microbes and Infection* 9, 214-223.

**Donnenberg, M.S. (2002).** *Escherichia coli* Virulence Mechanisms of a Versatile Pathogen. Elsevier Sci., (USA).

- Doulgere, J., Otto, B., Nassour, M., Wolters-Eisfeld, G., Rohde, H., Magnus, T., Wagener, C. and Streichert, T. (2015).** Soluble plasma VE-cadherin concentrations are elevated in patients with STEC infection and haemolytic uraemic syndrome: a case control study. *BMJ Open*; 5: 3 e005659 doi:10.1136/bmjopen-2014-005659
- Dow, M.A., Toth, I., Malik, A., Herpay, M., Nogrady, N., Ghenghesh, K.S. and Nagy, B. (2006).** Phenotypic and genetic characterisation of enteropathogenic *Escherichia coli* (EPEC) and entero-aggregative *E.coli* (EAEC) from diarrhoeal and non-diarrhoeal children in Libya. *Comparative Immunology, Microbiology and Infectious Diseases* 29, 100-113.
- Duda-Madej, A., Gosciniak, G., Andrzejewska, B., Duda, A.K. and Sobieszczanska, B. (2013).** Associations of untypeable enteropathogenic *Escherichia coli* (EPEC) strains with persistent diarrhoea in children from the region of lower Silesia in Poland. *Polish Journal of Microbiology* 62, 461-464.
- DuPont, H.L., Formal, S.B., Hornick, R.B., Snyder, M.J., Libonati, J.P., Sheahan, D.G., LaBrec, E.H. and Kalas, J.P. (1971).** Pathogenesis of *Escherichia coli* diarrhea. *The New England Journal of Medicine* 285, 1-9.
- Dutta, S., Guin, S., Ghosh, S., Pazhani, G.P., Rajendran, K., Bhattacharya, M.K., Takeda, Y., Nair, G.B. and Ramamurthy, T. (2013).** Trends in the Prevalence of Diarrhoeagenic *Escherichia coli* among Hospitalized Diarrhoeal Patients in Kolkata, India. *PLoS ONE* 8(2): e56068.
- Effler, E., Isaacson, M., Arntzen, L., Heenan, R., Canter, P., Barrett, T., Lee, L., Mambo, C., Levine, W., Zaidi, A. and Griffin, P.M. (2001).** Factors contributing to

the emergence of *Escherichia coli* O157 in Africa. *Emerging Infectious Diseases* 7, 812–819.

**Elias, W.P., Barros, S.F., Moreira, C.G., Trabulsi, L.R. and Gomes, T.A.T. (2002).** Enteroaggregative *Escherichia coli* strains among classical enteropathogenic *Escherichia coli* O serogroups. *Journal of Clinical Microbiology* 40, 3540-3541.

**Eifan, S.A. (2013).** Enteric viruses and aquatic environment. *The Internet Journal of Microbiology* 12(1). Available on: <http://ispub.com/IJMB/12/1/2926> (Accessed on 21st December 2015).

**Elsinghorst, E.A. and Weitz, J.A. (1994).** Epithelial cell invasion and adherence directed by the enterotoxigenic *Escherichia coli* tib locus is associated with a 104-kilodalton outer membrane protein. *Infection and Immunity* 62, 3463–3471.

**Erume, J., Berberov, E.M., Kachman, S.D., Scott, M.A. Zhou, Y. Francis, D.H. and Moxley, R.A. (2008).** Comparison of the contribution of heat-labile enterotoxin and heat-stable enterotoxin b to the virulence of enterotoxigenic *Escherichia coli* in F4ac receptor-positive young pigs. *Infection and Immunity* 76, 3141–3149.

**Estrada-Garcia, T. and Navarro-Garcia, F. (2012).** Enteroaggregative *Escherichia coli* pathotype: a genetically heterogeneous emerging foodborne enteropathogen. *FEMS Immunology and Medical Microbiology* 66, 281-298.

**European Centre for Disease Prevention and Control (ECDC) (2011).** Annual epidemiological report 2011. Reporting on 2009 surveillance data and 2010 epidemic intelligence data. European Centre for Disease Prevention and Control, Stockholm, Sweden.

- European Food Safety Authority (EFSA) (2013).** Scientific opinion on VTEC-serovars and scientific criteria regarding pathogenicity assessment. EFSA J. 11:3138 <http://www.efsa.europa.eu/en/efsajournal/pub/3138.htm> (Accessed 10th October 2015).
- Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.-S., Luchansky, J.B., and Kaspar, C.W. (1996).** Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Applied and Environmental Microbiology* 62, 1519-1525.
- Farmer, J.J., Janda, J.M., Brenner, F.W., Cameron, D.N. and Birkhead, K.M. (2005).** Genus *Vibrio*. In *Bergey's Manual of Systematic Bacteriology*, 2nd ed.; Brenner, D.J., Krieg, N.R., Staley, J.T., Eds.; Springer: New York, NY, USA, 2005; Volume 2, Part B, pp. 494–546.
- Farthing, M.J.G. (1993).** Pathophysiology of infective diarrhoea. *European Journal of Gastroenterology & Hepatology* 5, 796–807.
- Fernandez, D., Kruger, A., Polifroni, R., Bustamante, A.V., Sanso, A.M., Etcheverria, A.I., Lucchesi, P.M.A., Parma, A.E. and Padola, N.L. (2013).** Characterization of Shiga toxin-producing *Escherichia coli* O130:H11 and O178:H19 isolated from dairy cows. *Frontiers in Cellular and Infection Microbiology* 3(9), 44-49.
- Fernandez-Jauregui, C. (2010).** Water resources in developing countries: the millennium development goals in the 21st century. In *Water Engineering and Management through Time: Learning from History* ed. Cabrera, E. pp 291. Boca Raton, FL. CRC Press.



- Fleckenstein JM, Kopecko DJ, Warren RL and Elsinghorst EA (1996).** Molecular characterization of the tia invasion locus from enterotoxigenic *Escherichia coli*. *Infection and Immunity* 64, 2256–2265.
- Fleckenstein, J.M, Hardwidge, P.R., Munson, G.P., Rasko, D.A., Sommerfelt, H. and Steinland, H. (2010).** Molecular mechanisms of enterotoxigenic *Escherichia coli* infection. *Microbes and Infection* 12, 89–98.
- Fleckenstein, J.M, Lindler, L.E., Elsinghorst, E.A. and Dale, J.B. (2000).** Identification of a gene within a pathogenicity island of enterotoxigenic *Escherichia coli* H10407 required for maximal secretion of the heat-labile enterotoxin. *Infection and Immunity* 68, 2766–2774.
- Fleckenstein, J.M., Roy, K., Fischer, J.F. and Burkitt, M. (2006).** Identification of a two-partner secretion locus of enterotoxigenic *Escherichia coli*. *Infection and Immunity* 74, 2245–2258.
- Fong, T.T. and Lipp, E.K. (2005).** Enteric viruses of human and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Review* 69(2), 357-371.
- Food and Drug Administration (FDA) (2012).** Bad Bug Book, Foodborne pathogenic microorganisms and natural toxins. Second Edition. Available on: <http://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf> (Accessed 26th October 2015).
- Fosshage, M. (2014).** A new wastewater treatment technology for developing countries. *Filtration + Separation* 51(5), 14-17.

- Friesema, I.H., Keijzer-Veen, M.G., Koppejan, M., Schipper, H.S., van Griethuysen, A.J., Heck, M.E. and van Pelt, W. (2015).** Hemolytic uremic syndrome associated with *Escherichia coli* O8:H19 and Shiga toxin 2f gene. *Emerging Infectious Diseases* 21(1), 168-169.
- Gaastra, W. and Svennerholm, A.M. (1996).** Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). *Trends in Microbiology* 4, 444–452.
- Garcia-Armisen, T., Vercammen, K., Passerat, J., Triest, D., Servais, P. and Cornelis, P. (2011).** Antimicrobial resistance of heterotrophic bacteria in sewage-contaminated rivers. *Water Research* 45, 788-796.
- Gaschignard, J., Levy, C., Bingen, E. and Cohen, R. (2012).** Epidemiology of *Escherichia coli* neonatal meningitis. *Archives de Pediatrie* 19, S129-S134.
- George, D.B. and Manges, A.R. (2010).** A systematic review of outbreak and non-outbreak studies of extraintestinal pathogenic *Escherichia coli* causing community-acquired infections. *Epidemiology & Infection* 138, 1679-1690.
- Germani, Y., Soro, B., Vohito, M., Morel, O. and Morvan, J. (1997).** Enterohaemorrhagic *Escherichia coli* O157:H7 outbreaks in Central African Republic. *Lancet* 349, 1670.
- Giron, J.A., Jones, T., Millan-Velasco, F., Castro-Munoz, E., Zárate, L., Fry, J., Frankel, G., Moseley, S.L., Baudry, B. and Kaper, J.B. (1991).** Diffuse-adhering *Escherichia coli* (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. *Journal of Infectious Diseases* 163, 507-513.
- Goel, A.K., Ponmariappan, S., Kambol, D.V., Singh, L. (2007).** Single multiplex polymerase chain reaction for environmental surveillance of toxigenic-pathogenic O1 and non-O1 *Vibrio cholerae*. *Folia Microbiologica* 52, 81-85

- Goldwater, P.N. and Bettelheim, K.A. (2012).** Treatment of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic uremic syndrome (HUS). *BMC Medicine* 10, 1-8.
- Gomes, T.A., Vieira, M.A., Abe, C.M., Rodrigues, D., Griffin, P.M. and Ramos, S.R. (1998).** Adherence patterns and adherence-related DNA sequences in *Escherichia coli* isolates from children with and without diarrhea in São Paulo city, *Brazilian Journal Clinical of Microbiology* 36, 3609 –3613.
- Gomez-Duarte, O.G., Arzuza, O., Urbina, D., Bai, J., Guerra, J., Montes, O., Puello, M., Mendoza, K. and Castro, G.Y. (2010).** Detection of *Escherichia coli* enteropathogens by multiplex polymerase chain reaction from children’s diarrheal stools in two Caribbean-Colombian cities. *Foodborne Pathogens and Diseases* 7, 199 –206.
- Gomez-Duarte, O.G., Romero-Herazo, Y.C., Peaz-Canro, C.Z., Eslava-Schmalbach, J.H. and Arzuza, O. (2013).** Enterotoxigenic *Escherichia coli* associated with childhood diarrhoea in Colombia, South America. *The Journal of Infection in Developing Countries* 7(5): 372-381.
- Gorbach, S.L., Banwell, J.G., Chatterjee, B.D., Jacobs, B. and Sack, R.B. (1971).** Acute undifferentiated human diarrhea in the tropics. I. Alterations in intestinal microflora. *The Journal of Clinical Investigation* 50, 881-889.
- Grad, Y.H., Lipsitch, M., Feldgarden, M., Arachchi, H.M., Cerqueira, G.C., Fitzgerald, M., Godfrey, P., Haas, B.J., Murphy, C.I., Russ, C., Sykes, S., Walker, B.J., Wortman, J.R., Young, S., Zeng, Q., Abouelleil, A., Bochicchio, J., Chauvin, S., Desmet, T., Gujja, S., McCowan, C., Montmayeur, A., Steelman, S., Frimodt-Moller, J., Petersen, A.M., Struve, C., Krogfelt, K.A., Bingen, E.,**

- Weill, F-X., Lander, E.S., Nusbaum, C., Birren, B.W., Hung, D.T. and Hanage, W.P. (2012).** Genomic epidemiology of the *Escherichia coli* O104:H4 outbreaks in Europe, 2011. *Proceedings of the National Academy of Sciences of the United States of America* 109, 3065–3070.
- Grandy, G. Medina, M., Soria, R., Teran, G.C. and Araya, M. (2010).** Probiotic in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. *BMC Infectious Diseases* 10, 1-7.
- Greenberg, H.B. and Estes, M.K (2009).** Rotaviruses: from pathogenesis to vaccination. *Gastroenterology* 136(6), 1939-1951.
- Guerrant, R.L., Kosek, M., Moore, S., Lorntz, B., Brantley, R. and Lima, A.A. (2002).** Magnitude and impact of diarrhoeal diseases. *Archives of Medical Research* 33, 351-355.
- Gunzburg, S.T., Chang, B.J., Elliott, S.J., Burke, V. and Gracey, M. (1993).** Diffuse and enteroaggregative patterns of adherence of enteric *Escherichia coli* isolated from aboriginal children from the Kimberley region of Western Australia. *Journal of Infectious Diseases* 167, 755-758.
- Gupta, S.K., Keck, J., Ram, P.K., Crump, J.A., Miller, M.A and Mintz, E.D. (2008).** Part III. Analysis of data gaping pertaining to enterotoxogenic *Escherichia coli* infections in low and the medium human development index countries, 1984-2005. *Epidemiology and Infection* 136, 721-738.
- Gyles, C.L. and Barnum, D.A. (1969).** A heat-labile enterotoxin from strains of *Escherichia coli* enteropathogenic for pigs. *Journal of Infectious Diseases* 120, 419-426.

- Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T.A., Fairbrother, J., Harel, J., Maynard, C., Masson, L. and Brousseau, R. (2007).** Occurrence of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from different aquatic ecosystems within the St. Clair River and Detroit River Areas. *Applied and Environmental Microbiology* 73, 477-484.
- Health Canada (2006b).** Guidelines for Canadian drinking water quality: Guideline Technical Document —*Escherichia coli*. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
- Hedberg, C.W., Savarino, S.J., Besser, J.M., Besser, J.M., Paulus, C.J., Thelen, V.M., Myers, L.J., Cameron, D.N., Barrett, T.J., Kaper, J.B., Osterholm M.T. (1997).** An outbreak of foodborne illness caused by *Escherichia coli* O39:NM, an agent not fitting into the existing scheme for classifying diarrhoeagenic *E. coli*. *The Journal of Infect Diseases* 176, 1625-1628.
- Henderson, I.R., Navarro-Garcia, F. and Nataro, J.P. (1998).** The great escape: structure and function of the autotransporter proteins. *Trends in Microbiology* 6, 370–378.
- Herzer, P.J., Inouye, S., Inouye, M. and Whittam, T.S. (1990).** Phylogenetic distribution of branched RNS-linked multicopy single-stranded DNA among natural isolates of *Escherichia coli*. *Journal of Bacteriology* 172, 6175-6181.
- Hodges. K and Gill. R. (2010).** Infectious diarrhoea: Cellular and molecular mechanisms. *Gut Microbes* 1, 4-21.
- Huang, D.B., Mohanty, A., DuPont, H.L., Okhuysen, P.C. and Chiang, T. (2006).** A review of an emerging enteric pathogen: Enteroaggregative *Escherichia coli*. *Journal of Medical Microbiology* 55, 1303-1311.

- Iguchi, A., Thomson, N.R., Ogura, Y., Saunders, D., Ooka, T., Henderson, I.R., Harris, D., Asadulghani, M., Kurokawa, K., Dean, P., Kenny, B., Quali, M.A., Thurston, S., Dougan, G., Hayashi, T., Parkhill, J. and Frankel, G. (2009).** Complete Genome Sequence and Comparative Genome Analysis of Enteropathogenic *Escherichia coli* O127:H6 Strain E2348/69. *Journal of Bacteriology* 191, 347-54.
- Isaacson, M., Canter, P.H., Effeler, P., Arntzen, L., Bomans, P. and Heenan, R. (1993).** Haemorrhagic colitis epidemic in Africa. *Lancet* 341, 961.
- Islam, A., Labate, M., Djordjevic, S.P., Alam, M., Darling, A., Melvold, J., Stokes, H.W. (2013).** Indigenous *Vibrio cholerae* strains from a non-endemic region are pathogenic. *Open Biology* 3(2), 120181. <http://doi.org/10.1098/rsob.120181>
- Itoh, Y., Nagano, I., Kunishima, M., Ezaki, T. (1997).** Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable: H10 associated with a massive outbreak of gastrointestinal illness. *Journal of Clinical Microbiology* 35, 2546-2550.
- Iyoda, S., Tamura, K., Itoh, K., Izumiya, H., Ueno, N., Nagata, K., Togo, M., Terajima, J. and Watanabe, H. (2000).** Inducible *stx2* phages are lysogenised in the enteroaggregative and other phenotypic *Escherichia coli* O86:HNM isolated from patients. *FEMS Microbiology Letters* 191, 7-10.
- Jafari, A., Aslani, M.M. and Bouzari, S. (2013).** Enteroaggregative *Escherichia coli*, a heterogeneous, underestimated and underdiagnosed *E. coli* pathotype in Iran. *Gastroenterology and Hepatology from Bed to Bench* 6, 71-79.
- Jafari, A., Aslani, M.M., and Bouzari, S. (2012).** *Escherichia coli*: a brief review of diarrhoeagenic pathotypes and their role in diarrhoeal diseases in Iran. *Iran Journal of Microbiology* 4, 102-117.

- Jensen, B.H., Olsen, K.E.P., Struve, C., Krogfelt, K.A. and Petersen, A.M. (2014).** Epidemiology and clinical manifestation of Enteroaggregative *Escherichia coli*. *Clinical Microbiology Reviews* 27, 614-630.
- Jofre, J. and Blanch, A.R. (2010).** Feasibility of methods based on nucleic acid amplification techniques to fulfil the requirements for microbiological analysis of water quality. *Journal of Applied Microbiology* 109, 1853-1867.
- Johnson, J.R. and Russo, T.A. (2005).** Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. *International Journal of Medical Microbiology* 295, 383-404.
- Kaper, J.B. (1996).** Defining EPEC. *Rev Microbiol* 27, 130-133.
- Kaper, J.B., Nataro, J.P. and Mobley, H.L. (2004).** Pathogenic *Escherichia coli*. *Nature Reviews Microbiology* 2, 123–140.
- Karch, H. (2001).** The role of virulence factors in enterohemorrhagic *Escherichia coli* (EHEC)-associated hemolytic-uremic syndrome. *Seminars in Thrombosis and Hemostasis* 27, 207-213.
- Kaur, P., Chakraborti, A. and Asea, A. (2010).** Enteroaggregative *Escherichia coli*: an emerging enteric food borne pathogen. *Interdisciplinary Perspective on Infectious Diseases*, Article ID 254159, 10 pages.
- Keene, W.E., McAnulty, J.M., Hoesley, F.C., Williams, L.P., Hedberg, K., Oxman, G.L., Barrett, T.J., Pfaller, M.A., and Fleming, D.W. (1994).** A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *The New England Journal of Medicine* 331, 579-584.

- Kehl, S.C (2002).** Role of the laboratory in the diagnosis of enterohaemorrhagic *Escherichia coli* infections. *Journal of Clinical Microbiology* 40, 2711-2715.
- Kelly, P.J., Meads, N., Theobald, A., Fournier, P.E. and Raoult, D. (2004).** Enterohemorrhagic *Escherichia coli* O157, Kinshasa. *Emerging Infectious Diseases*. 10, 967-968.
- Kim, J.K. and Harrison, M.A. (2007).** Transfer of *Escherichia coli* O157:H7 to Romaine lettuce due to contact water from melting ice. *J. Food Prot.* 71, 252-256.
- Kiser, J.D., Paulson, C.P. and Brown, C. (2008).** Clinical inquires. What's the most effective treatment for giardiasis? *The Journal of Family Practice* 57, 270-272.
- Knutton, S., Shaw, R., Phillips, A.D., Smith, H.R., Willshaw, G.A., Watson, P. and Price, E. (2001).** Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* isolated from children in the United Kingdom. *Journal of Pediatric Gastroenterology and Nutrition* 33, 32-40.
- Kohler, C.D. and Dobrindt, U. (2011).** What defines extraintestinal pathogenic *Escherichia coli*? *International Journal of Medical Microbiology* 301, 642-647.
- Koo, H.L., Jiang, Z.D., Brown, E., Garcia, C., Qi, H. and DuPont, H.L. (2008).** Coliform contamination of vegetables obtained from popular restaurants in Guadalajara, Mexico, and Houston, Texas. *Clinical Infectious Diseases* 47, 218-221.
- Kosek, M., Bern, C. and Guerrant, R.L. (2003).** The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organisation* 81, 197-204.



- Kotloff, K.L., Nataro, J.P., Blackwell, W., Nasrin, D. and Farag, T. (2013).** Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicentre Study, GEMS): a prospective, case-control study. *Lancet* 382, 209-222.
- Lampel, K. (2014).** Enteroinvasive *Escherichia coli*: introduction and detection by classical cultural and molecular techniques. *In*, Encyclopedia of Food Microbiology Second Edition. Batt, C.A. and Tortorello, M. (Eds.). Academic Press, Oxford, UK.
- Lamprecht, C., Romanis, M. Huisamen, N., Carinus, A., Schoeman, N., Sigge, G.O., and Britz, T.J. (2014).** *Escherichia coli* with virulence factors and resistance in the Plankenburg. *South African Journal of Science* 110, 1-6.
- La Rosa, G., Fratini, M., della Libera, S., Iaconelli, M. and Muscillo, M. (2012).** Emerging and potentially emerging viruses in water environments. *Annali dell'Istituto Superiore di Sanita* 48, 397-406.
- Lawson, J.M. (2004).** Update on *Escherichia coli* O157:H7. *Current Gastroenterology Reports* 6, 297-301.
- Le Bouguenec, C., and Servin, A.L. (2006).** Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognised pathogens. *Microbiology Letters* 256, 185-194.
- Le Bouguenec, C., Lalioui, L., du Merle, L., Jouve, M., Courcoux, P., Bouzari, S., Selvarangan, R., Nowicki, B.J., Germani, Y., Andremont, A., Gounon, P. and Garcia, M-I. (2001).** Characterisation of AfaE adhesins produced by extraintestinal and intestinal human *Escherichia coli* isolates: PCR assays for detection of Afa

adhesins that do or do not recognize Dr blood group antigens. *Journal of Clinical Microbiology* 39, 1738–1745.

**Levine, M.M. and Edelman, R. (1984).** Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhoea: epidemiology and pathogenesis. *Epidemiology Reviews* 6, 31-51.

**Levine, M.M., Ferreccio, C., Prado, V., Cayazzo, M., Abrego, P., Martinez, J., Maggi, L., Baldini, M.M., Martins, W. and Maneval, D. (1993).** Epidemiology studies of *Escherichia coli* diarrhoeal infections in a low socioeconomic level peri-urban community in Santiago, Chile. *American Journal of Epidemiology* 138, 849-869.

**Lienemann, T., Salo, E., Rimhanen-Finne, R., Ronnholm, K., Taimisto, M., Hirvonen, J.J., Tarkka, E., Kuusi, M. and Siitonen, A. (2012).** Shiga toxin-producing *Escherichia coli* serotype O78:H in family, Finland, 2009. *Emerging Infectious Diseases* 18, 577-581.

**Lin, Z., Kurazono, H., Yamasaki, S. and Takeda, Y. (1993).** Detection of various variant verotoxin genes in *Escherichia coli* by polymerase chain reaction. *Microbiology and Immunology* 37, 543–548.

**Lopes, L.M., Fabbriotti, S.H., Ferreira, A.J.P., Kato, M.A.M.F., Michalski, J. and Scaletsky, I.C.A. (2005).** Heterogeneity among strains of diffusely adherent *Escherichia coli* isolated in Brazil. *Journal of Clinical Microbiology* 43, 1968-1972.

**Maguire, A.L., Brown, D.F.J., Gray, J.J. and Desselberger, U. (2001).** Rapid screening technique for class 1 integrons in *Enterobacteriaceae* and nonfermenting gram-negative bacteria and its use in molecular epidemiology. *Antimicrobial Agents and Chemotherapy* 45, 1022–1029.

- Maltby, R., Leatham-Jensen, M.P., Gibson, T., Cohen, P.S. and Conway, T. (2013).** Nutritional basis for colonization resistance by human commensal *Escherichia coli* strains HS and Nissle 1917 against *E. coli* O157:H7 in the mouse intestine. *PLoS ONE* 8(1), e53957.
- Manges, A.R., Johnson, J.R., Foxman, B., O'Bryan, T.T., Fullerton, K.E. and Riley, L.W. (2001).** Widespread distribution of urinary tract infections caused by multidrug-resistant *Escherichia coli* clonal group. *The New England Journal Medicine* 345, 1007-1013.
- Maiden, M.C.J., Bygraves, J.A., Feil, E., Morelli, G., Russell, J.E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D.A., Feavers, I.M., Achtman, M. and Spratt, B.G. (1998).** Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceeding of the National of Academy of Science of the United States of America* 95, 3140-3145.
- Mariani-Kurkdjian, P., Lemaitre, C., Bidet, P., Perez, D., Boggini, L., Kwon, T and Bonacorsi, S. (2014).** Haemolytic-uraemic syndrome with bacteraemia caused by new hybrid *Escherichia coli* pathotype. *New Microbes and New Infections* 2, 127-131.
- Martinez, J.L. (2009).** The role of natural of the natural environments in the evolution of resistance traits in pathogenic bacteria. *Proceeding of the Royal Society of B: Biological Science Journal* 276, 2521-2530.
- Mateo-Sagasta, J., Raschid-Sally, L. and Thebo, A. (2015).** Global wastewater and sludge production, treatment and use. In, *Wastewater: Economic Asset in an Urbanizing World*. Drechsel, P., Qadir, M. and Wichelns, D. (eds.), Springer, Netherlands.

- McFarland, L.V. (2007).** Meta-analysis of probiotics for the prevention of traveler's diarrhea. *Travel Medicine and Infectious Disease* 5, 97–105.
- Melnick, J.L. (1984).** Etiologic agents and their potential for causing waterborne virus diseases, p. 1-16. *In*, Enteric viruses in water, vol. 15. Melnick, J.L. (ed.), Karger, Basel, Switzerland.
- Mendez-Arancibia, E., Vargas, M., Soto, S., Ruiz, J., Kahigwa, E., Schellenberg, D., Urassa, H., Gascón, J, and Vila, J. (2008).** Prevalence of different virulence factors and biofilm production in enteroaggregative *Escherichia coli* isolates causing diarrhea in children in Ifakara (Tanzania). *The American Journal of Tropical Medicine and Hygiene* 78, 985-989.
- Menezes, F.G.R., Neves, S.S., Sousa, O.V., Vila-Nova, C.M.V.M., Maggioni, R., Theophilo, G.N.D., Hofer, E. and Vieira, R.H.S.F. (2014).** Detection of virulence genes in environmental strains of *Vibrio cholerae* from estuaries in Northeastern Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* 56, 427-432.
- Momtaz, H, Kaimian, A., Madani, M., Dehkordi, F.S., Ranjbar, R., Sarshar, M, and Souod, N. (2013).** Uropathogenic *Escherichia coli* in Iran: serogroup distribution, virulence factors and antimicrobial resistance properties. *Annals of Clinical Microbiology* 12, 8.
- Morabito, S., Karch, H., Mariani-Kurkdjian, P., Schmidt, H, Minelli, F., Bingen, E. and Caprioli, A. (1998).** Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of haemolytic-uremic syndrome. *Journal of Clinical Microbiology* 36, 840-842.

- Mossoro, C., Glaziou, P., Yassibanda, S., Lan, N.T.P., Bekondi, C., Minssart, P., Bernier, C., Le Bouguenec, C., and Germani, Y. (2002).** Chronic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome associated with HEp-2 adherent *Escherichia coli* in adults infected with human immunodeficiency virus in Bangui, Central African Republic. *Journal of Clinical Microbiology* 40, 3086–3088.
- Moyo, S.J., Maselle, S.Y., Matee, M.I., Langeland, N. and Mylvaganam, H. (2007).** Identification of diarrhoeagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infectious Diseases* 7, 92.
- Naidoo, S, and Olaniran, A.O. (2014).** Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. *International Journal of Environmental Research and Public Health* 11, 249-270.
- Nataro, J.P. and Kaper, J.B. (1998).** Diarrhoeagenic *Escherichia coli*. *Clinical Microbiology Reviews* 11(1), 142 – 201.
- Nataro, J.P., Mai, V., Jonhson, J., Blackwelder, W.C., Heimer, R. Tirrell, S., Edberg, S.C., Braden, C.R., Glenn Morris, J. Jr, and Hirshon, J.M. (2006).** Diarrhoea *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. *Clinical Infectious Diseases* 43, 402-407.
- National Enteric Surveillance Program (NESP) (2010).** National Enteric Surveillance Program (NESP) annual summary 2010. The National Microbiology Laboratory (NML) and Centre for Food-borne, Environmental and Zoonotic Infectious Diseases (CFEZID), Public Health Agency of Canada and Provincial Public Health Microbiology Laboratories, Winnipeg, Canada.

- National Research Council (NRC) (2012).** Water reuse: potential for expanding the nation's water supply through reuse of municipal wastewater. The National Academies Press, Washington, D.C.
- Navaneethan, U. and Giannella, R. (2010).** Definition, epidemiology, pathophysiology, clinical classification, and differential diagnosis of diarrhoea. *In*, Diarrhoea diagnostic and therapeutic advances. Guandalini, S. and Vaziri, H. (eds.). Springer Science+Business Media, LLC. P 1-16. e-ISBN 978-1-60761-183-7
- Navannethan, U. and Giannella, R.A. (2008).** Mechanisms of infectious diarrhoea. *Nature Clinical Practice Gastroenterology & Hepatology* 5, 637-647.
- Navarro, I. and Zagmutt, F.J. (2009).** Chemical health risks. In Urban Water Security: Managing Risks. Jimenez, B. and Rose, J. eds. CRC Press Boca Raton FL.
- Naylor, S.W., Low, J.C., Besser, T.E., Mahajan, A., Gunn, G.J., Pearce, M.C., Mckendrick, I.J., Smith, D.G. and Gally, D.L. (2003).** Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonisation of enterohaemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity* 71, 1505-1512.
- Nesme, J. and Simonet, P. (2015).** The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. *Environmental Microbiology* 17, 913-930.
- Nguyen, R.N., Taylor, L.S., Tauschek, M., Robins-Browne, R.M. (2006).** Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhoea in children. *Emerging Infectious Diseases* 12, 597-603.

- Nguyen, Y. and Sperandio, V. (2012).** Enterohemorrhagic *E. coli* (EHEC) pathogenesis. *Frontiers in Cellular and Infection Microbiology* 2, 37-43.
- Niyogi, S.K. (2005).** Shigellosis. *The Journal of Microbiology* 43, 133–143.
- Nweze, E.I. (2009).** Virulence properties of diarrhoeagenic *E. coli* and the aetiology of diarrhoea in infants, young children and other age groups in Southeast, Nigeria. *American-Eurasian Journal of Scientific Research* 4, 173-179.
- O’Sullivan, J., Bolton, D.J., Duffy, G., Baylis, C., Tozzoli, R., Wasteson, Y. and Lofdahl, S. (2006).** Methods for detection and molecular characterisation of pathogenic *Escherichia coli*. CO-ORDINATION ACTION FOOD-CT-2006-036256, Pathogenic *Escherichia coli* Network.
- Ochoa, T.J. and Cleary T.G. (2015).** *Escherichia coli*. In, Nelson Textbook of Pediatric 20th Edition. Kliegman, R., Stanton, B., St. Geme, J. and Schor, N. (Eds.). Elsevier.
- Ochoa, T.J. and Contreras, C.A. (2011).** Enteropathogenic *E. coli* (EPEC) infection in children. *Current Opinion in Infectious Diseases* 24(5), 478-483.
- Ochoa, T.J., Barletta, F., Contreras, C. and Mercado, E. (2008).** New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 102(9), 852-856.
- Ochoa, T.J., Rivera, F.P., Bernal, M., Meza, R., Ecker, L., Gil, A.I., Cepeda, D., Mosquito, S., Mercado, E., Maves, R.C., Hall, E.R., Svennerholm, A-M., McVeigh, A., Savarino, S. and Lanata, C.F. (2010).** Detection of the CS20 colonization factor antigen in diffuse-adhering *Escherichia coli* strains. *FEMS Immunology and Medical Microbiology* 60, 186-189.

- Odonkor, S.T. and Ampofo, J.K. (2013).** *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research* 4, 5-11.
- Ogasawara, T., Ito, K., Igarashi, K., Yutsudo, T, Nakabayashi, N. and Takeda, Y. (1988).** Inhibition of protein synthesis by Vero toxin (VT2 or Shiga-like toxin II) produced by *Escherichia coli* O157:H7 at the level of elongation factor 1-dependent aminoacyl-tRNA binding to ribosomes. *Microbial Pathogenesis* 4, 127-135.
- Okeke, I.N. (2009).** Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *The Journal of Infection in Developing Countries* 3(11), 817-842.
- Okeke, I.N., Lamikanra, A., Steinruck, H. and Kaper, J.B. (2000).** Characterisation of *Escherichia coli* strains from cases of childhood diarrhoea in provincial Southwestern Nigeria. *Journal of Clinical Microbiology* 38(1): 7.
- Okeke, I.N., Macfarlane-Smith, L.R., Fletcher, J.N., Snelling, A.M. (2011).** IS3 profiling identifies the enterohaemorrhagic *Escherichia coli* O-island 62 in a distinct enteroaggregative *E. coli* lineage. *Gut Pathogens* 3:4.
- Okeke, I.N., Ojo, O., Lamikanra, A. and Kaper, J.B. (2003).** Etiology of acute diarrhoea in adults in Southwestern Nigeria. *Journal of Clinical Microbiology* 41, 4525-4530.
- Okeke, I.N., Wallace-Gadsden, F., Simons, H.R., Matthews, N., Labar, A.S., Hwang, J. and Wain, J. (2010).** Multi-locus sequence typing of Enteroaggregative *Escherichia coli* isolates from Nigeria children uncovers multiple lineages. *PLoS One* 5, e14093
- Okhuysen, P.C. and DuPont, H.L. (2010).** Enteroaggregative *Escherichia coli* (EAEC): A cause of acute and persistent diarrhoea of worldwide importance. *The Journal of Infectious Diseases* 202, 503–505.



- Olive, M. (1989).** Detection of enterotoxigenic *Escherichia coli* after polymerase chain reaction amplification with a thermostable DNA polymerase. *Journal of Clinical Microbiology* 27, 261-265.
- Olsen, B., Kolmos, H.J., Orskoy, F. and Orskov, I. (1994).** Cluster of multiresistant *Escherichia coli* O78:H10 in great Copenhagen. *Scandinavian Journal of Infectious Diseases* 26, 406-410.
- Onanuga, A., Igbeneghu O. and Lamikanra, A. (2014).** A study of the prevalence of diarrhoeagenic *Escherichia coli* in Gwagwalada, Federal Capital Territory of Nigeria. *The Pan Africa Medical Journal* 17, 1-8.
- Osode, A.N. and Okoh, A.I. (2010).** Survival of free-living and plankton-associated *Escherichia coli* in the final effluents of a waste water treatment facility in a peri-urban community of the Eastern Cape Province of South Africa. *African Journal of Microbiology Research* 4, 1424-1432.
- Osode, A.N. and Okoh, A.I. (2008).** Enterotoxigenic *Escherichia coli* (ETEC): A recurring decimal in infants and traveler's diarrhea. *Reviews on Environmental Health* 23, 135-148.
- Otranto, D. and Eberhard, M.L. (2011).** Zoonotic helminths affecting the human eye. *Parasit Vectors* 4, 1-21.
- Pai, M., Kang, G., Ramarishna, B.S., Venataraman, A. and Muliyl, J. (1997).** AN epidemic of diarrhoea in south India caused by enteroaggregative *Escherichia coli*. *Indian Journal Medical Research* 106, 7-12.

- Palaniappan, R.U.M., Zhang, Y.X., Chiu, D.T., Torres, A., DebRoy, C., Whittam, T.S. and Chang, Y.F. (2006).** Differentiation of *Escherichia coli* pathotypes by oligonucleotide spotted array. *Journal of Clinical Microbiology* 44, 1495-1501
- Panos, G.Z., Betsi, G.I. and Falagas, M.E. (2006).** Systemic review: are antibiotics detrimental or beneficial for the treatment of patients with *Escherichia coli* O157:H7 infection? *Alimentary Pharmacology & Therapeutical* 24, 731-742.
- Pass, M.A., Odedra, R. and Batt, R.M. (2000).** Multiplex PCRs for identification of *Escherichia coli* virulence genes. *Journal of Clinical Microbiology*, 38, 2001-2004.
- Pastorello, I., Paccani, S.R., Rosini, R., Mattera, R., Navarro, M.F., Urosev, D., Nesta, B., Surdo, P.L., Vecchio, M.D., Ripa, V., Bertoldi, I., Moriel, D.G., Laarman, A.J., van Strijp, J.A.G., Daura, X., Pizza, M., Serino, L., Soriani, M. (2013).** EsiB, a novel pathogenic *Escherichia coli* secretory immunoglobulin A-binding protein impairing neutrophil activation. *mBio* 4, 206-213.
- Patel, M.M., Hall, A.J., Vinje, J. and Parashar, U.D. (2009).** Noroviruses: a comprehensive review. *Journal of Clinical Virology* 44, 1-8.
- Patel, S.K., Dotson, J., Allen, K.P. and Fleckenstein, J.M. (2004).** Identification and molecular characterization of EatA, an autotransporter protein of enterotoxigenic *Escherichia coli*. *Infection and Immunity* 72, 1786–1794.
- Perez, C., Gomez-Duarte, O.G. and Arias, M.L. (2010).** Diarrhoeagenic *Escherichia coli* in children from Costa Rica. *The American Journal of Tropical Medicine and Hygiene* 83, 292-297.

- Petrovic, T. (2013). Prevalence of viruses in food and the environment.** *In*, Viruses in food and water: risks, surveillance and control. Cook, N. (ed.). Woodhead Publishing Limited, Cambridge CB22 3HJ, United Kingdom. P 19.
- Phillips, I., Eykyn, S. King, A., Gransden, W.R., Rowe, B. and Frost, J.A. (1988).** Epidemic multiresistant *Escherichia coli* infection in West Lambeth Health District. *Lancet* 331, 1038-1041.
- Pimentel, D., Berger, B., Filiberto, D., Newton, M., Wolfe, B., Karabinakis, E., Clark, S., Poon, E., Abbett, E., and Nandagopal, S. (2004).** Water resources: agricultural and environmental issues. *Bioscience*, 54(10), 909-918.
- Pitout, J.D., Gregson, D.B., Church, D.L., Elsaved, S. and Laupland, K.B. (2005).** Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. *Journal of Clinical Microbiology* 43, 2844-2849.
- Pitout, J.D.D. (2012).** Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. *Frontier in Microbiology* 3, 1-7.
- Poolmani, J.T. and Wacker, M. (2015).** Extraintestinal Pathogenic *Escherichia coli*, a Common Human Pathogen: Challenges for Vaccine Development and Progress in the Field. *The Journal of Infectious Diseases* 213, 6-13.
- Port, J.A., Cullen, A.C., Wallace, J.C., Smith, M.N. and Faustman, E.M. (2014).** Metagenomic frameworks for monitoring antibiotic resistance in aquatic environments. *Environmental Health Perspectives* 122, 222–228.
- Postel, S.L., Daily, G.C. and Ehrlich, P.R. (1996).** Human appropriation of renewable fresh water. *Science* 271, 785–787.

- Pourakbari, B., Heydari, H., Mahmuodi, S., Sabouni, F., Teymuri, M., Ferdosian, F., Ashtiani, M.T.H. and Mamishi, S. (2013).** Diarrhoeagenic *E. coli* pathotypes in children with and without diarrhoea in an Iranian referral paediatrics centre. *Eastern Mediterranean Health Journal* 19, 617-621.
- Prescott, L.M., Harley, J.P. and Klein, D.A. (2008).** Microbiology 7<sup>th</sup> edition McGraw-Hill International Edition p. 939.
- Pruimboom-Brees, I.M., Morgan, T.W., Ackermann, M.R., Nystrom, E.D., Samuel, J.E., Cornick, N.A. and Moon, H.W. (2000).** Cattle lack vascular receptor for *Escherichia coli* O157:H7 Shiga toxin. *Proceedings of the National Academy of Sciences of the United States of America* 97, 10325-10329.
- Qadri, F., Svennerholm, A.M., Faruque, A.S.G. and Sack, R.B. (2005).** Enterotoxogenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical Microbiology Reviews* 18, 465-483.
- Quiroga, M., Oviedo, P., Chinen, I., Pegels, E., Husulak, E., Binztein, N., Rivas, M., Schiavoni, L., and Vergara, M. (2000).** Asymptomatic infections by diarrheagenic *Escherichia coli* in children from Misiones, Argentina, during the first twenty months of their lives. *Revista do Instituto de Medicina Tropical de São Paulo* 42, 9-15.
- Qureshi, A.L., Mahessar, A.A., Leghari, M.E., Lashari, B.K. and Mari, F.M. (2015).** Impact of releasing wastewater of sugar industries into drainage system of LBOD, Sindh, Pakistan. *International Journal of Environmental Science and Development* 6, 381-386.
- Rahal, E.A., Kazzi, N., Kanbar, A., Abdelnoor, A.M. and Matar, G.M. (2011).** Role of rifampicin in limiting *Escherichia coli* O157:H7 Shiga-like toxin expression and

enhancement of survival of infected BALB/c mice. *International Journal of Antimicrobial Agents* 37, 135-139.

**Rajendran, P., Ajjampur, S.S., Chidambaram, D., Chandrabose, G., Thangaraj, B., Sarkar, R., Samuel, P., Rajan, D.P. and Kang, G. (2010).** Pathotypes of diarrhoeagenic *Escherichia coli* in children attending a tertiary care hospital in South India. *Diagnostic Microbiology and Infectious Disease* 68: 117–122.

**Raji, M.A., Minga, U. and Machangu, R. (2006).** Current epidemiological status of enterohaemorrhagic *Escherichia coli* O157:H7 in Africa. *Chinese Medical Journal* 119, 217-222.

**Ramamurthy, T. and Albert M.J. (2012).** Epidemiology, pathogenesis and genetics of diarrhoeagenic *Escherichia coli* infections. In, Foodborne and Waterborne Bacterial Pathogens: Epidemiology, Evolution and Molecular Biology. Faruque, S.M. (Ed.). Caister Academic Press, Norfolk, UK.

**Ramanaiah, V.D., Salam, N.M.A., Arumugam, A. and Prabhu, R.G. (2015).** Sociodemographic profile of acute diarrhoeal diseases in a tertiary care hospital, Tirupati. *IOSR Journal of Dental and Medical Sciences* 14, 29-32.

**Ramirez-Castillo, F.Y., Harel, J., Moreno-Flores, A.C., Loera-Muro, A., Guerrero-Barrera, A. L. and Avelar-Gonzalez, F.J. (2014).** Antimicrobial resistance: the role of aquatic environments. *International Journal of Current Research and Academic Review* 2, 231-246.

**Ranjbar, R., Rahbar, M., Naghoni, A., Farshad, S., Davari, A., and Shahcheraghi, F. (2011).** A cholera outbreak associated with drinking contaminated well water. *Archives of Iranian Medicine*, 14, 339-340.

- Rapa, R.A., Islam, A., Monahan, L.G., Mutreja, A., Thomson, N., Charles, I.G., Stokes, H.W. and Labbate, M. (2015).** A genomic island protection integrated into recA of *Vibrio cholerae* contains a divergent recA and provides multi-pathway protection from DNA damage. *Environmental Microbiology* 17, 1090-1102.
- Rasko, D.A., Rosovitz, M.J., Myers, G.S.A., Mongodin, E.F., Fricke, W.F., Gajer, P., Crabtree, J., Sebaihia, M., Thomson, N.R., Chaudhuri, R., Henderson, I.R., Sperandio, V. and Ravel, J. (2008).** The pangenome structure of *Escherichia coli*: comparative genomic analysis of *E. coli* commensal and pathogenic isolates. *Journal of Bacteriology* 190, 6881-6893.
- Redwan, N.A., Al-Fassi, F.A and Ali, M.A. (2008).** Health aspect of Virological water quality: an overview review. *Journal of Applied Science research* 4, 1205-1215.
- Rehydration Project (2014).** Pathophysiology of water diarrhoea: dehydration and rehydration. Available on: [www.rehydrate.org/diarrhoea/tmsdd/2med.htm](http://www.rehydrate.org/diarrhoea/tmsdd/2med.htm) (Accessed 09 October 2015).
- Reischl, U., Linde, H.J., Metz, M., Leppmeier, B. and Lehn, N. (2000).** Rapid identification of methicillin-resistant *Staphylococcus aureus* and simultaneous species confirmation using real-time fluorescence. *Journal of Clinical Microbiology* 38, 2429-2433.
- Reischl, U., Youssef, M.T., Kilwinski, J., Lehn, N., Zhang, W.L., Karch, H. and Strockbine, N.A. (2002).** Real-time fluorescence PCR assays for the detection and characterization of Shiga toxin, intimin, and enterohemolysin genes from Shiga toxin-producing *Escherichia coli*. *Journal of Clinical Microbiology* 40, 2555–2565.

- Reischl, U., Youssef, M.T., Wolf, H., Hyytia-Tress, E. and Strockbine, N.A. (2004).** Real-Time fluorescence PCR assays for detection and characterization of heat-Labile 1 and heat-Stable 1 enterotoxin genes from Enterotoxigenic *Escherichia coli*. *Journal of Clinical Microbiology* 42, 4092-4100.
- Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, H.M., Johnson, L.M., Hargrett, N.T., Blake, P.A., and Cohen, M.L. (1983).** Haemorrhagic colitis associated with a rare *Escherichia coli* serotype. *The New England Journal of Medicine* 308, 681-685.
- Riley, W.B. (1968).** An outbreak of diarrhoea in an infants' home due to a *Shigella*-like organism. *Medical Journal of Australia* 2, 1175-1176.
- Rizzo, L., Manaia, C., Schwartz, T., Dagot, C. Ploy, M.C., Michael, I. and Fatta-Kassinos, D. (2013).** Urban wastewater treatment plants as hotspot for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total Environment* 447, 345-360.
- Robins-Browne, R.M., Still, C.S., Miliotis, M.D., Richardson, N.J., Koornhof, H.J., freiman, I., Schoub, B.D., Lecatsas, G. and Hartman, E. (1980).** Summer diarrhoea in African infants and children. *Archives of Diseases in Childhood* 55, 923-928.
- Roy, K., Bartels, S., Qadri, F. and Fleckenstein, J.M. (2010).** Enterotoxigenic *Escherichia coli* elicits immune responses to multiple surface proteins. *Infection and Immunity* 78, 3027-3035.
- Roy, K., Hamilton, D., Allen, K.P., Randolph, M.P. and Fleckenstein, J.M. (2008).** The EtpA exoprotein of enterotoxigenic *Escherichia coli* promotes intestinal colonization

and is a protective antigen in an experimental model of murine infection. *Infection and Immunity* 76, 2106–2112.

**Roy, K., Hamilton, D., Ostmann, M.M. and Fleckenstein, J.M. (2009).** Vaccination with EtpA glycoprotein or flagellin protects against colonization with enterotoxigenic *Escherichia coli* in a murine model. *Vaccine* 27, 4601–4608.

**Roy, K., Hillard, G.M., Hamilton, D.J., Luo, J., Ostmann, M.M. and Fleckstein, J.M. (2009a).** Enterotoxigenic *Escherichia coli* EtpA mediates adhesion between flagella and host cells. *Nature* 457, 594–598.

**Runft, D.L., Mitchell, K.C., Abuaita, B.H., Allen, J.P., Bajer, S., Ginsburg, K., Neely, M.N. and Withey, J.H. (2014).** Zebrafish as a natural host model for *Vibrio cholerae* colonization and transmission. *Applied and Environmental Microbiology* 80, 1710–1717.

**Russo, T.A. and Johnson, J.R. (2000).** Proposal for a New Inclusive Designation for Extraintestinal Pathogenic Isolates of *Escherichia coli*: ExPEC. *The Journal of Infectious Diseases* 181, 1753–1754.

**Russo, T.A. and Johnson, J.R. (2003).** Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and Infection* 5, 449–456.

**Sack, D.A., Shimko, J., Torres, O., Bourgeois, A.L., Francia, D.S., Gustafsson, B., Kärnell, A., Nyquist, I. and Svennerholm, A.M. (2007).** Randomised, double-blind, safety and efficacy of a killed oral vaccine for enterotoxigenic *E. coli* diarrhoea of travellers to Guatemala and Mexico. *Vaccine* 25, 4392–4400.



- Sack, R.B.** (1968). Proceedings of the 4th Joint Conference, Japan-U.S. Cooperative Medical Science Program, Unzen, Japan, p. 23-25.
- Sack, R.B., Hirschhorn, N., Brownlee, R.A. Jacobs, B., Chatterjee, B.D. and Mitra, R.C.** (1971). Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. *Journal of Infectious Diseases* 123, 378 –385.
- Sahl, J.W. and Rasko, D.A.** (2012). Analysis of Global Transcriptional Profiles of Enterotoxigenic *Escherichia coli* Isolate E24377A. *Infection and Immunity* 80, 1232 -1242.
- Sahl, J.W., Steinsland, H., Redman, J.C., Angiuoli, S.V. Nataro, J.P. Sommerfelt, H. and Rasko, D.A.** (2011). A comparative genomic analysis of diverse clonal types of enterotoxigenic *Escherichia coli* reveals pathovar-specific conservation. *Infection and Immunity* 79, 950 –960.
- Sato, T., Qadir, M., Yamamoto, S., Endo, T. and Zahoor, A.** (2013). Global, regional, and country level need for data on wastewater generation, treatment, and use. *Agricultural Water Management* 130, 1-13.
- Saulnier, P., Chachaty, E., Hilali, F. and Andremont, A.** (1997). Single-step polymerase chain reaction for combined gene detection and epidemiological typing in three bacterial models. *FEMS Microbiology Letters* 150, 311–316.
- Savarino, S.J., Fasano, A., Robertson, D.C. and Levine, M.M.** (1991). Enterotoxigenic *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. *Journal of Clinical Investigation* 87, 1450 –1455.
- Schiller, L.R.** (1999). Secretory diarrhoea. *Curr Gastroenterol Rep* 1, 389-397.

- Schlipkoter, U. and Flahault, A. (2010).** Communicable diseases: achievements and challenges for public health. *Public Health Reviews* 35, 90-119.
- Schultsz, C., Pool, G.J., van Ketel, R., de Wever, B., Speelman, P. and Dankert, J. (1994).** Detection of enterotoxigenic *Escherichia coli* in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. *Journal of Clinical Microbiology* 32, 2393–2397.
- Schwarzenbach, R.P., Egli, T., Hofstetter, T.B., Gunten, UV and Wehril, B. (2010).** Global water pollution and human health. *Annual Review of Environment and Resources* 35, 109-36.
- Selander, R.K., Caugant, D.A. and Whittam, T.S. (1987).** Genetic structure and variation in natural populations of *Escherichia coli*. In: Neidhardt, F., Ingraham, J.L., Low, K.B., Magasanik, B., Schaechter, M., Umberger, H.E. (eds.). *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. Washington, DC: American Society for Microbiology, 1987:1625–1648.
- Shah, S. (2004).** Evaluation of diarrhoea: The challenge continues! Part-I. *Indian Journal of Medical Science* 58, 75-78.
- Shannon, K.L., Lawrence, R.S. and McDonald, D. (2011).** Anthropogenic Sources of Water Pollution: Parts 1 and 2. In, Water and Sanitation-Related Diseases and the Environment: Challenges, Intervention and Preventive Measures. Selendy, J.M.H. (Ed.). John Willey & Sons. Hoboken, NJ, USA. Doi: 10.1002/9781118148594.ch24
- Shmilovitz, M., Kretzer, B., and Levy, E. (1974).** The anaerobic serotype 147 as an etiologic agent of dysentery in Isearl. *Israel Journal of Medical Sciences* 10, 1425-1429.

- Shoener, B.D., Bradley, I.M., Cusick, R.D. and Guest, J.S. (2014).** Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. *Environmental Science: Processes and Impacts* 16, 1204-1222.
- Smith, H.W. and Linggood, M.A. (1971).** The transmissible nature of enterotoxin production in a human enteropathogenic strain of *Escherichia coli*. *Journal of Medical Microbiology* 4, 301-305.
- Smith, J.L. and Fratamico, P.M. (2015).** *Escherichia coli* and other enterobacteriaceae: food poisoning and health effects. In, Encyclopedia of Food and Health. Caballero, B., Finglas, P.M. and Toldra, F. (Eds.). Academic Press, Waltham MA.
- Smith, J.L., Fratamico, P.M. and Gunther, N.W. (2007).** Extraintestinal pathogenic *Escherichia coli*. *Foodborne Pathogens and Disease* 4, 134-163.
- Snelling, A.M., Macfarlane-Smith, L.R., Flether, J.N. and Okeke, I.N. (2009).** The commonly-used DNA probe for diffusely-adherent *Escherichia coli* cross-reacts with a subset of enteroaggregative *E. coli*. *BMC Microbiology* 9, 269.
- Sousa, C.P. (2006).** The versatile strategies of *Escherichia coli* pathotypes: a mini review. *Journal of Venomous Animals and Toxins including Tropical Diseases* 12, 363-373.
- Souza, V., Castillo, A. and Eguiarte, L. (2002).** The evolutionary ecology of *Escherichia coli*. *American Scientist* 90(4), 332. URL: [www.americanscientist.org/issues/pub/the-evolutionary-ecology-of-escherichia-coli/1](http://www.americanscientist.org/issues/pub/the-evolutionary-ecology-of-escherichia-coli/1)
- Stenstrom, T.A., Boisen, F., Georgsen, F., Lahti, K., Lund, V., Anderson, Y. and Omerod, K. (1994).** Waterborne Infections in the Nordic countries. *Tema Nord*, 585.

- Sung, J.J.Y. (2002).** Introduction to gastroenterology. *In*, Principles and practice of clinical medicine in Asia: treating the Asian patient. Sung, J.J.Y., Wong, L.K.S., Li, P.K.T., Sanderson, J. and Kwok, T.C.Y (eds.). Lippincott Williams & Wilkins Asia Ltd, HKSAR, China.
- Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S., Donnell, H.D. Jr., Gelreich, H.D., Payne, B.J., Meyer, A. Jr., Wells, J.G., Greene, K.D., Bright, M., Bean, N.H., and Blake, P.A. (1992).** A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Annals of Internal Medicine* 117, 812-819
- Taddei, C.R., Fasano, A., Ferreira, A.J., Trabulsi, L.R. and Martinez, M.B. (2005).** Secreted autotransporter toxin produced by a diffusely adhering *Escherichia coli* strain causes intestinal damage in animal model assays. *FEMS Microbiology Letters* 250, 263–269.
- Tarr, P.I., Gordon, C.A. and Chandler, W.L. (2005).** Shiga-toxin-producing *Escherichia coli* and haemolytic uremic syndrome. *Lancet* 365, 1073-1086.
- Tau, N.P., Meidany, P., Smith, A.M., Sooka, A. and Keddy, K.H. (2012).** *Escherichia coli* O104 associated with human diarrhoea, South Africa, 2004–2011. *Emerging Infectious Diseases* 18, 1314-1317.
- Tennant, S.M., Tauschek, M., Azzopardi, K., Biahm, A., Bennett-Wood, V., Hartland, E.L., Qi, W., Whittam, T.S. and Robins-Browne, R.M. (2009).** Characterisation of atypical enteropathogenic *E. coli* strains of clinical origin. *BMC Microbiology* 9, 1-11.

- Thevenon, F., Adatte, T, Wildi, W. and Pote, J. (2012).** Antibiotic resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). *Chemosphere* 86, 468-476.
- Tokuda, K., Nishi, J., Imuta, N., Fujiyama, R., Kamenosono, A., Manago, K. and Kawano, Y. (2010).** Characterization of typical and atypical enteroaggregative *Escherichia coli* in Kagoshima, Japan: biofilm formation and acid resistance. *Microbiology and Immunology* 54, 320-329.
- Trabulsi, L.R., Keller, R. and Tardelli Gomes, T.A. (2002).** Typical and atypical enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases* 8, 508-513.
- Tsen, H.Y. and Jian, L.Z. (1998).** Development and use of a multiplex PCR system for the rapid screening of heat-labile toxin I, heat-stable toxin II and Shiga-like toxin I and II genes of *Escherichia coli*. *Journal of Applied Microbiology* 84, 585–592.
- Turner, S.M., Scott-Tucker, A., Cooper, L.M. and Henderson, I.R. (2006).** Weapons of mass destruction: virulence factors of the global killer enterotoxigenic *Escherichia coli*. *FEMS Microbiology Letters* 263, 10–20.
- United Nations Children’s Education Fund (UNICEF) (2013).** Press release, New York, 22 March 2013. [http://www.unicef.org/media/media\\_68359.html](http://www.unicef.org/media/media_68359.html) (Accessed 20 September 2015).
- United Nations Children’s Fund (UNICEF) (2012).** Diarrhoea: acute diarrhoea still a major cause of child death. [http://www.unicef.org/health/index\\_43834.html](http://www.unicef.org/health/index_43834.html) (Accessed 20 September 2015).
- United Nations Educational, Scientific and Cultural Organisation (UNESCO) (2013).** UN World Water Day 2013: International Year of Water Cooperation.

<http://www.unwater.org/water-cooperation-2013/water-cooperation/facts-and-figures/en/> (Accessed 08 September 2015).

**United Nations World Water Assessment Programme. (UN WWAP) (2003).** The World Water Development Report 1: Water for People, Water for Life. UNESCO: Paris, France.

**United States Environmental Protection Agency (US EPA) (2013).** Emerging Technologies for Wastewater Treatment and In-Plant Wet Weather Management. EPA 832-R-12-011.

**Unterweger, D., Miyata, S.T., Bachmann, V., Brooks, T.M., Mullins, T., Kostiuk, B., Provenzano, D. and Pukatzki, S. (2014).** The *Vibrio cholerae* type VI secretion system employs diverse effector modules for intraspecific competition. *Nature Communications* 5, 3524.

**Vallance, B.A., Chan, C., Robertson, M.L. and Finlay, B.B. (2002).** Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: emerging themes in pathogenesis and prevention. *Canadian Journal of Gastroenterology and Hepatology* 16, 771-778.

**Vally, H., Hall, G., Dyda, A., Raupach, J., Combs, B. and Desmarchelier, P. (2012).** Epidemiology of Shiga toxin producing *Escherichia coli* Australia, 2000-2010. *BMC Public Health* 12:63.

**Veenstra, S., Alaerts, G.J. and Bijlsma, M. (1997).** *In: Water Pollution Control – A Guide to the Use of Water Quality Management Principles.* Published on behalf of the United Nations Environment Programme, the Water Supply & Sanitation Collaborative Council and the World Health Organisation by E. & F. Spon.

- Viboud, G.I., Jouve, M.J., Binsztein, N., Vergara, M., Rivas, M., Quiroga, M. and Svennerholm, A. (1999).** Prospective cohort study of enterotoxigenic *Escherichia coli* infections in Argentinean children. *Journal of Clinical Microbiology* 37, 2829-2833.
- Vidal, J.E., Canizalez-Roman, A., Gutierrez-Jimenez, J. and Navarro-Garcia, F. (2007).** Molecular pathogenesis, epidemiology and diagnosis of enteropathogenic *Escherichia coli*. *Salud Publica de Mexico* 49, 376-386.
- Vidal, M., Kruger, E., Duran, C., Lagos, R., Lavine, M., Prado, V., Toro, C. and Vidal, R. (2005).** Single multiplex PCR assay to identify simultaneously the six categories of diarrhoeagenic *Escherichia coli* associated with enteric infections. *Journal of Clinical Microbiology* 43, 5362-5365.
- Vieira, N., Bates, S.J., Solberg, O.D., Ponce, K., Howsmon, R. Cavellos, W., Trueba, G, Riley, L. and Eisenberg, J.N. (2007).** High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. *The American Journal of Tropical Medicine and Hygiene* 76, 528-533.
- Vigil, K.J., Jiang, Z.D., Chen, J.J., Palumbo, K.L., Galbadage, T., Brown, E.L., Yiang, J., Koo, H., DuPont, M.W., Ericsson, C., Adachi, J.A. and DuPont, H.L. (2009).** Coliform and *Escherichia coli* Contamination of Desserts Served in Public Restaurants from Guadalajara, Mexico, and Houston, Texas. *The American Journal of Tropical Medicine and Hygiene* 80, 606-608.
- Vogt, R.L. and Dippold, L. (2005).** *Escherichia coli* O157:H7 outbreak associated with the consumption of ground beef, June-July 2002. *Public Health Reports* 120, 174-178.

- von Sperling, M. and Chernicharo, C.A.L. (2000).** A comparison between wastewater treatment processes in term of compliance with effluent quality standards. In XXVII Congresso Interamericano de Engenharia Sanitaria e Ambiental, *Anais*, pp 1 – 12. Porto Alegre. Asociacion Interamericana de Ingenieria Sanitaria y Ambiental.
- Weintraub, A. (2007).** Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *Journal of Medical Microbiology*, 56, 4-8.
- Welinder-Olsson, C. and Kaijser, B. (2005).** Enterohemorrhagic *Escherichia coli* (EHEC). *Scandinavian Journal of Infectious Diseases* 37, 405-416.
- Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., Thomas, C.M. and Williams, A.P. (2013).** The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *The Lancet Infectious Diseases* 13, 155–165.
- Wilshaw, G.A., Cheasty, T., Smith, H.R. (2000).** *Escherichia coli*. In: Lund, Baird-Parker, Gould, (eds.). The microbiological safety and quality of food II. Maryland, USA: Aspen Press, pp. 1136-77.
- Wolf, M.K. (1997).** Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. *Clinical Microbiology Reviews* 10, 569-584.
- World Health Organisation (WHO) (2001).** Water Quality: Guidelines, Standards and health. Assessment of Risk and Risk Management for Water-Related Infectious Disease. 1st ed. World Health Organization, Geneva.



**World Health Organisation (WHO) (2002).** Global defense against the infectious disease threat. In *Emerging and Epidemic-Prone Diseases*, ed. MK Kindhauser, pp. 56–103. Geneva, Switz.: WHO

**World Health Organisation (WHO) (2010).** Enterotoxigenic *E. coli*. URL: [www.who.int/vaccine\\_research/diseases/diarrhoeal/en/index4.html](http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index4.html) (Accessed 20 September 2015)

**World Health Organisation (WHO) (2015a).** Diarrhoea disease. <http://www.who.int/mediacentre/factsheets/fs330/en/> (Accessed 20 September 2015).

**World Health Organisation (WHO) (2015b).** Water-related disease: cholera. [http://www.who.int/water\\_sanitation\\_health/diseases/cholera/en/](http://www.who.int/water_sanitation_health/diseases/cholera/en/) (Accessed 29 September 2105).

**World Health Organisation (WHO) (2015c).** Global Health Observatory (GHO) data: cholera. [http://www.who.int/gho/epidemic\\_diseases/cholera/en/](http://www.who.int/gho/epidemic_diseases/cholera/en/) (Accessed 29 September 2105).

**World Health Organisation (WHO) (2015d).** Media centre: cholera. <http://www.who.int/mediacentre/factsheets/fs107/en/> (Accessed 30 September, 2015).

**World Health Organisation (WHO) (2015e).** Enterohaemorrhagic *Escherichia coli* (EHEC). <http://www.who.int/mediacentre/factsheets/fs125/en/> (Accessed 19 October, 2015).

**World Health Organization (WHO) (2005).** Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. WHO Press, Geneva, Switzerland.

**World Health Organisation/ United Nations Environmental Programme (WHO/UNEP)**

(1997). Water pollution control - a guide to the use of water quality management principles. Edited by Richard Helmer and Ivanildo Hespanhol Published on behalf of the United Nations Environment Programme, the Water Supply & Sanitation Collaborative Council and the World Health Organization by E. & F. Spon

**Wray, C., McLaren, I.M., Randall, L.P. and Pearson G.R. (2000).** Natural and experimental infection of normal cattle with *Escherichia coli* O157. *Vet. Rec.* 147, 65-68.

**Wright, A.C., Hill, R.T., Johnson, J.A., Roghman, M.C., Colwell, R.R. and Morris, J.G. (1996).** Distribution of *Vibrio vulnificus* in the Chesapeake Bay. *Applied and Environmental Microbiology* 62, 717-724.

**Wright, G.D. (2010).** Antibiotic resistance in the environment: a link to the clinic? *Current Opinion in Microbiology* 13, 589-594.

**Wyn-Jones, A.P., Carducci, A., Cook, N., D'Agostino, M., Divizia, M., Fleischer, J., Gantzer, C., Gawler, A., Girones, R., Holler, C., de Roda Husman, A.M., Kay, D., Kozyra, I., Lopez-Pila, J., Muscillo, M., Nascimento, M.S., Papageorgiou, G., Ruties, S., Sellwood, J., Szewzyk, R. and Wyer, M. (2011).** Surveillance of adenovirus and noroviruses in European recreational waters. *Water Research* 45, 1025-1038.

**Yamamoto, T. and Echeverria, P. (1996).** Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic *E. coli* strains pathogenic for humans. *Infection and Immunity* 64, 1441-1445.

- Yang, B., Feng, L., Wang, F. and Wang, L. (2015).** Enterohemorrhagic *Escherichia coli* senses low biotin status in the large intestine for colonization and infection. *Nature Communications* 6 Article number 6592, 1-12.
- Yang, J.R., Wu, F.T., Tsai, J.L., Mu, J.J., Lin, L.F., Chen, K.L., Kuo, S.H., Chiang, C.S. and Wu, H.S. (2007).** Comparison between O serotype method and multiplex real-time PCR to identify diarrhoeagenic *Escherichia coli* Taiwan. *J. Clin. Microbiol.* 45, 3620-3625.
- Yang, X. and Wang, H. (2014). Pathogenic *E. coli* (Introduction).** In, Encyclopedia of Food Microbiology Second Edition. Batt, C.A. and Tortorello, M. (Eds.). Academic Press, Oxford, UK.
- Yavzori, M., Porath, N., Ochana, O., Dagan, R., Orni-Wasserlauf, R. and Cohen, D. (1998).** Detection of enterotoxigenic *Escherichia coli* in stool specimens by polymerase chain reaction. *Diagnostic Microbiology and Infectious Diseases* 31, 503-509.
- Young, H.K. (1993).** Antimicrobial resistance spread in aquatic environments. *Journal of Antimicrobial Chemotherapy* 31, 627-635.
- Zhang, L. and Foxman, B. (2003).** Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. *Frontiers in Bioscience* 8, e235–244.
- Zuber, M. (1999).** *E. coli*: Serotypes other than 0157:H7. MSPH DOH, Regional Epidemiologist; Tallahassee FL, USA.

## **CHAPTER THREE**

**Seasonal variation in the physicochemical quality  
indices of the final effluents of two wastewater  
treatment plants in Amathole District Municipality  
Area of South Africa.**

---

This Chapter together with data from chapter four has been submitted for publication in

CLEAN – Soil, Air, Water

---

### 3.1 Abstract

We assessed the physicochemical qualities of the discharged final effluents of two wastewater treatment plants in the Eastern Cape Province of South Africa. A total of 144 wastewater final effluent samples were collected in triplicate over a twelve-month sampling period (September 2012 to August 2013) from the final effluents tanks (FE) and the discharge points (DP). Wastewater qualities including pH, temperature, turbidity, TDS, DO, EC and free chlorine were determined *on site* while BOD,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^-$  and COD were determined in the laboratory. Sample characteristics ranged as follows: pH (6.5 – 7.6), TDS (95 – 171 mg/L), EC (134 – 267  $\mu\text{S}/\text{cm}$ ), temperature (12 – 27 °C), turbidity (1.5 – 65.7 mg/L), free chlorine (0.08 – 0.72 mg/L), DO (2.06 – 9.81), BOD (0.13 – 9.81),  $\text{NO}_3^-$  (0 – 21.5 mg/L),  $\text{NO}_2^-$  (0 – 0.76 mg/L),  $\text{PO}_4^-$  (0 – 18.3 mg/L) and COD (27 – 680 mg/L). Parameters such as pH, TDS, EC, Temperature, nitrite and on most occasions DO complied with set guidelines. Other parameters, however, including turbidity, BOD, nitrate, phosphate and COD fell short of recommended guidelines. Our findings highlight the detrimental impacts the discharged effluents may have on the receiving watersheds overtime and, necessitates the need for consistent monitoring of the treatment processes and facilities to ensure the protection of public and environmental health especially the fresh-water resource of the Eastern Cape Province.

**Keywords:** Wastewater, final effluent, physicochemical quality, environmental health, watershed.

### **3.2 Introduction**

The water resources of any country constitute one of its precious assets and need to be protected and conserved (Kumar *et al.*, 2005). Clean water availability is the main driver of economic growth and development since it plays an important role in sustenance of healthy livelihoods and industry, and guarantees food security. Problems associated with scarcity of water could be an ingredient of tension among water-stressed nations (Vidyasagar, 2007). In view of its innumerable uses, great importance must be placed on improvement and conservation of water resources in the communities (United Nations' Millennium Development Goal number 7; Kumar *et al.*, 2005; Vidyasagar, 2007).

Increasing global human use and pollution of freshwater sources have reached levels where water scarcity potentially affects world economics and ecosystem balance (Jury and Vaux, 2007). Water quality degradation has also enormously contributed to the shrinkage of available freshwater resources for human consumption and species survival (Jury and Vaux, 2007). Water-related human morbidity and mortality, resulting from widely divergent levels of both water quality and quantity, is already widespread, with about 80% of the global human population facing exposure to high threat levels of water insecurity (Vorosmarty *et al.*, 2010). Khan and Javed (2007) reported that an estimated 1.1 billion people, mostly in the developing world did not have access to improved drinking water sources, and more than 2.6 billion did not have good sanitation services. Consequently, a global estimate of 1.6 million people die yearly from diarrheal diseases mainly linked to lack of access to safe drinking water and basic sanitation, 90% of which are children under the age of 5 (WHO, 2013).

Liquid wastes such as untreated or inadequately treated domestic or industrial sewage are major sources of pollutants in developing countries. Municipal wastewaters may contain dissolved and suspended organic matter, organic and inorganic chemicals, pharmaceuticals, heavy metals, radio-active wastes, substances with genotoxic properties, and, disease causing agents such as bacteria and viruses (FAO, 1992; Picardi, 2002; CCME, 2006; Abor, 2007; Abdel-Raouf *et al.*, 2012). These are frequently discharged into aquatic environments (oceans, rivers, lakes, wetlands), sometimes without adequate treatment (Lagerblad, 2010). Uncontrolled discharge of poorly treated municipal effluents results in contamination of surface water, making such water unsuitable for human consumption, crop irrigation, fish production or recreation (Kivaisi, 2001). Discharged effluents from sewage treatment plants represent point sources of pollution to surface water. While some of the chemicals discharged from these sources are not harmful, others may be toxic to humans and aquatic life (NOAA, 2008). These may endanger public health contributing to high oxygen demand and eutrophication of receiving water bodies, promoting toxic algal blooms which may result in destabilization of aquatic ecosystems (WHO, 1985; Jackson *et al.*, 1989; NSFC, 1996; Ojo and Adeniyi, 2012).

The potential hazardous impacts of pollutants on the receiving watershed quality are manifold and depend on volume of the discharge, its chemical composition and concentrations (Owili, 2003). The time of discharge, weather conditions, as well as the type of the discharge are also pertinent, as also are the characteristic features of the receiving water bodies such as flow volume and dilution capacity (Nemerow and Dasgupta, 1991; Canter, 1996; NOAA, 2008).

A number of studies in the past have highlighted the detrimental impacts of inadequately treated effluents on freshwater resources in the Eastern Cape Province of South Africa (Igbinosa and Okoh, 2009; Osode and Okoh, 2009; Odjadjare and Okoh, 2010). Those studies, however have reported the qualities of discharged effluents of a few treatment plants and their harmful impacts on the freshwater resources in the province. Vital information regarding the current performance efficiencies of wastewater treatment plants in the province, and the attendant negative consequences on the limited available freshwater resources continues to be insufficient. In order to ensure protection of public health and maintenance of the integrity of the natural environment, it becomes imperative that constant evaluation of the current operational efficiencies of the selected treatment plants in relation to their compliances with recommended limits for discharged final effluents be carried out before the effluents are released back into the receiving watershed. This study therefore evaluated the physicochemical qualities of the discharged final effluents of two wastewater treatment facilities in South Africa over a twelve month sampling period, how these qualities vary with seasons, and their possible impacts to receiving watershed.

### **3.3 Materials and Methods**

#### **3.3.1 Description of study sites**

The two wastewater treatment facilities assessed in this study are both located in the Amathole District Municipality of the Eastern Cape Province of South Africa. Table 3.1 below gives detailed description of the facilities. Both treatment plants use the activated sludge technology to treat their wastewater. The activated sludge system uses combination of oxygen and biological process (microorganisms) for the biodegradation of the oxygen-demanding organic pollutants from the wastewater. The mixture of the wastewater and the



microorganisms at this stage is referred to as mixed liquor. Treated effluent from this stage is discharged into the settling tanks or secondary clarifier for further treatment (such as disinfection) before discharge into the receiving watershed. A well-managed activated sludge system can removed about 85% of suspended and BOD content of the wastewater (World Bank Group, 2016).

**Table 3.1:** Description of the study sites

<b>Assessment Area</b>	<b>SWWTP</b>	<b>KWWTP</b>
Geographical coordinates	32°34.285´S, 27°26.095´E	32°41.519´S, 27°08.615´E
Technology	Activated sludge and sludge drying beds	Activated sludge and sludge drying beds
Designs capacity (Megalitre/day)	4.0	0.67
Operational % to Design Capacity	62.5%	Nil
Microbiological compliance	5%	70.0%
Chemical compliance	5%	70.0%
Physical Compliance	5%	56.7%
Annual Average Effluent Quality Compliance	5%	65.6%
Wastewater Risk rating (%CRR*/CRRmax)	76.5%	88.2%
Highest Risk Area	Poor effluent compliance	No influent monitoring, poor effluent compliance
Discharge Point (River)	Cumakala River	Keiskamma River

*Source:* DWAF, Green Drop Progress Report 2012. \*Cummulative Risk Rate

### **3.3.2 Sample Collection and Analytical Procedures**

Wastewater sample collection was done monthly for a period of 12 months (from September 2012 to August 2013). The pH, electrical conductivity (EC), temperature and total dissolved solids (TDS) of the effluents were measured *on site* using a Crison Multimeter MM40. Dissolved oxygen (DO), turbidity and free chlorine were also determined *on site* using an HQ40d BOD meter (HACH Company), a microprocessor turbidity (HACH Company, model 2100P) and a free and total ion specific meter (Hanna-BDH laboratory supplies) respectively. Nutrient concentrations (phosphate, nitrate and nitrite) and chemical oxygen demand (COD) were determined in the laboratory by standard photometric methods (DWAF, 1999) using a UV/Vis spectrophotometer (Spectroquant-Pharo 100, Merck Pty Ltd). The biochemical oxygen demand (BOD) of the samples was also determined in the laboratory by calculating the difference in the dissolved oxygen concentration of the samples on day 1 and after 5 days incubation period using the HACH BOD meter (HACH Company, model 2943900) (APHA/AWWA/WEA, 1998). Samples for laboratory analysis were collected in duplicate (total of 96 samples) from the final effluent tanks (FE) and the discharge points (DP) of the wastewater treatment plants (these was done to compare changes in wastewater characteristics between the final effluent and the discharge point particularly increased chlorine contact time). The samples for laboratory analysis were transported in cooler boxes containing ice to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice for analysis within 6 h of collection. A total of twelve physicochemical parameters were assessed.

### **3.3.3 Analytical Quality Control**

All equipment including the Crison Multimeter MM40, the HACH-2100P microprocessor turbidimeter, the Merck-Spectroquant Pharo 100 spectrophotometer, the Hanna-BDH free

and the total ion specific meter, HACH-HQ40d BOD meter and Merck-TR300 thermoreactor were appropriately calibrated according to the respective manufactures' instructions before use for analyses. Ready to use analytical grade standard solutions were used throughout the study. Spectrophotometers were blanked using blank solution provided by the manufacturer or distilled water subjected to the same treatment as the sample. All analyses were carried out in triplicates and the values reported as means  $\pm$  the standard deviation of the triplicate measurements.

### **3.3.4 Statistical Analysis**

Both descriptive and inferential statistical techniques were used to analyse the data obtained in the study. The means and standard deviations were used to estimate the average values of the variables (parameters) of interest and the degrees of variability of these variables. This was done using One-way ANOVA (SPSS 20.0 version for Windows program). Tests of significance employed were the Paired-Samples T-test and the ANOVA F-test. The coefficients of Correlation amongst the physicochemical parameters were calculated using the Pearson correlations procedure. Statistical significance was set at  $P$  values  $<0.05$ .

## **3.4 Results and Discussion**

The seasonal means  $\pm$  the standard deviations and variations in the physicochemical qualities of the wastewater treatment plants studied are comprehensively presented in Table 3.2 below. Table 3.3 shows the seasonal ranges of the physicochemical qualities at SWWTP and KWWTP. The pH regimes observed did not vary significantly ( $P>0.05$ ) at the final effluent tanks (FE) and discharge points (DP) between SWWTP and KWWTP across the four seasons of the year. The lowest pH values were recorded at SWWTP in the months of spring while

the highest pH values were observed in the winter months. The pH values observed in this study complied with South Africa's recommended limit of 5.5 to 9.5 for discharged treated effluent (DWAF, 2004). The pH ranges also fell within the World Health Organisation recommended limit of 6.5 to 8.5 for water meant for full contact recreation (WHO, 1984; 1989; Igbiosa and Okoh, 2009) and the European Union (EU) pH protection limit of 6.0 to 9.0 for fishes and aquatic life (Chapman, 1996). The neutral and alkaline pH reported in this study is similar to those reported by other authors (Obi *et al.*, 2004; Odjadjare and Okoh, 2010; Imoobe and Koye, 2011).

The total dissolved solids (TDS) profile of samples from both SWWTP and KWWTP ranged between 95 mg/L and 171 mg/L throughout the sampling period. Total dissolved solids are a measure of the amount of all soluble substances, most of which carry electrical charges. The TDS profile did not significantly vary ( $P>0.05$ ) between the FE and DP samples from both SWWTP and KWWTP except in the months of spring and autumn. Also, the observed TDS did not vary significantly ( $P>0.05$ ) among the seasons at both SWWTP and KWWTP, an indication of the consistency of the concentration/removal of the dissolved solids from the wastewater. The TDS profile obtained in the study was in compliance with the recommended TDS limit of  $\leq 450$  mg/L for discharged treated effluent (DWAF, 2004).

The electrical conductivity (EC) profile in this study ranged between 134 and 267  $\mu\text{S}/\text{cm}$  over the sampling period. The measured EC varied significantly ( $P<0.05$ ) between FE and DP samples at both SWWTP and KWWTP. The ranges of values of the EC obtained in this study fell within the stipulated EC general limit of 70 mS/m (700  $\mu\text{S}/\text{cm}$ ) above intake water to a maximum of 150 mS/m (1500  $\mu\text{S}/\text{cm}$ ) and EC special limit of 50 mS (500  $\mu\text{S}/\text{cm}$ ) above

background receiving water to a maximum of 100 mS/cm (1000  $\mu$ S/cm) as recommended by DWAF (2004). Electrical conductivity (EC) measures the capacity of water (or other electrolyte) to conduct an electrical current. The EC of water is a function of the types and quantities of dissolved substances in water. Although Radtke *et al.* (2005) reported that there is no universal linear relation between total dissolved substances and conductivity, EC has often been used as an indicator of TDS, because current conduction by an electrolyte solution is basically dependent on the concentration of ionic species (Hayashi, 2004).

The temperature regimes observed in the study varied significantly ( $P < 0.05$ ) between SWWTP and KWWTP samples, and ranged between 12°C and 27°C through the sampling months. The minimum temperature (12°C) was observed at the DP samples of KWWTP in June 2013 (winter) while the highest temperature (27°C) was observed at the DP samples of SWWTP in February 2013 (summer). The temperature ranges did not vary significantly between the FE and the DP samples within a particular season at each treatment plant. However, the observed ranges varied significantly ( $P < 0.05$ ) among the seasons over the sampling period. The observed temperature regimes of the DP effluents did not pose any danger to the receiving water bodies since they were in compliance with the stipulated general limit of  $\leq 35^\circ\text{C}$  (Government Gazette, 1984). The temperature profile shows that effluent temperature was largely determined by season. Temperature is a fundamental and important water quality parameter driving all biochemical activities in the water column (UNEP, 2008). Wastewater from municipal treatment plants is probably the largest anthropogenic heat source for urban fresh waters (Chapman, 1996). Its effect on stream water temperature depends on the temperature and volume of wastewater added to the stream. Understanding how fresh water temperatures are affected by various anthropogenic heat input from wastewater in particular is, therefore, important (Kinouchi *et al.*, 2007).

**Table 3.2:** Seasonal variations in physicochemical characteristics of the two selected wastewater treatment plants.

Parameters	Season	SWWTP				KWWTP			
		STF*	STD*	T-Value	Pr >  T	KHF*	KHD*	T-value	Pr >  T
<b>pH</b>	Spring	7.0 ± 0.1 <sup>a</sup>	7.3 ± 0.3 <sup>a</sup>	-3.309	.011	7.0 ± 0.2 <sup>a</sup>	7.0 ± 0.2 <sup>a</sup>	-1.493	.174
	Summer	7.2 ± 0.2 <sup>ab</sup>	7.2 ± 0.2 <sup>a</sup>	-2.063	.073	7.2 ± 0.2 <sup>ab</sup>	7.2 ± 0.2 <sup>ab</sup>	-1.403	.198
	Autumn	7.3 ± 0.0 <sup>bc</sup>	7.3 ± 0.0 <sup>a</sup>	-8.000	.000	7.3 ± 0.0 <sup>bc</sup>	7.3 ± 0.0 <sup>bc</sup>	-3.833	.005
	Winter	7.3 ± 0.0 <sup>bc</sup>	7.3 ± 0.0 <sup>a</sup>	-0.887	.401	7.3 ± 0.0 <sup>bc</sup>	7.3 ± 0.0 <sup>bc</sup>	-1.333	.219
	<b>F-Value; Pr &gt;F:</b>	17.565; .000	1.646; .198			23.401; .000	11.866; .000		
<b>TDS (mg/L)</b>	Spring	136 ± 21 <sup>b</sup>	140 ± 15 <sup>b</sup>	-1.398	.200	159 ± 9 <sup>b</sup>	158 ± 10 <sup>b</sup>	2.697	.027
	Summer	127 ± 15 <sup>ab</sup>	125 ± 15 <sup>ab</sup>	1.757	.117	128 ± 12 <sup>a</sup>	126 ± 16 <sup>a</sup>	.831	.430
	Autumn	111 ± 16 <sup>a</sup>	108 ± 9 <sup>a</sup>	1.129	.291	127 ± 10 <sup>a</sup>	124 ± 10 <sup>a</sup>	2.834	.022
	Winter	130 ± 23 <sup>ab</sup>	125 ± 23 <sup>ab</sup>	1.896	.095	131 ± 17 <sup>a</sup>	131 ± 17 <sup>a</sup>	-.018	.986
	<b>F-Value; Pr &gt;F:</b>	2.869; .052	6.277; .002			14.007; .000	12.559; .000		
<b>EC (µS/cm)</b>	Spring	213 ± 32 <sup>b</sup>	219 ± 23 <sup>b</sup>	-1.439	.188	249 ± 14 <sup>b</sup>	247 ± 15 <sup>b</sup>	2.054	.074
	Summer	198 ± 23 <sup>ab</sup>	196 ± 23 <sup>b</sup>	1.540	.162	200 ± 19 <sup>a</sup>	197 ± 24 <sup>a</sup>	.805	.444
	Autumn	173 ± 25 <sup>a</sup>	163 ± 16 <sup>a</sup>	1.576	.154	199 ± 15 <sup>a</sup>	194 ± 15 <sup>a</sup>	2.014	.079
	Winter	202 ± 37 <sup>ab</sup>	196 ± 35 <sup>b</sup>	1.779	.113	204 ± 26 <sup>a</sup>	204 ± 26 <sup>a</sup>	-.084	.935
	<b>F-Value; Pr &gt;F:</b>	2.873; .052	7.585; .001			14.290; .000	12.597; .000		
<b>Temp (°C)</b>	Spring	20 ± 3 <sup>b</sup>	20 ± 4 <sup>b</sup>	-.027	.979	19 ± 3 <sup>b</sup>	18 ± 3 <sup>b</sup>	1.448	.186
	Summer	23 ± 1 <sup>c</sup>	24 ± 2 <sup>c</sup>	-1.650	.138	22 ± 1 <sup>c</sup>	21 ± 1 <sup>d</sup>	2.728	.026
	Autumn	20 ± 4 <sup>b</sup>	20 ± 4 <sup>b</sup>	-2.137	.065	19 ± 4 <sup>b</sup>	19 ± 4 <sup>bc</sup>	1.105	.301
	Winter	15 ± 1 <sup>a</sup>	15 ± 1 <sup>a</sup>	-1.917	.092	13 ± 1 <sup>a</sup>	13 ± 0 <sup>a</sup>	3.418	.009
	<b>F-Value; Pr &gt;F:</b>	20.292; .000	13.926; .000			19.834; .000	22.003; .000		
<b>Turbidity (NTU)</b>	Spring	5.47 ± 3.31 <sup>a</sup>	6.48 ± 2.06 <sup>a</sup>	-1.414	.195	44.07 ± 15.73 <sup>b</sup>	42.88 ± 17.06 <sup>b</sup>	1.136	.289
	Summer	7.09 ± 5.38 <sup>a</sup>	7.18 ± 5.50 <sup>a</sup>	-.532	.609	14.67 ± 4.81 <sup>a</sup>	15.87 ± 3.89 <sup>a</sup>	-1.637	.140
	Autumn	8.96 ± 6.40 <sup>a</sup>	9.71 ± 10.20 <sup>a</sup>	-.401	.699	17.51 ± 12.58 <sup>a</sup>	26.96 ± 26.17 <sup>ab</sup>	-1.931	.090
	Winter	6.04 ± 1.65 <sup>a</sup>	5.55 ± 1.58 <sup>a</sup>	.760	.469	45.10 ± 12.12 <sup>b</sup>	30.71 ± 11.94 <sup>ab</sup>	3.867	.005
	<b>F-Value; Pr &gt;F:</b>	1.014; .399	.830; .487			17.020; .000	3.939; .017		
<b>Free Cl (mg/L)</b>	Spring	0.22 ± 0.10 <sup>a</sup>	0.30 ± 0.13 <sup>b</sup>	-2.433	2.081	0.28 ± 0.16 <sup>a</sup>	0.24 ± 0.13 <sup>a</sup>	2.081	.071
	Summer	0.22 ± 0.04 <sup>a</sup>	0.19 ± 0.03 <sup>a</sup>	1.846	.102	0.37 ± 0.20 <sup>a</sup>	0.41 ± 0.21 <sup>a</sup>	-1.167	.277
	Autumn	0.24 ± 0.08 <sup>a</sup>	0.16 ± 0.04 <sup>a</sup>	2.303	.050	0.29 ± 0.25 <sup>a</sup>	0.28 ± 0.22 <sup>a</sup>	.716	.494
	Winter	0.19 ± 0.09 <sup>a</sup>	0.19 ± 0.09 <sup>a</sup>	-.074	.943	0.28 ± 0.09 <sup>a</sup>	0.28 ± 0.06 <sup>a</sup>	.000	1.000
	<b>F-Value; Pr &gt;F:</b>	.659; .583	4.957; .006			.580; .632	1.578; .214		

**Table 3.2: continued...**

<b>DO (mg/L)</b>	Spring	5.30 ± 0.40 <sup>a</sup>	8.12 ± 0.33 <sup>b</sup>	-51.226	.000	2.39 ± 0.25 <sup>a</sup>	5.51 ± 0.42 <sup>a</sup>	-31.119	.000
	Summer	5.19 ± 0.51 <sup>a</sup>	7.52 ± 0.29 <sup>a</sup>	-24.161	.000	4.35 ± 2.63 <sup>b</sup>	6.25 ± 1.46 <sup>a</sup>	-4.785	.001
	Autumn	5.18 ± 0.70 <sup>a</sup>	7.57 ± 0.48 <sup>a</sup>	-21.922	.000	7.82 ± 0.53 <sup>c</sup>	8.40 ± 0.64 <sup>b</sup>	-6.815	.000
	Winter	5.37 ± 0.24 <sup>a</sup>	8.35 ± 0.15 <sup>b</sup>	-31.375	.000	9.12 ± 0.25 <sup>c</sup>	9.63 ± 0.13 <sup>c</sup>	-7.291	.000
	<b>F-Value; Pr &gt;F:</b>	.381; .812	13.576; .000			47.812; .000	47.721; .000		
<b>BOD (mg/L)</b>	Spring	2.78 ± 1.35 <sup>a</sup>	2.92 ± 3.10 <sup>a</sup>	-.173	.867	1.48 ± 0.37 <sup>a</sup>	4.59 ± 0.28 <sup>a</sup>	-18.325	.000
	Summer	2.69 ± 1.57 <sup>a</sup>	4.96 ± 1.45 <sup>a</sup>	-2.291	.051	1.84 ± 0.51 <sup>a</sup>	2.92 ± 1.46 <sup>a</sup>	-3.199	.013
	Autumn	2.95 ± 2.05 <sup>a</sup>	2.76 ± 2.78 <sup>a</sup>	.239	.819	2.74 ± 1.99 <sup>a</sup>	3.99 ± 3.19 <sup>a</sup>	-1.896	.095
	Winter	1.91 ± 2.43 <sup>a</sup>	3.20 ± 3.83 <sup>a</sup>	-2.738	.026	4.28 ± 4.08 <sup>a</sup>	4.00 ± 4.34 <sup>a</sup>	1.039	.329
	<b>F-Value; Pr &gt;F:</b>	.526; .667	1.088; .368			2.704; .062	.559; .646		
<b>NO<sub>3</sub> (mg/L)</b>	Spring	2.46 ± 1.92 <sup>a</sup>	2.41 ± 1.90 <sup>a</sup>	.438	.673	5.52 ± 1.13 <sup>a</sup>	5.38 ± 1.09 <sup>a</sup>	.389	.707
	Summer	4.81 ± 0.64 <sup>b</sup>	5.79 ± 1.81 <sup>b</sup>	-1.972	.084	10.57 ± 6.65 <sup>ab</sup>	10.67 ± 5.72 <sup>b</sup>	-.232	.823
	Autumn	5.21 ± 1.40 <sup>b</sup>	4.37 ± 0.96 <sup>b</sup>	1.726	.123	14.33 ± 5.69 <sup>bc</sup>	14.88 ± 5.70 <sup>bc</sup>	-.617	.555
	Winter	4.41 ± 0.71 <sup>b</sup>	4.21 ± 0.49 <sup>ab</sup>	.665	.525	17.87 ± 1.50 <sup>c</sup>	16.69 ± 1.03 <sup>c</sup>	1.730	.122
	<b>F-Value; Pr &gt;F:</b>	8.222; .000	8.535; .000			12.562; .000	13.495; .000		
<b>NO<sub>2</sub> (mg/L)</b>	Spring	0.13 ± 0.08 <sup>a</sup>	0.16 ± 0.03 <sup>a</sup>	-1.389	.202	0.32 ± 0.80 <sup>a</sup>	0.34 ± 0.09 <sup>a</sup>	-2.234	.056
	Summer	0.20 ± 0.03 <sup>ab</sup>	0.20 ± 0.03 <sup>b</sup>	-.316	.760	0.38 ± 0.24 <sup>a</sup>	0.37 ± 0.25 <sup>a</sup>	.236	.820
	Autumn	0.22 ± 0.06 <sup>c</sup>	0.19 ± 0.03 <sup>ab</sup>	1.492	.174	0.24 ± 0.04 <sup>a</sup>	0.22 ± 0.02 <sup>a</sup>	1.280	.236
	Winter	0.22 ± 0.05 <sup>c</sup>	0.21 ± 0.03 <sup>b</sup>	.138	.894	0.22 ± 0.02 <sup>a</sup>	0.24 ± 0.03 <sup>a</sup>	-1.281	.236
	<b>F-Value; Pr &gt;F:</b>	5.275; 0.005	5.600; 0.003			2.898; .050	2.763; .058		
<b>PO<sub>4</sub> (mg/L)</b>	Spring	5.73 ± 6.63 <sup>ab</sup>	6.19 ± 7.11 <sup>ab</sup>	-2.357	.046	3.27 ± 0.10 <sup>a</sup>	3.15 ± 1.04 <sup>a</sup>	1.280	.236
	Summer	4.84 ± 3.94 <sup>ab</sup>	4.71 ± 3.81 <sup>ab</sup>	1.105	.301	6.49 ± 5.74 <sup>a</sup>	6.43 ± 5.76 <sup>a</sup>	.766	.466
	Autumn	8.14 ± 5.84 <sup>b</sup>	7.87 ± 5.46 <sup>b</sup>	.787	.454	14.48 ± 2.40 <sup>b</sup>	14.14 ± 1.16 <sup>b</sup>	.463	.656
	Winter	1.07 ± 0.93 <sup>a</sup>	1.06 ± 0.96 <sup>a</sup>	.073	.943	3.38 ± 0.33 <sup>a</sup>	3.30 ± 0.32 <sup>a</sup>	1.674	.133
	<b>F-Value; Pr &gt;F:</b>	3.287; .033	3.157; .038			25.077; .000	26.755; .000		
<b>COD (mg/L)</b>	Spring	183 ± 120 <sup>a</sup>	213 ± 219 <sup>a</sup>	-.734	.484	322 ± 150 <sup>b</sup>	260 ± 90 <sup>c</sup>	1.070	.316
	Summer	104 ± 61 <sup>a</sup>	112 ± 54 <sup>a</sup>	-.512	.622	98 ± 33 <sup>a</sup>	90 ± 50 <sup>a</sup>	.526	.613
	Autumn	135 ± 90 <sup>a</sup>	99 ± 73 <sup>a</sup>	2.613	.031	163 ± 81 <sup>a</sup>	169 ± 71 <sup>ab</sup>	-.316	.760
	Winter	100 ± 39 <sup>a</sup>	110 ± 43 <sup>a</sup>	-.590	.571	200 ± 19 <sup>a</sup>	198 ± 44 <sup>bc</sup>	.118	.909
	<b>F-Value; Pr &gt;F:</b>	1.881; .153	1.770; .173			10.426; .000	10.142; .000		

Values are means of triplicate measurements across a season ± Standard deviations (SD); Means with the same superscript are not significantly different (P>0.05)

**Key:** \*STF- SWWTP final effluent; \*STD- KWWTP discharge point; \*KHF- KWWTP final effluent; \*KHD- KWWTP discharge point; TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO<sub>3</sub>- Nitrate; NO<sub>2</sub>- Nitrite; PO<sub>4</sub>- Phosphate; COD- Chemical oxygen demand.

**Seasons:** Spring (September, October, November); summer (December, January, February); autumn (March, April, May); winter (June, July, August).

**Table 3.3:** Seasonal ranges of the physicochemical qualities of SWWTP and KWWTP plants

Parameter	Season	Range per study site		Regulatory guideline
		SWWTP	KWWTP	
pH	Spring	6.7 – 7.6	6.5 – 7.1	5.5 – 9.5 (DWAF 1999)
	Summer	6.9 – 7.3	6.9 – 7.3	
	Autumn	7.2 – 7.3	7.2 – 7.3	
	Winter	7.1 – 7.4	7.2 – 7.4	
TDS (mg/L)	Spring	95 – 155	146 – 171	450 mg/L (DWAF 1996b)
	Summer	107 – 145	107 – 146	
	Autumn	100 – 148	111 – 142	
	Winter	105 – 165	113 – 161	
EC ( $\mu\text{S}/\text{cm}$ )	Spring	148 – 242	228 – 267	700 $\mu\text{S}/\text{cm}$ above intake to a maximum of 1500 $\mu\text{S}/\text{cm}$ (DWAF 1999)
	Summer	167 – 226	167 – 228	
	Autumn	134 – 231	176 – 222	
	Winter	164 – 257	177 – 249	
Temp ( $^{\circ}\text{C}$ )	Spring	15 – 25	15 – 23	Maximum of 37 $^{\circ}\text{C}$ (APHA 1975) <sup>a</sup>
	Summer	21 – 27	20 – 23	
	Autumn	16 – 26	15 – 25	
	Winter	13 – 17	12 – 14	
Turbidity (NTU)	Spring	2.51 – 10.60	21.80 – 58.80	<5 NTU (WHO 2008) <sup>a</sup>
	Summer	3.18 – 14.30	9.80 – 22.30	
	Autumn	1.50 – 24.70	6.13 – 65.70	
	Winter	3.00 – 9.00	21.00 – 59.00	
Free Cl (mg/L)	Spring	0.11 – 0.49	0.12 – 0.54	0.25 mg/L (DWAF 1999)
	Summer	0.15 – 0.29	0.13 – 0.72	
	Autumn	0.10 – 0.38	0.08 – 0.68	
	Winter	0.10 – 0.39	0.15 – 0.39	



DO (mg/L)	Spring	4.80 – 8.55	2.06 – 2.60	≥5 mg/L (WHO 2006) <sup>a</sup>
	Summer	4.45 – 7.91	2.47 – 8.22	
	Autumn	4.22 – 8.08	7.10 – 9.17	
	Winter	5.06 – 8.63	8.88 – 9.81	
BOD <sub>5</sub> (mg/L)	Spring	0.13 – 7.14	0.88 – 5.03	3-6 mg/L (EU standard) (Chapman 1996) <sup>a</sup>
	Summer	0.60 – 6.88	1.15 – 4.77	
	Autumn	0.13 – 6.47	0.19 – 8.15	
	Winter	0.24 – 8.43	0.38 – 9.81	
NO <sub>3</sub> <sup>-</sup> (mg/L)	Spring	0 – 4.50	4.30 – 7.40	15 mg/L (DWAF 1999)
	Summer	3.18 – 7.91	5.50 – 19.60	
	Autumn	3.50 – 8.10	7.40 – 21.50	
	Winter	3.30 – 5.50	14.90 – 19.70	
NO <sub>2</sub> <sup>-</sup> (mg/L)	Spring	0 – 0.22	0.22 – 0.45	15 mg/L (DWAF 1999)
	Summer	0.16 – 0.24	0.16 – 0.76	
	Autumn	0.15 – 0.36	0.19 – 0.31	
	Winter	0.17 – 0.33	0.19 – 0.29	
PO <sub>4</sub> <sup>-</sup> (mg/L)	Spring	1.03 – 16.10	2.09 – 4.52	10 mg/L (DWAF 1999)
	Summer	1.63 – 10.20	2.32 – 14.70	
	Autumn	1.09 – 15.40	11.60 – 18.30	
	Winter	0 – 2.41	2.89 – 3.83	
COD (mg/L)	Spring	73 – 680	148 – 650	75 mg/L after removal of algae (DWAF 1999)
	Summer	32 – 222	32 – 176	
	Autumn	27 – 266	92 – 318	
	Winter	61 – 195	143 – 277	

<sup>a</sup> International guidelines were used where limit does exist in DWAF's guidelines

**Key:** SWWTP – Stutterheim wastewater treatment plant; KWWTP – Keiskammahoek wastewater treatment plant. **Seasons:** Spring (September, October, November); summer (December, January, February); autumn (March, April, May); winter (June, July, August).

Although there is no specific limit for turbidity in the South African Guideline for General and Special Standards Requirements for the Purification of Wastewater or Effluent (Gazette No. 9225, Regulation No 991, 18 May 1984; Osode, 2010), the South African Target Water Quality Range for turbidity in water for domestic water supply is 0 to 1 nephelometric turbidity unit (NTU) (DWAF, 1996b) while the WHO standard is 5 NTU (WHO, 2004). The turbidity levels of the discharged effluents in this study did not comply with either of these standards for most part of the sampling period particularly at KWWTP, which was largely expected because the volume of wastewater treated at this plant was above the operational capacity of the plant.

Turbidity is an important operational parameter in process control and can indicate problems with treatment processes, particularly coagulation or sedimentation and filtration (WHO, 2004). It is caused by suspended matter or impurities that interfere with the clarity of the water. These impurities may include clay, silt, finely divided inorganic and organic matter, soluble coloured organic compounds, and plankton and other microscopic organisms (US EPA, 1999a; DWAF, 2002). One major implication of excessive turbidity in water is reduction in light penetration which may result in the decline of the rate of photosynthesis by the aquatic plants, and this may lead to less food being available for the aquatic animals (Palmer *et al.*, 2004). Also, high turbidity increases water temperature because suspended particles absorb more heat. This, in turn, reduces the concentration of dissolved oxygen (DO) because warm water holds less DO than cold (US EPA, 2012c).

Turbidity in water directly correlates with the microbial load within water resources. Excessive turbidity can affect the effectiveness of chlorination during disinfection in

wastewater treatment, resulting in the failure of the removal of microorganisms (Fatoki *et al.*, 2001; Obi *et al.*, 2007). The ineffectiveness of chlorination may also increase the chances of trihalomethane (THM) precursors forming in the wastewater effluent (Fatoki *et al.*, 2001). THM are carcinogenic compounds that are formed as a by-product from chlorine and organic matter reaction, which may result in serious health implications for the aquatic life and humans exposed to it (Odjadjare and Okoh, 2010).

The dissolved oxygen levels (DO) recorded in the study varied significantly ( $P < 0.05$ ) between the FE and the DP samples at both treatment plants throughout the sampling period as shown in Table 1. Dissolved oxygen values largely ranged between 2.06 mg/L and 9.81 mg/L and also varied significantly ( $P < 0.05$ ) among the seasons except in STF samples with F-value and P-Value of 0.381 and 0.812 respectively.

DO is an important parameter used in water quality control and it is critical for the survival and reproduction of fish and other aquatic organisms that cannot obtain oxygen directly from the atmosphere. Although DWAF does not have a recommended limit for DO in discharged effluent, the observed DO levels were, for most part of the sampling in compliance with the World Health Organisation (WHO) limit of  $\geq 5$  mg/L of dissolved oxygen in drinking water (WHO, 2006). Also, the higher DO levels recorded at the discharge points compared to the final effluents may be as a result of the mixing of the effluents as it flows into the receiving water bodies in comparison with the final effluent tanks where there was relatively less mixing of the effluent. The dissolved oxygen measurements obtained in the study were comparable to those reported elsewhere (Obire *et al.*, 2003; Jaji *et al.*, 2007; Igbiosa and Okoh, 2009).

An indication of the organic oxygen demand of wastewater can be obtained by measuring the amount of oxygen required for its stabilisation either as biological oxygen demand (BOD) or chemical oxygen demand (COD). Whilst BOD measurements determine the amount of oxygen required by microorganisms in breaking down organic matter, COD determination measures the amount of oxygen required for chemical decomposition of organic and inorganic contaminants, dissolved or suspended in water (Salem *et al.*, 2011). A general yardstick of evaluating the performance of sewage treatment plants is the degree of reduction of BOD (often determined as 5-day, 20 °C BOD) and the performance efficiency of treatment plants depends, not only on proper design and construction, but also on good operation and maintenance (Kumar *et al.*, 2010). The BOD profile at both treatment plants did not vary significantly ( $P>0.05$ ) between FE and DP samples and among the seasons (Table 1), and ranged between 0.13 mg/L and 9.81 mg/L throughout the sampling period. The determination of BOD and COD is useful in evaluating the compliance of effluents with water quality requirements standards and also in the estimation of the potential of organic waste present in such effluent to deplete oxygen (DWAF, 1996a). Since BOD measures the amount of oxygen required by bacteria to break down the organic matter present in the water, the greater the biodegradable organic matter present in water (effluent), the greater the BOD values (Akinlua and Asubiojo, 2006; Akan *et al.*, 2008). Continuous discharge of effluent with high BOD into freshwater systems can have negative consequences on such water systems and may cause harm to the aquatic life of such water systems (Morrison *et al.*, 2001; Akinlua and Asubiojo, 2006). Although DWAF does not have a set limit for BOD in discharged final effluent, the seasonal BOD values of treated final effluent complied with the European Union set limit of 3 – 6 mg/L. The BOD levels recorded in the study were similar to those reported elsewhere (Akinlua and Asubiojo, 2006).

The COD of the samples varied between 27 and 680 mg/L over the sampling months. The COD values at SWWTP did not vary significantly ( $P>0.05$ ) among the seasons while significantly high variations in the COD levels were observed at KWWTP. The observed COD levels were above the DWAF recommended limit of 75 mg/L (DWAF, 2004) for most part of the sampling period. The reported COD levels at KWWTP in this study were similar to a previous study carried out on the wastewater treatment plant (Morrison *et al.*, 2001).

The free chlorine measurements of the discharged wastewater effluents ranged between 0.08 and 0.72 mg/L over the sampling period at both study sites. The difference in free chlorine concentration between the FE and the DP samples were not significant ( $P>0.05$ ). The seasonal free chlorine concentrations were also not significantly different except in the DP samples at WWTP1, with an F-value of 4.957 and a P-Value of 0.006 as shown in Table 1. Chlorine is an extremely active oxidizing chemical that reacts with many substances in wastewater (Hung *et al.*, 2012). If added to wastewater in inadequate quantities, it reacts rapidly and is thus consumed. The addition of chlorine dosage to a level where free chlorine is formed is often referred to as “breakpoint” chlorination (Hung *et al.*, 2012). Disinfection of public water and wastewater systems through the use of chlorine has been practiced for almost a century and although the advantages and the disadvantages of disinfection with chlorine have been extensively highlighted, it remains the most widely used chemical for disinfection of water (Harp, 2002). Chlorine application in wastewater treatment may be done by the addition of gaseous and liquid chlorine to the treated wastewater, and is not only limited to disinfection purposes but may also involve odour and corrosion control, greases removal, BOD reduction and oxidation of ferrous sulphate and ammonia (Hung *et al.*, 2012). The amount of reducing organic and inorganic matter in wastewater varies, therefore, the amount of chlorine that has to be added to wastewater for different purposes will also vary.

The chlorine used by these organic and inorganic substances is often termed chlorine demand. For effective chlorine disinfection, both sufficient chlorine dosages as well as contact time are necessary. Among the principal factors that affect the effectiveness of chlorine are pH, temperature, and contacting conditions (Hung *et al.*, 2012). High levels of variations from DWAF's recommended limit of 0.25 mg/L were observed in the free chlorine regimes of the discharged effluents in this study. Inadequate levels of free chlorine in the discharged effluent may allow for the survival of bacteria including pathogenic ones in the discharged final effluents while chlorine over dose may equally be detrimental to the aquatic lives in the receiving water bodies. Therefore, adequate measures must be taken to ensure that sufficient amounts of chlorine are used in the treatment of effluents (US EPA, 2004).

One important characteristic of discharged wastewater effluents that often impacts receiving waters is its nutrient content. Excessive nutrient loading, especially nitrogen and phosphorus, is a major on-going threat to freshwater quality worldwide, particularly in water-scarce countries like South Africa (Carey and Migliaccio, 2009; Muller *et al.*, 2009). Many aquatic systems have very low ambient nutrient concentration and small shifts in the nutrient load can result in dramatic changes in the aquatic community structure (Miltner and Rankin 1998; Dodds and Welch, 2000; Rabalais, 2002). The nitrate ( $\text{NO}_3^-$ ) concentration in this study did not vary significantly ( $P>0.05$ ) between the final effluents and the discharge point samples at both study sites over the sampling period. However, the concentrations varied significantly with season and ranged between 0 and 21.3 mg/L. Nitrate concentrations at WWTP1 were within the DWAF limit of 15 mg/L for discharged effluent (DWAF, 2004). Conversely high levels of nitrates above this limit were recorded at KWWTP, particularly in winter. The observed phenomenon could have arisen due to the small aerator used at this site which is below the capacity of the volume of wastewater treated at the site. High nitrate levels in

discharged effluents have been reported to lead to eutrophication in receiving water bodies (Morrison *et al.*, 2001).

The nitrite ( $\text{NO}_2^-$ ) concentration profile exhibited a similar pattern with that of nitrate. The observed seasonal mean values were within the recommended limit of 15 mg/L for discharged final effluents as stipulated by DWAF (DWAF, 2004) and would unlikely be a threat to the receiving watershed.

The orthophosphate ( $\text{PO}_4^-$ ) contents of the discharged effluents ranged between 0 mg/L and 18.3 mg/L and did not vary significantly ( $P>0.05$ ) between the FE and the DP samples at both study site. However, variations were observed in the samples among the seasons. High values above the recommended limit of 10 mg/L for discharged final effluents (DWAF, 2004) were recorded mostly in the autumn months at KWWTP.

Many researchers investigating nutrient pollution from nonpoint sources have severally discovered that nutrient loads were often more strongly influenced by discharged effluent from wastewater treatment plants than the nonpoint sources (Ahearn *et al.*, 2005; Popova *et al.*, 2006; Migliaccio *et al.*, 2007). One major implication of the excessive release of nutrients into freshwater bodies is eutrophication (DWAF, 2009). Uncontrolled inputs of phosphorus and/or nitrogen to aquatic environments leads to increased rates of eutrophication with the attendant problems.

Nitrogen in wastewater derives from breakdown products of proteins found in urine and faeces (Svirejeva-Hopkins and Reis, 2011). These products are very soluble and often pass through sewage treatment process, and are subsequently discharged into rivers as a component of sewage treatment effluents. Nitrogen may exist in the form of nitrate, nitrite, ammonia or ammonium salts in wastewater. All these forms of nitrogen can be used by macrophytes and algae thereby leading to increased rates of eutrophication. The differing forms of nitrogen are relatively stable in most river systems with nitrite slowly transforming into nitrate in well oxygenated rivers and ammonia transforming into nitrite and/or nitrate (Florescu *et al.*, 2011). Studies have suggested that nitrate and nitrite ions act on many systems and could serve as direct precursors for the production of nitric oxide, a potent physiological regulator in vertebrates (Avery, 1999).

Nitrate and nitrite have been reported to be toxic in humans and animals for decades (Avery, 1999; Guillette and Edwards, 2005). As early as 1945, methemoglobinemia (Blue Baby syndrome) was associated with drinking nitrate-contaminated well water on farms from the Midwest USA (Guillette and Edwards, 2005). Methemoglobinemia is formed during nitrate-induced oxidation of haemoglobin. This prevents normal oxygen binding and leads to hypoxia. Methemoglobinemia, as well as additional concerns, continue today with increasing nitrate contamination of water bodies, ammonium ions have also been reported to have toxic effects on fish (Avery, 1999; Porter *et al.*, 1999; Guillette and Edwards, 2005). Uncontrolled discharge of phosphorus can also encourage excessive growths of aquatic plants and algae and thus contribute to eutrophication. Orthophosphates are the sole form of soluble inorganic phosphorus which can be directly utilised by the aquatic organisms, mainly algae and other plants. Together with nitrates and nitrites, orthophosphates are found in high levels in discharged effluents of wastewater treatment plants and are important growth limiting factors



in eutrophication, which result in undesirable ecological effects within the receiving water resources (DWAF, 1996a; Morrison *et al.*, 2001).

Ekholm and Krogenus (1998) reported that municipal wastewater contains substantial amounts of phosphorus contributed by human urine and detergents. High levels of phosphates have been known to contribute to increased growth of vegetation in water systems with elevated oxygen demand in such water systems (McEldowney *et al.*, 1993). However, apart from its contribution to eutrophication and toxic algal blooms, phosphates do not have any other notable effects on human health.

Although there have been increased access to potable water and improved sanitation over the last two decades; with more than 1.8 million people gaining access to improved water source and sanitation facilities (Naidoo and Olaniran, 2014). Nonetheless, in spite of the remarkable global progress to improve access to drinking water facilities, at present there are some 884 million and an additional 2.5 billion people lacking improved water sources and sanitation respectively (UNGLAAS, 2012). With the projection that the world population is heading to 8.3 billion by the year 2025, water pollution issues are anticipated to get worse, most importantly in developing countries with rapid urbanisation which further overburdens existing water supplies (Kraemer, *et al.*, 2001). Despite the fact that South Africa projects at spending 0.5% of its gross domestic product on improving sanitation, other issues such as hydrological unpredictability and growing agricultural needs have further increased reliance on its finite freshwater resource (Naidoo and Olaniran, 2014). Increasing pressure on current sewage and wastewater treatment facilities has led to the discharge of inefficiently treated

effluent, emphasising the need to advance and assume more stringent approaches for monitoring discharged effluent and receiving watersheds.

While the discharged effluents in this study largely complied with recommended guidelines for pH, TDS, EC, temperature, nitrate and on most occasions DO, they fell short of the guideline limits in terms of the turbidity, BOD, nitrate, phosphate and COD. High levels of inconsistency in the free chlorine concentration were observed in the discharged effluents at both sites throughout the sampling period. This calls for attention as chlorination remains the commonly used disinfectant for sewage treatment and its effectiveness particularly in removing biological contaminants such as pathogens is necessary. High levels of chlorine concentration in some of the months can equally be detrimental to aquatic lives in the receiving watersheds of these discharged effluents.

To determine the statistical relationships among the various physicochemical parameters measured in the study; correlation analysis was carried out on these parameters. Table 3.4 shows the correlation coefficient for the various parameters measured. There was a positive significant correlation between pH and each of nitrate ( $r = 0.313$ ;  $P < 0.01$ ) and DO ( $r = 0.537$ ;  $P < 0.01$ ) while TDS ( $r = -0.359$ ;  $P < 0.01$ ), EC ( $r = -0.360$ ;  $P < 0.01$ ), temperature ( $r = -0.180$ ;  $P < 0.05$ ), turbidity ( $r = -0.215$ ;  $P < 0.01$ ), free chlorine ( $r = -0.206$ ;  $P < 0.05$ ) and COD ( $r = -0.258$ ;  $P < 0.01$ ) were inversely correlated to pH. TDS showed a positive linear relationship with EC ( $r = 1.000$ ;  $P < 0.01$ ). This agrees with the knowledge that TDS is a measure of dissolved minerals (salts) in water, most of which carry electrical charges (DWAF, 1996a; Salem *et al.*, 2011; Rout and Sharma, 2011). TDS also showed positive correlation with turbidity ( $r = 0.417$ ;  $P < 0.01$ ), nitrite ( $r = 0.170$ ;  $P < 0.05$ ) and COD ( $r =$

**Table 3.4:** Correlation half-matrix of physicochemical wastewater quality parameters.

Parameters	pH	TDS	EC	Temp	Turb	Free Cl	DO	BOD <sub>5</sub>	NO <sub>3</sub> <sup>-</sup>	NO <sub>2</sub> <sup>-</sup>	PO <sub>4</sub> <sup>-</sup>	COD
pH	<b>1.000</b>											
TDS	<b>-.359**</b>	<b>1.000</b>										
EC	<b>-.360**</b>	<b>1.000**</b>	<b>1.000</b>									
Temp	<b>-.180*</b>	.018	.001	<b>1.000</b>								
Turbidity	<b>-.215**</b>	<b>.417**</b>	<b>.409**</b>	<b>.220**</b>	<b>1.000</b>							
Free Cl	<b>-.206*</b>	.137	.147	<b>.165*</b>	.098	<b>1.000</b>						
DO	<b>.537**</b>	<b>-.332**</b>	<b>-.325**</b>	<b>-.392**</b>	<b>-.021*</b>	<b>-.204*</b>	<b>1.000</b>					
BOD <sub>5</sub>	.076	.158	.137	<b>.212*</b>	<b>.285**</b>	<b>-.329**</b>	.104	<b>1.000</b>				
NO <sub>3</sub> <sup>-</sup>	<b>.313**</b>	-.085	-.076	<b>-.329**</b>	<b>.353**</b>	-.126	<b>.523**</b>	.124	<b>1.000</b>			
NO <sub>2</sub> <sup>-</sup>	-.040	<b>.170*</b>	<b>.168*</b>	-.003	<b>.189*</b>	<b>.443**</b>	<b>-.352**</b>	.011	.071	<b>1.000</b>		
PO <sub>4</sub> <sup>-</sup>	.124	-.014	-.028	<b>.344**</b>	.038	-.009	<b>.199*</b>	.103	<b>.355**</b>	-.142	<b>1.000</b>	
COD	<b>-.258**</b>	<b>.261**</b>	<b>.259**</b>	<b>-.283**</b>	<b>.416**</b>	.018	-.048	-.095	.023	.032	.063	<b>1.000</b>

\*Correlation is significant at  $P < 0.05$  level (2-tailed); \*\*. Correlation is significant at  $P < 0.01$  level (2-tailed).

**Key:** TDS- Total dissolved solid; EC- Electrical conductivity; Temp- Temperature; Turb- Turbidity; Free Cl- Free chlorine; DO- Dissolved oxygen; BOD- Biological oxygen demand; NO<sub>3</sub><sup>-</sup>- Nitrate; NO<sub>2</sub><sup>-</sup>- Nitrite; PO<sub>4</sub><sup>-</sup>- Phosphate; COD- Chemical oxygen demand.

0.261;  $P < 0.01$ ) while it was inversely correlated with DO ( $r = -0.332$ ;  $P < 0.01$ ).

The temperature of the samples positively correlated with each of turbidity ( $r = 0.220$ ;  $P < 0.01$ ), BOD<sub>5</sub> ( $r = 0.212$ ;  $P < 0.05$ ) and PO<sub>4</sub><sup>-</sup> ( $r = 0.344$ ;  $P < 0.01$ ) while it showed an inverse correlation with dissolved oxygen DO ( $r = -0.392$ ;  $P < 0.01$ ), nitrate ( $r = -0.329$ ;  $P < 0.01$ ) and COD ( $r = -0.283$ ;  $P < 0.01$ ). Excessive levels of TDS have been shown to elevate biological and chemical oxygen demand in water systems (Jonnalagadda and Mhere, 2001). Temperature in water systems has been shown to be inversely correlated to DO; which often decreases with higher temperature and *vice versa* (Onuoha and Nwadukwe, 1989; Francis *et al.*, 2007).

Turbidity of samples in the study exhibited positive correlation with BOD ( $r = 0.285$ ;  $P < 0.01$ ), nitrate ( $r = 0.353$ ;  $P < 0.01$ ), nitrite ( $r = 0.189$ ;  $P < 0.05$ ) and COD ( $r = 0.416$ ;  $P < 0.01$ ) while it exhibited an inverse correlation with DO ( $r = -0.021$ ;  $P < 0.05$ ). Higher levels of turbidity have been known to increase water temperatures because suspended particles absorb more heat. Increased temperatures, in turn, reduce the amount of DO in water systems because warm water holds less DO than cold. Higher turbidity also decreases the intensity of light penetrating the water, which reduces photosynthesis and the production of DO (USEPA, 2012).

### **3.5 Conclusions**

An essential role of wastewater treatment is the removal hazardous components from wastewater, in order to protect public health and conserve freshwater sources that often serve

as receiving bodies for discharged effluents. Although, the negative impact of inadequately treated effluents on surface water has received attention in recent years, the production of effluents of high quality remains a major challenge with increasing detrimental impacts on freshwater ecosystems, notably, eutrophication in many rivers. The unabated practises of the discharge of inadequately treated effluents from these wastewater treatment facilities may alter the ecosystem balance of the receiving freshwater bodies over time. The findings of this investigation highlight the potential harmful impact of the discharged effluents on the receiving watersheds over time. There is need for proper and consistent monitoring of the treatment processes and facilities as well as the receiving watersheds, in order to ensure the protection of public and environmental health. The result also underscore the importance of monitoring of the discharged effluent for other important and emerging pollutants of public health concerns as a panacea for the incessant pollution of the limited available fresh water resources in South Africa.

## References

- Abdel-Raouf, N., Al-Homaidan, A.A., Ibraheem, I.B.M. (2012).** Microalgae and wastewater treatment. *Saudi Journal of Biological Science* 19(3), 257-275.
- Abor, P.A. (2007).** Medical waste management at Tygerberg hospital in the Western Cape, South Africa. Dissertation, Cape Peninsula University of Technology. Available on: <http://digitalknowledge.cput.ac.za/>. (Accessed 15 February 2014).
- Ahearn, D.S., Sheibley, R.W., Dahlgren, R.A., Anderson, M.M., Johnson, J., Tate, K.W. (2005).** Land use and land cover influence on water quality in the last free flowing river draining the western Sierra Nevada, California. *Journal of Hydrology* 312, 234-247.
- Akan, J.C., Abdulrahman, F.I., Dimari, G.A., Ogugbuaja, V.O. (2008).** Physicochemical determination of pollutants in wastewater and vegetable samples along the Jakara Wastewater Channel in Kano Metropolis, Kano State, Nigeria. *European Journal of Scientific Research* 23(1), 122-133.
- Akinlua, A. and Asubiojo, O.I. (2006).** Physicochemical and trace metal characterization of battery factory wastewater. *Journal of Applied Science* 6(7), 1456-1462.
- Avery, A.A. (1999).** Infantile methemoglobinemia: Reexamining the role of drinking water nitrates. *Environmental Health Perspectives* 107, 583-586.
- Canadian Council of Ministers of the Environment (CCME) (2006).** Municipal waste effluent in Canada. Municipal Wastewater Effluent Development Committee December 2006.
- Canter, L.W. (1996).** Prediction and assessment of impacts on the surface-water environment. Second Edition. University of Oklahoma. Mc Graw-Hill Inc.

**Carey, R.O. and Migliaccio, K.W. (2009).** Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. *Environment Management* 44, 205-217.

**Chapman, D. (1996).** Water quality assessments: A guide to the use of biota, sediments and water in environmental monitoring 2nd. Ed. UNESCO, World Health Organization, United Nations Environment Programme, London.

**Department of Water Affairs and Forestry Republic of South Africa (DWAF) (2004).** Government Gazette No. 20526, 8th October 1999. Revision of General Authorisations in Terms of Section 39 of the National Water Act, 1998 (Act no. 36 of 1998). Available on: <http://www.ewisa.co.za/misc/WWManage/defaultStandards.htm>. (Accessed 31 December 2013).

**Department of Water Affairs and Forestry Republic of South Africa (DWAF) (1996a).** South African Water Quality Guidelines (Volume 7): Aquatic Ecosystems (1st Edition): Department of Water Affairs and Forestry, Pretoria.

**Department of Water Affairs and Forestry, Republic of South Africa (DWAF). (1996b).** South African Water Quality Guidelines, (Volume 2), Recreational Water Use (2nd Ed.). Department of Water Affairs and Forestry, Pretoria.

**Department of Water Affairs and Forestry, Republic of South Africa (DWAF) (2002).** Cabinet memorandum: Submission of a co-ordinated progress report on the Government's Domestic National Sanitation Programme, Pretoria.

**Department of Water Affairs Republic of South Africa (DWA) (1999).** Resource directed measures for protection of water resources. Volume 3: River Ecosystem Versio 1.0. Pretoria.

**Department of Water Affairs Republic of South Africa (DWA) (2009).** Executive summary municipal wastewater treatment base information for targeted risk-based regulation Eastern Cape Province status at June 2009.

**Department of Water Affairs Republic of South Africa (DWA) (2012).** Green Drop Progress Report 2012.

**Dodds, W.K. and Welch, E.B. (2000).** Establishing nutrient criteria in streams. *Journal of the North American Benthological Society* 19, 186-196.

**Ekholm, P. and Krogenus, K. (1998).** Bioavailability of phosphorus in purified municipal wastewater. *Water Research* 32, 343-351.

**Fatoki, O.S., Muyima, N.Y.O. and Lujiza, N. (2001).** Situation analysis of water quality in the Umtata River catchment. *Water South Africa* 27(4), 467-473.

**Florescu, D., Ionete, R.E., Sandru, C., Iordache, A. and Culea, M. (2011).** The influence of pollution monitoring parameters in characterizing the surface water quality from Romania Southern area. *Romanian Journal of Physics* 56(7–8), 1001–1010.

**Food and Agriculture Organization of the United Nations (FAO) (1992).** Wastewater treatment and use in agriculture – irrigation and drainage paper 47. Produced by Natural Resources Management and Environmental Department. Available on: <http://www.fao.org/docrep/T0551E/t0551e00.htm> (Accessed 11 January 2014).

**Francis, A., Sikoki, F.D. and Ansa, E.J. (2007).** Physicochemical parameters of the Andoni River System-Niger Delta, Nigeria. *Journal of Fisheries International* 2(1), 27-31.

**Government Gazette (1984).** Requirements for the purification of wastewater or effluent. Gazette no. 9225, Regulation No 991, 18 May 1984.



- Guillette, L.J. and Edwards, T.M. (2005).** Is nitrate an ecologically relevant endocrine disruptor in vertebrates? *Integrative and Comparative Biology* 45, 19-27.
- Harp, D.L. (2002).** Current Technology of Chlorine Analysis for Water and Wastewater. Technical information Series – Booklet No. 17. Printed in USA, HACH Company, 2002. Available on: [http://www.hach.com/cms\\_portals/hach\\_com/cms/documents/pdf/LIT/L7019-ChlorineAnalysis.pdf](http://www.hach.com/cms_portals/hach_com/cms/documents/pdf/LIT/L7019-ChlorineAnalysis.pdf). (Accessed 10 January 2014).
- Hayashi, M. (2004).** Temperature-electrical conductivity relation of Water for environmental monitoring and geophysical Data inversion. *Environmental Monitoring and Assessment* 96, 119–128.
- Hung, Y.T., Wang, L.K. and Shammas, N.K. (2012).** Handbook of environment and waste management, air and water pollution control. World Scientific Co. Pte. Ltd. 5 Toh Tuck link, Singapore.
- Igbiosa, E.O. and Okoh, A.I. (2009)** Impact of discharge wastewater effluents on the physico-chemical qualities of a receiving watershed in a typical rural community. *International Journal of Environmental Science and Technology* 6 (2), 175-182.
- Imoobe, T.O.T. and Koye, P.I.O. (2011).** Assessment of the impact of effluent from a soft drink processing factory on the physico-chemical parameters of Eruvbi stream Benin City, Nigeria. *Bayero Journal of Pure and Applied Sciences* 4(1), 126 – 134.
- Jackson, M.K., Morris, G.P., Smith, P.G. and Crawford, J.F. (1989).** Environment Health Reference Book. London: Butterworth-Heinemann.

- Jaji, M.O., Bamgbose, O., Odukoya, O.O. and Arowolo, T.A. (2007).** Water quality assessment of Ogun River, South West Nigeria. *Environmental Monitoring and Assessment* 133 (1-3), 447-482.
- Jonnalagada, S.B. and Mhere, G. (2001).** Water quality of the Odzi River in the eastern highlands of Zimbabwe. *Water Research* 35, 2371-2376.
- Jury, W.A. and Vaux, H.J. (2007).** The emerging global water crisis: managing scarcity and conflict between water users. *Advances in Agronomy* 95:1-76.
- Khan, F.J. and Javed, Y. (2007).** Delivering access to safe drinking water and adequate sanitation in Pakistan. Pakistan Institute of Development Economics, Islamabad, Pakistan. Working Paper No 21.
- Kinouchi, T., Yagi, H. and Miyamoto, M. (2007).** Increase in stream temperature related to anthropogenic heat input from urban wastewater. *Journal of Hydrology* 335(1-2), 78-88.
- Kivaisi, A.K. (2001).** The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review. *Ecological Engineering* 16 (4), 545–560.
- Kraemer, R.A., Choudhury, K. and Kampa, E. (2001).** Protecting water resources: pollution prevention. Thematic Background Paper. International Conference on Freshwater, Bonn 2001.
- Kumar, K.S., Kumar, P.S. and Babu, M.J.R. (2010).** Performance evaluation of Waste water treatment plant. *International Journal of Engineering Science and Technology* 2(12): 7785-7796.

- Kumar, R., Singh, R.D. and Sharma, K.D. (2005).** Water resources of India. *Current Science* 89(5), 794-811.
- Lagerblad, L. (2010).** Wastewater treatment wetlands – A case study in Colombo, Sri Lanka. Minor Field Study 146 Committee of Tropical Ecology Uppsala University, Sweden. Available on: [http://www.ibg.uu.se/digitalAssets/106/106214\\_lagerblad-lovisa.pdf](http://www.ibg.uu.se/digitalAssets/106/106214_lagerblad-lovisa.pdf). (Accessed 12 February 2014).
- McEldowney, S., Hardman, D.J. and Waite, S. (1993).** Pollution and Biotreatment. Longman Group Ltd., Essex., England.
- Migliaccio, K.W., Haggard, B.E., Chaubey, I. and Matlock, M.D. (2007).** Linking watershed sub-basin characteristics to water quality parameter in War eagle Creek watershed. *Transaction of ASAE* 50:2007-2016.
- Miltner, R.J. and Rankin, E.T. (1998).** Primary nutrients and the biotic integrity of rivers and streams. *Freshwater Biology* 40, 145-158.
- Morrison, G., Fatoki, O.S., Persson, L. and Ekberg, A. (2001).** Assessment of the impact of point source pollution from the Keiskammahoek Sewage Treatment Plant on the Keiskamma River - pH, electrical conductivity, oxygen-demanding substrate (COD) and nutrients. *Water SA* 27(4), 475-480.
- Muller, M., Schreiner, B., Smith, L., Koppen, B., Sally, H., Aliber, M., Cousins, B., Tapela, B., Merwe-Botha, M., Karar, E. and Pietersen, K. (2009).** Water security in South Africa. Development Planning. Working paper Series No.12, DBSA: Midrand.

**Naidoo, S, and Olaniran, A.O. (2014).** Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. *International Journal of Environmental Research and Public Health* 11, 249-270.

**National Oceanic and Atmospheric Administration (NOAA) (2008).** Categories of pollution: Point source. Available on: <http://oceanservice.noaa.gov/education/kits/pollution/03pointsource.html>. (Accessed 31 October 2013).

**National Small Flow Clearinghouse (NSFC) (1996).** On site wastewater disposal and public health. Pipeline, summer 1996, Vol. 7, No.3. Available on: <http://www.nesc.wvu.edu/>. (Accessed 11 May 2014).

**Nemerow, N.L. and Dasgupta, A. (1991).** Industrial and hazardous waste treatment. Van Nostrand Reinhold, New York, NY.

**Obi, C.L., Bessong, P.O., Momba, M.N.B., Potgieter, N., Samie, A. and Igumbor, E.O. (2004).** Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa. *Water SA* 30(4), 515-519 .

**Obi, C.L., Momba, M.N.B., Samie, A., Igumbor, J.O., Green, E. and Musie, E. (2007).** Microbiological, physicochemical and management parameters impinging on the efficiency of small water treatment plants in Limpopo and Mpumalanga Provinces of South Africa. *Water SA* 33(2), 229-237.

**Obire, O., Tamuno, D.C. and Wemedo, S.A. (2003).** Physico-chemical quality of Elechi Creek in Port Harcourt, Nigeria. *Journal of Applied Science and Environmental Management.* 7 (1), 43-49.

- Odjadjare, E.E. and Okoh, A.I. (2010).** Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment. *Environmental Monitoring and Assessment* 17(1-4), 393-394.
- Ojo, A.O. and Adeniyi, I.F. (2012).** The Impacts of Hospital Effluent Discharges on the Physico-chemical Water Quality of a Receiving Stream at Ile-Ife, Southwestern Nigeria. *Journal of Sustainable Development* 5(11), 82-92.
- Onuoha, G.C. and Nwadukwe, F.O. (1989).** Water quality monitoring. In Ayinla OA (ed) Proceeding of the Aquaculture Training programme (ATP). African regional Aquaculture centre (ARAC) Aluu, pp.: 12-23.
- Osode, A.N. and Okoh, A.I. (2010).** Survival of free-living and plankton-associated *Escherichia coli* in the final effluents of a waste water treatment facility in a peri-urban community of the Eastern Cape Province of South Africa. *African Journal of Microbiology Research* 4(13), 1424-1432.
- Osode, A.N. and Okoh, A.I. (2009).** Impact of discharged wastewater final effluent on the physicochemical qualities of a receiving watershed in a suburban community of the Eastern Cape Province. *Clean – Soil, Air, Water* 37 (12), 938-944.
- Owili, M.A. (2003).** Assessment of impact of sewage effluents on coastal water quality in Hafnarfjordur, Iceland. The United Nations University Fishries Training Programme, Final Report, Reykjavik, Iceland.
- Palmer, C.G., Berold, R.S. and Muller, W.J. (2004).** Environmental water quality in water resources management. WRC Report No TT 2217/04, Water Research Commission, Pretoria, South Africa.

- Picardi, A. (2002).** Tonga health care project health care management feasibility study. E728 Volume 2. Available on: <http://www-wds.worldbank.org/>. (Accessed 15 February 2014).
- Popova, Y.A., Keyworth, V.G., Haggard, B.E., Storm, D.E., Lynch, R.A. and Payton, M.E. (2006).** Streams nutrient limitation and sediment interactions in the Eucha-Spavinaw Basin. *Journal of Soil and Water Conservation* 61, 105-115.
- Porter, W.P., Jaeger, J.W. and Carlson, I.H. (1999).** Endocrine, immune, and behavioural effects of aldicarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixture at groundwater concentration. *Toxicology and Industrial Health* 15, 133-150.
- Rabalais, N.N. (2002).** Nitrogen in aquatic ecosystems. *Ambio* 31, 102-112.
- Radtke, D.B., Davis, J.V. and Wilde, F.D. (2005).** Specific Electrical Conductance. U.S. Geological Survey TWRI Book 9. Available on: <http://water.usgs.gov/owq/FieldManual/Chapter6/Final508Chapter6.3.pdf>. (Accessed 5 September 2013).
- Rout, C. and Sharma, A. (2011).** Assessment of drinking water quality: A case study of Ambala cantonment area, Haryana, India. *International Journal of Environmental Sciences* 2(2), 933-945.
- Salem, I.B., Ouardani, I., Hassine, M. and Aouni, M. (2011).** Bacteriological and physicochemical assessment of wastewater in different region of Tunisia: impact on human health. *BMC Research Notes* 4:144.
- Svirejeva-Hopkins, A. and Reis, S. (2011).** The European nitrogen assessment (ed) Sutton MA, Howard CM, Erisman JW, Billen G, Bleeker A, Rennfelt P, Grinsven HV, Grizzetti B. Cambridge University Press.

**United Nations Environment Programme Global Environment Monitoring System**

**(GEMS)/Water programme (UNEP) (2008).** Water quality for Ecosystem and human health Second edition. Available on: <http://www.unep.org/>. (Accessed 8 August 2013).

**United Nations Water Global Annual Assessment of Sanitation and Drinking Water**

**(UNGLAAS) (2012).** The Challenge of Extending and Sustaining Services; World Health Organization: Geneva, Switzerland, 2012.

**United State Environmental protection Agency (USEPA) (2004).** Primer for municipal

wastewater treatment systems. Office of wastewater management Washington DC 20460. Document no. EPA 832-R-04-001.

**United States Environmental protection Agency (US EPA) (1999a).** EPA guidance

manual turbidity provision, April 1999. Available on:

[http://www.epa.gov/ogwdw/mdbp/pdf/turbidity/chap\\_07.pdf](http://www.epa.gov/ogwdw/mdbp/pdf/turbidity/chap_07.pdf). (Accessed 28 December 2013).

**United States Environmental protection Agency (US EPA) (2012).** Water: Turbidity.

Available on: <http://water.epa.gov/type/rsl/monitoring/vms55.cfm>. (Accessed 12 July 2012).

**Vidyasagar, D. (2007).** Global minute: water and health – walking for water and water wars.

*Journal of Perinatology* 27, 56–58.

**Vorosmarty, C.J., McInyre, P.B., Gessener, M.O., Dudgeon, D., Prusevich, A., Green,**

**P., Gidden, S., Bunn, S.E., Sullivan, C.A., Reidy Liermann C. and Davies PM (2010).** Global threats to human water security and river biodiversity. *Nature* 467, 551-561.

**World Bank Group (2016).** Water. Available on: <http://water.worldbank.org/shw-resource-guide/infrastructure/menu-technical-options/wastewater-treatment> (Accessed 10 March 2016).

**World Health Organisation (WHO) (1984).** Guideline for Drinking Water Quality Recommendation. World Health Organization, Geneva, Vol. 1, 130.

**World Health Organisation (WHO) (1985).** Management of wastewater from hospital and other health care establishments, report on a meeting, Bergen, 28 June- 1 July 1983. Copenhagen, World Health Organisation Regional office for Europe. (Euro Reports and Studies, No 97).

**World Health Organisation (WHO) (2004).** Guidelines for Drinking Water Quality 3<sup>rd</sup> edition. Volume 1 Recommendations. World Health Organization, Geneva.

**World Health Organisation (WHO) (2006).** Guidelines for Drinking Water Quality Vol. 1 Geneva, Switzerland.

**World Health Organisation (WHO) (2008).** Guidelines for Drinking Water Quality. Third edition. Geneva, Switzerland.

**World Health Organisation (WHO) (2013).** Health through safe drinking water and basic sanitation. Available on: [http://www.who.int/water\\_sanitation\\_health/mdg1/en/print.html](http://www.who.int/water_sanitation_health/mdg1/en/print.html). (Accessed 10 January 2014).



## **CHAPTER FOUR**

**Assessment of the microbiological qualities of  
discharged final effluents of two wastewater treatment  
facilities located in Amathole District Municipality of  
the Eastern Cape Province, South Africa.**

---

Some data from this Chapter was published in

Environmental Science and Pollution Research

---

#### 4.1 Abstract

In this chapter, we report on the microbiological qualities of the final effluents of two wastewater treatment facilities in the Eastern Cape Province, South Africa over a twelve month sampling period (September 2012 to August 2013) pursuant to determining their operational efficiencies. The final effluent samples were collected monthly and in triplicates to assess seasonal variations on the distribution of these microbiological indicators. Bacteriological analysis (faecal indicator bacteria (*E. coli*) and *Vibrio* species) was done using standard membrane filtration (MF) technique. All samples analysed were positive for the bacterial groups with significant ( $P \leq 0.05$ ) seasonal variation in their densities. Presumptive *E. coli* counts ranged generally between 1 CFU/100ml –  $1.4 \times 10^5$  CFU/100ml, while counts of presumptive *Vibrio* species ranged between 4 CFU/100ml –  $1.4 \times 10^4$  CFU/100ml. Molecular identification of the presumptive isolates by PCR yielded positive reaction rates of 76.2% (381/500) and 69.8% (279/400) for *E. coli* and *Vibrio* species respectively. The antibiotic susceptibility profiles of 205 confirmed *Vibrio* isolates against 18 commonly used antibiotics revealed resistance frequencies ranging from 0.5% (imipenem) to 96.1% (penicillin G) based on recommended breakpoint concentrations. Eighty one percent (166/205) of the *Vibrio* isolates showed multidrug resistance (resistance to 3 or more antibiotics), while the most common multiple antibiotic resistance phenotype (MARP) was AP-T-TM-SMX-PG-NI-PB, occurring in 8 isolates. Multiple antibiotic resistance indices (MARI) estimates at both sites were 0.35 and 0.33 at plants SWWTP and KWWTP respectively. The finding of this study reiterates the ability of potentially pathogenic bacteria to circumvent wastewater treatment processes and be released directly into surface water with the attendant risk associated upon human exposure. We conclude that inadequately treated municipal effluents are potential contributors to water pollution problems in the Eastern Cape. The need for the relevant authorities to deploy well-coordinated efforts towards

curbing indiscriminate discharge of poor quality effluents into the aquatic milieu of the Eastern Cape in the interest of public health and freshwater ecosystem conservation becomes imperative.

**Keywords:** Wastewater, final effluents, *E. coli*, *Vibrio* species, PCR, multidrug resistance

## 4.2 Introduction

Human and animal faecal materials from wastewater are major sources of enteropathogenic microbes in the freshwater environments (Frigon *et al.*, 2013). Numerous species of enteric pathogens of human and animal origins capable of causing viral, bacterial and parasitic infections may be present in environmental water including discharged wastewater effluents (Gerba and Smith, 2005; Godfree and Farrell, 2005). The range and numbers of pathogens in municipal wastewater may differ with the levels of prevalent diseases in the community, commercial and industrial discharges and seasonal factors (Godfree and Farrell, 2005).

Microbial pathogens in wastewater discharged effluents present a risk and may cause infection in human via direct or indirect contamination of food crops, seafood, drinking water, zoonoses or by means of a vector. The detrimental effects of waterborne outbreaks have often been estimated by risk rates for morbidity among exposed individual. Nonetheless, the effects of infection from contaminated water on acute and cumulative illness absences may equally have a long-term health effect, and the relative socioeconomic lost due to waterborne illness have hardly ever been estimated (Halonen, *et al.*, 2012). Concerns about health risks associated with the discharge of inadequately treated wastewater effluents into freshwater bodies have renewed awareness in the effects of treatments on microbiological pathogens (Godfree and Farrell, 2005).

Present-day knowledge about the inadequacies of indicator microorganisms as representatives of the wide range of potential pathogens that may be present in the environment has necessitated assessment of environmental matrices including surface water and wastewater for specific pathogen of public health importance (Berg, 1978; Grabow,

1996; Toze 1999; Ashbolt *et al.*, 2001; Horman *et al.*, 2004; Masters *et al.*, 2012). Diverse pathogenic bacteria of enteric origins such as pathogenic strains of *E. coli*, *Pseudomonas aeruginosa*, *Salmonella* species, *Shigella* species, *Staphylococcus aureus* and *Vibrio* species may be present in wastewater effluents presenting public and environmental health risks (Shannon *et al.*, 2007). Viruses, protozoa and helminths are other pathogens of enteric origins that have been found in treated and untreated wastewater effluents (Toze, 2006). Waterborne infection risks associated with these pathogens depend on a number of factors such as prevalence and distribution of the pathogens in water, the infective doses required, health status of exposed population and the chances of faecal contamination of water sources (Haas *et al.*, 1999).

*Escherichia coli* is an important cause of human illness associated with food and waterborne outbreaks every year in the United States and other parts of the world (CDC, 2014a). The bacterium has long been used as specific indicator of faecal contamination in water due to its close association with the human digestive tract (Scott *et al.*, 2002; Dungeni *et al.*, 2010; McAllister and Topp, 2012; Odonkor and Ampofo, 2013). Although, most *E. coli* strains are non-pathogenic, some strains of the bacteria have acquired virulence capabilities and can cause gastrointestinal illnesses, along with other more serious health complications (Vidal *et al.*, 2005; Health Canada, 2006b; Prescott *et al.*, 2008). The pathogenic strains of *E. coli* that are capable of causing enteric infections are designated diarrheagenic *E. coli* (DEC), a group that includes emerging pathogen of public health importance worldwide (Nataro and Kaper, 1998; Vidal *et al.*, 2005). Six categories of DEC that possess different virulence factors including: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAaggEC) and

diffusely adhering *E. coli* (DAEC) have been identified (Vidal *et al.*, 2005). Uropathogenic *E. coli* (UPEC), Neonatal Meningitis *E. coli* (NMEC) and Avian pathogenic *E. coli* (APEC) are three other extraintestinal pathogenic *E. coli* (ExPEC) strains that have also been characterised (Wiles *et al.*, 2008; Dubois *et al.*, 2009). UPEC and NMEC are able to cause infections in the urinary tract, bloodstream (bacteremia) and in the central nervous system (sepsis and meningitis) (Nataro and Kaper, 1998), while APEC strains have poultry animals as their major reservoir (Collingwood *et al.*, 2014).

A novel multidrug-resistant strain of *E. coli* designated enteroaggregative, Shiga toxin-producing *E. coli* O104:H4 was identified in Germany in 2011 (Frank *et al.*, 2011; Bielaszewska, 2011; Wu *et al.*, 2011, Mellmann *et al.*, 2011). This strain was linked to a bloody diarrhoea and haemolytic uremic syndrome (HUS) outbreak, with confirmed cases of over 3816 infected individuals across Europe and the United States; 53 deaths, 51 of which occurred in Germany (WHO, 2011; ECDC, 2011; EFSA, 2012; ECDC, 2013). The HUS-associated strain was thought to have emerged from strains of enteroaggregative *E. coli* (EAEC) that have acquired the capability of producing Shiga toxin because they were found to carry genes typically found in two types of pathogenic *E. coli*: enteroaggregative *E. coli* (EAEC) and Enterohemorrhagic *E. coli* (EHEC) (Mellmann *et al.*, 2011).

*Vibrio* species are gram-negative, curved, motile, rod-shaped mesophilic facultative bacteria that are known natural inhabitants of marine and estuarine environments (Morris and Black, 1985; McLaughlin, 1995; Thompson *et al.*, 2005; Newton *et al.*, 2012). About a dozen pathogenic species of vibrio can cause human infections including cholera and vibriosis (Janda *et al.*, 1988; Holmberg, 1992; Austin, 2009; CDC, 2014b). Vibriosis is characterised

by diarrhoea, gastroenteritis, wound infections, primary septicaemia and other extraintestinal infections (Altakruse *et al.*, 2000; Dechet *et al.*, 2008; Horseman and Surani, 2010; Newton *et al.*, 2012). Infection caused by *Vibrio* is commonly contracted via ingestion of contaminated food or water, or by direct colonisation through wounds. *Vibrio cholerae* is the most studied and the most medically significant member of the genus, comprising numerous strains classified according to O group (Hlady and Klontz, 1996; Heidelberg *et al.*, 2000; Austin, 2009). *Vibrio cholerae* and other epidemiological important strains constitute a major public health challenge worldwide, and particularly in developing countries. Oral rehydration or intravenous fluid therapies are the primary treatments for cholera. Antibiotic therapy may also reduce the severity and duration of the vibrio-induced cholera. Numerous studies, however, have documented increasing resistance to established antibiotics among different *Vibrio* strains (Yu *et al.*, 2012; Al-Othubi *et al.*, 2014; Scarano *et al.*, 2014; Costa *et al.*, 2015; Zavala-Norzagaray *et al.*, 2015). Other high risk vibrio pathogens include *V. parahaemolyticus* and *V. vulnificus* while low risk pathogenic species of public health significance include *V. alginolyticus*, *V. fluvialis*, *V. furnissi*, *V. mimicus*, *V. metschnikovii* and *V. harveyi* (Austin, 2009).

Interest in the occurrence and prevalence of pathogens in treated effluent discharges is of epidemiological importance. Over the past few years, there have been increased frequencies of waterborne outbreaks in South Africa (Bateman, 2008; Igbiosa *et al.*, 2009; Blumberg *et al.*, 2011). Large number of South Africa wastewater treatment infrastructures are dilapidated leading to discharge of large volume of sewage into surface water (Bateman, 2009; Bateman, 2010) thus, calling for regulatory authorities' attention to prevent the spread of potentially contagious and deadly waterborne diseases. Paucity of well documented information on the

potential contributions of discharged wastewater effluents to the impairment of the microbiological qualities, as well as the risk associated with the discharge of pathogenic bacteria into the surface waters of the Eastern Cape, South Africa is the rationale for this current study. In this study, we reported on the microbiological qualities of the final effluents of two wastewater treatment facilities in terms of the prevalence and distribution of faecal indicator bacteria (*E. coli*) and *Vibrio* species as well as their correlation with selected physicochemical qualities of the effluents.

### **4.3 Materials and Methods**

#### **4.3.1 Description of study sites**

The two wastewater treatment facilities assessed in this study are both located in the Amathole District Municipality of the Eastern Cape, South Africa. One of the treatment plants (SWWTP) is located within geographical coordinates 32°34'17''S, 27°26'95''E. It uses activated sludge and drying beds technology to treat its wastewater, and discharges its final effluents into the Cumakala River (DWA, 2009; DWAF, 2012). The plant has a design capacity of 4.0 Mℓ/day and an operational capacity of 62.5%. The second plant (KWWTP) is located within the geographical coordinates 32°41'31''S, 27°08'36''E and has a design capacity of 0.67 Mℓ/day. It uses activated sludge and sludge lagoon treatment technology and discharges its final effluents into the Keiskamma River (DWAF, 2012).

#### **4.3.2 Sample collection and treatment**

Wastewater final effluent samples were collected in duplicates over a twelve month sampling period (from September 2012 to August 2013, between 8 a.m. and 1 p.m.), from the final effluent/chlorination tanks (FE) and the discharge points (DP) (in order to assess the



microbiological significance of increased contact time between the final effluent tanks and the points of discharge into the receiving water bodies). All samples were collected aseptically using sterile 1.7 L screw-capped bottles. De-chlorination of the samples for bacteriological analysis was done by adding 0.1M Sodium thiosulphate to give a final concentration of 100 mg/L and ample air space was left in the bottles to aid homogenisation by shaking before analysis. The samples were immediately placed in light proof insulated cooler boxes containing ice-packs and transported to the Applied Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, South Africa for analysis within 6 h of collection as recommended by American Public Health Association (APHA, 1998). A total of forty eight wastewater samples (each in duplicate) were collected over the sampling period.

#### **4.3.3 Detection and enumeration of *E. coli* and *Vibrio* spp.**

Presumptive *E. coli* and *Vibrio* spp. were analysed by membrane filtration (MF) techniques according to standard methods (APHA, 1998). Appropriate serial dilutions of each of the collected samples were prepared and 100 ml from each of the dilutions was filtered through membrane filters (47-mm diameter, 0.45 µm pore size; Pall Corporation, Ann Arbor, Michigan) with the aid of vacuum pump. Thereafter, the membrane filters were aseptically transferred onto prepared Petri-dishes containing the appropriate medium for the target bacterial groups. Presumptive *E. coli* density was detected and enumerated by placing the membrane filters on *E. coli*-Coliforms Chromogenic medium (Laboratorios CONDA, South Africa) and incubated at 37 °C for 24 h. All colonies that appear as blue to dark violet were counted and recorded as presumptive *E. coli* (CFU/100 ml of wastewater sample analysed). *E. coli* ATCC 29522 strain was used as the control strain for *E. coli* tests. Total presumptive

Vibrios (yellow and green colonies) were detected and enumerated by placing the membrane filters on thiosulfate-citrate–bile salt-sucrose (TCBS) agar (Laboratorios CONDA, South Africa) and incubated at 37 °C for 24 to 48 h. Reference strain DSM 19283 was used as positive control for the detection of *Vibrio* spp.

#### **4.3.4 Molecular confirmation of presumptive *E. coli* and *Vibrio* isolates**

Prior to molecular identification of isolates, single colonies of putative *E. coli* and *Vibrio* spp. were picked from the MF plates and purified further using *E. coli*-Coliforms Chromogenic medium and TCBS agar respectively until pure cultures were achieved. Pure tentative bacterial isolates were transferred on to nutrient agar (Merck, Wadeville, South Africa) plates and incubated overnight at 35 °C preparatory for preliminary identification (Gram staining and oxidase test). For *E. coli*, Gram-negative and oxidase negative isolates were selected and stored in 20% glycerol for further analyses while Gram-negative and oxidase positive isolates of *Vibrios* were stored for further analyses. The identities of the presumptive isolates were confirmed by polymerase chain reactions (PCR) using specific oligonucleotide primers (Table 4.1). DNA extraction was done following the description of Medici *et al.* (2003) and Queipo-Ortuno *et al.* (2008) with some modifications. Single colonies of freshly grown bacterial cultures (18 to 24 h) were picked using sterile loop avoiding agar contamination, an important cause of erratic amplification and suspended in 200 µL of sterile deionised nucleases-free water (Thermo Scientific Inc.). The suspensions were vortexed and the cells were lysed by heating for 10 min at 100°C using an Accu Block digital dry bath (Labnet International, Inc.). The suspensions were allowed to cool and centrifuged at 13 000 rpm for 10 min to pellet the cell debris. Thereafter, the lysate supernatant was carefully pipetted into new nuclease free Eppendorf tubes and used immediately for PCR assays or stored at -20 °C

until use. The PCR components included 5.0  $\mu\text{L}$  of DNA template and 20.0  $\mu\text{L}$  mix containing (12.5  $\mu\text{L}$  of PCR Master Mix (2X) (Thermo Scientific Inc.), 1.0  $\mu\text{L}$  each of forward and reverse primers (10 picomole) and 5.5  $\mu\text{L}$  of sterile deionised nucleases-free water (Thermo Scientific Inc.)) to make a final volume of 25.0  $\mu\text{L}$  per reaction. The PCR assays were carried out using MyCycler™ thermal cycler. For *E. coli*, the cycling conditions were initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 90 s, annealing at 60 °C for 90 s and extension at 72 °C for 90 s and a final extension at 72 °C for 10 min. The amplicon were held at 4 °C after the completion of the cycles.

For *Vibrio* spp., the reaction condition included initial denaturation at 93 °C for 15 min followed by 35 cycles of denaturation at 92 °C for 40 s, annealing at 57 °C for 60 s and extension at 72 °C for 90 s and a final extension at 72 °C for 7 min. The amplicon were held at 4 °C after the completion of the cycles. *Escherichia coli* ATCC 29522 strain and *Vibrio* strain reference DSM 19283 were included in the PCR assays as positive controls for *E. coli* and *Vibrio* respectively. The negative control included the PCR mix and nuclease-free water.

**Table 4.1:** Primer sequences and sizes of PCR-amplified target genes of the *E. coli*. and *Vibrio* spp. isolates.

Target strains	Primer	Primer sequence (5'→3')	Target genes	Amplicon size	References
<i>E. coli</i>	<i>UAL754</i> <i>UAR900</i>	AAA ACG GCA AGA AAA AGC AG ACG CGT GGT TAC AGT CTT GCG	<i>uidA</i>	147 bp	Bej <i>et al.</i> , 1991; Maheux <i>et al.</i> , 2009
<i>Vibrio</i> spp.	V.16S-700F V.16S-1325R	CGG TGA AAT GCG TAG AGA T TTA CTA GCG ATT CCG AGT TC	16SrR	663 bp	Kwok <i>et al.</i> , 2002

The PCR products were resolved by gel electrophoresis (1.5% agarose, 0.5 mg/L ethidium bromide, 0.5X TBE buffer) at 100 V for 60 min then visualised using UV transilluminator.

#### **4.3.5 Antibiogram of PCR-confirmed *Vibrio* spp. isolates**

Antibiogram profiling of the PCR-confirmed *Vibrio* isolates was done by standard disc diffusion (Kirby-Bauer disc agar diffusion) method following the description of Clinical and Laboratory Standards Institute (CLSI, 2011). The susceptibility/resistance of the isolates was evaluated against 18 commercial antibiotic discs (Mast Diagnostics, Merseyside, United Kingdom). The antibiotic selection was based on some drugs used in vibriosis treatment and veterinary medicine. These include ampicillin, amikacin, imipenem, meropenem, streptomycin, chloramphenicol, ciprofloxacin, nalidixic, tetracycline, trimethoprim, norfloxacin, sulfamethoxazole, gentamycin, neomycin, penicillin G, nitrofurantoin, polymyxin B and cefuroxime. The CLSI interpretative chart was used to determine the susceptibility/resistance of the test isolates by comparing the diameter of the zones of inhibition of the isolates against the test antibiotics (CLSI, 2011). Multiple antibiotic resistance phenotypes (MARP) profiles were generated for the isolates exhibiting resistances to three or more test antibiotics while the estimation of multiple antibiotic resistance indices (MARI) for the multidrug resistant isolates were estimated for the two sampling sites by the formula previously described by Krumperman (1983) to assess the health risk of such isolates to the environment. The formula is given as:  $MAR\ index\ (MARI) = a / (b \times c)$ ; where, a = the aggregate antibiotic resistance score of isolates, b = number of antibiotics, and c = number of isolates.

#### **4.3.6 Analysis of selected physicochemical variables**

Some important physicochemical/environmental variables which may impact the disinfection (chlorination) process as well as the microbiological quality of the wastewater samples including turbidity and free chlorine were determined as described in section 3.3.2. All analyses were carried out in triplicates.

#### **4.3.7 Statistical analysis**

One-way ANOVA (SPSS 23.0 version for Windows program) was used for calculation of means and standard deviations. Comparison of seasonal means and differences between FE and DP samples was done using the Paired-Samples T-test and the ANOVA F-test respectively. The relationship between faecal indicator bacteria and physicochemical (free chlorine and turbidity) measured was established by Pearson correlation procedure. All statistical significance was set at  $P$  values  $<0.05$ .

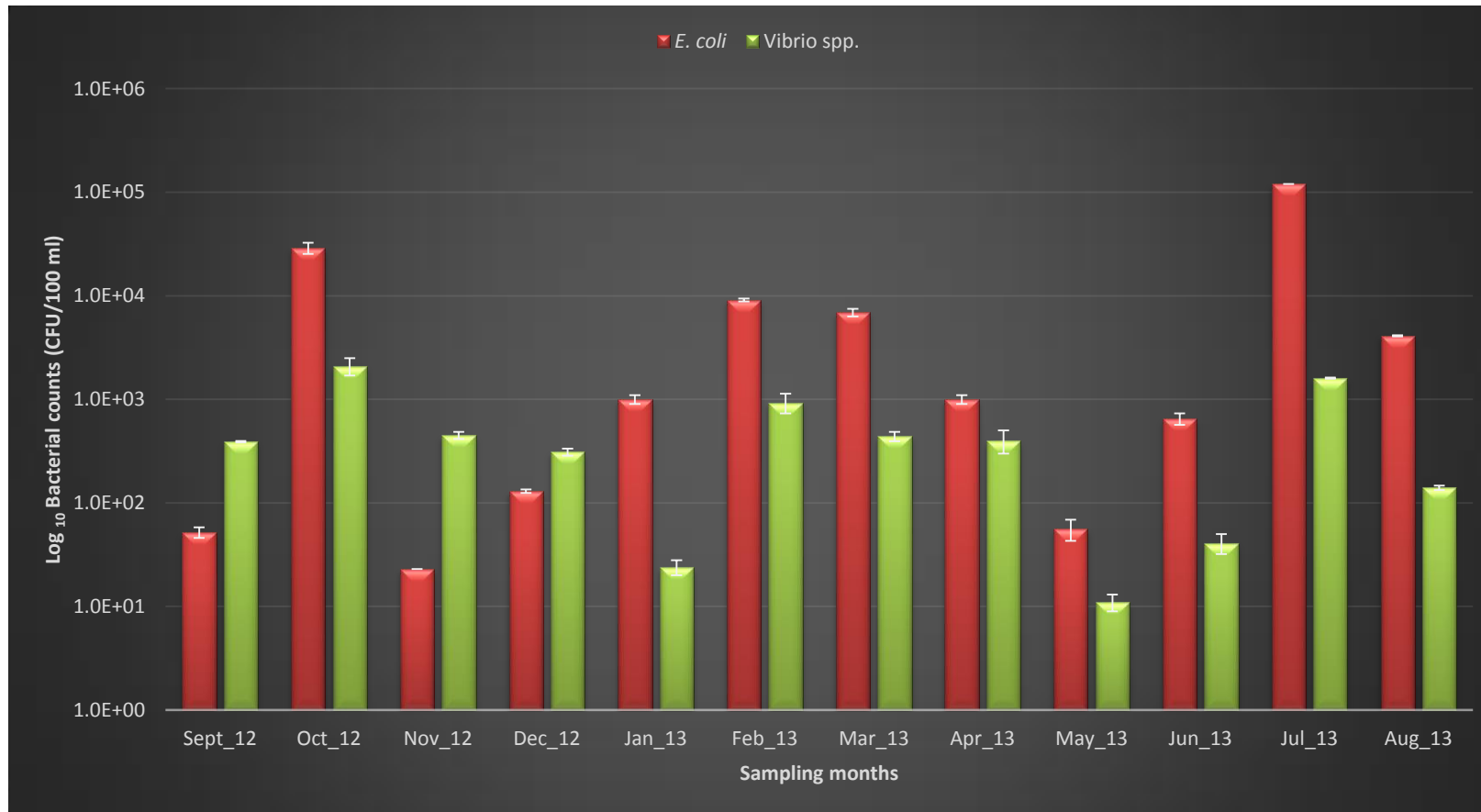
### **4.4 Results**

#### **4.4.1 Prevalence and distribution of *E. coli* and *Vibrio* species in the final effluents**

The prevalence and distribution patterns obtained for *E. coli* and *Vibrio* bacteria across the four sampling points are shown in Figures 4.1 to 4.4 below. Generally, the prevalence for the bacteria varied widely across the twelve month sampling period as shown in the figures. Across all sampling points, *E. coli* and *Vibrio* species densities ranged from  $1 - 1.2 \times 10^5$  CFU/100 ml and  $4 - 1.4 \times 10^4$  CFU/100 ml respectively. Presumptive *E. coli* and *Vibrio* species were detected in all 48 (100%) samples throughout the sampling period.

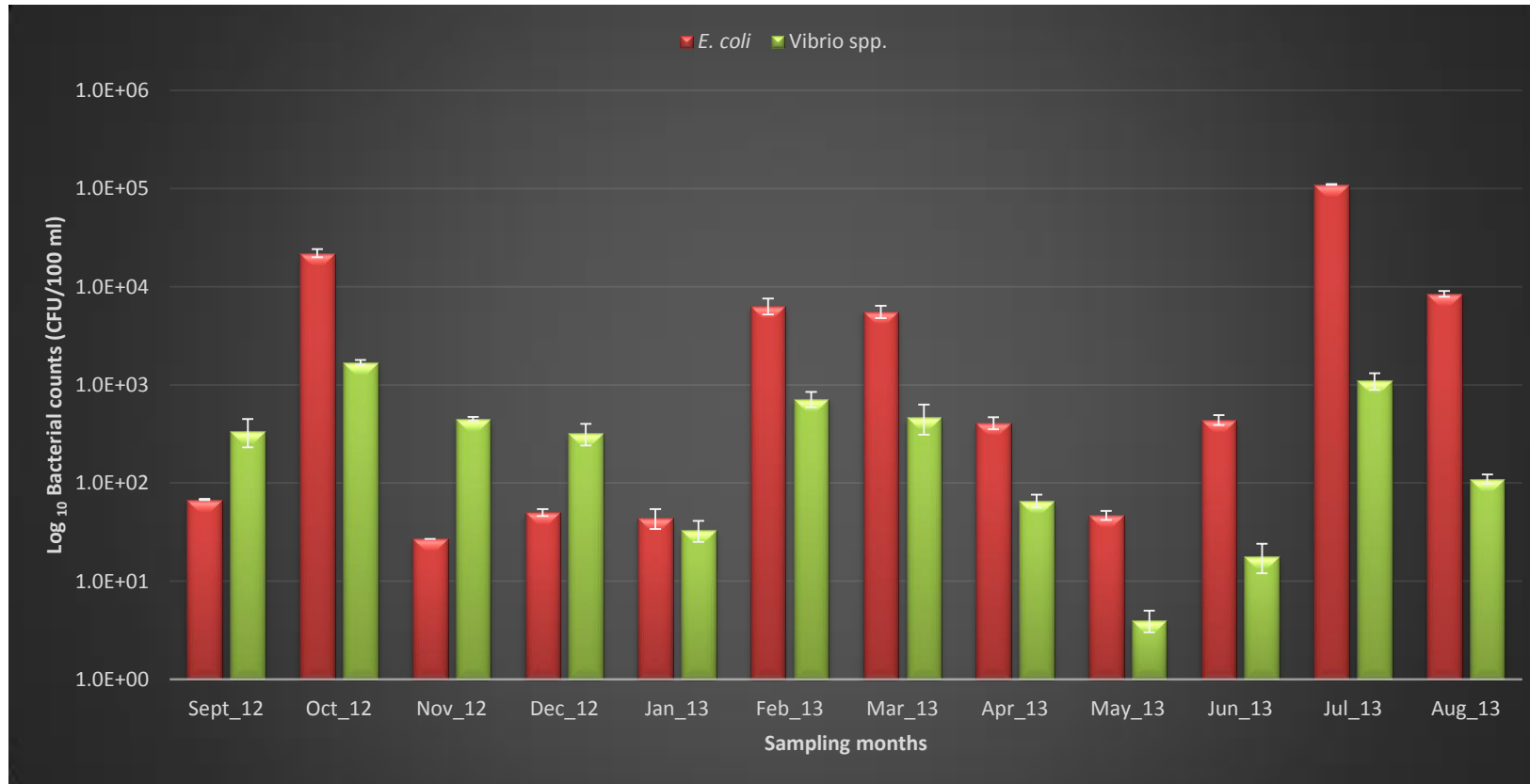
At SWWTP, the lowest (23 CFU/100 ml) and the highest ( $1.2 \times 10^5$  CFU/100 ml) densities of *E. coli* were observed in the FE samples in the months of November 2012 and July 2013 respectively, while the lowest (4 CFU/100 ml) and the highest ( $2.1 \times 10^3$  CFU/100 ml) counts of *Vibrio* species were recorded in the months of May 2013 and October 2012 respectively. Similarly, at KWWTP. The least count of *E. coli* (1 CFU/100 ml) was recorded at the DP in the summer month of January 2013 while, the highest *E. coli* count ( $9.2 \times 10^3$  CFU/100 ml) was also recorded at the DP in November 2012. The least detection of *Vibrio* spp. (7 CFU/100 ml) at the discharge point of KWWTP was in February 2013, while the highest detection was in the final effluent samples in November 2012.

Seasonally, the bacterial densities and distribution varied significantly ( $P \leq 0.05$ ). Highest seasonal means of *E. coli* (SWWTP,  $9.1 \times 10^3$  CFU/100 ml; KWWTP,  $3.6 \times 10^3$  CFU/100 ml) and presumptive *Vibrio* spp. (SWWTP,  $8.8 \times 10^2$  CFU/100 ml; KWWTP,  $4.9 \times 10^3$  CFU/100 ml) were observed in spring (September, October, November) at both sampling sites. *E. coli* was also highest in spring at KWWTP ( $4.1 \times 10^3$  CFU/100 ml) while it was highest in winter (June, July, August) at SWWTP ( $4.2 \times 10^4$  CFU/100 ml). The lowest seasonal mean for presumptive *E. coli* ( $2.1 \times 10^2$  CFU/100 ml) and *Vibrio* spp. ( $2.9 \times 10^1$  CFU/100 ml) were observed in summer (December, January, February) at KWWTP while at SWWTP, the lowest seasonal mean (*E. coli*,  $2.0 \times 10^3$  CFU/100 ml and *Vibrio* spp.,  $1.8 \times 10^2$  CFU/100 ml) were all observed in autumn (March, April, May).

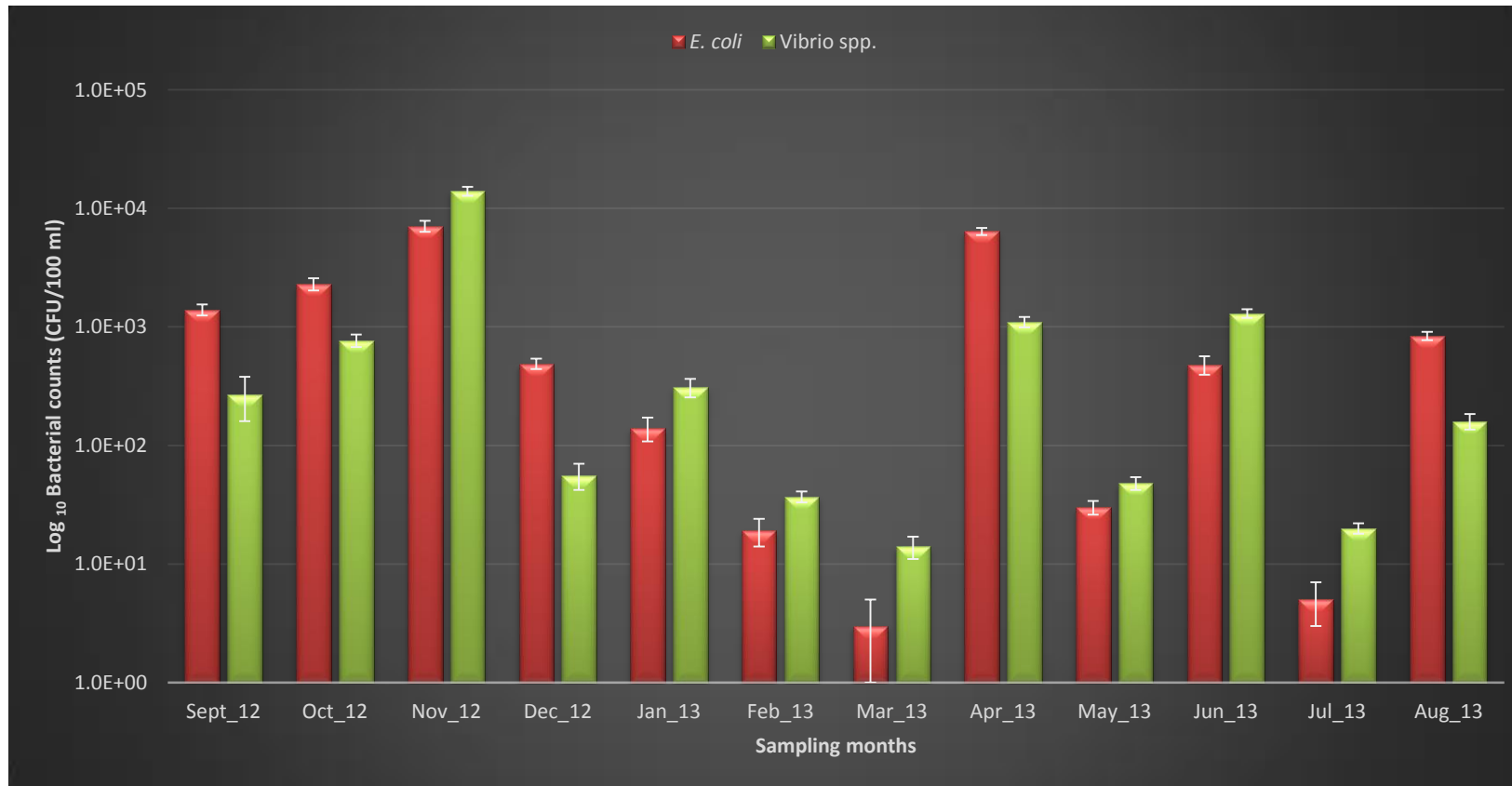


**Figure 4.1:** Prevalence and distribution of bacterial groups at SWWTP final effluent (SFE).

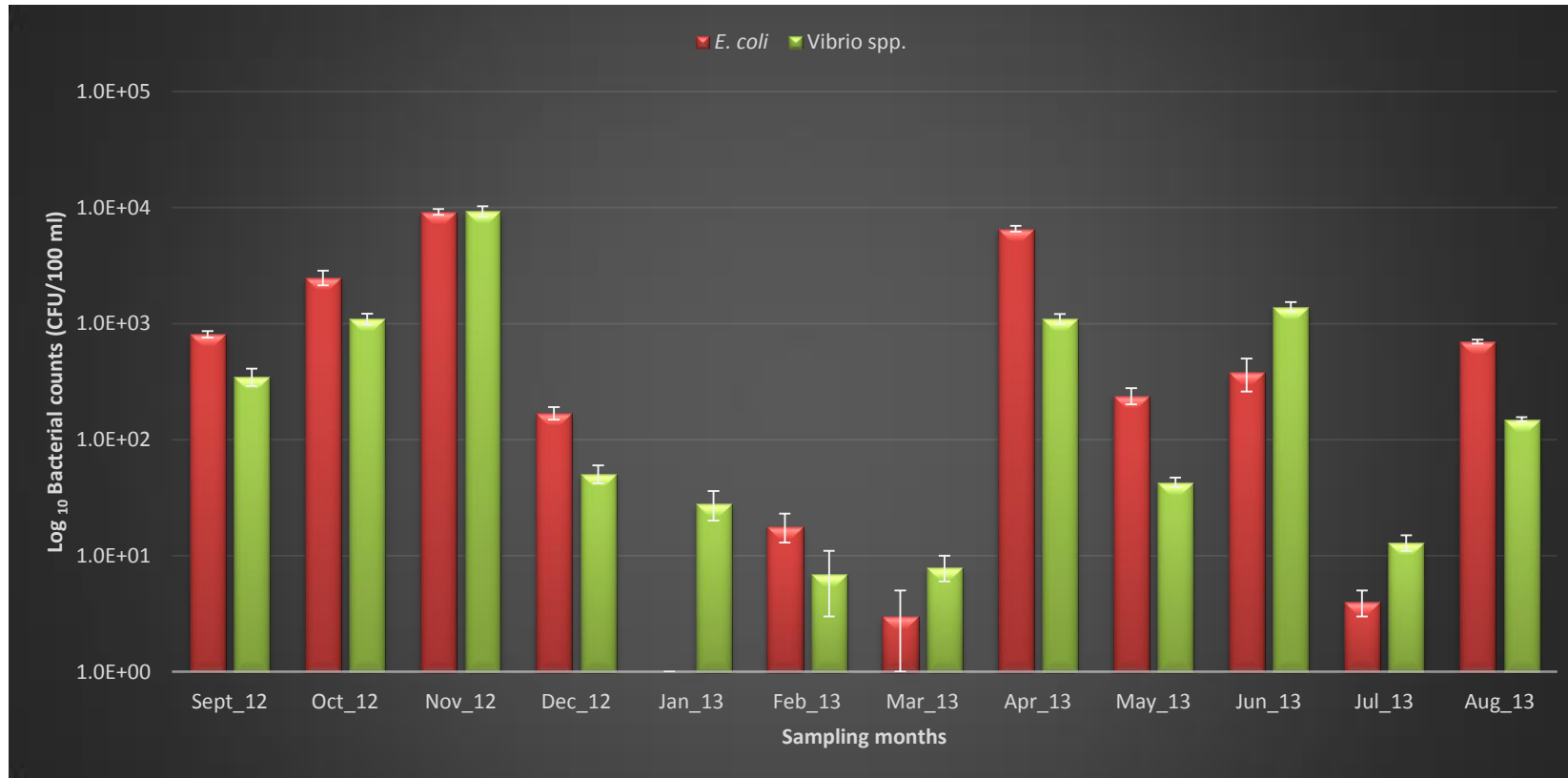




**Figure 4.2:** Prevalence and distribution of bacterial groups at SWWTP discharge point (SDP).



**Figure 4.3:** Prevalence and distribution of bacterial groups at KWWTP final effluents (KFE).

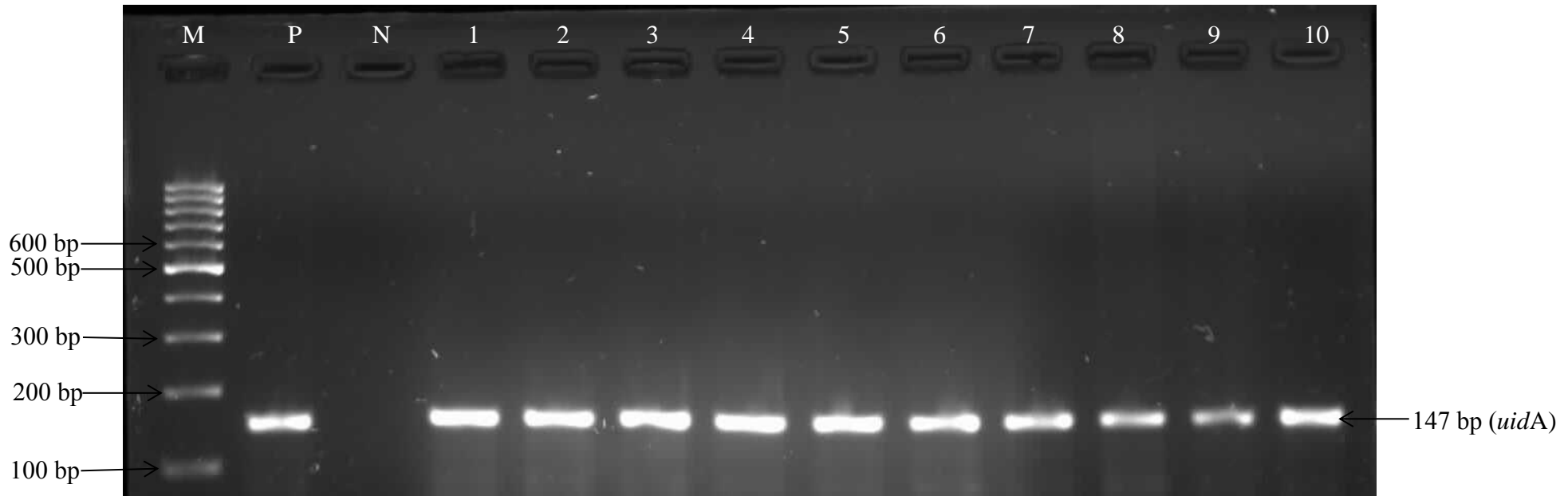


**Figure 4.4:** Prevalence and distribution of bacterial groups at KWWTP discharge point (KDP).

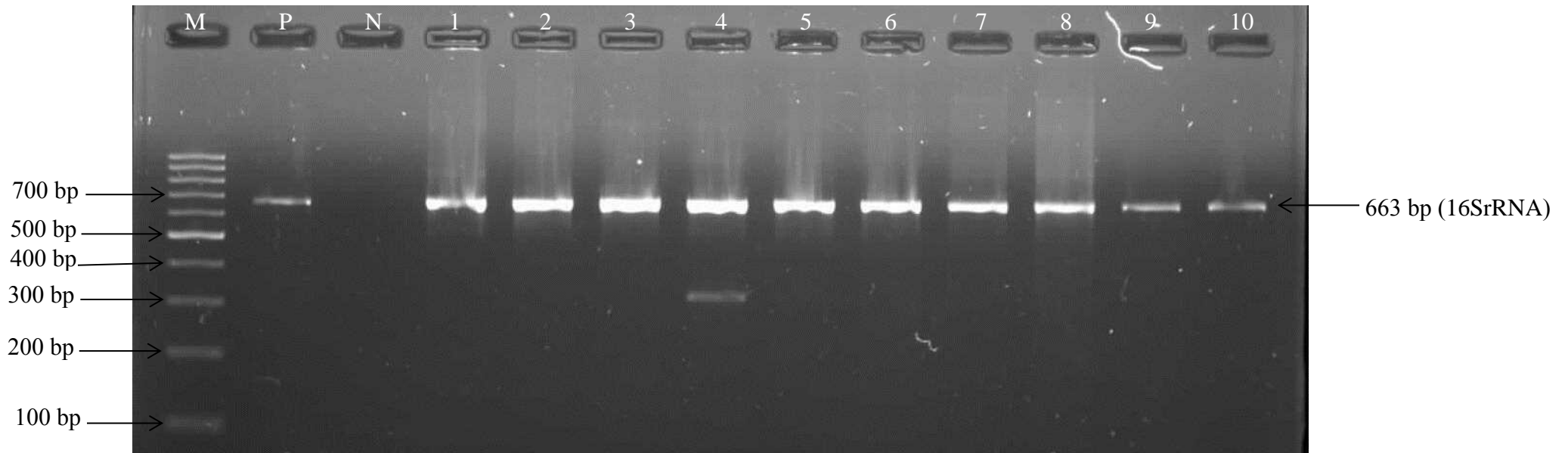
#### 4.4.2 Molecular identification of *E. coli* and *Vibrio* isolates

The molecular identification of the presumptive *E. coli* isolates using the housekeeping *uidA* gene shows a positive reaction rate of 76.2% (381/500) for isolates recovered from both study sites. Of the total isolates positive for *uidA* gene, 77.6% (194/250) were from SWWTP, while 74.8% (187/250) of the isolates were recovered from KWWTP. Figure 4.5 below shows a representative agarose gel electrophoresis picture of some of the confirmed *E. coli* isolates.

For the presumptive *Vibrio* species, a positive PCR reaction rate of 69.8% (279/400) was observed for the presence of 16SrRNA (a variable region around the positions of 700 and 1325 within the 16SrRNA for *Vibrio* genus identification). At study site 1 (SWWTP), 66.2% (157/237) of the tested isolates were positive for the 16SrRNA gene, while 74.9% (122/163) isolates tested positive from KWWTP. Figure 4.6 below shows the gel electrophoresis images of some of the positive isolates.



**Figure 4.5:** Molecular confirmation of *E. coli* isolates. Legend: Lane M: 100 bp Molecular weight marker; lane P: Positive control (*E. coli* ATCC 25922 strain); lane N: Negative control; lanes 1 to 10 *E. coli* isolates.



**Figure 4.6:** Molecular confirmation of *Vibrio spp.* Isolates. Legend: Lane M: 100 bp Molecular weight marker; lane P: Positive control (DSM 11058 strain); lane N: Negative control; lanes 1 to 10 *Vibrio spp.* isolates.

#### **4.4.3 Antibiotic susceptibility pattern of *Vibrio* spp.**

The antibiotic susceptibility profiles of 205 randomly selected PCR-confirmed *Vibrio* species isolates against 18 different antibiotics showed marked susceptibility ( $\geq 95$ ) to 4 of the antibiotics these include imipenem, meropenem, trimethoprim and sulfamethoxazole while the isolates also exhibited susceptibility greater than  $>90$  to amikacin and ciprofloxacin (Table 4.2). None of the test antibiotics was completely potent against all the isolates with the expression of resistance ranging between 0.5% (imipenem) to 96.1% (penicillin G). Approximately 81% (166/205) of the tested isolates showed multiple antibiotic resistance phenotype (MARP) against 3 or more antibiotics. The commonest MARP was AP-T-TM-SMX-PG-NI-PB occurring in 8 isolates. The highest number of MARP observed in a single isolate was 11 (that is, MARP 11), with 3 isolates found in this category. MARP 10 was also observed in 6 of the test isolates. The multiple resistance antibiotic indices (MARI) estimated for both sites are 0.35 (SWWTP) and 0.33 (KWWTP).

#### **4.4.4 Free chlorine and turbidity profiles of the discharged effluent samples.**

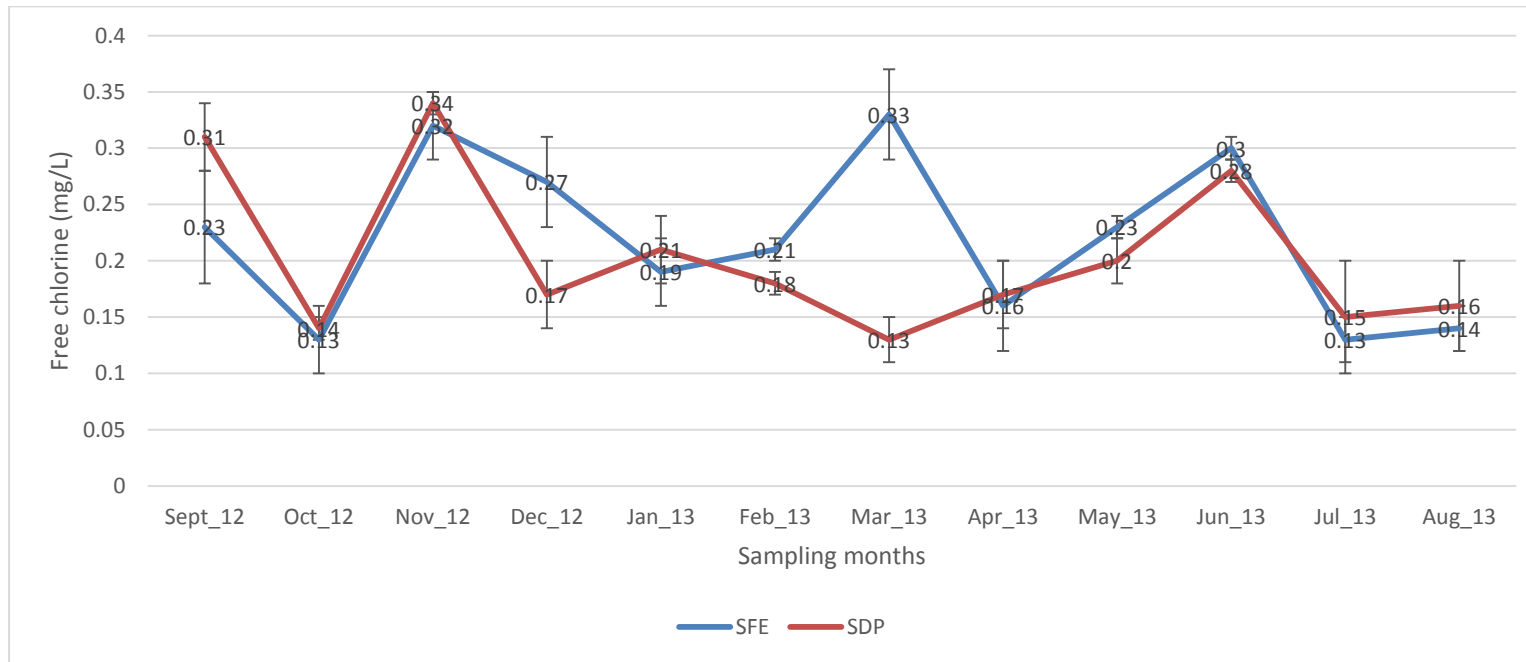
The profiles of the free chlorine observed in the discharged effluents at SWWTP and KWWTP over the sampling period are shown in Figures 4.7 and 4.8 below respectively, while the turbidity profiles over discharged effluents over the sampling period are shown in Figure 4.9. The concentration of the free chlorine varied significantly ( $P < 0.05$ ) at SWWTP ranging from 0.13 to 0.34 mg/L and at KWWTP it ranged between 0.09 and 0.65.

**Table 4.2.** Antibiogram of randomly selected PCR-confirmed *Vibrio* species isolates.

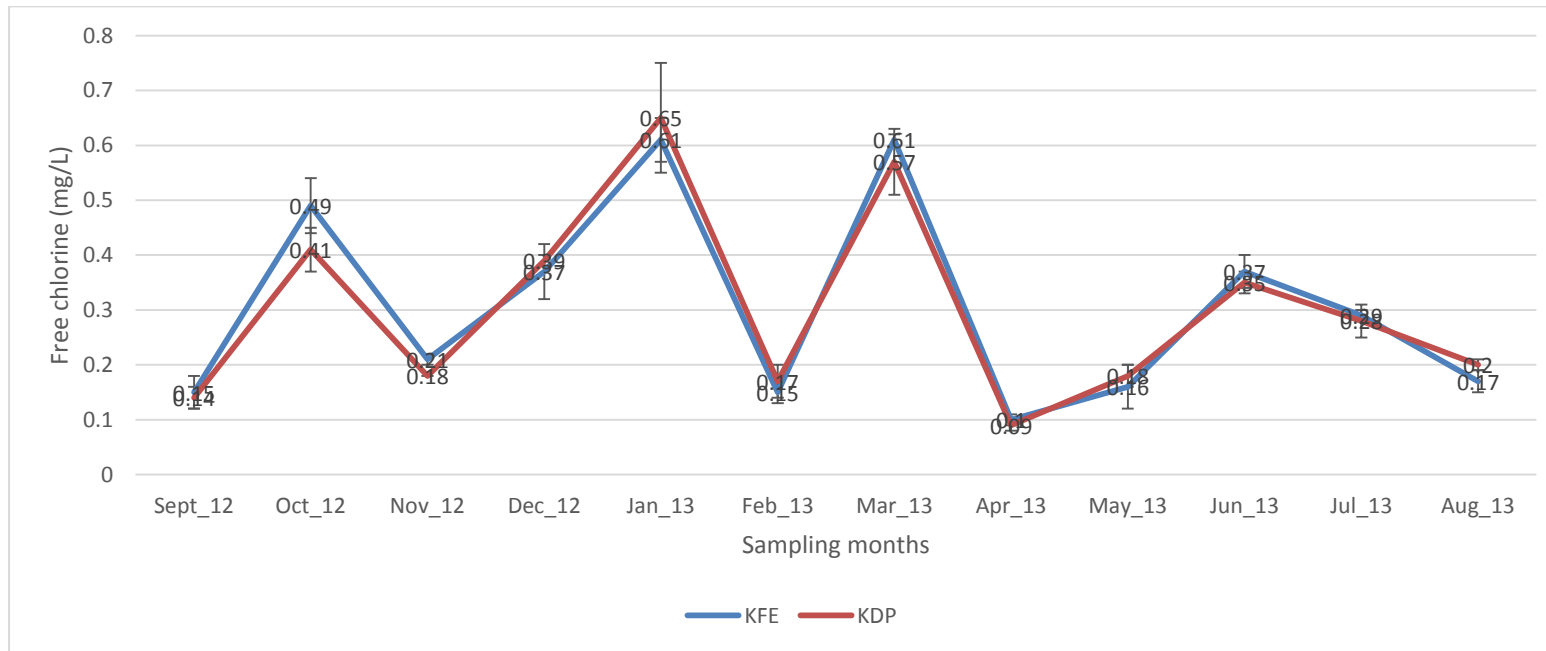
<b><u>N = 205</u></b>			
<b>Antibiotic</b>	<b>Sensitive</b>	<b>Intermediate</b>	<b>Resistant</b>
	<b>% (n)</b>	<b>% (n)</b>	<b>% (n)</b>
Ampicillin [AP] 25 µg	60 (123)	34.1 (70)	5.9 (12)
Amikacin [AK] 30 µg	90.7 (186)	2.4 (5)	6.8 (14)
Imipenem [IMI] 10 µg	98.5 (202)	1 (2)	0.5 (1)
Meropenem [MEM] 10 µg	95 (192)	2.4 (5)	2.4 (5)
Streptomycin [S] 10 µg	81 (166)	7.3 (15)	11.7 (24)
Chloramphenicol [C] 10 µg	41 (84)	17.6 (36)	41.5 (85)
Ciprofloxacin [CIP] 5 µg	90.2 (185)	7.8 (16)	2 (4)
Nalidixic [NA] 30 µg	66.3 (136)	6.3 (13)	27.3 (56)
Tetracycline [T] 30 µg	49.3 (101)	2 (4)	48.8 (100)
Trimethoprim [TM] 5 µg	52.2 (107)	2.9 (6)	44.9 (92)
Norfloxacin [NOR] 10 µg	96.1 (197)	1.5 (3)	2.4 (5)
Sulfamethoxazole [SMX] 25 µg	22 (45)	3.9 (8)	74.1 (152)
Gentamycin [GM] 120 µg	96.6 (198)	1.5 (3)	2 (4)
Neomycin [NE] 10 µg	85.4 (175)	2.4 (5)	12.2 (25)
Penicillin G [PG] 10 units	3.9 (8)	-	96.1 (197)
Nitrofurantoin [NI] 300 µg	46.8 (96)	3.4 (7)	49.8 (102)
Polymyxin B [PB] 300 units	49.3 (101)	1 (2)	49.8 (102)
Cefuroxime [CXM] 30 µg	69.8 (143)	18.5 (38)	11.7 (24)

Number of antibiotics = 18

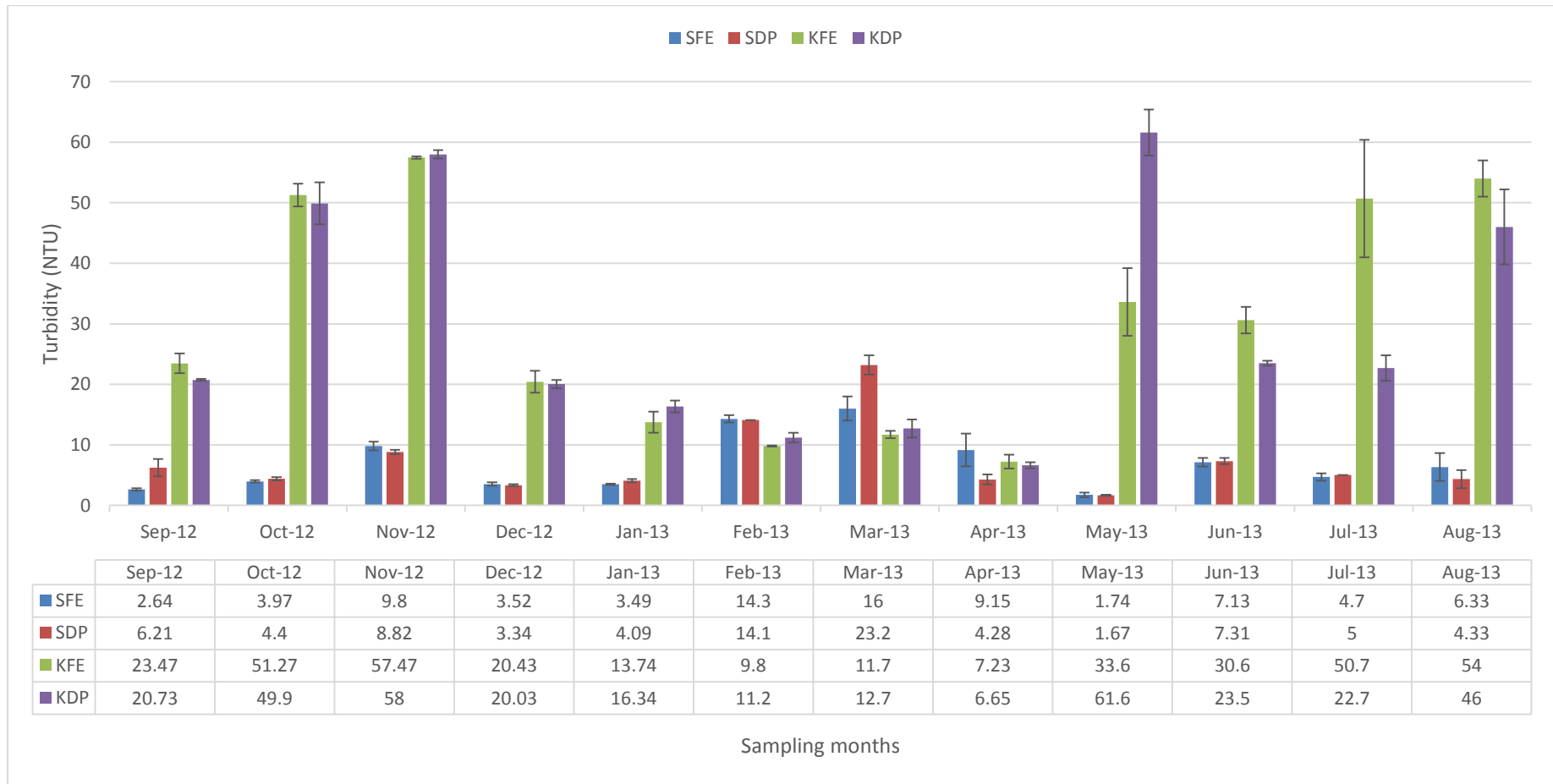




**Figure 4.7:** Concentration of free chlorine in the discharged effluent at SWWTP over the 12 month sampling period



**Figure 4.8:** Concentration of free chlorine in the discharged effluent at KWWTP over the 12 month sampling period.



**Figure 4.9:** Turbidity profiles of the discharged final effluents over the sampling period.

#### 4.5 Discussion

Considering the high incidences of deaths and debilitating illnesses caused by human contact with polluted water, it is important to understand factors controlling the spread of waterborne pathogens and their respective indicators. Pathogenic microorganisms of enteric origin are one of the most common pathogens encountered in the aquatic environments including discharged municipal wastewater effluent. They often get into the environment either by direct discharge and defaecation or through runoff from land surfaces, and can infect living terrestrial and aquatic organisms in the environment, leading to waterborne diseases. The densities of these organisms found in treated wastewater effluents largely depend on factors such as treatment levels (to which the wastewater has been previously subjected to), treatment technology employed for treatment, the competence of individuals handling the treatment processes, and the regulatory legislation governing discharged effluent quality.

In the current study, faecal indicator bacteria (*E. coli*) and *Vibrio* species were detected in all samples analysed over the sampling period. Even though faecal indicator bacteria may not be pathogenic themselves, their presence in water system are universally accepted to indicate faecal contamination of food and water sources, and probably the corresponding presence of other pathogenic organisms (Reynolds, 2003). *E. coli* is a subgroup of faecal coliforms used as an indicator of faecal contamination (Ji, 2008). Although vast majority of *E. coli* are completely harmless and might even be beneficial in the human colon by protecting against virulent organisms such as *Salmonella* and *Shigella*, some strains of the bacteria have acquired genetic elements that encode virulence factors (Ingraham, 2010). Pathogenic *E. coli* strains cause diverse forms of bacterial induced illnesses with symptoms ranging from mild diarrhoea to severe complication and even death (Rocourt, 2013). As observed in the study,

the counts of the presumptive faecal indicator bacteria complied with the DWAF limit of  $10^3$  CFU/100 ml for most part of the sampling period. However, there were months during the sampling period where the counts were far above this limit. About 37.5% (18/48) of the total effluent sample analysed had counts above the set limit. Similar findings to this have been reported by other studies with faecal indicator bacteria surviving wastewater treatment processes and were detected in count above the  $10^3$  CFU/100 ml set limit for discharged final effluent (Anastasi *et al.*, 2012; Hendricks and Pool, 2012).

Even though there is no specific set guideline for *Vibrio* spp. density in discharged effluent in South Africa, however, using the faecal coliform as the base limit for the evaluation of Vibrios, we observed that about 77.1% (37/48) of the samples analysed for presumptive *Vibrio* spp. complied with the general limit permissible for discharged final effluent in South Africa over the sampling period. Similarly, other studies have reported the detection of *Vibrio* in discharged effluents in South Africa and have emphasised the possibility of their survival in the environment (Igbinosa *et al.*, 2009; Dungeni *et al.*, 2010; Wennberg *et al.*, 2013; Ye and Zhang, 2013; Okoh *et al.* 2015)

Chlorination has been the most widely used disinfection method in wastewater treatment. Disinfection of effluents prior to their discharge into the environment helps in deactivating faecal indicator bacteria and other potentially harmful organisms that may have escaped the initial treatment stages. It helps to provide a degree of protection from direct or indirect contacts with such discharged effluents. Both treatment facilities assessed in this study utilise automated chlorination system for dosing their final effluents before discharging into the receiving watershed. As shown in Figures 4.7 and 4.8 above, the concentrations of free

chlorine in the discharged effluent at SWWTP were for most part of the sampling period in line with the limit of 0.25 mg/L for discharged final effluents. The turbidity measurements at this site were also considerably low compared to what obtains at KWWTP. KWWTP was particularly noted for high free chlorine concentrations and high turbidity levels for most part of the study (Figures 4.8 & 4.9). The high free chlorine levels observed at this site may partly be due to the incessant breakdown of the automated chlorination system which leads to manual dosing on several occasion during the study. There was notably high turbidity in the discharged effluent also at this site in the months of October and November 2012 which correlates with high bacterial count at this site for that sampling month. This could be as a result of the aerators not function at the time of sampling. High turbidity has been shown to hinder the effectiveness of disinfection in water, and often correlates with microbial load within water resources (Obi *et al.*, 2007). However there was an improvement in the turbidity and bacterial count levels after the aerators were fixed. ANOVA F-test showed no significant difference ( $p \leq 0.05$ ) in the seasonal mean of bacteria counts at SWWTP while there was slight variation in the seasonal means at KWWTP. The Paired-Samples T-test comparison of final effluent and discharge point samples revealed no significant differences ( $p \leq 0.05$ ) in seasonal mean bacterial counts.

The discovery of antibiotics has always been considered as one of the major scientific breakthroughs of the 20th century. However, the increasing incidences of microbial resistance have become a major global concern to public health since the phenomenon was first reported in the 1940's. Today, a wide range of newer generations of antimicrobials have been reportedly impotent against previously susceptible microorganisms (Jindal *et al.*, 2015). Different studies have documented the rising incidences of antibiotic resistances in

potentially pathogenic *Vibrio* species (Jury *et al.*, 2010; Reboucas *et al.*, 2011; Nsofor *et al.*, 2014; Letchumanan *et al.*, 2015). In this study, all PCR-confirmed *Vibrio*-test isolates exhibited resistance to at least one of the 18 antibiotics used in the study (Table 4.3). The highest frequency of resistant isolates was recorded against Penicillin G (96.1%) followed by Sulfamethoxazole with a resistance frequency of 74.1%. Resistance frequencies displayed by the isolates against other antibiotic were <50% as shown in Table 4.3, while only 1 isolates showed resistance against Imipenem. Our finding was similar to that of Letchumanan *et al.*, (2015), who reported a susceptibility of 98% for Imipenem against *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. However, contrary to their record of  $\geq 82\%$  susceptibility rates for chloramphenicol, trimethoprim and tetracycline, our test isolates exhibited susceptibility rate of 41%, 52.2% and 49.3% respectively to these antibiotics. Several MARP combinations were exhibited against the PCR-confirmed *Vibrio* spp. isolates indication the ineffectiveness or lack of sensitivity of the isolates to the antibiotic and about 81% (166/205) of the *Vibrio* spp. isolate exhibited multiple antibiotic resistance (resistance against 3 or more antibiotics). The multiple antibiotic resistance indices (MARI) estimates for both study site (SWWTP, 0.35 and KWWTP, 0.33) suggest that the isolates might have originated from sources with high contamination of antibiotics.

#### **4.6 Conclusion**

The major concern related to the presence of pathogenic microorganisms in water is the risk associated with illnesses upon exposure of human and animals to contaminated water sources. The finding of this study reaffirms the ability of potentially pathogenic bacteria to circumvent the various wastewater treatment processes and be discharged directly into freshwater sources which often serve as the receiving watershed for discharged municipal

effluents. We conclude that relevant authorities should deploy proactive measures towards curbing indiscriminate discharge of poor quality effluent into the aquatic milieu of the Eastern Cape in the interest of public health and freshwater ecosystem conservation.



## References

**Al-Othrubí, S.M.Y., Kqueen, C.Y., Mirhosseini, H., Hadi, Y.A. and Radu, S. (2014).**

Antibiotic resistance of *Vibrio parahaemolyticus* isolated from cockles and shrimp sea food marketed in Selangor, Malaysia. *Clinical Microbiology* 3, 148.

**Altekruse, S.F., Bishop, R.D., Baldy, L.M., Thompson, S.G., Wilson, S.A., Ray, B.J. and**

**Griffin, P.M. (2000).** *Vibrio* gastroenteritis in the US Gulf of Mexico region: the role of raw oysters. *Epidemiology and Infection* 124, 489–95.

**American Public Health Association (APHA) (1998).** Standard methods for the

examination of water and wastewater. 20thEdn. American Public Health Association, American Water Works Association, Water Environment Federation: Washington DC, USA

**Anastasi, E.M., Matthews, B., Stratton, H.M. and Katouli, M. (2012).** Pathogenic

*Escherichia coli* found in sewage treatment plants and environmental waters. *Applied and Environmental Microbiology* 78, 5536-5541.

**Ashbolt, N.J., Grabow, W.O.K and Snozzi, M. (2001).** Indicators of microbial water

quality. Water Quality: Guidelines, Standards and Health. World Health Organization (WHO). Chapter 13: Indicators of Microbial Water Quality. Edited by Lorna Fewtrell and Jamie Bartram. Published by IWA Publishing, London, UK.

**Austin, B. (2009).** Vibrios as causal agents of zoonoses. *Veterinary Microbiology* 140, 310

-317.

**Bateman, C. (2008).** Incompetent maintenance/inept response- eighty more Eastern Cape

babies die. *South African Medical Journal* 98(4), 342-344.

- Bateman, C. (2009).** Up to its eyeballs in sewage-government pleads for help. *South African Medical Journal* 99(5), 556-560.
- Bateman, C. (2010).** Second report slams crippling neglect of water/sanitation systems. *South African Medical Journal* 100(6), 556-560.
- Bej, A.K., McCarty, S.C. and Atlas, R.M. (1991).** Detection of coliform bacteria and *Escherichia coli* by multiplex polymerase chain reaction: comparison with defined substrate and plating methods for water quality monitoring. *Applied and Environmental Microbiology* 57, 2429–2432.
- Berg, G. (1978).** The indicator system. In *Indicators of Viruses in Water and Food* (ed. G. Berg), pp. 1–13, Ann Arbor Science Publishers, Ann Arbor, MI.
- Bielaszewska, M., Mellmann, A., Zhang, W., Kock, R., Fruth, A., Bauwens, A., Peters, G. and Karch, H. (2011).** Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *The Lancet Infectious Diseases* 11(9):671-676.
- Blumberg, L., de Jong, G., Thomas, J., Archer, B.N., Cengimbo, A. and Cohen, C. (2011).** Outbreaks in South Africa 2004-2011, the outbreak response unit of the NICD, and the vision of an inspired leader. *South African Journal of Epidemiology and Infection* 26(4), 195- 197.
- Centers for Disease Control and Prevention (CDC) (2014a).** Reports of selected *E. coli* outbreak investigations 2014. <http://www.cdc.gov/ecoli/outbreaks.html> (Accessed 1st August 2014).

**Centers for Disease Control and Prevention (CDC) (2014b).** National Enteric Disease Surveillance: COVIS Annual Summary, 2012. <http://www.cdc.gov/> (Accessed 4th August 2014).

**Clinical and Laboratory Standards Institute (CLSI) (2011).** Performance standards for antimicrobial susceptibility testing; 21st informational supplement, M100-S21. Wayne, PA: CLSI.

**Collingwood, C., Kemmett, K., Williams, N. and Wigley, P. (2014).** Is the concept of avian pathogenic *Escherichia coli* as a single pathotype fundamentally flawed? *Frontier in Veterinary Science* 1, 1-4.

**Costa, R.A., Araujo, R.L., Souza, O.V. and Vieira, R.H.S. (2015).** Antibiotic-resistant Vibrios in farmed shrimps. *BioMed Research International*, Article ID 505914, 5 pages.

**Dechet, A.M., Yu, P.A., Koram, N. and Painter, J. (2008).** Nonfoodborne Vibrio infections: an important cause of morbidity and mortality in the United States, 1997–2006. *Clinical Infectious Diseases* 46:970–6.

**Department of Water Affairs Republic of South Africa (DWA) (2009).** Executive summary municipal wastewater treatment base information for targeted risk-based regulation Eastern Cape Province status at June 2009.

**Department of Water Affairs Republic of South Africa (DWAF) (2012).** Green Drop Progress Report 2012.

**Dubois, D., Prasadaraao, N.V., Mittal, R., Bret, L., Roujou-Gris, M. and Bonnet, R. (2009).** CTX-M  $\beta$ -Lactamase production and virulence of *Escherichia coli* K1. *Emerging Infectious Diseases*. [www.cdc.gov/eid](http://www.cdc.gov/eid). Vol. 15, No. 12, December 2009.

Available on: <http://wwwnc.cdc.gov/eid/article/15/12/pdfs/09-0928.pdf>. (Accessed 6th August 2014).

**Dungeni, M., van der Merwe, R.R. and Momba, M.N.B. (2010).** Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water SA* 36(5), 513-529.

**European Centre for Disease Prevention and Control (ECDC) (2011).** Shiga toxin-producing *E. coli* (STEC): Update on outbreak in the UK. <http://www.ecdc.europa.eu/> (Accessed 1st August 2014).

**European Centre for Disease Prevention and Control (ECDC) (2013).** Annual Epidemiological Report 2013. Reporting on 2011 surveillance data and 2012 epidemic intelligence data. <http://www.ecdc.europa.eu/> (Accessed 1st August 2014).

**European Food Safety Authority (EFSA) (2012).** *E. coli*: rapid response in a crisis. <http://www.efsa.europa.eu/en/press/news/120711.htm> (Accessed on 1st August 2014).

**Frank, C., Werber, D., Cramer, J.P., Askar, M., Faber, M., Heiden, M.A., Bernard, H., Fruth, A., Prager, R., Spode, A., Wadl, M., Zoufaly, A., Jordan, A., Kemper, M.J., Follin, P., Muller, L., King, L.A., Rosner, B., Buchholz, U., Stark, K. and Krause, G. (2011).** Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *The New England Journal of Medicine* 365, 1771-1780.

**Frigon, D., Biswal, B.K., Mazza, A., Masson, L. and Gehr, R. (2013).** Biological and physicochemical wastewater treatment processes reduced the prevalence of virulent *Escherichia coli*. *Applied and Environmental Microbiology* 79(3):835-844.

- Gerba, C.P. and Smith, J.E. (2005).** Sources of pathogenic microorganisms and their fate during land application of wastes. *Journal of Environmental Quality* 34:42–48.
- Godfree, A. and Farrell, J. (2005).** Process for managing pathogens. *Journal of Environmental Quality* 34(1): 105-513.
- Grabow, W.O.K. (1996).** Waterborne diseases: Update on water quality assessment and control. *Water SA* 22, 193–202.
- Haas, C.N., Rose, J.B. and Gerba, C.P. (1999).** Quantitative Microbial Risk Assessment. John Wiley and Sons, New York.
- Halonen, J.I., Kivimäki, M., Oksanen, T., Virtanen, P., Virtanen, M.J., Pentti, J. and Vahtera, J. (2012).** Waterborne Outbreak of Gastroenteritis: Effects on Sick Leaves and Cost of Lost Workdays. *PLoS ONE* 7(3): e33307.
- Health Canada (2006b).** Guidelines for Canadian drinking water quality: Guideline Technical Document —*Escherichia coli*. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
- Heidelberg, J.F., Eisen, J.A., Nelson, W.C., Clayton, R.A., Gwinn, M.L., Dodson, R.J., Haft, D.H., Hickey, E.K., Peterson, J.D., Umayam, L., Gill, S.R., Nelson, K.E., Read, T.D., Tettelin, H., Richardson, D., Ermolaeva, M.D., Vamathevan, J., Bass, S., Qin, H., Dragoi, I., Sellers, P., McDonald, L., Utterback, T., Fleishmann, R.D., Nierman, W.C., White, O., Salzberg, S.L., Smith, H.O., Colwell, R.R., Mekalanos, J.J., Venter, J.C. and Fraser, C.M. (2000).** DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406, 477-483.

- Hendricks, R. and Pool, E.J. (2012).** The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering.*
- Hlady, W.G. and Klontz, K.C. (1996).** The epidemiology of *Vibrio* infections in Florida, 1981–1993. *Journal of Infectious Diseases* 173, 1176–1183.
- Holmberg, S.D. (1992).** *Vibrio*. In: Gorbach SL, Bartlett JG, Blacklow NR (eds.) *Infectious Diseases*. Philadelphia: WB Saunders Company; 14931502.
- Horman, A., Rimhanen-Finne, R., Maunula, L., von Bonsdorff, C.H., Torvela, N., Heikinheimo, A. and Hanninen, M.L. (2004).** *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., noroviruses, and indicator organisms in surface water in Southwestern Finland, 2000-2001. *Applied and Environmental Microbiology* 70(1): 87-95.
- Horseman, M.A. and Surani, S. (2010).** A comprehensive review of *Vibrio vulnificus*: an important cause of severe sepsis and skin and soft-tissue infection. *International Journal of Infectious Diseases* 5, e157–66.
- Igbinosa, E.O., Obi, L.C. and Okoh, A.O. (2009).** Occurrence of potential pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. *Research in Microbiology* 160, 531-537.
- Ingraham, J.L. (2010).** *March of the microbes: sighting the unseen*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts, London, England.
- Janda, J.M., Powers, C., Bryant, R.G. and Abbott, S. (1988).** Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clinical Microbiology Reviews* 1, 245-267.

- Ji, Z-G. (2008).** Hydrodynamics and water quality: modelling rivers, lakes and estuaries. John Wiley & Sons, Inc., New Jersey, United States.
- Jindal, A.K., Pandya, K. and Khan, I.D. (2015).** Antimicrobial resistance: a public health challenge. *Medical Journal Armed Forces India* 71, 178-181.
- Jury, K.L., Vancov, T., Stuetc, R.M. and Khan, S.J. (2010).** Antibiotic resistance dissemination and sewage treatment plants. *Current research, technology and education Topics in Applied Microbiology and Medical Biotechnology*, 509-519.
- Krumperman, P.H. (1983).** Multiple Antibiotic Resistance Indexing of *Escherichia coli* to identify high-risk of sources of faecal contamination of foods. *Applied and Environmental Microbiology* 46(1):165–170.
- Kwok, A.Y., Wilson, J.T., Coulthart, M., Ng, L.K., Mutharia, L. and Chow, A.W. (2002).** Phylogenetic study and identification of human pathogenic *Vibrio* species based on partial hsp60 gene sequences. *Canadian Journal of Microbiology* 48, 903-910.
- Letchumanan, V., Yin, W., Lee, L and Chan, K. (2015).** Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. *Frontiers in Microbiology* 6, 1-11.
- Maheux, A.F., Picard, F.J., Boissinot, M., Bissonnette, L, Paradis, S. and Bergeron, M.G. (2009).** Analytical comparison of nine PCR primer sets designed to detect the presence of *Escherichia coli/Shigella* in water samples. *Water Research* 43: 3019–3028

- Masters, N., Wiegand, A., Ahmed, W. and Katouli, M. (2012).** *Escherichia coli* virulence genes profile of surface waters as an indicator of water quality. *Water Research* 45, 6321 – 6333.
- McAllister, T.A. and Topp, E. (2012).** Role of livestock in microbiological contamination of water: Commonly the blame, but not always the source. *Animal Frontier* 2(2):17-27.
- McLaughlin, J.C. (1995).** Vibrio. In: Murray PR, Baron EJ, Pfaller MA, et al, (eds.) Manual of Clinical Microbiology. 6th ed. Washington, DC: ASM Press; 1995:465-476.
- Medici, D.D., Croci, L., Delibato, E., Pasquale, S.D., Filetici, E. and Toti, L. (2003).** Evaluation of DNA extraction methods for use in combination with SYBT Green I real-time PCR to detect *Salmonella enterica* serotype enteritidis in poultry. *Applied and Environmental Microbiology* 69(6): 3456-3461.
- Mellmann, A., Harmsen, D., Cummings, C.A., Zentz, E.B., Leopold, S.R., Rico, A., Prior, K., Szczepanowski, R., Ji, Y., Zhang, W., McLaughlin, S.F., Henkhaus, J.K., Leopold, B., Bielaszewska, M., Prager, R., Brzoska, P.M., Moore, R.L., Guenther, S., Rothberg, J.M. and Karch, H. (2011).** Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology. *PLoS ONE* 6(7): e22751.
- Morris, J. and Black, R. (1985).** Cholera and other vibrioses in the United States. *The New England Journal of Medicine* 312, 343-350.
- Nataro, J.P. and Kaper, J.B. (1998).** Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews* 11, 142–201.



- Newton A, Kendall M, Vugia DJ, Henao OL and Mahon BE (2012).** Increasing rates of vibriosis in the United States, 1996–2010: Review of Surveillance Data From 2 Systems. *Clinical Infectious Diseases* 54(S5):S391–395.
- Nsofor, C.A., Kemajou, S.T. and Nsofor, C.M. (2014).** Incidence and antibiotic susceptibility pattern of *Vibrio* species from sea foods sold in Port-Harcourt, Nigeria. *Journal of Bacteriology Resaerch* 6, 13-16.
- Obi, C.L., Momba, M.N.B., Samie, A., Igumbor, J.O., Green, E. and Musie, E. (2007).** Microbiological, physicochemical and management parameters impinging on the efficiency of small water treatment plants in Limpopo and Mpumalanga Provinces of South Africa. *Water South Africa* 33(2):229-237.
- Odonkor, S.T. and Ampofo, J.K. (2013).** *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research* 4(2):5-11.
- Okoh, A.I., Sibanda, T., Nongogo, V., Adefisoye, M.A., Olayemi, O.O. and Nontongana, N. (2015).** Prevalence and characterisation of non-cholerae *Vibrio* spp. in the final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. *Environmental Science and Pollution Reseasrch International* 22(3), 2008-2017.
- Prescott, L.M., Harley, J.P. and Klein, D.A. (2008).** Microbiology 7th edition McGraw-Hill International Edition pp 939.
- Queipo-Ortuno, M.I., Colmenero, J.D.D., Macias, M., Bravo, M.J. and Morata, P. (2008).** Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. *Clinical Vaccine Immunology* 15(2):293-296.

- Reboucas, R.H., de Sousa, O.V., Lima, S.A., Vasconcelos, F.R., de Carvalho, P.B. and Vieira, R.H. (2011).** Antimicrobial resistance profile of *Vibrio* species isolated from marine shrimps farming environments (*Litopenaeus vannamei*) at Ceara, Brazil. *Environmental Research* 111, 21-24.
- Reynolds, K.A. (2003).** Coliforms bacteria: a failed indicator of water quality? Available on: <http://archive.wcponline.com/colum.cfm?T=T&ID=2349&AT=T> (Accessed 08 January 2016).
- Rocourt, J. (2013).** Foodborne diseases and vulnerable groups. *In*, Encyclopedia of food safety. Motarjemi, Y., Moy, G. and Todd, E. (Eds.). Academic Press
- Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Viridis, S. and De Santis, E.P. (2014).** Antibiotic resistance of *Vibrio* species isolated from *Sparus aurata* reared in Italian mariculture. *New Microbiologica* 37, 329-337.
- Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, T.M. and Lukasik, J. (2002).** Microbial Source Tracking: Current Methodology and Future Directions. *Applied and Environmental Microbiology* 68(12):5796-5803.
- Shannon, K.E., Lee, D.Y., Trevors, J.T. and Beaudette, L.A. (2007).** Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. *Science of the Total Environment* 382:121-129.
- Thompson, F.L., Gevers, D., Thompson, C.C., Dawyndt, P., Naser, S., Hoste, B., Munn, C.B. and Swings, J. (2005).** Phylogeny and Molecular Identification of Vibrios on the Basis of Multilocus Sequence Analysis. *Applied and Environmental Microbiology* 71(9), 5107–5115.

- Toze, S. (1999).** PCR and the detection of microbial pathogens in water and wastewater. *Water Research* 33(17), 3545 – 3556.
- Toze, S. (2006).** Reuse of effluent water—benefits and risks. *Agricultural Water Management* 80:147-159.
- Vidal, M., Kruger, E., Duran, C., Lagos, R., Lavine, M., Prado, V., Toro, C. and Vidal, R. (2005).** Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *Journal of Clinical Microbiology* 43(10):5362- 5365.
- Wennberg, A.C., Tryland, I., Ostensvik, O., Secic, I., Monshaugen, M. and Liltved, H. (2013).** Effect of water treatment on the growth potential of *Vibrio cholerae* and *Vibrio parahaemolyticus* in seawater. *Marine Environmental Research* 83, 10–15.
- Wiles, T.J., Kulesus, R.R. and Mulvey, M.A. (2008).** Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Experimental and Molecular Pathology* 85, 11–19.
- World Health Organisation (WHO) (2011).** Outbreaks of *E. coli* O104:H4 infection: update 30. <http://www.euro.who.int/> (Accessed 1st August 2014).
- Wu, C.J., Hsueh, P.R. and Ko, W.C. (2011).** A new health threat in Europe: Shiga toxin producing *Escherichia coli* O104:H4 infections. *Journal of Microbiology, Immunology and Infections* 44: 390-393.
- Ye, L. and Zhang, T. (2013).** Bacterial communities in different sections of a municipal wastewater treatment plant revealed by 16S rDNA 454 pyrosequencing. *Applied Microbiology and Biotechnology* 97, 2681–2690.

**Yu, L., Zhou, Y., Wang, R., Lou, J., Zhang, L., Li, J., Bi, Z and Kan, B. (2012).** Multiple antibiotic resistance of *Vibrio cholerae* serogroup O139 in China from 1993 to 2009. *PLoS* 7, e38633.

**Zavala-Norzagaray, A.A., Aguirre, A.A., Velazquez-Roman, J., Flores-Villasenor, H., Leon-Sicairos, N., Ley-Quinonez, C.P., Hernandez-Diaz, L. J. and Canizalez-Roman, A. (2015).** Isolation, characterisation and antibiotic resistance of *Vibrio* spp. in sea turtles from Northwestern Mexico. *Frontiers in Microbiology* 6, 1-10.

## CHAPTER FIVE

### **Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa**

---

This Chapter was published in

*MicrobiologyOpen*

---

## 5.1 Abstract

Antimicrobial resistance (AMR) is a global problem impeding the effective prevention/treatment of an ever-growing array of infections caused by pathogens; a huge challenge threatening the achievements of modern medicine. In this paper, we report the occurrence of multidrug resistance (MDR) in *Escherichia coli* pathotypes isolated from discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. Standard disc diffusion method was employed to determine the antibiotic susceptibility profile of 223 PCR-confirmed *E. coli* isolates against 17 common antibiotics in human therapy and veterinary medicine. Seven virulence associated and fourteen antibiotic resistance genes were also evaluated by molecular methods. Molecular characterisation revealed five pathotypes of *E. coli* in the following proportions: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%), UPEC (41.7%) and others (26.9%). Isolates showed varying (1.7% – 70.6%) degrees of resistance to 15 antibiotics. Multidrug resistance was exhibited by 32.7% of the isolates with commonest multiple antibiotic resistant phenotype (MAR<sub>P</sub>) being AP-T-CFX (12 isolates), while multiple antibiotic resistant indices (MAR<sub>I</sub>) estimated are 0.23 (Site 1) and 0.24 (Site 2). Associated antibiotic resistance determinants detected in the isolates include: *strA* (88.2%), *aadA* (52.9%), *cat I* (15%), *cmlA1* (4.6%), *bla*<sub>TEM</sub> (56.4%), *tetA* (30.4%), *tetB* (28.4%), *tetC* (42.2%), *tetD* (50%), *tetK* (11.8%) and *tetM* (68.6%). We conclude that municipal wastewater effluents are important reservoirs for the dissemination of potentially pathogenic *E. coli* (and possibly other pathogens) and antibiotic resistance determinants in the aquatic milieu of the Eastern Cape Province and a risk to public health.

**Keywords:** *E. coli* pathotypes; antibiotic-resistance; MAR<sub>P</sub>; MAR<sub>I</sub>; public health; MDR

## 5.2 Introduction

Antimicrobial resistance (AMR) is a global health concern responsible for increasing incidences of both debilitating and lethal diseases resulting from enhanced virulence and limited available therapy to treat common infectious diseases (Michael *et al.*, 2014; WHO, 2015). Increasing development of resistance to established antibiotics has taken a center stage in prophylactic and curative medicine worldwide and more importantly in low-income African countries (Ndiokubwayo *et al.*, 2013). Understanding the various mechanisms including the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon (Ahmed *et al.*, 2010).

The proliferation of antibiotic resistance is currently outpacing the development of novel antibiotics (Fahrenfeld *et al.*, 2013), calling for effective approaches to alleviate the spread of antibiotic resistance (Carlet *et al.*, 2012). The rising resistance in bacteria has been reported mainly due to mobile genetic elements that can be readily spread through bacterial populations (Kumarasamy *et al.*, 2010). Bacterial resistance to antibiotics is partly conferred by antibiotic resistance genes, which encode specific antidrug functions such as efflux pumps, production of enzymes that destroy antibiotics, modification of drug's target site or the production of alternative pathways that bypass drug's action (Webber and Piddock, 2003; Tenover, 2006). Changes in bacteria genome can either be by *de novo* mutation or via the horizontal acquisition of resistant genetic materials, which expands the genome of such bacteria, and causing mutation by altering the pre-existing DNA of the cell (Tenover, 2006; Bennet, 2008). Health problems associated with antibiotic resistant bacteria is less of disease pathology, but more of limited therapeutic remedies, particularly in much of the developing

countries lacking access to good quality treatments, and consequently infections continue to be an important cause of morbidity and mortality (WHO, 1999; Samie *et al.*, 2012).

Antibiotic resistance genes have been detected and quantified from different environmentally relevant matrices, including treated wastewater effluents, which are known to contribute to resistance genes loadings in surface waters (Storteboom *et al.*, 2010; LaPara *et al.*, 2011; Fahrenfeld *et al.*, 2013; Pruden *et al.*, 2013). Wastewater treatment plants (WWTPs) have been recognised as important reservoirs for antibiotic resistance genes associated with human and animal pathogens (Schlüter *et al.*, 2007; Rahube and Yost, 2010; LaPara *et al.*, 2011; Rizzo *et al.*, 2013; Rahube *et al.*, 2014). Antibiotic resistant bacteria and unabsorbed antibiotic residues are often excreted in urine and faeces, and ultimately travel to WWTPs via domestic sewer lines (Rizzo *et al.*, 2012). Classes of antibiotic residues that are frequently detected in municipal WWTPs effluents include  $\beta$ -lactam (penicillin G, oxacilline); macrolides (erythromycin, clarithromycin, roxithromycin); lincosamide (lincomycin); tetracyclines (tetracycline, oxytetracycline); sulphonamides (sulfamethazine, sulfamethoxazole); and fluoroquinolones (ciprofloxacin, norfloxacin) (Giger *et al.*, 2003; Benito-Pena *et al.*, 2006; Karthikeyan and Meyer, 2006; Rahube *et al.*, 2014).

Environmental bacteria communities, including *Escherichia coli* has been associated with recognised antibiotic-resistant gene pools that have been transferred into the normal humans and animal flora, where they exert strong selective pressure for the emergence and spread of resistance (Kinge *et al.*, 2010; Alves *et al.* 2014). Even though, *E. coli* has been a subject of scientific investigations for over a century, and interestingly takes a central stage in genetic and molecular manipulations, this bacteria continues to startle human knowledge of



adaptation and pathogenicity in their host organisms (Souza *et al.*, 2002). While most *E. coli* are commensal member of the normal intestinal flora, some highly adapted strains of the bacteria cause diarrhoeal disease and other gastrointestinal infections along with other serious extraintestinal health problems (Health Canada, 2006b; Prescott *et al.*, 2008; Stecher and Hardt 2008; Katouli 2010).

The pathogenic types (pathotypes) of *E. coli* have been broadly categorised as intestinal pathogenic *E. coli* (InPEC), the main cause of gastrointestinal infections or as extraintestinal pathogenic *E. coli* (ExPEC), the etiological agents of urinary tract infection (UTIs), newborn meningitis and abdominal sepsis and septicaemia (Russo and Johnson, 2000; Kaper *et al.*, 2004; Nesta *et al.*, 2014). At least six of the InPEC pathotypes including enteropathogenic *E. coli* (EPEC), a leading cause of infantile diarrhoea in developing countries (Donnenberg *et al.*, 1993), enterohaemorrhagic *E. coli* (EHEC), the aetiological agent of sporadic cases and outbreaks of haemorrhagic colitis and haemolytic-uremic syndrome (Tzipori *et al.*, 1995), enterotoxigenic *E. coli* (ETEC), which causes traveller and infantile diarrhoea, enteroaggregative *E. coli* (EAEC), a major cause of persistent gastroenteritis and diarrhoea in infants mostly in developing countries (Cerna *et al.*, 2003), enteroinvasive *E. coli* (EIEC), which produces dysentery (Levine, 1987), and diffusely adherent *E. coli* (DAEC), a potential cause of diarrhoea in children (Scaletsky *et al.*, 2002; Kaper *et al.*, 2004) have been well described, while ExPEC are typically divided into three groups namely avian pathogenic *E. coli* (APEC), uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) (Ewers *et al.*, 2007; Johnson *et al.*, 2007). ExPEC strains (uropathogenic *E. coli* and neonatal meningitis *E. coli*) are causative agents of infections in anatomical sites outside of the

gastrointestinal tract, and are associated with urinary tract infections, neonatal meningitis and septicaemia (Lamprecht *et al.*, 2014).

*E. coli* can be used as surrogate for surveillance of antibiotic resistance because it is found more commonly in diverse hosts and environments, it acquires resistance easily (Erb *et al.*, 2007), and is a reliable indicator of resistance in other pathogenic bacteria such as *Salmonellae* spp. (McEgan *et al.*, 2013; Nsofor *et al.*, 2013). Therapeutic options for treating antibiotic resistant *E. coli* vary depending on the type of infection. For instance, urinary tract infections (UTIs) are best treated with trimethoprim/sulfamethoxazole and fluoroquinolones (Taur and Smith, 2007), whereas for Shiga toxin-producing *E. coli* infections, antimicrobial drug therapy is not recommended (Igarashi, 1999).

Nonetheless, numerous studies on the multidrug resistance (MDR) profiling of bacteria have focused mostly on isolates from clinical and food sources, with little information available on the MDR profiles of potentially pathogenic bacteria from final effluents in South Africa. Considering the importance of wastewater effluents as hotspot or potential reservoirs for the dissemination of pathogens and antibiotic resistance genes in the environment hence, the need for such information becomes imperative. Consequently, this study aimed at, investigating the prevalence and antimicrobial resistance patterns of pathogenic *E. coli* strains isolated from treated final effluents in the Eastern Cape, South Africa.

## **5.3 Materials and Methods**

### **5.3.1 Study design and source of samples**

Wastewater final effluent samples were collected monthly; two samples per site (a total of 48 samples), over a twelve month sampling period (September 2012 to August 2013) from two wastewater treatment facilities in Amathole District Municipality, Eastern Cape, South Africa. The two facilities are located within the geographical coordinates 32°34'17''S, 27°26'95''E (Site 1) and 32°41'31''S, 27°08'36''E (Site 2) respectively. Both treatment plants use the activated sludge and drying beds technology, and disinfect their final effluents by chlorination before discharging into the receiving watersheds.

### **5.3.2 Molecular confirmation and characterisation of *E. coli* pathotypes**

The identities of 223 presumptive *E. coli* isolates previously recovered from treated wastewater final effluents on *E. coli*-Coliforms Chromogenic medium (Laboratorios CONDA, South Africa) were confirmed by polymerase chain reaction (PCR) method using the house-keeping gene (*uidA* [ $\beta$ -D glucuronidase] 147 bp) (Janezic *et al.*, 2013). The confirmed isolates were further delineated by PCR analysis into different *E. coli* pathotypes following after the descriptions of (Cebula *et al.*, 1995; Vidal *et al.*, 2005) with modifications. The pathotypes assayed in this study included: EPEC, ETEC, EAEC, EIEC, DAEC, UPEC and NMEC. Bacterial DNA extraction was done by boiling method (De Medici *et al.*, 2003; Queipo-Ortuno *et al.*, 2008). The PCR consisted of 5.0  $\mu$ L of bacterial DNA template, 12.5  $\mu$ L of PCR Master Mix (2X) (Thermo Scientific Inc.), 10 picomole each of forward and reverse primers and 5.5  $\mu$ L of nucleases-free water in a final reaction volume of 25.0  $\mu$ L. *E. coli* DSM reference strains (DSM 8695, DSM 10973, DSM 10974, DSM 4816, DSM 10819, DSM 9025) were included in the assays as internal positive controls for the targeted *E. coli* pathotypes while the negative control consisted of the PCR cocktail and

**Table 5.1.** Primer sequences and sizes of PCR-amplified target genes of *E. coli* pathotypes.

Target Strains	Target Genes	Primer Sequence (5'→3')	Reaction Conditions (°C)	Cycles	Amplicon Size (bp)	Positive Control	Reference
EPEC	<i>eae</i>	TCAATGCAGTTCCGTTATCAGTT GTAAAGTCCGTTACCCCAACCTG	95°, 94°, 55°, 68°, 72° 15', 45'', 45'', 2', 5'	35	482	DSM 8695	Stanilova <i>et al.</i> , 2011
ETEC	<i>lt</i>	GCACACGGAGCTCCTCAGTC TCC TTCATCCTTTCAATGGCTTT	95°, 94°, 58°, 72°, 72° 3', 30'', 1', 1', 10'	35	218	DSM 10973	Stacy-Phipps <i>et al.</i> , 1995
	<i>st</i>	AAAGGAGAGCTTCGTCACATTTT AATGTCCGTCTTGCGTTAGGAC	94°, 94°, 58°, 72°, 72° 3', 1', 1', 1', 5'	35	129	DSM 10973	Stacy-Phipps <i>et al.</i> , 1995
EAEC	<i>eagg</i>	AGACTCTGGCGAAAGACTGTATC ATGGCTGTCTGTAATAGATGAGAAC	95°, 94°, 55°, 68°, 72° 15', 45'', 45'', 2', 5'	35	194	DSM 10974	Pass <i>et al.</i> , 2000
UPEC	<i>papC</i>	GACGGCTGTACTGCAGGGTGGCG ATATCCTTTCTGCAGGGATGCAATA	94°, 94°, 55°, 72°, 72° 2', 1', 1', 1', 5'	30	382	DSM 4816	Hilali <i>et al.</i> , 2000
NMEC	<i>ibeA</i>	TGGAACCCGCTCGTAATATAC CTGCCTGTTCAAGCATTGCA	95°, 94°, 58°, 72°, 72° 3', 30'', 1', 1', 10'	30	342	DSM 10819	Cebula <i>et al.</i> , 1995
DAEC	<i>daaE</i>	GAACGTTGGTTAATGTGGGGTAA TATTCACCGGTCGGTTATCAGT	94°, 92°, 59°, 72°, 72° 2', 30'', 30'', 30'', 5'	30	542	-	Vidal <i>et al.</i> , 2005
EIEC	<i>ipaH</i>	CTC GGCACGTTTTAATAGTCTGG GTGGAGAGCTGAAGTTTCTCTGC	95°, 95°, 55°, 72°, 72° 5', 45'', 45'', 45'', 10'	30	320	DSM 9025	Vidal <i>et al.</i> , 2005

nuclease-free water in place of bacterial DNA templates. Table 5.1 lists primers sequences and PCR conditions for the pathotyping. We could not obtain the reference strain for DAEC at the time of carrying out this study.

### **5.3.3 Antibiotic resistance profiling of confirmed *E. coli* isolates**

The antibiotic resistance/susceptibility profile of the confirmed isolates was determined using the disc-agar diffusion method (CLSI, 2011). Seventeen (17) commercial antibiotic discs (Mast Diagnostics, Merseyside, United Kingdom) which include: ampicillin, amikacin, imipenem, meropenem, streptomycin, chloramphenicol, ciprofloxacin, cephalexin, nalidixic acid, tetracycline, norfloxacin, gentamicin, cefuroxime, cefotaxime, polymyxin B, colistin sulphate and nitrofurantoin were employed for the susceptibility profiling. The selection of the antibiotics used in the study was based on those antibiotic commonly used in human therapy and veterinary medicine. The susceptibility profile of the isolates was determined by measuring the diameters of zones of inhibition and then comparing with the CLSI interpretative charts to determine the sensitivity of the isolates to the test antibiotics.

### **5.3.4 Multiple Antibiotic Resistance Phenotypes and Multiple Antibiotic Resistance**

#### **Indexing**

Multiple antibiotic resistance (MAR) phenotypes profiles were generated for the various pathotypes detected based on exhibition of resistances to three or more antibiotics while multiple antibiotic resistance indices (MAR index) of the isolates were estimated by the formula given below as previously described by Krumperman (1983) to evaluate their health risks to the environment.

$$MAR\ index\ (MARI) = a / (b \times c)$$

where; a = the aggregate antibiotic resistance score of isolates,

b = number of antibiotics and,

c = number of isolates

### **5.3.5 Detection of Antibiotic Resistant Determinants Genes (ARDs)**

The ARDs in the confirmed isolates showing resistant to the various test antibiotics were assayed by PCR using specific oligonucleotide primers that detect genes conferring resistance on the bacterial isolates. A total of 14 ARDs cutting across four classes of antibiotics to which the isolates exhibited resistance were assayed. These included aminoglycosides (*strA*, *aadA*), phenicols (*cat I*, *cat II*, *cmlA1*),  $\beta$ -lactam (*ampC*, *blaZ*, *blaTEM*) and tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetK*, *tetM*). Table 5.2 shows the ARDs, the oligonucleotide primers sequences and the PCR protocols for the target genes.

**Table 5.2.** PCR conditions for antibiotic resistant determinants assayed.

Target	Primer Sequence	Amplicon Size (bp)	PCR Condition	Cycles
<i>strA</i>	F: CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	546	94°, 94°, 68°, 72°, 72° 4', 45'', 45'', 45'', 7'	30
<i>aadA</i>	F: GTGGATGGCGGCCTGAAGCC R: AATGCCAGTCGGCAGCG	525	94°, 94°, 68°, 72°, 72° 4', 45'', 45'', 45'', 7'	30
<i>ampC</i>	F: TTCTATCAAMACTGGCARCC R: CCYTTTTATGTACCCAYGA	550	94°, 94°, 60°, 72°, 72° 4', 45'', 45'', 45'', 7'	30
<i>cat I</i>	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547	94°, 94°, 50°, 72°, 72° 5', 30'', 30'', 90'', 5'	30
<i>cat II</i>	F: AACTTTGCCCTTTATCGTC R: TGAAAGCCATCACATACTGC	543	94°, 94°, 50°, 72°, 72° 5', 30'', 30'', 90'', 5'	30
<i>cmlA1</i>	F: CACCAATCATGACCAAG R: GGCATCACTCGGCATGGACATG	115	94°, 94°, 50°, 72°, 72° 5', 30'', 30'', 90'', 5'	30
<i>blaZ</i>	F: ACTTCAACACCTGCTGCTTTC R: TGACCACTTTTATCAGCAACC	490	94°, 94°, 60°, 72°, 72° 5', 30'', 30'', 90'', 5'	30
<i>blaTEM</i>	F: TTTCGTGTCCGCCCTTATTCC R: CCGGCTCCAGATTTATCAGC	690	94°, 94°, 60°, 72°, 72° 5', 30'', 30'', 90'', 5'	30
<i>tetA</i>	F: GCTACATCCTGCTTGCCCTTC R: CATAGATCGCCGTGAAGAGG	210	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35
<i>tetB</i>	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCAATAACACCG	659	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35
<i>tetC</i>	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	418	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35
<i>tetD</i>	F: AAACCATTACGGCATTCTGC R: GACCGGATACACCATCCATC	787	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35
<i>tetK</i>	F: GTAGCGACAATAGGTAATAGT R: GTAGTGACAATAAACCTCCTA	460	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35
<i>tetM</i>	F: AGTGGAGCGATTACAGAA R: CATATGTCCTGGCGTGTCTA	158	94°, 94°, 55°, 72°, 72° 5', 60'', 60'', 90'', 5'	35

## **5.4 Results**

### **5.4.1 Molecular Confirmation and Characterisation of *E. coli* Isolates into various Pathotypes**

The identities of 223 presumptive *E. coli* isolates were confirmed by the presence of housekeeping *uidA* gene (147 bp). A representative agarose gel electrophoresis picture of some of the confirmed *E. coli* isolates is shown in Figure III a in the Appendix section.

Molecular characterisation of the confirmed *E. coli* isolates revealed the following pathotypes distribution patterns: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%), UPEC (41.7%) and 26.9% of the isolates were not positive for any of the tested pathotypes. Representative gel electrophoresis pictures of the detected pathotypes are shown in Figures III b – f in the Appendix section.

### **5.4.2 Antibigram profiling of confirmed *E. coli* isolates**

The antibiotic resistance/susceptibility profiles of all confirmed pathotypes are summarised in Table 5.3. All isolates were susceptible to two or more test antibiotics. All were susceptible to meropenem and imipenem while only one isolate was resistant to each of gentamycin and amikacin. Of the test antibiotics, tetracycline is the antibiotic to which the highest frequencies of resistance (60.1%) were shown; this is followed by ampicillin with frequencies of 55.6% and cephalexin 51.1% (Table 5.3). About 33% of the ETEC strains showed resistance to each of streptomycin, chloramphenicol, cefotaxime, and nitrofurantoin while 5.9% and 70.6% of the EPEC strains were resistant to ciprofloxacin and tetracycline respectively. Also, the EAEC strains exhibited resistance responses ranging between 5.9% and 58.8% to



ciprofloxacin, ampicillin, chloramphenicol, tetracycline, nalidixic acid, polymyxin B, colistin sulphate, cephalixin and nitrofurantoin. For the NMEC strains, 63.6% of the isolates showed resistance against tetracycline, while 54.6% and 33.3% were resistant to ampicillin and nalidixic acid respectively. UPEC strains showed highest frequency of resistance (63.4%) against tetracycline, while 55.9% and 48.4% of the isolates were resistant to cephalixin and ampicillin respectively. About 1.7% of uncharacterised pathotypes were resistant to norfloxacin, gentamycin and amikacin, while 68.3% showed resistance against ampicillin.

#### **5.4.3 Multiple antibiotic resistance phenotypes and multiple antibiotic resistance indexing**

Two different multiple antibiotic resistant phenotypes (MARP) that is, AP-T-CFX-NI and S-AP-CXM-T-CTX-CFX were observed among the ETEC strains, both at a frequency of 33.3% (1/3) (Table 5.4). Similarly, seven different MAR phenotypes were observed for the EPEC pathotypes all at a frequency of 5.9% (1/17). The predominant MAR phenotype for the EAEC pathotypes was AP-T-NA-CFX at 17.7% (3/17) while S-CIP-AP-NOR-T-NA (9.1%) was the mostly observed phenotypes for NMEC pathotype. For UPEC pathotype, AP-T-CFX was the most prevalent (7.5%) MAR phenotype. For the uncharacterised (Others) *E. coli* isolates, MARP, AP-C-NA-PB was the most predominant, occurring at a frequency of 8.3% (5/6). The multiple antibiotic resistance indices (MARI) estimated for the two sampling sites are 0.23 (site 1) and 0.24 (site 2).

**Table 5.3.** Antibiotic resistance profiles of *E. coli* pathotypes showing percentages of resistance to the test antibiotics.

Antibiotic	<u>Resistance profiles (%)</u>						Frequency of resistance (n = 223)
	ETEC (n = 3)	EPEC (n = 17)	EAEC (n = 17)	NMEC (n = 33)	UPEC (n = 93)	Others (n = 60)	
Amikacin [AK] 30 µg	-	-	-	-	-	1 (1.7%)	1 (0.5%)
Ampicillin [AP] 25 µg	2 (66.7%)	9 (52.9%)	9 (52.9%)	18 (54.6%)	45 (48.4%)	41 (68.3%)	124 (55.6%)
Ceforoxime [CXM] 30 µg	-	-	-	6 (18.2%)	4 (4.3%)	4 (6.7%)	14 (6.3%)
Cefotaxime [CTX] 30 µg	1 (33.3%)	1 (5.9%)	-	-	6 (6.5%)	2 (3.3%)	10 (4.5%)
Cephalexin [CFX]	2 (66.7%)	7 (41.2%)	8 (47.1%)	-	52 (55.9%)	45 (75%)	114 (51.1%)
Chloramphenicol [C] 10 µg	1 (33.3%)	3 (17.7%)	1 (5.9%)	8 (24.2%)	13 (14.0%)	25 (41.7%)	51 (22.9%)
Ciprofloxacin [CIP] 5 µg	-	1 (5.9%)	2 (11.8%)	4 (12.1%)	2 (2.2%)	-	9 (4%)
Colistin sulphate [CO] 10 µg	-	1 (5.9%)	1 (5.9%)	-	7 (7.5%)	5 (8.3%)	14 (6.3%)
Gentamycin [GM] 120 µg	-	-	-	-	-	1 (1.7%)	1 (0.5%)
Imipenem [IMI] 10 µg	-	-	-	-	-	-	-
Meropenem [MEM] 10 µg	-	-	-	-	-	-	-
Nalidixic [NA] 30 µg	-	5 (29.4%)	7 (41.2%)	11 (33.3%)	21 (22.6%)	26 (43.3%)	70 (31.4%)
Nitrofurantoin [NI] 300 units	1 (33.3%)	-	2 (11.8%)	2 (6.1%)	1 (1.1%)	6 (10%)	12 (5.4%)
Norfloxacin [NOR] 10 µg	-	1 (5.9%)	-	6 (18.2%)	3 (3.2%)	1 (1.7%)	11 (4.9%)
Polymyxin B [PB] 300 units	-	3 (17.6%)	1 (5.9%)	-	5 (5.4%)	3 (5%)	12 (5.4%)
Streptomycin [S] 10 µg	1 (33.3%)	3 (17.7%)	-	5 (15.2%)	10 (10.8%)	-	18 (8.1%)
Tetracycline [T] 30 µg	2 (66.7%)	12 (70.6%)	10 (58.8%)	21 (63.6%)	59 (63.4%)	30 (50%)	134 (60.1%)

**Table 5.4.** The various multiple antibiotic resistant (MARP) phenotypes of *E. coli* pathotypes.

<b>ETEC pathotype (n = 3)</b>		
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
AP-T-CFX-NI	1	33.3
S-AP-CXM-T-CTX-CFX	1	33.3
<b>EPEC pathotype (n = 17)</b>		
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
AP-C-CFX	1	5.9
AP-C-T	1	5.9
S-AP-T	1	5.9
S-AP-T-CFX	1	5.9
AP-C-T-PB-CFX	1	5.9
S-CIP-AP-NOR-T-NA	1	5.9
AP-T-CTX-NA-PB-CO-CFX	1	5.9
<b>EAEC pathotype (n = 17)</b>		
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
AP-T-CFX	1	5.9
S-AP-CFX	1	5.9
AP-T-NA-CFX	3	17.7
AP-T-CFX-NI	1	5.9
AP-C-T-NA-CFX	1	5.9
<b>NMEC pathotype (n = 33)</b>		
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
AP-T-CFX	1	3.0
C-T-CFX	2	6.1
AP-NA-CFX	1	3.0
AP-T-CFX	2	6.1
T-NA-CFX	1	3.0
S-T-NA-CFX	1	3.0
AP-C-NOR-NA	1	3.0
AP-T-CFX-NI	1	3.0
AP-T-NA-CFX	1	3.0
AP-C-NOR-NA-CFX	1	3.0
S-CIP-AP-NOR-T-NA	3	9.1
AP-C-NOR-T-NA-CFX-NI	1	3.0
<b>UPEC pathotype (n = 93)</b>		
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
C-T-CFX	1	1.1
AP-T-CFX	7	7.5
AP-T-NA	2	2.2
PB-CO-CFX	1	1.1
AP-CO-CFX	1	1.1
S-AP-CFX	2	2.2
C-T-CFX	3	3.2
AP-NA-CFX	1	1.1
S-AP-T	1	1.1
T-NA-CFX	2	2.2
CIP-T-NA	1	1.1
C-T-NA-CFX	1	1.1
AP-T-NA-CFX	6	6.5
S-AP-T-CFX	3	3.2
AP-CXM-T-CFX	1	1.1
AP-NA-CO-CFX	1	1.1

AP-CXM-T-NA-CFX	2	2.2
AP-C-T-PB-CFX	1	1.1
C-T-NA-CFX-NI	1	1.1
CIP-NOR-T-NA-PB	1	1.1
S-AP-CXM-T-NA-CFX	1	1.1
AP-C-NOR-T-NA-CFX-NI	1	1.1

**OTHERS (n = 60)**

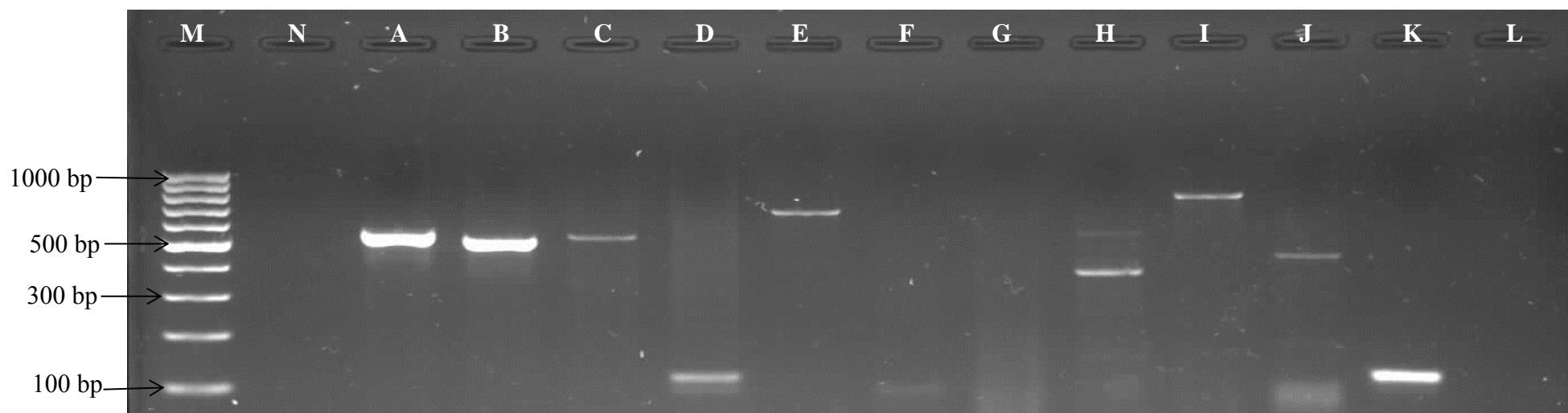
<b>MAR phenotypes</b>	<b>Number observed</b>	<b>Percentage (%)</b>
C-NA-CFX	2	3.3
CXM-T-NA	1	1.7
AP-CTX-CFX	1	1.7
AP-T-CO	1	1.7
AP-C-CFX	3	5.0
T-CFX-NI	1	1.7
T-NA-CFX	1	1.7
AP-T-CFX	3	5.0
AP-NA-CFX	1	1.7
AP-C-CFX	1	1.7
AP-T-NA-CO	1	1.7
AP-CXM-NA-CFX	1	1.7
C-T-NA-CFX	1	1.7
CIP-AP-NA-CFX	1	1.7
AP-C-NA-PB	5	8.3
C-NA-CFX-NI	1	1.7
AP-C-CFX-NI	2	3.3
AP-T-NA-CFX	4	6.7
AP-C-T-CFX	1	1.7
CIP-CXM-NOR-T-PB	1	1.7
CIP-AP-T-CO-CFX	1	1.7
AP-C-T-NA-CFX	4	6.7
AP-C-T-CFX-NI	2	3.3
AP-C-AK-T-NA-CFX	1	1.7
CIP-AP-C-CXM-CTX-PB-CFX	1	1.7

#### 5.4.4 Detection of antibiotic resistant determinants (ARDs)

Molecular detection of ARDs in isolates that exhibited multidrug resistance to the test antibiotics revealed the presence of 11 out of the 14 antibiotic resistant genes assayed, while *cat II*, *ampC* and *blaZ* genes were not detected in any of the isolates even though, some isolates showed resistance to these classes of antibiotic. The distribution of ARDs detection in the various resistant strains generally ranged between *cmlA1* (4.6%) and *strA* (88.2%) as shown in Table 5.5. Some isolates were found to harbour ARDs for two or more classes of antibiotics. For instance, six isolates belonging to different pathotypes carried *strA* and *aadA* (both conferring resistance to aminoglycosides) as well as *blaTEM* (conferring resistance to  $\beta$ -Lactam). Figure 5.1 below shows a representative gel electrophoretic image of some of the detected ARDs in the isolates.

**Table 5.5.** Distribution of antibiotic resistant genes *E. coli* isolates.

<b>Class of antibiotic</b>	<b>Antibiotic resistant genes</b>	<b>Number (%) of positive isolates</b>
Aminoglycosides	<i>strA</i>	15 (88.2)
	<i>aadA</i>	9 (52.9)
Phenicol	<i>cat I</i>	7 (15.9)
	<i>cat II</i>	0
	<i>cmlA1</i>	2 (4.6)
β-Lactam	<i>ampC</i>	0
	<i>blaZ</i>	0
	<i>blaTEM</i>	53(56.4)
Tetracycline	<i>tetA</i>	31 (30.4)
	<i>tetB</i>	29 (28.4)
	<i>tetC</i>	43 (42.2)
	<i>tetD</i>	51 (50)
	<i>tetK</i>	12 (11.8)
	<i>tetM</i>	70 (68.6)



**Figure 5.1.** Antibiotic resistant determinants. Lane M: 100 bp molecular weight marker; lane N: negative control; lane A: *strA* (546 bp); lane B: *aadA* (525 bp); lane C: *cat I* (547 bp); lane D: *cmlA1* (115 bp); lane E: *blaTEM* (690 bp); lane H: *tetC* (385 bp); lane I: *tetD* (787 bp); lane J: *tetK* (460 bp); lane K: *tetM* (125 bp).

## 5.5 Discussion

Whereas abundance of information exists in the literature of studies on the multiple drug resistance profiles of pathogenic microorganisms including *E. coli* pathotypes from clinical and food sources, adequate information continues to be lacking on the antibiotic resistance/susceptibility profiles of *E. coli* strains (pathotypes) from discharged final effluents in many developing countries such as South Africa. Considering the strategic importance of sewage or wastewater treatment plants as hotspots/reservoir, and point-source for the dissemination of ARDs in the environment, there is need to investigate WWTPs for their possible roles in contributing to the spread of drug resistance in potentially pathogenic microorganism in the environment (Samie *et al.*, 2012; Nsofor *et al.*, 2013). Besides, a study carried out on surface water in Australia suggested that more than 50% of *E. coli* isolates in surface water environment are likely to have originated from wastewater effluents (Anastasi *et al.*, 2012), underlining the importance of municipal wastewater treatment plants as potential point sources of pathogens into freshwater environment.

In this study, five different groups of pathogenic *E. coli* belonging to the two broad categories, that is, InPEC (ETEC, EPEC and EAEC) and ExPEC (NMEC and UPEC) with frequencies of detection ranging between 1.4% and 41.7% were isolated from treated final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. Intestinal pathogenic *E. coli* (InPEC) also known as diarrhoeagenic *E. coli* (DEC) are major etiological agents of paediatric diarrhoea which continues to be the most common cause of infantile morbidity and mortality most especially in developing countries. DEC can be transmitted via the oral-faecal route by ingesting food or water contaminated by human or animal faeces.



The pathogenesis of ETEC involves the establishment of adherence to the epithelium of the small intestine by means of colonization factors (CF) (Oh *et al.*, 2014). This is followed by the production of enterotoxins (a heat-stable toxin (ST) and/or a heat-labile toxin (LT)) which stimulate the lining of the intestine causing secretion of excessive fluid, often leading to diarrhoea (CDC, 2014). ETEC strains are responsible for millions of infections cases worldwide, and it is one of the most important pathogens associated with death following moderate to severe diarrhea in children (Luo *et al.*, 2014). Clinical symptoms of ETEC infection include profuse watery diarrhoea with abdominal cramps which is usually self-limited while fever, headache, nausea and vomiting are less common symptoms (Yoder *et al.*, 2006). The non-detection of the *st* gene as observed in our study might have resulted from the loss of the plasmid encoded gene (Taxt *et al.* 2010).

EPEC strains are classified as either typical or atypical, and are described as attaching and effacing pathogens due to their ability to form distinctive lesions on the surfaces of intestinal epithelial cells. Typical EPEC strains have been recognised as important agents of diarrhoea in developing countries while atypical strains are commonly isolated in developed countries (Santona *et al.*, 2013). These two subgroups of EPEC are differentiated based on the presence or absence of a bundle forming pili gene (*bfpA* gene) (Santona *et al.*, 2013). Clinical presentation of EPEC infection includes watery diarrhoea that is often accompanied by fever, vomiting and dehydration in children under 2 years of age. Persistent cases, lasting more than 2 weeks, have also been reported (Ochoa, 2011; Lai *et al.*, 2013). EAEC does not produce enterotoxins; however, it causes acute or persistent diarrhoea among infants and has been responsible for large outbreaks of diarrhoea in Europe, the United Kingdoms, Switzerland and Japan as well as in developing countries.

EAEC causes acute or persistent diarrhoea among infants, and has been responsible for large outbreaks in Europe, the United Kingdoms, Switzerland and Japan as well as in developing countries (Kaur *et al.*, 2010). EAEC adhere to the epithelial cells in a distinctive ‘stacked-brick’ pattern and also has the ability to form biofilms (Weintraub, 2007). Even though different studies searching for specific virulence factor in EAEC have been documented, the overall pathogenesis mechanisms of this pathogen remain unclear and largely unknown (Arenas-Hernandez, 2012). Some manifestations of infection by EAEC include watery diarrhoea, abdominal pain, nausea, vomiting and low-grade fever (Vaishnavi, 2013).

One of the most relevant diseases caused by ExPEC strains in animals is systemic colibacillosis leading to significant economic losses in the poultry industry (Ewers *et al.*, 2007). Neonatal meningitis *E. coli* (NMEC) and Uropathogenic *E. coli* (UPEC) establish infections in extraintestinal habitats. NMEC are the second most common cause of neonatal bacterial meningitis with a mortality rate of more than 10% and are responsible for about 20-50% cases of sequelae (Gaschignard *et al.*, 2012). UPEC is involved in a large number of urinary tract infections (UTIs), including cystitis and pyelonephritis, and infectious complications, which may lead to acute renal failure in healthy individuals as well as in renal transplant patients (Bien *et al.*, 2012). UTIs are considered to be the most common infections in humans and their development depend on anatomical factors, the integrity of host defence mechanisms, and the virulence of the infecting organisms (Nicolle, 2002). UTIs are classified into disease categories according to the site of infection: cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (Foxman, 2003; Bien *et al.*, 2012).

ExPEC are the predominant strains detected in this study, with frequency of detection ranging from 14.8% to 41.7%. They account for 56.5% of the total isolates, while InPEC strains with

frequencies of detection ranging from 1.4% to 7.6% accounted for 16.6% of the isolates. Uncharacterised strains made up 26.9% of the total isolates. Higher prevalence of ExPEC compared to InPEC as recorded in this study is corroborated by other different studies of municipal WWTPs with similar trends (Anastasi *et al.*, 2010; Mokracka *et al.*, 2011; Frigon *et al.*, 2013). In their report focusing on the prevalence and persistence of *E. coli* strains in four sewage treatment plants in a subtropical region of Queensland, Australia, Anastasi *et al.*, (2010) documented predominance (59.5%) of ExPEC strains. They concluded that ExPEC (uropathogenic) strains can survive all wastewater treatment processes thus, increases the chances of their release in surface water and constituting significant public health risk. In a similar study, Mokracka *et al.* (2011), reported a higher proportion (50.5%) of ExPEC compared to InPEC (21%) strains while investigating the phylogenetic groups, virulence genes and quinolone resistance of integrin-bearing *E.coli* strains isolated from a wastewater treatment plant. Similarly, Frigon *et al.* (2013) recorded an abundance of ExPEC (24%) over InPEC (10%) strains *E.coli* while investigating the removal of virulent *E.coli* by biological and physicochemical wastewater treatment processes. These reports are in line with our observation for the predominance of ExPEC associated strains in treated wastewater effluent compared to InPEC.

The antibiogram of the confirmed isolates in this study showed marked susceptibility ( $\geq 98.3\%$ ) to four (meropenem, imipenem, gentamycin and amikacin) of the test antibiotics. This observation is similar to the findings of Joly-Guillou *et al.* (2010), who reported 100% susceptibility of *E.coli* isolates recovered from French hospitals to meropenem and imipenem, and also consistent with the findings of Rocha *et al.* (2014) who recorded lack of resistance against imipenem, gentamycin and amikacin. Based on our observation, these four antibiotics showing marked potency against our test isolates could be very useful as drugs of

choice for therapeutic purposes in the event of waterborne outbreaks in South Africa and particularly in the immediate communities of the study area. The isolates exhibited varying levels of responses to other classes of antibiotic with tetracycline being the least potent with resistance  $\geq 50\%$ , this is followed by ampicillin with resistance frequency of  $\geq 47.8\%$ . Similar findings to our observation have been reported by Momtaz *et al.* (2012). Also, Tadesse *et al.* (2012) had a similar observation and noted that the high frequency of resistance to tetracycline (introduced in 1948), ampicillin (introduced in 1961) and other older drugs was as a result of the long term use of these drugs in human therapy.

Multiple antibiotic resistance (MAR) which was defined as the exhibition of resistance to three or more test antibiotics was observed in 32.7% of the isolates with different MAR phenotypes (MARP) (Table 5.4). The predominant MAR phenotype was AP-T-CFX (MARP 3) found in 12 (5.4%) isolates cutting across different pathotypes. Other MARP showing resistance against 4 to 7 different antibiotic were also detected at different frequencies. One major implication of multiple antibiotic resistance in *E. coli* and other pathogens is failure in treatment of some bacterial infections or illness that were thought to be curable previously with a huge public health burden. Multidrug (multiple antibiotics) resistance has led to the reclassification of certain diseases as re-emerging with associated health implications such as prolonged illness period, higher cost for therapy and increased risk of death.

Multiple antibiotic resistance index (MARI) has been used as a tool to estimate health risk associated with the spread of bacterial resistance to antimicrobial drugs in a given population. It is calculated as the number of antibiotics to which test isolates displayed resistance divided by total number of antibiotics to which the test organism has been evaluated for sensitivity. MARI value of 0.2 (arbitrary) is used to differentiate between low and high health risk, and

MARI greater than 0.2 suggests that a strain(s) of bacteria originate from an environment high contamination or antibiotics usages (Christopher *et al.*, 2013). The MARI estimates obtained for isolates from the two study sites (site 1 (0.23) and site 2 (0.24)) were similar and both greater than 0.2 which suggests that the isolates originated from environments with high use or contamination of antibiotics. The high MARI values obtained in this study may suggest exposure of our isolates to antibiotics pressure which might have resulted from inappropriate use of antibiotic among the population in the study area, and may lead further to increase in the development of multidrug resistance overtime if appropriate measures are not put in place.

Molecular analysis of ARDs reveals the presence of eleven genes conferring resistance to different classes of antibiotics. Selection of ARDs assayed for was based on the result of the MAR phenotypic analysis of the isolates. Six different ARDs conferring resistance against tetracycline were mostly detected at frequencies ranging from 11.8% (*tet K*) to 68.8% (*tetM*), suggesting resistance to this antibiotic may be genetically mediated as a result of long-term use. Similarly, high detection of *bla*TEM conferring resistance to  $\beta$ -lactam was observed at a frequency of 56.4% and supports the finding of Momtaz *et al.* (2012), who reported high rate of detection of antibiotic resistance determinants conferring resistance to tetracycline and ampicillin. Some of the isolates in this study were found to harbour multiple resistant genes conferring resistance to two or more different classes of antibiotics. The repertoire of antibiotic resistant genes found in the *E.coli* pathotypes might serve as a pointer to the possible presence of other antidrug resistant genes conferring resistance to other classes of antibiotics that were not targeted in this study, and our finding is in line with other reports on the detection of multiple antibiotic resistance genes in some commensal and pathogenic strains of *E. coli* (Smith *et al.*, 2007; Bailey *et al.*, 2010; Karczmarczyk *et al.*, 2011).

## 5.6 Conclusions

The results obtained in the study clearly showed that the final effluents of wastewater treatment plants are reservoir of antibiotic resistant *E. coli* pathotypes (and possibly other pathogens) and potential point sources of antibiotic resistant genes which might be transferred to other pathogens in the receiving watersheds, thus presenting a public health risk in the Eastern Cape Province of South Africa. The combination of the deadly duo of drug resistance and emerging virulence in pathogenic bacteria brings about a worrisome situation of possible lack of therapeutic options for some severe bacterial infections in the nearest future. This phenomenon coupled with high number of immunocompromised individuals in the Southern African region calls for priority attention to bring under control the spread of antibiotic resistance in order to safeguard the health of the general public.

## References

- Ahmed, M.O., Clegg, P.D., Williams, N.J., Baptiste, K.E. and Bennett, M. (2010).** Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. *Annals of Clinical Microbiology and Antimicrobials* 9, 12.
- Alves, M., Pereira, S.A., Araujo, S.M., Castro, B.B., Correia, A.C. and Henriques, I. (2014).** Seawater is a reservoir of multi-resistant *Escherichia coli*, including strains hosting plasmid-mediated quinolones resistance and extended-spectrum beta lactamases genes. *Frontiers in Microbiology* 5, 42610.3389/fmicb.2014.00426
- Anastasi, E.M., Matthews, B., Stratton, H.M. and Katouli, M. (2012).** Pathogenic *Escherichia coli* found in sewage treatment plants and environmental waters. *Applied and Environmental Microbiology* 78, 5536-5541.
- Anastasi, E.M., Matthews, B., Gundogdu, A., Vollmerhausen, T.L., Ramos, N.L., Stratton, H. Ahmed, W. and Katouli, M. (2010).** Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants. *Applied and Environmental Microbiology* 76, 5882–5886.
- Arenas-Hernández, M.M.P., Martínez-Laguna, Y. and Torres, A.G. (2012).** Clinical implications of enteroadherent *Escherichia coli*. *Current Gastroenterology Reports* 14(5), 386–394.
- Bailey, J.K., Pinyon, J.L., Anantham, S. and Hall, R.M. (2010).** Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. *Journal of Medical Microbiology* 59, 1331–1339.
- Benito-Pena, E., Partal-Rodera, A.I., Leon-Gonzalez, M.E. and Moreno-Bondi, M.C. (2006).** Evaluation of mixed mode solid phase extraction cartridges for the preconcentration of beta-lactam antibiotics in wastewater using liquid chromatography with UV-DAD detection. *Analytica Chimica Acta* 556, 415-422.

- Bennett, P.M. (2008).** Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British Journal of Pharmacology* 153 (Suppl 1), S347–S357.
- Bien, J., Sokolova, O. and Bozko, P. (2012).** Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *International Journal of Nephrology*, 2012, Article ID 681473, 15 pages.
- Carlet, J., Jarlier, V., Harbarth, S., Voss, A., Goossens, H. and Pittet D. (2012).** Ready for a world without antibiotics? The penières antibiotic resistance call to action, Participants of the 3rd World Healthcare-Associated Infections Forum. *Antimicrobial Resistance and Infection Control* 1(1), 11.
- Cebula, T.A., Payne, W.L. and Feng, P. (1995).** Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. *Journal of Clinical Microbiology* 33, 248–250.
- Centres for Disease Control and Prevention (CDC) (2014).** *Escherichia coli*. Centers for Disease Control and Prevention. Available online: <http://www.cdc.gov/ecoli/etec.html> (Accessed 11/12/2014).
- Cerna, J.F., Nataro, J.P. and Estrada-Garcia, T. (2003).** Multiplex PCR for detection of three plasmid-borne genes of enteroaggregative *Escherichia coli* strains. *Journal of Clinical Microbiology* 41, 2138–2140.
- Christopher, A.F., Hora, S. and Ali, Z. (2013).** Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Annals of Tropical Medicine and Public Health* 6, 285–289.



- Clinical and Laboratory Standards Institute (CLSI) (2011).** Performance standards for antimicrobial susceptibility testing; 21st informational supplement, M100-S21. Wayne, PA: CLSI.
- De Medici, D., Croci, L., Delibato, E., Di Pasquale, S., Filetici, E. and Toti, L. (2003).** Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype enteritidis in poultry. *Applied and Environmental Microbiology* 69, 3456–3461.
- Donnenberg, M.S., Tacket, C.O., James, S.P., Losonsky, G., Nataro, J.P. Wasserman S.S., Kaper, J.B and Levine, M.M. (1993).** Role of the *eaeA* gene in experimental enteropathogenic *Escherichia coli* infection. *The Journal of Clinical Investigation* 92(3), 1412–1417.
- Erb, A., Stürmer, T., Marre, R. and Brenner, H. (2007)** Prevalence of antibiotic resistance in *Escherichia coli*: overview of geographical, temporal, and methodological variations. *European Journal of Clinical Microbiology and Infectious Diseases* 26, 83–90.
- Ewers, C., Li, G., Wilking, H., Kiessling, S., Alt, K., Antao, E.M. Laturus, C., Diehl, I., Glodde, S., Homeier, T., Bohnke, U., Steinruck, H., Philipp, H.C. and Wieler, L.H. (2007).** Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they? *International Journal of Medical Microbiology* 297, 163–176. doi:10.1016/j.ijmm.2007.01.003.
- Fahrenfeld, N., Ma, Y., O'Brien, M. and Pruden, A. (2013).** Reclaimed water as a reservoir of antibiotic resistance genes: distribution system and irrigation implications. *Frontiers in Microbiology* 4, 130.
- Foxman, B. (2003).** Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Disease-a-Month* 49(2), 53–70.

- Frigon, D., Biswal, B.K., Mazza, A., Masson, L., Gehra R. (2013).** Biological and physicochemical wastewater treatment processes reduce the prevalence of virulent *Escherichia coli*. *Applied and Environmental Microbiology* 79(3), 835–844.
- Gaschignard, J., Levy. C., Bingen, E., Cohen, R. (2012).** Epidemiology of neonatal meningitis *Escherichia coli*. *Pediatrics Archives* 19(3), S129–S134.
- Giger, W., Alder, A.C., Golet, E.M., Kohler, H.P.E., McArdell, C.S., Molnar, E., Molnar, E., Siegrist, H., Suter, M.J.F. (2003).** Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. *Chimia* 57, 485–491.
- Health Canada. (2006b).** Guidelines for Canadian drinking water quality: Guideline Technical Document —*Escherichia coli*. Water Quality and Health Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
- Hilali, F., Ruimy, R., Saulnier, P., Barnabe, C., Lebouguenec, C., Tibayrenc, M., Andremont, A. (2000).** Prevalence of virulence genes and clonality in *Escherichia coli* strains that cause bacteraemia in cancer patients. *Infection and Immunity* 68(7), 3983–3989.
- Igarashi, T., Inatomi, J., Wake, A., Takamizawa, M., Katayama, H., Iwata, T. (1999).** Failure of pre-diarrheal antibiotics to prevent haemolytic uremic syndrome in serologically proven *Escherichia coli* O157:H7 gastrointestinal infection. *Journal of Pediatrics* 135, 768–769. doi: 10.1016/S0022-3476(99)70100-9.
- Janezic, K.J., Ferry, B., Hendricks, E.W., Janiga, B.A., Johnson, T., Murphy, S., Roberts, M.E., Scott, S.M., Theisen, A.N., Hung, K.F. and Daniel, S.L. (2013).** Phenotypic and genotypic characterisation of *Escherichia coli* isolated from untreated surface waters. *The Open Microbiology Journal* 7, 9–19.

- Johnson, T.J., Kariyawasam, S., Wannemuehler, Y., Mangiamele, P., Johnson, S.J. (2007).** The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. *Journal of Bacteriology* 189, 3228–3236.
- Joly-Guillou, M.L., Kempf, M., Cavallo, J.D., Chomarar, M., Dubreuil, L., Maugein J., Muller-Serieys, C. and Roussel-Delvallez, M (2010).** Comparative *in vitro* activity of Meropenem, Imipenem and Piperacillin/tazobactam against 1071 clinical isolates using 2 different methods: a French multicentre study. *BMC Infectious Diseases* 10, 72.
- Kaper, J.B., Nataro, J.P., Mobley, H.L. (2004).** Pathogenic *Escherichia coli*. *Nature Reviews Microbiology* 2, 123–140. doi:10.1038/nrmicro818.
- Karczmarczyk, M., Abbott, Y., Walsh, C., Leonard, N., Fanning, S. (2011).** Characterization of multidrug-resistant *Escherichia coli* isolates from animals presenting at a university veterinary hospital. *Applied and Environmental Microbiology* 77(2), 7104–7112. doi: 10.1128/AEM.00599-11.
- Karthikeyan, K.G., Meyer, M.T. (2005).** Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Science of Total Environmental* 361, 196–207.
- Katouli, M. (2010).** Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iran Journal of Microbiology* 2, 59-72.
- Kaur, P., Chakraborti, A. and Asea, A. (2010).** Enteroaggregative *Escherichia coli*: an emerging enteric food borne pathogen. *Interdisciplinary Perspective on Infectious Diseases* Volume 2010, Article ID 2541159, 10 pages.
- Kinge, N.W., Ateba, C.N., Kawadza, D.T. (2010).** Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, North-West province, South Africa. *South Africa Journal of Science* 106, 44–49.

- Krumperman, P.H. (1983).** Multiple Antibiotic Resistance Indexing of *Escherichia coli* to identify high-risk of sources of faecal contamination of foods. *Applied and Environmental Microbiology* 46(1), 165–170.
- Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S., Krishnan, P., Kumar, A.V., Maharjan, S., Mushtaq, S., Noorie, T., Paterson, D.L., Pearson, A., Perry, C., Pike, R., Rao, B., Ray, U., Sarma, J.B., Sharma, M., Thirunarayan, M.A., Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore, D.M. and Woodford, N. (2010).** Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infectious Diseases* 10, 597–602
- Lai, Y., Rosenshine, I., Leong, J.M. and Frankel, G. (2013).** Intimate host attachment: enteropathogenic and enterohaemorrhagic *Escherichia coli*. *Cellular microbiology* 15, 1796–1808. doi: 10.1111/cmi.12179.
- Lamprecht, C., Romanis, M., Huisamen, N., Carinus, A., Schoeman, N., Sigge, G.O. and Britz, T.J. (2014).** *Escherichia coli* with virulence factors and multidrug resistance in the Plankenburg River. *South African Journal of Science* 110, 1-6
- LaPara, T.M., Burch, T.R., McNamara, P.J., Tan, D.T., Yan, M., Eichmiller, J.J. (2011).** Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science Technology* 45(22), 9543–9549.
- Levine, M.M. (1987).** *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *The Journal of Infectious Diseases* 155(3), 377–389. doi: 10.1093/infdis/155.3.377.
- Luo, Q., Kumar, P., Vickers T.J., Sheikh, A., Lewis, W.G., Rasko, D.A., Sistrunk, J. and**

- Fleckenstein, J.M. (2014).** Enterotoxigenic *Escherichia coli* secretes a highly conserved Mucin-degrading metalloprotease to effectively engage intestinal epithelial cells. *Infection and Immunity* 82(2), 509–521..
- McEgan, R., Mootian, G., Goodridge, L.D., Schaffner, D.W., Danyluk, M.D. (2013).** Predicting *Salmonella* populations from biological, chemical, and physical indicators in Florida surface waters. *Applied and Environmental Microbiology* 79(13), 4094–4105.
- Michael, C.A., Dominey-Howes, D. and Labbate, M. (2014).** The antimicrobial resistance crisis: causes, consequences, and management. *Front Public Health* 6, 2:145.
- Mokracka, J., Koczura, R.; Jablonska, L., Kaznowski, L.A. (2011).** Phylogenetic groups, virulence genes and quinolone resistance of integron-bearing *Escherichia coli* strains isolated from a wastewater treatment plant. *Antonie Van Leeuwenhoek* 99, 817–824.
- Momtaz, H., Rahimi, E. and Moshkelani, S. (2012).** Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinarni Medicina* 57(4), 193–197.
- Ndihokubwayo, J.B., Yahaya, A.A, Desta, A.T., Ki-Zerbo, G., Asamoah-Odei, E., Keita, B., Pana, A.P. and Nkhoma, W. (2013).** Antimicrobial resistance in the African region: issues, challenges and actions proposed. Available online: <https://www.aho.afro.who.int/en/ahm/issue/16/reports/antimicrobial-resistance-african-region-issues-challenges-and-actions-proposed> (Accessed 31 May 2015).
- Nesta, B., Valeri, M., Spagnuolo, A., Rosini, R., Mora, M., Donato, P., Alteri, C.J., Vecchio, M.D., Buccato, S., Pezzicoli, A., Bertoldi, I., Buzzigoli, L., Tuscano, G., Falduto, M., Rippa, V., Ashhab, Y., Bensi, G., Fontana, M.R., Seib, K.L., Mobley, H.L.T., Pizza, M., Soriani, M. and Serino, L. (2014).** SsIE elicits functional antibodies that impair in vitro Mucinase activity and in vivo colonization

- by both intestinal and extraintestinal *Escherichia coli* strains. *PLoS Pathogens* 10(5), e1004124. doi:10.1371/journal.ppat.1004124.
- Nicolle, L.E. (2002).** Urinary tract infection in geriatric and institutionalized patients. *Current Opinion in Urology* 12(1), 51–55.
- Nsofor, C.A., Iroegbu, C.U., Call, D.R. and Davies, M.A. (2013).** The genetic relatedness of drug resistant *E. coli* isolates of human and animal origin in Nigeria. *International Journal of Genetic and Molecular Biology* 5(3), 37–41.
- Ochoa, T.J. and Contreras, C.A. (2011).** Enteropathogenic *Escherichia coli* infection in children. *Current Opinion in Infectious Diseases* 24, 478–483.
- Oh, K.H., Kim, D.W., Jung, S.M. and Cho, S.H. (2014).** Molecular characterization of enterotoxigenic *Escherichia coli* strains isolated from diarrheal patients in Korea during 2003–2011. *PLoS ONE* 9(5), e96896. doi:10.1371/journal.pone.0096899.
- Pass, M.A., Odedra, R. and Batt, R.M. (2000).** Multiplex PCRs for identification of *Escherichia coli* virulence genes. *Journal of Clinical Microbiology* 38, 2001–2004.
- Prescott, L.M., Harley, J.P. and Klein, D.A. (2008).** *Microbiology*, 7<sup>th</sup> edition McGraw-Hill International Edition, 2008; p. 939.
- Pruden, A., Edwards, M. and Falkinham, J.O. (2013).** State of the Science of opportunistic pathogens in premise plumbing: methodology, microbial ecology, and epidemiology. Water Research Foundation Project 4379 Final Report Denver, CO: Water Research Foundation.
- Queipo-Ortuno, M.I., Colmenero, J.D., Macias, M., Bravo, M.J. and Morata, P. (2008).** Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. *Clinical and Vaccine Immunology* 15(2), 293–296. doi:10.1128/CVI.00270-07.
- Rahube, T.O., Viana, L.S., Koraimann, G. and Yost, C.K. (2014).** Characterization and

comparative analysis of antibiotic resistance plasmids isolated from a wastewater treatment plant. *Frontiers in Microbiology* 5, 558. doi:10.3389/fmicb.2014.00558.

**Rahube, T.O and Yost, C.K. (2010).** Antibiotic resistance plasmids in wastewater treatment plants and their possible dissemination into the environment. *African Journal of Biotechnology* 9, 9183–9190.

**Rizzo, L., Fiorentino, A. and Anselmo, A. (2012).** Effect of solar radiation on multidrug resistant *E. coli* strains and antibiotic mixture photodegradation in wastewater polluted stream. *Science of Total Environmental* 427–428, 263-268. doi:[10.1016/j.scitotenv.2012.03.062](https://doi.org/10.1016/j.scitotenv.2012.03.062).

**Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I., Fatta-Kassinos, D. (2013).** Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of Total Environmental* 447, 345–60. doi: 10.1016/j.scitotenv.2013.01.032. Epub 2013 Feb 7.

**Rocha, R.S., Leite, L.O., Sousa, O.V. and Vieira, R.H.S. (2014).** Antimicrobial susceptibility of *Escherichia coli* isolated from fresh-marketed Nile Tilapia (*Oreochromis niloticus*). *Journal of Pathogens* 2014, Article ID 756539, 1–5

**Russo, T.A. and Johnson, J.R. (2000).** Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. *The Journal of Infectious Diseases* 181, 1753–1754.

**Samie, A., Guarrant, R.L., Barrett, L., Bessong, P.O., Igumbor, E.O. and Obi, C.L. (2012).** Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarrhoeal human stools from Vhembe district, South Africa. *Journal of Health Population and Nutrition* 27, 739–745.

**Santona S., Diaz N., Fiori P.L., Francisco, M., Sidat M., Cappuccineli, P., Rappeli P.**

- (2013). Genotypic and phenotypic features of enteropathogenic *Escherichia coli* isolated in industrialized and developing countries. *The Journal of Infection in Developing Countries* 2013, 7(3), 214–219.
- Scaletsky, I.C.A., Fabbriotti, S.H., Carvahlo, R.L.B., Nunes, C.R., Maranhao, H.S., Morais, M.B., Fagundes-Neto, U. (2002).** Diffusely adherent *Escherichia coli* as a cause of acute diarrhoea in young children in Northern Brazil: a case-control study. *Journal of Clinical Microbiology* 40(2), 645–648.
- Schlüter, A., Szczepanowski, R., Pühler, A. and Top, E.M. (2007).** Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. *FEMS Microbiology Reviews* 31(4), 449–77.
- Smith, J.L., Drum, D.J.V., Dai, Y., Kim, J.M., Sanchez, S., Maurer, J.J., Hofacre, C.L. and Lee, M.D. (2007).** Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. *Applied and Environmental Microbiology* 73(5), 1404–1414.
- Souza, V., Castillo, A. and Eguiarte, L. (2002).** The evolutionary ecology of *Escherichia coli*. *American Scientist* 90(4), 332.
- Stacy-Phipps, S., Mecca, J.J. and Weiss, J.B. (1995).** Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during the course of infection. *Journal of Clinical Microbiology* 33, 1054–1059.
- Stanilova, S., Rusenova, N., Petrova, D. and Stoyanchev T. (2011).** Polymerase reaction assay for detection of enteropathogenic *Escherichia coli* strain in meat. *Trakia Journal of Science* 9(3), 51–57.
- Stecher, B. and Hardt, W. (2008).** The role of microbiota in infectious disease. *Trends Microbiol.* 16, 107-114.



- Storteboom, H., Arabi, M., Davis, J.G., Crimi, B. and Pruden, A. (2010).** Tracking antibiotic resistance genes in the South Platte River basin using molecular signatures of urban, agricultural, and pristine sources. *Environmental Science and Technology* 44(19), 7397–7404.
- Tadesse, D.A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M.J., McDermott, P.F. (2012).** Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerging Infectious Diseases* 18, 5.
- Taur, Y. and Smith, M.A. (2007).** Adherence to the Infectious Diseases Society of America guidelines in the treatment of uncomplicated urinary tract infection. *Clinical Infectious Diseases* 44(6), 769–774.
- Taxt, A., Aasland, R., Sommerfelt, H., Nataro, J. and Puntervoll, P. (2010).** Heat-stable enterotoxin of enterotoxigenic *Escherichia coli* as a vaccine target. *Infect. Immun.* 78, 1824 – 1831.
- Tenover, F.C. (2006).** Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control* 34(5), S3–S10.
- Tzipori, S., Guner, F., Donnenberg, M.S., Montigny, L., Kaper, J.B. and Donohue-Rolfe, A. (1995).** The role of the *eaeA* gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic *Escherichia coli* infection. *Infection and Immunity* 6(9), 3621–3627.
- Vaishnavi, C. (2013).** Infection of the gastrointestinal system. Jaypee Brothers Medical Publisher (P) Ltd. New Delhi, India.
- Vidal, M., Kruger, E., Duran, C., Lagos, R., Lavine, M., Prado, V., Toro, C. and Vidal, R. (2005).** Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *Journal of Clinical Microbiology* 43(10), 5362–5365.

- Webber, M.A. and Piddock, L.J.V. (2003).** The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy* 51, 9–11.
- Weintraub, A. (2007).** Enteroaggregative *Escherichia coli*: epidemiology, virulence and detection. *Journal of Medical Microbiology* 56, 4–8. doi: 10.1099/jmm.0.46930-0.
- World Health Organisation (WHO) (2015).** Antimicrobial resistance. Available on: <http://www.who.int/mediacentre/factsheets/fs194/en/>. (Accessed 29th May 2015).
- World Health Organization (WHO) (1999).** New frontiers in the development of vaccines against enterotoxigenic (ETEC) and enterohemorrhagic (EHEC) *E. coli* infections. *Weekly Epidemiological Record* 74(13), 98–100.
- Yoder, J.S., Cesario, S., Plotkin, V., MA, X., Shannon, K.K., Dworkin, M.S. (2006).** Outbreak of Enterotoxigenic *Escherichia coli* infection with an unusually long duration of illness. *Clinical Infectious Diseases* 42(11), 1513–1517. doi: 10.1086/503842.

## **CHAPTER SIX**

**Faecal coliform abundance and qPCR detection of human adenovirus, hepatitis A  
and rotavirus in discharged effluents of two wastewater treatment facilities in the  
Eastern Cape Province, South Africa**

---

This chapter was published in

Food and Environmental Virology

---

## 6.1 Abstract

The incidence of enteric viruses in reclaimed wastewater, their removal by efficient treatment processes, and the public health hazards associated with their release into aquatic environments are pertinent issues in environmental microbiology. In this study we assessed the incidences of faecal coliforms and enteric viruses including human adenovirus (HAdV), hepatitis A virus (HAV) and rotavirus (RoV) in the final effluents of two wastewater treatment plants in the Eastern Cape Province, South Africa over a period of twelve months. Faecal coliforms were detected in counts ranging from 1 CFU/100ml to  $2.7 \times 10^4$  CFU/100ml while HAdV was detected in 62.5% of the samples with concentration ranging from  $8.4 \times 10^1$  genome copies/L to  $1.0 \times 10^5$  genome copies/L. HAV and RoV were not detected in the samples analysed over the sampling period. Adenovirus species HAdV-B (serotype 2) and HAdV-F (serotype 41) were detected in 86.7 % and 6.7% of the 30 HAdV positive samples respectively. No consistent seasonal trend was observed in HAdV concentration, however, increased concentrations of HAdV was generally observed in the winter months. Also, there was no correlation between faecal coliforms counts and HAdV prevalence at both treatment plants. The persistent occurrence of HAdV in the discharged treated effluent points to the potential public health risk through the release of HAdV genomes into the receiving watersheds and the possibility of their transmission to human population.

**Keywords:** Adenovirus, faecal coliforms, environmental microbiology, wastewater, public health

## 6.2 Introduction

Globally, coliforms are still largely used as indicators of faecal contamination to assess microbiological safety and/or hence, the possible presence of pathogens in water systems. However, it is essential to note the lack of correlation that often occurs between the presence of bacterial and viral pathogens in water (DePaola *et al.*, 2010; He *et al.*, 2011) and traditional water quality indicators such as faecal coliforms give little or no information on the presence and concentrations of enteric viruses in water systems (Eifan, 2013). In spite of advances in water and wastewater treatment technologies, waterborne outbreaks still remain a major threat to human health worldwide (Okoh *et al.*, 2007; Amenu, 2014), most importantly, in developing countries where large portion of the populations still depend on untreated surface water for their immediate water needs. These surface waters are, in many instances, impacted by inadequately treated wastewater effluents, thus causing serious disturbance in their ecosystem functioning (Tyagi *et al.*, 2006). Direct and indirect exposure of populations to wastewater effluents remains a primary concern in many developing countries due to lack or non-implementation of directives regarding the microbiological quality of treated effluents (Koivunen *et al.*, 2003; Tyagi *et al.*, 2006).

The occurrence of enteric viruses in aquatic environment varied widely and depends largely upon human activities (Eifan, 2013). High concentrations of human enteric viruses are often excreted in the faeces and urine of infected individuals, and may find their ways into a variety of aquatic environment and food, particularly in areas with poor sanitary infrastructures (Cheong-Hoon and Kim, 2008; Sibanda and Okoh, 2012), and these can be transmitted back to susceptible persons to continue the cycle of disease (Rzezutka and Cook, 2004). Enteric viruses of public health importance include different groups of viruses present in the intestinal tracts of human and animals, and cause different illnesses in their host. Some

commonly studied enteric viruses and the diseases they cause are presented in Table 6.1 below.

Adenoviruses are non-enveloped icosahedral double stranded DNA virions with diameter ranging between 70-90 nm. The viral capsid consists of 240 hexons and 12 penton bases, each with a fibre protruding from the viral particle surface, giving it characteristic morphological appearance (ICTV, 2012). Human adenoviruses (HAdVs) have been linked to a wide range of community and institutional outbreaks and have been isolated from practically all human organ systems (Lynch *et al.*, 2011; Lu *et al.*, 2014). They are the second most important pathogenic viral agents of infantile gastroenteritis after rotavirus (Fong *et al.*, 2010; Haramoto *et al.*, 2010). There are presently 69 recognised genotypes of HAdV which are classified into seven species, designated species A through G based on biophysical, biochemical and genetic properties, and new genotypes are being recognised by using phylogenetic analysis based on complete genome sequencing (ICTV, 2012; Lu *et al.*, 2014).

Hepatitis A virus (HAV) is small, non-enveloped virus grouped into the genus *Hepatovirus* in the *Picornaviridae* family. HAV is a leading cause of acute viral hepatitis with annual estimated cases of about 1.5 million worldwide (Franco *et al.*, 2012). The complete genomic characterisation of HAV by sequencing of the VP1/2A junction and the VP1 gene indicated 3 genotypes (I, II and III) which can be divided into subtypes A and B (both have been described for human) while genotypes IV, V and VI have been described for primates (Coudray-Meunier *et al.*, 2014). Sanitation and socioeconomic development are two main factors determining the geographical distribution of HAV. HAV endemic region include

**Table 6.1:** Some commonly studied enteric viruses and the diseases caused.

<b>Family</b>	<b>Example</b>	<b>Disease</b>
<i>Parvoviridae</i>	Parvovirus	Gastroenteritis
<i>Coronaviridae</i>	Coronaviruses, Torovirus	Gastroenteritis, respiratory diseases
<i>Picornaviridae</i>	Poliovirus, Coxsackieviruses, Echovirus, Hepatitis A, Enteroviruses	Poliomyelitis, paralysis, meningitis, fever, Herpangina, respiratory diseases, hand- foot and mouth disease, rash, myocarditis, gastroenteritis, encephalitis, hepatitis and Guillain-Barre Syndrome
<i>Adenoviridae</i>	Adenoviruses	Gastroenteritis, conjunctivitis and respiratory diseases
<i>Reoviridae</i>	Orthoreoviruses, Rotaviruses, Orbivirus, Coltivirus	Gastroenteritis, respiratory diseases and fever
<i>Caliciviridae</i>	Noroviruses, Hepatitis E, Caliciviruses, Norwalk virus	Gastroenteritis, hepatitis and respiratory disease
<i>Astroviridae</i>	Astrovirus	Gastroenteritis
<i>Circoviridae</i>	Torque tenovirus, Torque tenoviurs-like virus	Respiratory diseases and liver diseases
<i>Picobirnaviridae</i>	Picobirnavirus	Gastroenteritis
<i>Polyomaviridae</i>	JC virus, BK virus and Simian virus 40	Leukoencephalopathy and haemorrhagic cystitis

Africa, the Middle East, India, Central and South America. HAV is mainly transmitted via faecal-oral route through human-to-human contact or by ingesting contaminated water and food, most especially shellfish, soft fruit and uncooked vegetable.

Rotavirus (RoV) is an infectious virus that causes damage to the lining of the small intestine leading to gastroenteritis. RoV is a double-stranded RNA virus belonging to the family *Reoviridae*. It is mainly transmitted through the faecal-oral route by ingesting contaminated food and fluid including water, airborne droplets and person-to-person contact. It is the most common aetiological viral agent of severe diarrhoea among infants and young children (Dennehy, 2000). Clinical presentation of rotavirus infection includes fever, vomiting and watery diarrhoea, severe dehydration and stomach pain that may last between 3 and 5 days. Based on the antigenicity and nucleotide sequence analysis of the VP6 gene, 8 different species of RoV have been identified designated species A through to G (Kindler *et al.*, 2013). RoV is shed in large concentrations by infected individual, and like other enteric viruses, is commonly found in domestic wastewater and can contaminate surface water sources. It is known to exhibit greater resistance to common disinfectant agents than most other enteric viruses and can survive in environmental water for day to weeks depending on the quality of the water and its temperature (Sattar *et al.*, 1984; Clark and Graz, 2010; Rigotto *et al.*, 2010).

The important roles water plays in the epidemiology of enteric viruses have well been acknowledged (van Heerden *et al.*, 2003; Jiang, 2006; Mena and Gerba, 2009). HAdV are present in sewage (Albinana-Gimenez *et al.*, 2009; Dong *et al.*, 2010) more than other enteric viruses, and can survive and remain infectious for prolonged period of time in environmental waters (Thurston-Enriquez *et al.*, 2003; Rodriguez-Lazaro *et al.*, 2012). HAV have also been



known to be stable in the environment and can resist disinfection, heating, pressure and low pH (Koopmans *et al.*, 2002; Koopmans and Dizer, 2004). Additionally, insufficient chlorination and reduced contact time may lead to failure in the removal of viral pathogens during disinfection due to increased resistant among enteric viruses (Fong and Lipp, 2005).

Currently, microbiological quality of water and wastewater is still largely monitored in South Africa using indicators bacteria while adequate information is lacking on the virological quality of surface and discharged wastewater effluents. Although, some studies have documented the virological qualities of some freshwater sources in other provinces like the Western Cape, Limpopo and KwaZulu Natal (Grabow *et al.*, 2004; van Heerden *et al.*, 2005, van Zyl *et al.*, 2006; Olaniran *et al.*, 2012), and a few reports on river water in the Eastern Cape Province (Sibanda and Okoh, 2012; Chigor and Okoh, 2012), while no similar report was found on discharged effluents in the province. In this paper, we reported on quantitative and qualitative detection of HAdV, HAV and RoV in the discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa and correlate the prevalence of HAdV and faecal indicator bacteria in the final effluents.

## **6.3 Materials and Methods**

### **6.3.1 Study site description and collection of samples**

The two wastewater treatment plants are located in Eastern Cape Province, South Africa and named Stutterheim Wastewater Treatment Works (SWTW) on geographical coordinates 32°34'17''S, 27°26'95''E and Keiskammahoek Wastewater Treatment Works (KWTW) on coordinates 32°41'31''S, 27°08'36''E . Both treatment plants utilise the activated sludge and sludge lagoon treatment technology, and discharge their final effluents into the Cumakala and

Keiskamma rivers respectively (DWAF, 1999). Collection of wastewater final effluents samples was done monthly (September 2012 to August 2013) from both the chlorination final effluent tanks (FE) and the discharge points (DP) of the two treatment plants. This was done to test the significance of increased chlorine contact time between the FE and DP. The distance between FE and DP at SWTW and KWTW are 23.3 m and 7.1 m respectively. The samples were collected in sterile 1.7 L bottles and transported in cooler boxes to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory, University of Fort Hare, Alice. Twenty four wastewater effluent samples were collected from each site giving a total of 48 samples over the sampling period.

### **6.3.2 Detection and enumeration of faecal coliforms**

Faecal coliforms (FC) densities were enumerated by using membrane filtration techniques according to standard methods (APHA, 1998). Appropriate serial dilutions of each of the samples were made and 100 ml from each of the dilutions was filtered through membrane filters (47-mm diameter, 0.45 µm pore size; Pall Corporation, Ann Arbor, Michigan) with the aid of a vacuum pump. The membrane filters were placed on m-FC agar (Merck, Wadeville, South Africa) and incubated at 44.5°C for 24 h. Colonies that exhibit various shades of dark blue were counted and reported as CFU/100 ml of wastewater sample analysed.

### **6.3.3 Concentration of viruses in wastewater samples and extraction of viral nucleic acids**

The adsorption-elution method previously described by Haramoto *et al.*, (2005) was used to concentrate the viral particles in the collected effluent samples with slight modifications. Samples were firstly pre-filtered using glass fibre (Millipore, Ireland) to remove debris and

reduce clogging of filter membranes. Briefly, 5 ml aliquot of 250 mM AlCl<sub>3</sub> was passed through an HA filter (0.45 µm pore size and 47 mm diameter; Millipore Ireland) to form a cation (Al<sup>3+</sup>)-coated filter, this was followed by filtering 1 L of the pre-filtered samples through the filter. Afterwards, 200 mL of 0.5 mM H<sub>2</sub>SO<sub>4</sub> (pH 3.0) was passed through the filter to remove Al<sup>3+</sup>, and the viruses were eluted with 10 mL of 1 mM NaOH (pH 10.8). The eluate was carefully recovered in a tube containing 50 µL of 100 mM of H<sub>2</sub>SO<sub>4</sub> (pH 1.0) and 100 µL of 100 × Tris-EDTA (TE) buffer for neutralisation before further concentration using Centriprep YM-50 ultrafiltration device (Millipore) to obtain a final volume of approximately 700 µL. Each final concentrated sample was aliquoted in 200 µL and stored at -80 °C until ready to use. Storing viruses at temperature below -60 °C has been proven to result in inconsequential loss of both titre and infectivity for periods longer than a decade (Gould, 1999; Merrill *et al.*, 2012). HAdV DNA was extracted from 200 µL of the concentrated samples using DNA extraction kits (Quick-gDNA™ MiniPrep; Zymo Research, USA), following the manufacturer's instruction. Purified viral DNA was eluted in 60 µL of DNA elution buffer. Extraction of the RNA viruses (HAV and RoV) was done using RNA purification kits (Quick-RNA™ MiniPrep; Zymo Research, Irvine, USA). 100 µL of the concentrated samples were extracted and eluted in a final volume of 10 µL elution buffer as instructed by the manufacturer.

#### **6.3.4 Reverse transcription of HAV and RoV genomes**

The eluted 10 µL RNA genomes were converted into complementary (cDNA) in a reverse-transcription step. The reverse-transcription step included a 20 µL (final volume) consisting of 10 µL RNA template, 1 µL of 100 µM Random Hexamer primer, 1 µL dNTP mix (10 mM each of GTP, ATP, CTP and TTP stock), 2.5 µL DEPC-treated water, 4 µL of 5 × RT buffer, 0.5 µL Ribolock RNase inhibitor and 1 µL of 200-U/µl RevertAid™ Premium reverse

transcriptase (Fermentas, Burlington, ON, Canada). The mixture was briefly vortexed and centrifuged, and the transcription was carried out in a Dri-Block DB.2A (Techne, SA) at 25 °C for 10 min followed by incubation at 60 °C for 30 min and final incubation at 85 °C for 5 min. For RoV, the RNA was initially subjected to denaturation for 5 min at 95 °C and flash chilling in ice for 2 min to separate the double-stranded of RoV prior to the reverse-transcription as previously described by Jothikumar *et al.*, (2009).

### **6.3.5 Construction of standard curves and qPCR sensitivity studies**

Standard curves were plotted following the descriptions of Haramoto *et al.* (2008). For HAdV, viral nucleic acid was extracted from ATCC VR-6 (Strain Tonsil 99) reference strain using DNA extraction kits (Quick-gDNA™ MiniPrep; Zymo Research, USA) while transcribed cDNAs from ATCC VR-1357 (Strain PA21) and ATCC VR-2274 (Strain 248) were used to construct the standard curves for HAV and RoV respectively. The extracted DNA/cDNA were quantified using a Qubit fluorometer (Invitrogen) followed by tenfold serial dilutions using nuclease-free water. The DNA/cDNA extracts from the samples and the positive control strains were subjected to qPCR simultaneously, each in triplicate. As previously described by Simmons and Xagorarakis (2011), the amplification efficiency and the detection limits of the qPCR assays were established and validated before their application to the sample extracts. The sensitivity and specificity the assays were established using nucleic acid from stock culture of HAV, RoV and HAdV DNA from seven-fold serial dilution of the genomic extracts, while a detection limit of 10 copies of target DNA per reaction was set for each qPCR assays.

### **6.3.6 TaqMan probe-based qPCR assays for the detection and quantification of HAdVs, HAV and RoV genomes**

TaqMan-based real-time PCR (qPCR) assays were run to determine the concentrations of viral in the extracted samples in a StepOnePlus System (OPTIPLEX 755, Applied Biosystems). The amplification and realtime qautification of HAdV genomes in the samples was done by amplifying the hexon gene of the virus (Xagorarakis *et al.*, 2007) while RoV detection and quantification was done by amplifying the inner capsid protein VP6 as described by Lai *et al.*, (2005). The qPCR was done in a 96-well plate by adding 5  $\mu$ L of the HAdV DNA extracts/transcribed cDNA of HAV/RoV to 20  $\mu$ L of PCR “cocktail” mixture (12.5  $\mu$ L of 2 $\times$  TaqMan universal PCR master mix, consisting of 0.05u/ $\mu$ L Taq DNA polymerase, reaction buffer, 4 mM MgCl<sub>2</sub> and 0.4 mM of each of dNTP; 400 nM forward primer; 400 nM reverse primer, 250 nM TaqMan probe and PCR grade water) in each well of the plate to make a final volume of 25  $\mu$ L per reaction (Haramoto *et al.*, 2008). All qPCR protocols were run for 45 cycles and florescence activity data was collected at the end of each PCR cycle. This was followed by SDS software (Applied Biosystems) analysis to obtain quantitative data on the concentration of viral DNA in each well. Samples’ positivity was defined by a threshold cycle (C<sub>T</sub>) value of  $\leq 35$  while the limit of detection was demonstrated to be less than 10 viral genome copies per reaction. The primer sets and probes as well as the qPCR protocols used for the detection and quantification of the viruses are given in Table 6.2.

**Table 6.2:** Specific oligonucleotide primers and probes for the qPCR detection and quantification viral genomes.

Enteric Virus	Primer Sequence (5'→3') and TaqMan Probe Label	Reaction Conditions (°C)	Reference	Control Strain
Adenovirus	JTVX (F): 5'-GGA CGC CTC GGA GTA CCT GAG-3' JTVX (R): 5'-ACI GTG GGG GTT TCT GAA CTT GTT-3' JTVX (P): 5'-FAM-CTG GTG CAG TTC GCC CGT GCC A-BHQ-3'	95°, 95°, 55°, 72° 15', 10'', 30'', 20''	Xagorarakis <i>et al.</i> , (2007)	ATCC VR-6
Hepatitis A Virus	HAV68 (F): 5'-TCA CCG CCG TTT GCC TAG-3' HAV240 (R): 5'-GGA GAG CCC TGG AAG AAA G-3' HAV150 (P): 5'-FAM-CCT GAA CCT GCA GGA ATT AA-MGBNFQ-3'	95°, 95°, 60°, 70° 10', 15'', 1', 1'	Pinto <i>et al.</i> , (2009)	ATCC VR-1357; Strain PA21
Rotavirus	JVK (F): 5'-CGA TGG TTG ATG CTC AAG ATG GA-3' JVK (R): 5'-TCA TTG TAA TCA TAT TGA ATA CCC A-3' JVK (P): 5'-FAM-ACA ACT GCA GCT TCA AAA GAA GWG T-BHQ-3'	95°, 95°, 55°, 72° 15', 15'', 30'', 30''	Jothikumar <i>et al.</i> , (2009)	ATCC VR-2274; Strain 248

Key: Abbreviations: F, forward/sense; R, reverse/antisense; p, probe; FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/nonfluorescent quencher; TAMRA, 6-carboxy-tetramethylrhodamine (quencher dye); BHQ, black hole quencher.

### **6.3.7 Characterisation of human adenovirus species and serotypes**

Samples that were positive for HAdV from the qPCR were further subjected to qualitative PCR to detect the epidemiologically important adenovirus species and serotypes. Adenovirus species and serotypes assayed for included: Ad40 and Ad41 (belonging to species F), Ad3, Ad7 and Ad21 (belonging to species B), Ad1, Ad2, Ad5 and Ad6 (belonging to species C) and Ad4 (belonging to species E). Serotype-specific PCR assays as described by Metzgar *et al.* (2005) were used to detect the various serotypes with some modifications. The PCR assays consisted of 5 µL of viral DNA added to 20 µL of reaction buffer (12.5 µL of 2× PCR master mix, 0.5 µL each of forward and reverse primers and 6.5 µL of nuclease free water) to make a final volume of 25 µL per reaction. The primer combinations and the molecular weight (in base pairs) used for the detection of the various species and serotypes are shown in Table 6.3. The amplicons were resolved on 1.5% agarose gel electrophoresis stained with ethidium bromide in TBE (Tris-borate-EDTA) buffer at 100 V for 1 h. The resolved amplicons were visualised and digitised using trans-illuminator (BioDoc-It System; UVP Upland, CA 91786, USA).

### **6.3.8 Quality Control**

Positive controls (spiked samples of known viral DNA/cDNA concentrations) and negative control (nuclease free water and PCR buffer) were included in all PCR assays. Due to extreme sensitive nature of qPCR, cross contamination of samples and amplified product were eliminated by carrying out DNA extraction and PCR assays in separate rooms. DNAZap™ (Ambion®) solution was always used to wash micropipettes before and after every PCR assay to completely degrade all DNA and RNA which might contaminate our samples, while pre-sterilised filtered racked micropipette tips were used throughout the assays.

Table 6.4 lists the adenovirus ATCC reference strains used in the study for the detection of HAdV species and serotypes.

### **6.3.9 Statistical Analysis**

Calculation of means and standard deviations were performed using One-way ANOVA (SPSS 22.0 version for Windows program). Comparison of differences in means between paired samples was done using the Paired-Samples T-test. The correlation between FC and HAdV concentrations was determined by Linear Regression analysis using HAdV concentration as dependent variable and FC count as the predictor at *P* values equal to 0.05.



**Table 6.3 Primer sets and amplicon sizes for the detection of HAdV species and serotypes**

Species	Serotypes	Primer	Sequence (5'-3')	Amplicon size	Target region	References
B	Ad3	Ad3F	GGTAGAGATGCTGTTGCAGGA	503 bp	Ad3 hexon	Metzgar <i>et al.</i> , 2005
		Ad3R	CCCATCCATTAGTGTCATCGGT			
	Ad7	Ad7F	GGAAAGACATTACTGCAGACA	311 bp	Ad7 hexon	
		Ad7R	AATTTTCAGGCGAAAAAGCGTCA			
	Ad21	Ad21F	GAAATTACAGACGGCGAAGCC	237 bp	Ad21 hexon	
		Ad21R	AACCTGCTGGTTTTGCGGTTG			
C	AdC	AdCF	TGCTTGCGCTHAAAATGGGCA		AdC fibre	Adhikary <i>et al.</i> , 2004
		Ad1R	CGAGTATAAGACGCCTATTTACA	630 bp	Ad1 fibre	
		Ad2R	CGCTAAGAGCGCCGCTAGTA	204 bp	Ad2 fibre	
	Ad5R	ATGCAAAGGAGCCCCGTAC	455 bp	Ad5 fibre		
	Ad6R	CTTGCAGTCTTTATCTGAAGCA	929 bp	Ad6 fibre		
	Ad4	Adeno4.U3	CAAGGACTACCAGGCCGTCA	254 bp	4 hexon	
F	Ad40	AdF1	ACTTAATGCTGACACGGGCAC	152 bp	AdF fibre	Xu <i>et al.</i> , 2000
		K402	CACTTAATGCTGACACG		Long fibre gene	
	Ad41	K403	ACTGGATAGAGCTAGCG			

**Table 6.4 Viral control strains used for HAdV species and serotype detection**

<b>Adenovirus serotype</b>	<b>Reference number</b>	<b>Strain</b>
Adenovirus T 21	ATCC(R) VR-256	Strain AV 1645
Human adenovirus 1	ATCC VR-1	Strain Adenoid 71
Human adenovirus 2	ATCC VR-846	Strain Adenoid 6
Human adenovirus 3	ATCC VR-3	Strain GB
Human adenovirus 40	ATCC VR-931	Strain Dugan
Human adenovirus 40	ATCC VR-1572	Strain R1-67
Human adenovirus 5	ATCC VR-1516	Type 5 Reference Material
Human adenovirus 6	ATCC VR-6	Strain Tonsil 99
Human adenovirus 7	ATCC VR-7	Strain Gomen
Human adenovirus 41	ATCC VR-930	Strain Tak (73-3544)

## **6.4 Result**

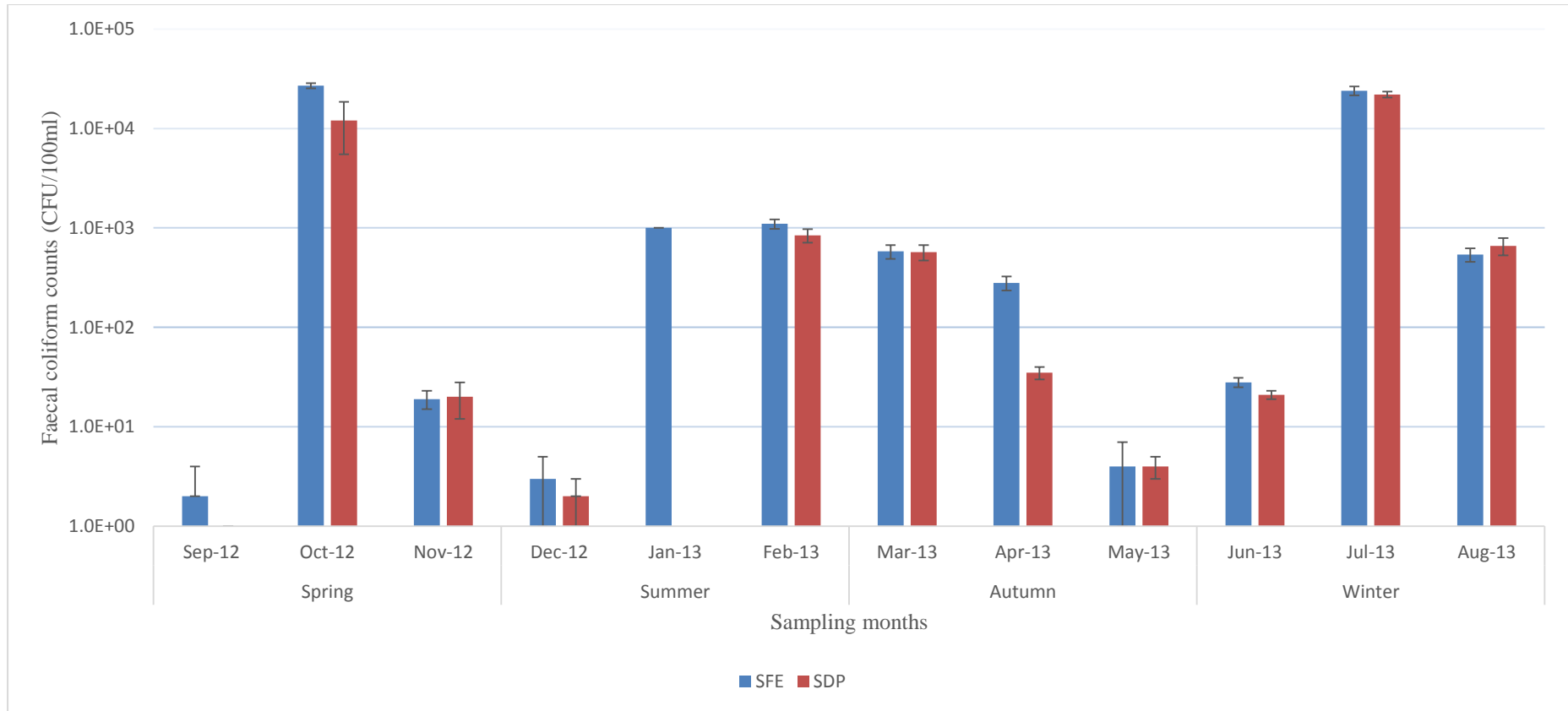
### **6.4.1 Detection and enumeration of faecal coliforms**

Ninety six percent (46/48) of the wastewater samples were positive for faecal coliforms (FC) at counts ranging from 1 CFU/100 ml to  $2.7 \times 10^4$  CFU/100 ml. The counts of FC in the samples varied widely and ranged at both sites as follows: SWTP (0 –  $2.7 \times 10^4$  CFU/100 ml) and KWTP (0 –  $8.5 \times 10^3$  CFU/100 ml). The counts also varied significantly ( $P < 0.05$ ) among the seasons with the highest FC counts (SWTP,  $2.7 \times 10^4$  CFU/100 ml and KWTP,  $8.5 \times 10^3$  CFU/100 ml) occurring in the spring months at both study sites as shown in Figures 6.1 and 6.2 below, while the lowest counts were observed in summer (January 2013) also at both sites. There appears to be a similar trend in the counts of FC at both treatment plants with the highest and the lowest FC counts occurring at spring and summer respectively. Of the 24 wastewater samples collected at SWTP, 25% (6/24) had FC counts above the 1000 CFU/100 ml for discharged final effluents as recommended by the Department of Water

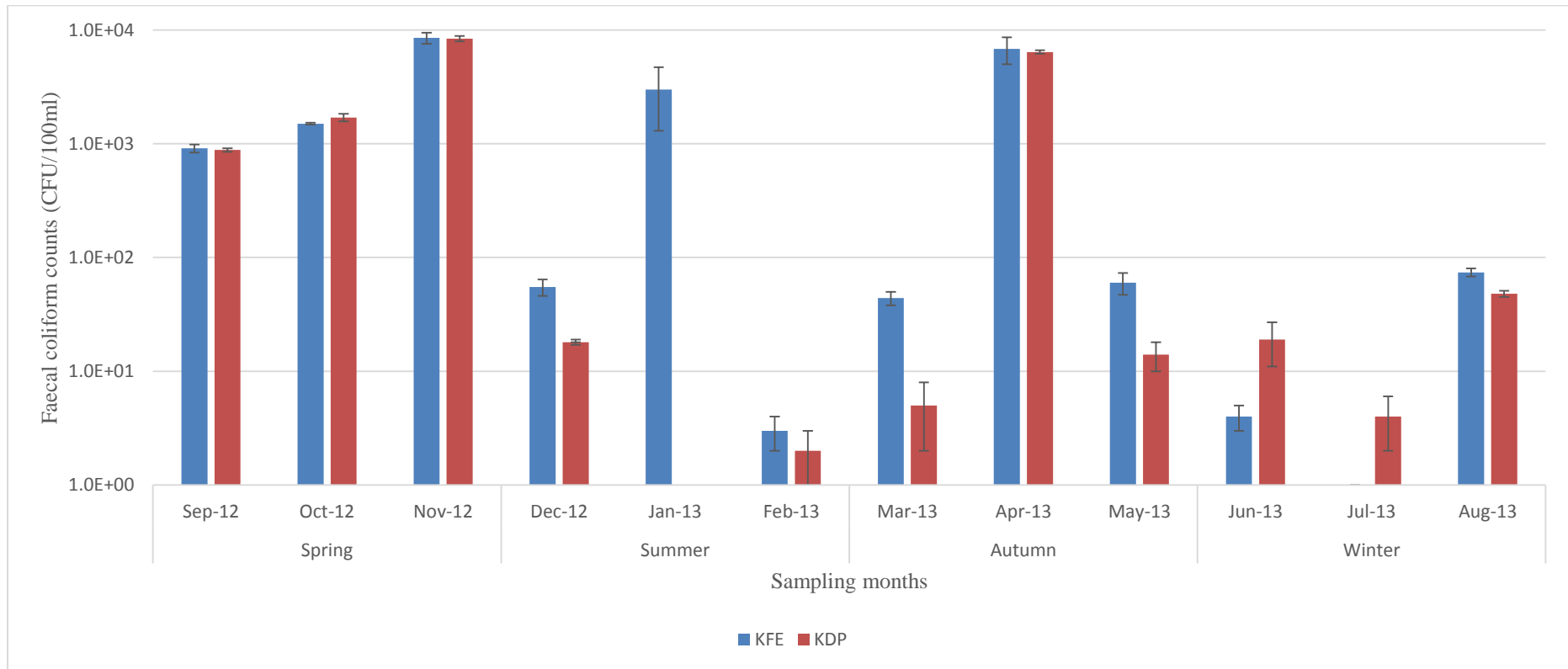
Affairs and Forestry, Republic of South Africa (DWAF, 1999) while 29.2% (7/24) were above this recommended limit at KWTP.

#### **6.4.2 Real-time PCR sensitivity and detection limits**

The sensitivity and specificity of the primers and probes used for the qPCR assays were validated using the respective viral nucleic acid extracts as templates. The control viral strains of HAdV (ATCC VR-6), HAV (ATCC VR-1357; strain PA 21) and RoV (ATCC VR-2274; strain 248) were all detected by qPCR. The resultant standard curves (HAdV, slope -3.53, Y-intercept 28.34; HAV, -2.94, Y-intercept 33.11 and RoV, slope -3.95, Y-intercept 38.94) showed strong correlation coefficients ( $R^2$ ) of 0.99 (for HAdV and HAV) and 0.97 (for RoV) respectively. Amplification efficiencies were >92% for all reactions, while no amplification was observed in the negative controls.



**Figure 6.1.** Monthly variation in FC counts in wastewater samples from SWTW (SFE: SWTW final effluent sample; SDP: SWTW discharge point sample).



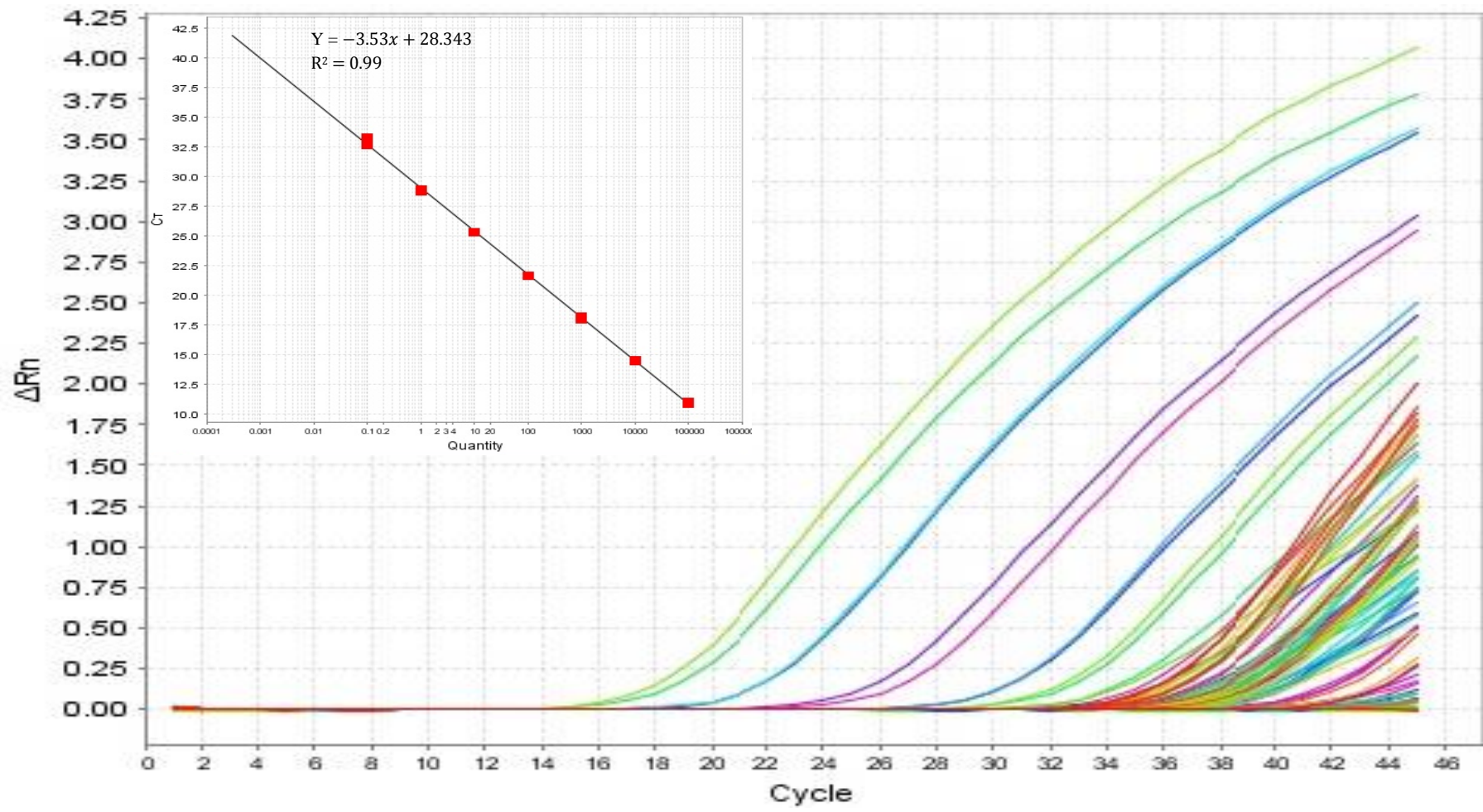
**Figure 6.2.** Monthly variation in FC counts in wastewater samples from KWTW (KFE: KWTW final effluent sample; KDP: KWTW discharge point sample).

### **6.4.3 Detection and quantification of human adenovirus genome**

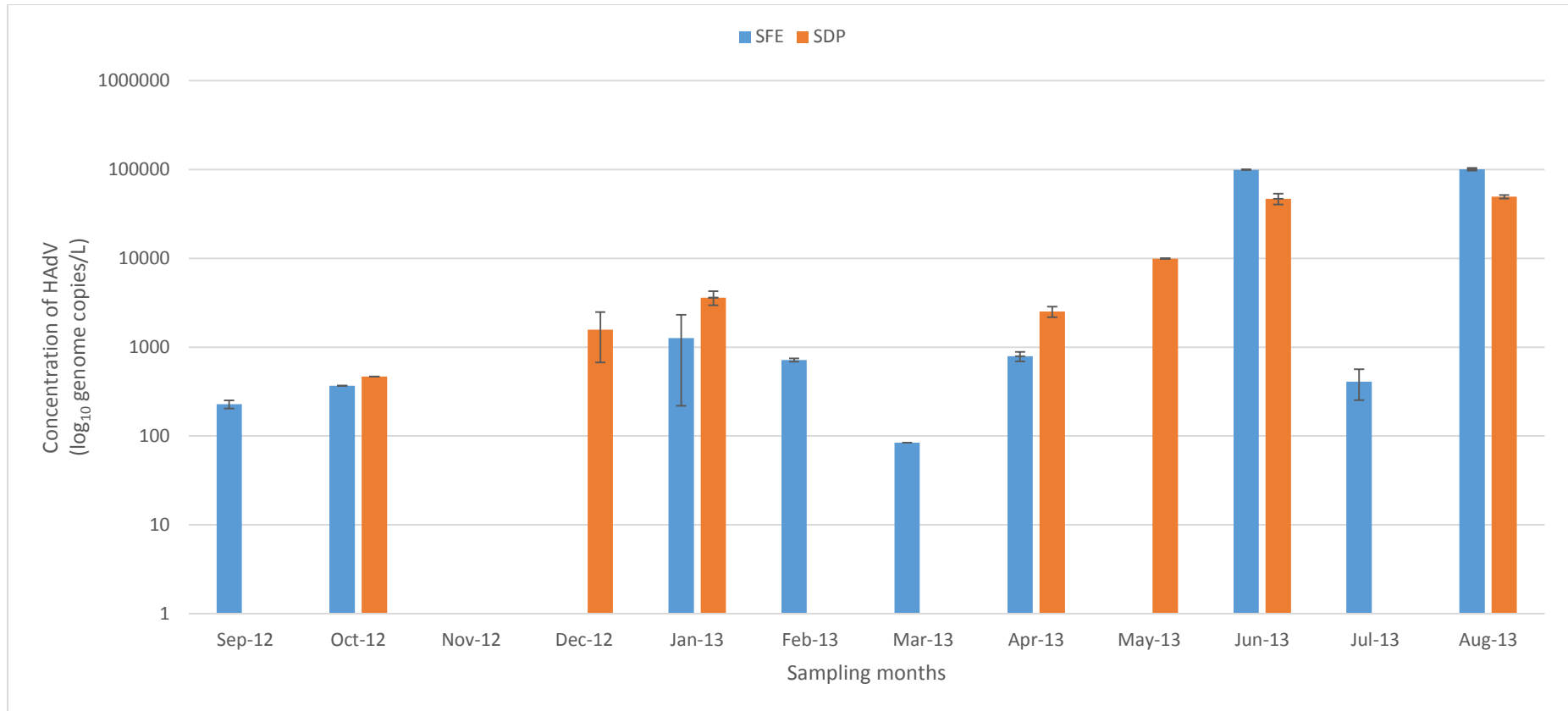
HAdV was detected in 30 of the 48 wastewater samples by qPCR giving a detection rate of 62.5% and with concentrations generally ranging between  $8.4 \times 10^1$  genome copies/L and  $1.0 \times 10^5$  genome copies/L. Of the 30 adenovirus positive samples, 16 (53.3%) were collected from SWTP (9 positive samples from SWTP final effluent (SFE) with concentration ranging between  $8.4 \times 10^1 - 1.0 \times 10^5$  genome copies/L and 7 positive samples from SWTP discharge point (SDP) ranging between  $4.7 \times 10^2 - 5.0 \times 10^4$  genome copies/L) while 14 (46.7%) were from KWTP (7 positive samples from KWTP final effluent (KFE) ranging between  $2.3 \times 10^2 - 6.6 \times 10^4$  genome copies/L and 7 positive samples from KWTP discharge point (KDP) ranging between  $2.8 \times 10^2 - 2.4 \times 10^4$  genome copies/L). Figure 6.3 shows the qPCR amplification plot for HAdV quantification together with the standard curve while the concentrations (in  $\log_{10}$  genome copies/L) of HAdV in the samples from STWP and KWTP are shown in Figures 6.4 and 6.5 respectively. No consistent seasonal trend was observed in the distribution of HAdV at both study sites over the sampling period. However, increased concentrations of HAdV were generally observed in winter at all sampling points (Figure 6.6).

### **6.4.4 Characterisation of human adenovirus virus species and serotypes**

Further characterisation of HAdV-positive samples by conventional PCR to detect epidemiologically important species and their serotypes including AdB (Ad3, Ad7, Ad21), AdC (Ad1, Ad2, Ad5, Ad6), AdE (Ad4) and AdF (Ad40, Ad41) showed predominance of species B (serotype Ad3) which was detected in 26 out of the 30 HAdV-positive samples, while species F (serotype Ad41) was detected in 2 samples (Table 6.4).

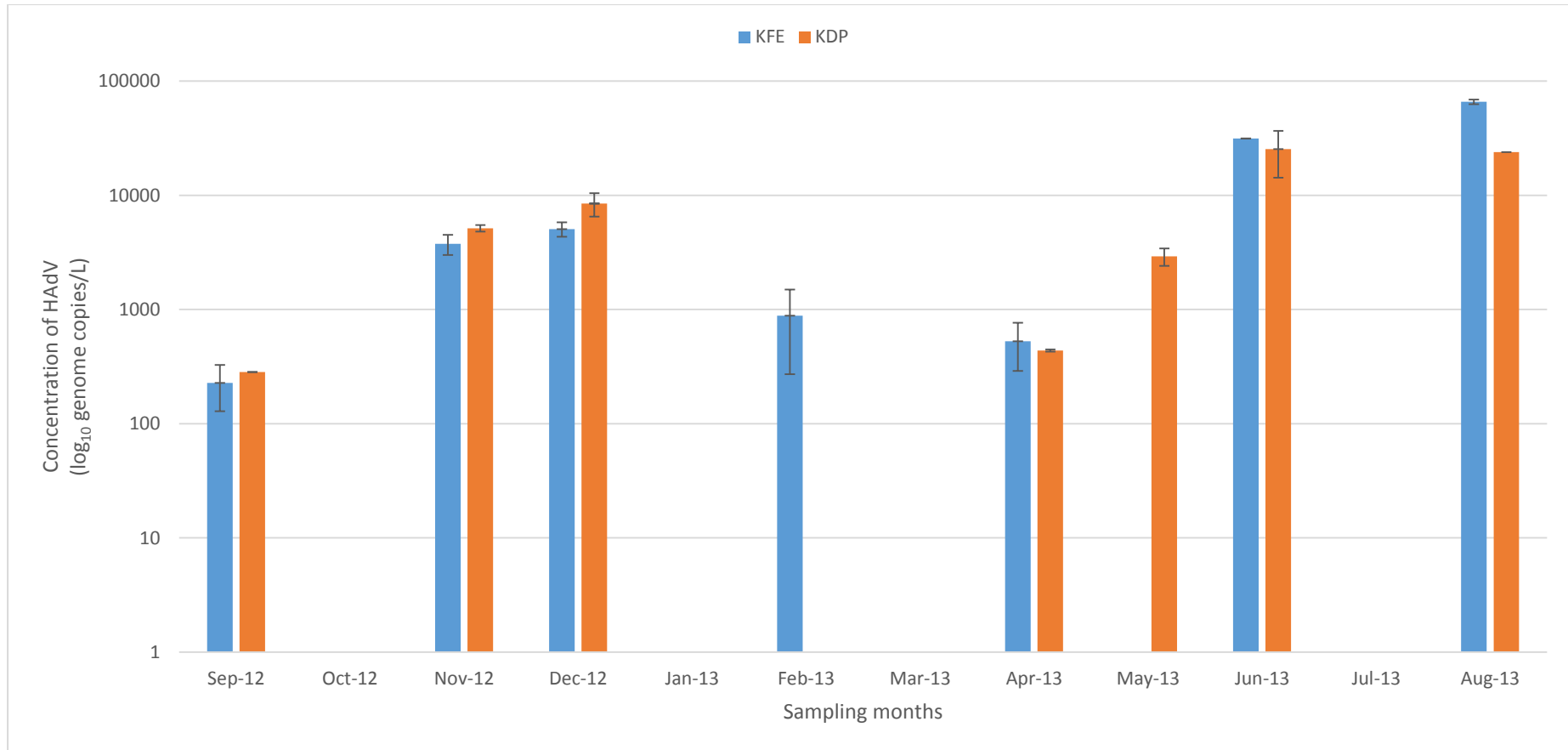


**Figure 6.3.** Amplification plot for HAdV detection and quantification from the discharged effluents samples.

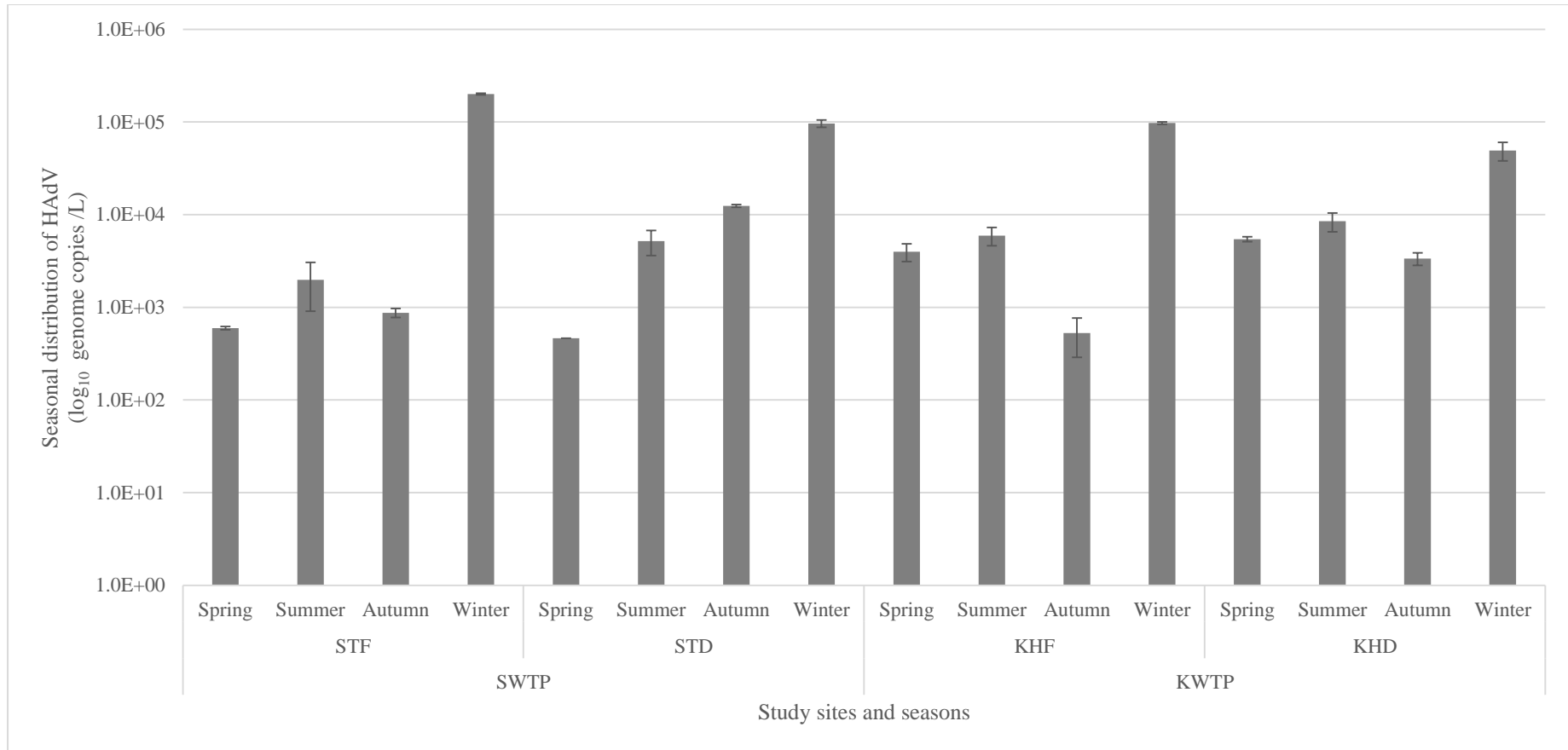


**Figure 6.4.** Monthly variation in HAdV concentrations in wastewater samples from SWTP (SFE: SWTP final effluent sample; SDP: SWTP discharge point sample).





**Figure 6.5.** Monthly variation in HAdV concentrations in wastewater samples from KWTP (KFE: KWTP final effluent sample; KDP: KWTP discharge point sample).



**Figure 6.6.** Seasonal distribution of HAdV concentration at all sampling points.

**Table 6.5** Characterisation of HAdV in the effluent samples into species and serotypes

Study site		Sampling month											
		Sept-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	May-13	Jun-13	Jul-13	Aug-13
<b>SWTP</b>	<b>STF</b>	AdB	AdB	-	-	+	AdB	AdB	+	-	AdB	AdB	AdB
	<b>STD</b>	-	AdB	-	AdB, AdF	AdB	-	-	AdB	AdB	AdB	-	AdB
<b>KWTP</b>	<b>KHF</b>	AdB	-	AdB	+	-	AdB	-	AdB, AdF	-	AdB	-	AdB
	<b>KHD</b>	AdB	-	+	AdB	-	-	-	AdB	AdB	AdB	-	AdB

Key: AdB human adenovirus serotype 3; AdF human adenovirus serotype 41; + HAdV detected by qPCR; - HAdV not detected by qPCR.

#### **6.4.5 Detection and quantification of hepatitis A virus and rotavirus**

The detection of HAV and RoV in the samples was insignificant. RoV was not detected in any of the samples throughout the sampling period while HAV were only detected at concentration  $<1$  genome copies/L, which was far below the detection limit set for viral genome amplification.

#### **6.5 Discussion**

Faecal coliforms (FC) include several bacteria genera mostly belonging to the *Enterobacteriaceae* family. A significant number of FC can circumvent wastewater treatment processes and survive for certain period of time in the aquatic environments (Kavka and Poetsch, 2002). Although, faecal indicator bacteria may not be pathogenic, their presence in water system often signify the probable presence of other faecal transmitted pathogens and reflects impairment in water quality, with increased risk of gastrointestinal and other waterborne illnesses (Ashbolt *et al.*, 2001; Bhandaram *et al.*, 2011; Sibanda *et al.*, 2013). Effluent discharges from wastewater treatment plants are the major sources of faecal indicators and other enteric pathogenic microorganisms in surface water. Growing awareness about the need for efficient use of water resources has placed emphasises on wastewater recycling as an important source of replenishing freshwater supply in South Africa (Dungeni *et al.*, 2010). However, different studies have revealed the contributions of inadequately treated effluents to the pollution of freshwater resources which sometimes serve as source of water for many rural dwellers still lacking access to pipe borne or improved water sources in South Africa (Momba *et al.*, 2006; Mema, 2009; CSIR, 2010; Naidoo and Olaniran, 2014).

Inefficient wastewater treatment process will lead to the discharge of final effluents with unacceptable microbiological quality into receiving watersheds with associated public health risk (Castro-Hermida *et al.*, 2008; Casadio *et al.*, 2010).

While our study showed higher prevalence of FC in spring than other seasons of the year, other studies have shown different prevalence and distributions of FC in some aquatic environment. In his report while working on the distribution of presumptive faecal coliforms around Rothera point, Antarctic Peninsula, Hughes (2003) reported low concentration of FC in summer and suggested this might have resulted from the biological damaging effect of solar radiation in summer. He observed high concentration of FC in winter and suggested this might be due to a combination of factors such as increased input by migrating wildlife, low solar radiation and sewage contribution. He concluded that environmental factors including solar radiation, water salinity, temperature, sea ice conditions and faecal input by human and local wildlife populations affect FC distribution. Wani *et al.* (2013) documented a greater efficiency in the removal of faecal indicator bacteria including faecal coliforms, *E. coli* and faecal streptococci in summer and autumn months, and least in winters while investigating the effect of seasonal change on the removal efficiency of a FAB (Fluidized Aerobic Bioreactor) based sewage treatment plant and the impact of the discharged effluent in the vicinity of Dal Lake.

Human adenoviruses are ubiquitous in environments where human faecal and sewage contamination has occurred and have been implicated as causative agents of persistent infections and outbreaks in drinking and recreational waters (WHO, 2005; Jiang, 2006). They have also been shown to be resistant to tertiary wastewater treatments and UV radiation, as well as chemical, physical and adverse pH conditions, which allows them to survive for a prolonged period in the environment (Thompson *et al.* 2003; Thurston-Enriquez *et al.* 2003; WHO, 2005). Proper monitoring of human enteric viruses in water system is very important, different reports have suggested that viral agents are the causative agents of approximately 50% of all known gastrointestinal infections (CDC, 1988; Choi and Jiang, 2005). In this current study, 62.5% of the wastewater effluent samples analysed was positive for HAdV with no consistent seasonal variations, however, adenovirus concentration increased generally in winter at all sampling points. The increased concentration observed in winter months may be due to favourable environmental conditions such as lower temperature compared to other seasons of the year. Lipp *et al.* (2001) reported that aquatic environment factors such as temperature, pH, ultraviolet light and salinity play important role in the survival of microorganisms and at higher temperatures inactivation of viruses occur due to the denaturation of its protein and nucleic acids while lower temperatures support viral survival for longer period.

Increased resistance of adenovirus to ultraviolet light may be as a result of the host cell DNA-repair mechanisms which tend to repair the viral double stranded DNA genome if damaged by ultraviolet light (Gerba *et al.*, 2002; Thurston-Enriquez *et al.*, 2003). Thus, HAdV may be

discharged into the aquatic environment from wastewater effluents that meet bacterial indicator standards (Jiang, 2006). The lack of seasonal variability as observed in this study is consistent with findings documented in other studies. Barrella *et al.* (2009) reported a lack of seasonal trend in the distribution of human adenovirus and hepatitis A virus in their study on the occurrence of adenovirus and hepatitis virus in raw sewage in Limeria, Sao Paulo. Other studies have equally documented high prevalence of adenoviruses from different environmental matrices (including human faeces, raw and treated wastewater, beach water, river water, seawater, shellfishes), and have suggested their use as index of faecal pollution from human sources because of their stability in the environment, host specificity, persistent infections and less or no variability in their seasonal occurrences (He and Jiang, 2005; Carducci *et al.*, 2006; Dey *et al.*, 2011; Vieira *et al.*, 2012; Bofill-Mas *et al.*, 2013).

Adenovirus serotype 3 (species B) was the predominant (86.7%) HAdV serotype detected by the qualitative PCR in this study while serotype 41 (species F) was only detected in two (6.7%) of the adenovirus positive samples and 13.3% (4/30) of the samples were not positive for any of the assayed species/serotypes. Even though, HAdV serotypes 40 and 41 have been reported as the most common and critical etiological agents of acute viral gastroenteritis in children throughout the world besides the group A rotavirus (Fukuda *et al.*, 2006; Logan *et al.*, 2006; Xagorarakis *et al.*, 2007; Carraturo *et al.*, 2008; Rezaei *et al.*, 2012). Also, in their report on the quantitative detection of adenovirus in some environmental waters in Michigan, Fong *et al.*, (2010) documented adenovirus type 3 as the least detected serotype from raw and primary effluent. They however concluded that surface water impacted by discharged

wastewater effluents may not be suitable for full-body recreational activities. The high prevalence of HAdV B as seen in this study could be an indicator of the predominant adenovirus species in circulation among the human population in our study area. Adenovirus serotype 3 together with other serotypes (including 5, 7 and 21) has been associated with adenoviral lower respiratory tract infection epidemic (LRTI) (WHO, 2005; Moro *et al.*, 2009; Alharbi *et al.*, 2012). Type 3 adenovirus has also been linked to outbreaks of conjunctivitis (Abelson and Shapiro, 2010). The detection of HAdV serotypes in the treated effluent suggests that the human population in our study area could have suffered adenovirus related illness most importantly during the sampling period.

The FC counts were not significantly ( $P < 0.05$ ) different between the FE and the DP samples at both treatment plants as shown by the Paired-Samples T-test analysis. This could be as a result of the relative short distances (23.3 m at SWTP and 7.1 m at KWTP) between the final effluent tanks and the discharge point allowing little or no further disinfection action of the residual chlorine before discharging the effluents into the receiving watershed. However, slight variations were observed for the adenovirus concentrations. The concentrations of HAdV in the samples did not correlate ( $P < 0.05$ ) with FC counts in the samples as shown by the linear regression analysis. This observation is not surprising as several other studies have highlighted the lack of correlation that exist occurs between faecal indicator bacteria and the presence of enteric viruses in water quality monitoring (Baggi *et al.*, 2001; Jiang and Chu, 2004; Jiang *et al.*, 2007; Ahmed *et al.*, 2008; Hata *et al.*, 2012). An important implication of this observed phenomenon will be that, while the discharged treated effluent complied with



recommended guideline for faecal coliform for most part of the sampling period, they however, carry high loads of human adenovirus which represent health risk to persons coming in contact direct or indirectly with these effluents.

The detection of HAV and RoV in the effluent samples was negligible over the sampling period, while RoV was not detected at all in any of the samples, HAV was only detected in two samples (4.1%) from SDP but at concentration  $<1$  genome copies/L, below our set detection limit. The nondetection of RoV as observed in this study is similar to the finding of Hot *et al.*, (2003) who reported 0% detection of rotavirus in 68 surface water samples while investigating the detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in four French rivers. A similar observation of nondetection of RoV was also made by Symonds *et al.*, (2009) while reporting of the detection of eukaryotic viruses in wastewater sample from the United State. The failure of RoV detection may suggest that individuals in the study area were not shedding this potentially pathogenic virus at the time of the study or that the RoV was completely inactivated by the disinfectant (chlorine) used at the treatment works since the viral genome were successfully amplified from the control strain. Nondetection of HAV as observed in this study is also comparable to the report of Prado *et al.*, (2012) who did not detect HAV in 24 treated effluent samples over the course of one year sampling period or urban wastewater from Rio de Janeiro, Brazil. Although HAV might have been present in our effluent samples, however their concentrations might be far below the detection limits of 10 genome copies/L set for the study.

## **6.6 Conclusion**

Monitoring of discharged final effluents of wastewater treatment plants could serve as an important approach to ensure the protection of surface waters which often serve as the receiving watershed from the impact of poorly treated effluents, give information about the prevalent pathogen(s) circulating among the human population in a given area and help in making informed decisions to protect public health. The presence of high concentrations of HAdV in the discharged final effluents signifies the inefficiency of the treatment process to adequately remove the potential pathogen and this constitutes a significant public health risk particularly among immunocompromised persons given that a significant number of rural dwellers in the study area still depend on untreated surface water for various domestic and agricultural uses. As demonstrated in this study and other related studies, real-time PCR is an important and powerful tool for rapid detection and quantification of viral nucleic acid in environmental samples. However, due to its inability to discriminate between infectious and non-infectious viral particles, it is imperative to carry out virus infectivity assays using appropriate techniques to ascertain the infectivity capabilities of the viral particles detected by the qPCR.

## References

- Abelson, M.B. and Shapiro, A. (2010).** A guide to understanding adenovirus, the diseases it cause and the best ways to treat these conditions. *Review of Ophthalmology*. Available on:  
[http://www.reviewofophthalmology.com/content/d/therapeutic\\_topics/i/1206/c/22739/](http://www.reviewofophthalmology.com/content/d/therapeutic_topics/i/1206/c/22739/)  
(Accessed 12th May 2015).
- Adhikary, A.K., Inada, T., Banik, U. and Okabe, N.J. (2004).** Identification of subgenus C adenoviruses by fibre-based multiplex PCR. *Journal of Clinical Microbiology* 42, 670-673.
- Ahmed, W., Goonetilleke, A. and Gardner, T. (2008).** Alternative indicators for detection And quantification of faecal pollution. Available on: <https://www.s3amazonaws.com>.  
(Accessed 15th May, 2015)
- Albinan-Gimenez, N., Miagostovich, M.P., Calua, B., Huguet, J.M., Matia, L. and Girones, R. (2009).** Analysis of adenoviruses and polymaviruses quantified by 1PCR as indicators of water quality in source and drinking-water treatment plants. *Water Research* 43, 1-9.
- Alharbi, S., Caesele, P.V., Consunji-Araneta, R., Zoubeidi, T., Fanella, S., Souid, A. and Alsuwaidi, A.R. (2012).** Epidemiology of severe pediatric adenovirus lower respiratory tract infections in Manitoba, Canada, 1991-2005. *BMC Infectious Diseases* 12, 55.
- Amenu, D. (2014).** Wastewater Treatment Plants As a Source of Microbial Pathogens in Receiving Watersheds. *Research Journal of Chemical and Environmental Sciences* 2, 11-19.

**American Public Health Association (APHA), American Water Works Association**

**(AWWA) and Water Environment Federation (WEA) (1998).** Standard methods for the examination of water and wastewater 20<sup>th</sup> ed. 1998. Published by American Public Health Association in Washington, DC.

**Ashbolt, N.J., Grabow, W.O.K. and Snozzi, M. (2001).** Indicators of microbial water quality, Water quality guidelines, standards and health, (L. Fewtrell and J. Bartram, Ed. IWA Publishing, London, pp 289-315.

**Baggi, F., Demarta, A. and Peduzzi, R. (2001).** Persistence of viral pathogens and bacteriophages during sewage treatment: lack of correlation with indicator bacteria. *Research in Microbiology* 152,173-751.

**Barrella, K.M., Garrafa, P., Monezi, T.A., Hársi, C.M., Salvi, C., Violante, C.P.A.B. and Mehnert, D.U. (2009).** Longitudinal study on occurrence of adenoviruses and hepatitis A virus in raw domestic sewage in the city of Limeira, São Paulo. *Brazilian Journal of Microbiology* 40(1), 102-107.

**Bhandaram, U., Guerra, A., Robertson, B., Slattery, H. and Tran, K. (2011).** Effect of urban runoff on water quality indicators in Ballona Creek, CA. UCLA Senior Practicum in Environmental Science. UCLA Institute of the Environment and Sustainability (pp. 1–28). <http://www.environment.ucla.edu/media/files/Effect-of-Urban-Runoff-ms-ncg.pdf> (Accessed 16th April 2015).

**Bofill-Mas, S., Rusinol, M., Fernandez-Cassi, X., Carratala, A., Hundesa, A. and Girones, R. (2013).** Quantification of human and animal viruses to differentiate the origin of the fecal contamination present in environmental samples. *BioMed Research*

- Carducci, A., Verani, M., Battistini, R., Pizzi, F., Rovini, E., Andreoli, E. and Casini, B. (2006).** Epidemiological surveillance of human enteric viruses by monitoring of different environmental matrices. *Water Science and Technology*, 54(3), 239-244.
- Carraturo, A., Catalani, V. and Tega, L. (2008).** Microbiological and epidemiological aspects of rotavirous and enteric adenovirus infections in hospitalized children in Italy. *New Microbiol.* 31, 329–336
- Casadio, A., Maglionico, M., Bolognesi, A. and Artina, S. (2010).** Toxicity and pollutant impact analysis in an urban river due to combined sewer overflows loads. *Water Sci Technol* 61, 207–215.
- Castro-Hermida, J.A., Garcia-Presedo, I., Almeida, A., Gonzalez-Warleta, M., Costa, J.M.C. and Mezo, M. (2008).** Contribution of treated wastewater to the contamination of recreational river area with *Cryptosporidium* spp. and *Gardia duodenalis*. *Water Research* 42(13):3528-3538.
- Centers for Disease Control and Prevention (CDC) (1988).** Water-related disease outbreaks, 1985. *Morbidity and Mortality Weekly Report* 37, 15-24.
- Cheong-Hoon, L. and Kim, S.J. (2008).** Molecular detection of human enteric viruses in urban rivers in Korea. *Journal of Microbiology and Biotechnology* 18(6), 1156-1163.
- Chigor, V.N. and Okoh, A.I. (2012).** Quantitative Detection and Characterization of Human Adenoviruses in the Buffalo River in the Eastern Cape Province of South Africa. *Food and Environmental Virology* 4, 198–208.
- Choi, S. and Jiang, S.C. (2005).** Real-time PCR quantification of human adenoviruses in

urban rivers indicates genome prevalence but low infectivity. *Applied and Environmental Microbiology* 71, 7426-7433.

**Clark, S. and Graz. M. (2010).** Rotavirus. Available on:

<http://waterbornepathogens.susana.org/menuviruses/rotaviruses> (Accessed 15th November 2015).

**Coudray-Meunier, C., Fraisse, A., Mokhtari, C., Martin-Latil, S., Roque-Afonso, A-M., and Perelle, S. (2014).** Hepatitis A virus subgenotyping based on RT-qPCR assays. *BMC Microbiology* 14, 296. doi:10.1186/s12866-014-0296-1

**CSIR (2010).** A CSIR perspective on water in South Africa – 2010. CSIR Report No.

CSIR/NRE/PW/IR/2011/0012/A. Available on:

[http://www.csir.co.za/nre/docs/CSIR%20Perspective%20on%20Water\\_2010.PDF](http://www.csir.co.za/nre/docs/CSIR%20Perspective%20on%20Water_2010.PDF)

(Accessed 17th April 2015).

**Dennehy, P.H. (2000).** Transmission of rotavirus and other enteric pathogens in the home. *Pediatr. Infect. Dis. J.* 19 (Suppl 10), S103-S105.

**DePaola, A., Jones, J.L., Woods, J., Burkhardt, W., Calci, K.R., Krantz, J.A., Bowers, J.C., Kasturi, K., Byars, R.H., Jacobs, E., Willaims-Hill, D. and Nabe, K. (2010).** Bacterial and viral pathogens in live oysters: 2007 United States Market survey. *Applied and Environmental Microbiology* 76(9), 2754-2768.

**Dey, R.S., Ghosh, S., Chawla-Sarkar, M., Panchalingam, S., Nataro, J.P., Sur, D., Manna, B. and Ramamurthy, T. (2011).** Circulation of a novel patten of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. *Journal of Clinical Microbiolgy*, 49(2), 500-505.

- Dong, Y., Kim, J. and Lewis, G.D. (2010).** Evaluation of methodology for detection of human adenoviruses in wastewater, drinking water, stream water and recreational waters. *Journal of Applied Microbiology* 108, 800-809.
- Dungeni, M., van der Merwe, R.R. and Momba, M.N.B. (2010).** Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water SA*, 36(5), 607-614.
- Department of Water Affairs and Forestry, Republic of South Africa. (DWAF) (1999).** Department of Water Affairs and Forestry, South Africa. General Authorisations in terms of Section 39 of the National Water Act, 1998 (Act No. 36 of 1998). *Government Gazette No. 20526*, 8 October 1999. DWAF, Pretoria. Available on: <http://www.dwaf.gov.za/Documents/Notices/GenAuthPublished-eng.pdf>. (Accessed 15th April 2015).
- Eifan, S.A. (2013).** Enteric viruses and aquatic environment. *The Internet Journal of Microbiology* 12(1). Available on: <http://ispub.com/IJMB/12/1/2926> (Accessed on 23rd March 2015).
- Fong, T.T. and Lipp, E.K. (2005).** Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiol. Molecular Biology Reviews* 69, 357–371.
- Fong, T.T., Phanikumar, M.S., Xagorarakis, I. and Rose, J.B. (2010).** Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. *Applied and Environmental Microbiology* 76(3), 715-723.

- Franco, E., Meleleo, C., Serino, L., Sorbara, D. and Zaratti, L. (2012).** Hepatitis A: epidemiology and prevention in developing countries. *World Journal Hepatology* 4, 68-73.
- Fukuda, S., Kuwayama, M., Takao, S. Shimazu, Y and Miyazaki, K. (2006).** Molecular epidemiology of subgenus F adenoviruses associated with pediatric gastroenteritis during eight years in Hiroshima Prefecture as a limited area. *Archive of Virology* 151, 2511-2517.
- Gerba, C.P., Gramos, D.M. and Nwachuku, N. (2002).** Comparative Inactivation of Enteroviruses and Adenovirus 2 by UV Light. *Applied and Environmental Microbiology*, 68(10), 5167-5169.
- Gould, E.A. (1999).** Methods for long-term virus preservation. *Molecular Biotechnology* 13(1), 57–66.
- Grabow, W.O.K., Taylor, M.B. and Ehlers, M.M. (2004).** Executive summary of research on assessment of the risk of infection associated with viruses in South African drinking water supplies. Water Research Commission. Available on: <http://www.wrc.org.za/> (Accessed 13th March 2015).
- Haramoto, E., Katayama, H., Oguma, K. and Ohgaki, S. (2005).** Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa River in Japan. *Applied and Environmental Microbiology* 71(5), 2403–2411.
- Haramoto, E., Katayama, H., Utagawa, E. and Ohgaki, S. (2008).** Development of sample storage methods for detecting enteric viruses in environmental water. *Journal of Virological Methods* 151, 1–6.



- Haramoto, E., Kitajima, M., Katayama, H. and Ohgaki, S. (2010).** Real-time PCR detection of adenoviruses, polyomaviruses, and torque teno viruses in river water in Japan. *Water Research* 44(6), 1747-1752.
- Hata, A., Kitajima, M. and Katayama, H. (2012).** Occurrence and reduction of human viruses, F-species RNA coliphage genogroups and microbial indicators at a full-scale wastewater treatment plant in Japan. *Journal of Applied Microbiology* 114, 545-554.
- He, J.W. and Jiang, S. (2005).** Quantification of enterococci and human adenoviruses in environmental samples by real-time PCR. *Applied and Environmental Microbiology*, 71(5), 2250–2255.
- He, X., Wei, Y., Cheng, L., Zhang, D. and Wang, Z. (2011).** Molecular detection of three gastroenteritis viruses in urban surface waters in Beijing and correlation with levels of fecal indicator bacteria. *Environmental Monitoring and Assessment* 184, 5563-5570.
- Hot, D., Legeay, O., Jacques, J., Gantzer, C., Caudrelier, Y., Guyard, K., Lange, M. and Andreoletti, L. (2003).** Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. *Water Research* 37, 4703-4710.
- Hough, H.S., Liang, S., Chen, C.M., Keith, J., Echavarria, M., Sanchez, J.L., Kolavic, S.A, Vaughn, D.W. and Binn, L.N. (2002).** Rapid type-specific diagnosis of adenovirus type 4 infection using a hexon-based quantitative fluorogenic PCR. *Diagnostic Microbiology and Infectious Diseases* 42, 227-236.
- Hughes, K.A. (2003).** Influence of seasonal environmental variables on the distribution of presumptive faecal coliforms around an Antarctic Research Station. *Applied and Environmental Microbiology* 69(8), 4884–4891.

- International Committee on Taxonomy of Viruses (ICTV) (2012).** King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. (eds): Virus Taxonomy 9th Report of the International Committee on Taxonomy of Viruses. New York: Elsevier, 2012; 131–132.
- Jiang, S.C. (2006).** Human adenovirus in water: occurrence and health implications: a critical review. *Environmental Science and Technology* 40, 7132-7140.
- Jiang, S.C. and Chu, W. (2004).** PCR detection of pathogenic viruses in southern California urban rivers. *Journal of Applied Microbiology*, 97, 17–28.
- Jiang, S.C., Chu, W. and He, J. (2007).** Seasonal detection of human viruses and coliphage in Newport Bay, California. *Applied and Environmental Microbiology* 73(20), 6468–6474.
- Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. (2005).** Quantitative real-time PCR assays for the detection of human adenovirus and identification of serotypes 40 and 41. *Applied and Environmental Microbiology* 71(6), 3131-3136.
- Kavka, G. and Poetsch, E. (2002).** Microbiology. In: Joint Danube Survey – Technical report of the International Commission for the Protection of the Danube River. 138-150.
- Kindler, E., Trojnar, E., Heckel, g., Otto, P.H. and Johne, R. (2013).** Analysis of rotavirus species diversity and evolution including the newly determined full-length genome sequences of rotavirus F and G. *Infection, Genetics and Evolution* 14, 58-67.

- Koivunen, J., Siitonen, A. and Tanski, H.H. (2003).** Elimination of enteric bacteria in biological chemical wastewater treatment and tertiary filtration units. *Water Research* 37, 690- 698.
- Koopmans, M. and Duizer, E. (2004).** Foodborne viruses: an emerging problem. *International Journal of Food Microbiology* 90, 23-41.
- Koopmans, M., von Bondorff C.H., Vinje, J., de Medici, D. and Monroe, S. (2002).** Foodborne viruses. *FEMS Microbiology Review* 26, 187-205.
- Lai, H.C., Lin, S.J., Lin, H.R., Ku, C.S., Wang, L. and Yang, C.C. (2005).** Phylogenetic analyses of human rotavirus in central Taiwan in 1996, 2001 and 2002. *Journal of Clinical Virology* 32(3), 199–217.
- Lipp, E., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S. and Rose, J. (2001).** The effects of seasonal variability and weather on microbial faecal pollution and enteric pathogens in a subtropical estuary. *Estuaries and Coasts*, 24:(2), 266-276. doi: 10.2307/1352950
- Logan, C., O’Leary, J., and O’Sullivan, N. (2006).** Real-time reverse transcription-PCR for detection of rotavirus and adenovirus as causative agents of acute viral gastroenteritis in children. *Journal of Clinical Microbiology* 44, 3189–3195.
- Lu, Q.B., Tong, Y.G., Wo, Y., Wang, H.Y., Liu, E.M., Gray, G.C., Liu, W. and Caob, W.C. (2014).** Epidemiology of human adenovirus and molecular characterization of human adenovirus 55 in China, 2009–2012. *Influenza and Other Respiratory Viruses* 8(3), 302–308.
- Lynch, J.P., Fishbein, M., Echavarria, M. (2011).** Adenovirus. *Seminars in Respiratory and Critical Care Medicine* 32, 494–511.

- Mema, V. (2009).** Impact of poorly maintained wastewater and sewage treatment plants: lessons from South Africa. Council for Scientific and industrial Research (CSIR) Pretoria, South Africa. Available on: [http://www.ewisa.co.za/literature/files/335\\_269%20Mema.pdf](http://www.ewisa.co.za/literature/files/335_269%20Mema.pdf) (Accessed 17 April 2015).
- Mena, K.D. and Gerba, C.P. (2009).** Waterborne adenovirus. *Reviews of Environmental Contamination and Toxicology* 298, 133-167.
- Merrill, D. R., Wade, C.D., Fahnestock, P. and R. O. Baker (2012).** Long-term and short-term stability of viruses depend on storage temperature and preservation methods. Available on: <http://www.beiresources.org/Portals/2/PDFS/Long-Term%20and%20Short-Term%20Stability%20of%20Viruses.pdf>. (Accessed 27th April 2015).
- Metzgar, D., Osuna, M., Yingst, S., Rakha, M., Earhart, K., Elyan, D., Esmat, H., Saad, M.D, Kajon, A, Wu, J., Gray, G.C., Ryan, M.A. and Russell, K.L. (2005).** PCR analysis of Egyptian respiratory adenovirus isolates, including identification of species, serotypes, and coinfections. *Journal of Clinical Microbiology*, 43(11), 5743-5752.
- Momba, M.N.B., Osode, A.N. and Sibewu, M. (2006).** The impact of inadequately wastewater treatment on the receiving water bodies – Case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *Water SA* 32(5), 687-692.
- Moro, M.R., Bonville, C.A., Suryadevara, M., Cummings, E., Faddoul, D., Kobayaa, H., Branigan, P.J. and Domachowske, J.B. (2009).** Clinical features, adenovirus types,

and local production of inflammatory mediators in adenovirus infections. *The Pediatric Infectious Diseases Journal* 28(5), 376-380.

**Naidoo, S. and Olaniran, A.O. (2014).** Treated wastewater effluent as a source of microbial pollution of surface water resources. *International Journal of Environmental Research and Public Health* 11(1), 249-270. doi: 10.3390/ijerph110100249.

**Okoh, A.I., Odjadjare, E.E., Igbiosa, E.O. and Osode, A.N. (2007).** Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. *African Journal of Biotechnology* 6(25), 2932-2944.

**Olaniran, A.O., Naidoo, S. and Pillay, B. (2012).** Surveillance of invasive bacterial pathogens and human enteric viruses in wastewater final effluents and receiving water bodies – a case study from Durban, South Africa. *Clean – Soil, Air, Water* 2012, 40 (7), 681–691.

**Pinto, R.M., Costafreda, M.I. and Bosch, A. (2009).** Risk assessment in shellfish-borne outbreaks of hepatitis A. *Applied and Environmental Microbiology*, 75(23), 7350-7355.

**Prado, T., Fumian, T.M., Miagostovich, M.P. and Gaspar, M. (2012).** Monitoring the hepatitis A virus in urban wastewater from Rio de Janeiro. *Transactions of Royal Society of Tropical Medicine and Hygiene* 106, 104-109.

**Rezaei M, Sohrabi A, Edalat R, Saidat SD, Gomari H, Rezaei M and gilani SM (2012).** Molecular epidemiology of acute gastroenteritis caused by subgenus F (40, 41) enteric adenoviruses in inpatient children. *LabMedicine*, 43, 10-15.

- Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C.K., Correa, A.A., Souza, D.S., Miagostovich, M.P., Simoes, C.M. and Barardi, C.R. (2010).** Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. *Journal of Applied Microbiology* 109, 1979-1987.
- Rodriguez-Lazaro, D., Cook, N., Ruggeri, F.M., Sellwood, J., Nasser, A., Nascimento, M.S., D'Agostino, M., Santos, R., Saiz, J.C, Rzezutka, A., Bosch, A., Girones, R., Carducci, A., Muscillo, M., Kovac, K., Diez-Valcarce, M., Vantarakis, A., von Bonsdorff, C.H., de Roda Husman, A.M., Hernandez, M. and van der Poel, W.H. (2012).** Virus hazards from food, water and other contaminated Environments. *FEMS Microbiol Reviews* 36, 786–814.
- Rzezutka, A. and Cook, N. (2004).** Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews* 28, 441–453.
- Sattar, S.A., Rapheal, R.A. and Springthorpe, V.S. (1984).** Rotavirus survival in conventionally treated drinking water. *Canadian Journal of Microbiology* 30(5), 653-656.
- Sibanda, T. and Okoh, A.I. (2012).** Assessment of the incidence of enteric adenovirus species and serotypes in surface water in the Eastern Cape Province of South Africa: Tyume River as a study case. *The Scientific World Journal* (2012), Article ID 949216, 9 pages doi:10.1100/2012/949216.
- Sibanda, T., Chigor, V.N. and Okoh, A.I. (2013).** Seasonal and spatio-temporal distribution of faecal-indicator bacteria in Tyume River in the Eastern Cape Province, South Africa. *Environmental Monitoring Assess* 185, 6579–6590. DOI 10.1007/s10661-012-3048-4.

- Simmons, F.J. and Xagorarakis, I. (2011).** Release of infectious human enteric viruses by full scale wastewater utilisties. *Water Research* 45, 3590-3598.
- Symonds, E.M., Griffin, D.W. and Breitbart, M. (2009).** Eukaryotic viruses in wastewater samples from the United State. *Applied Environmental Microbiology* 75, 1402-1409.
- Thompson, S.S., Jackson, J.L., Suva-Castillo, M., Yanko, W.A., El Jack, Z., Kuo, J.C.C., Williams, F.P. and Schnurr, D.P. (2003).** Detection of infectious human adenoviruses in tertiary-treated and ultraviolet-disinfected wastewater. *Water Environment Research* 75(2), 163–170.
- Thurston-Enriquez, J.A., Haas, C.N., Jacangelo, J., Riley, K. and Gerba, C.P. (2003).** Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology* 69, 577-582.
- Tyagi, V.K., Chopra, A.K., Kazmi, A.A., Kuma, A. (2006).** Alternative microbial indicators of faecal pollution curret perspective. *Iran Journal Environmental Health Science and Engineering*, 3(3), 205-216.
- van Heerden J, Ehlers MM, Heim A and Grabow WOK (2005).** Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Journal of Applied Microbiology* 99, 234–242.
- van Heerden J, Ehlers MM, Van Zyl WB and Grabow WOK (2003).** Incidence of adenovirus in raw and treated water. *Water Research* 37(15), 3704-3708.
- van Zyl, W.B., Page, N.A., Grabow, W.O.K., Steele, A.D. and Taylor, M.B. (2006).** Molecular epidemiology of group A rotaviruses in water sources and selected raw vegetables in Southern Africa. *Applied and Environmental Microbiology* 72, 4554–4560.

**Vieira CB, Mendes ACO, Guimaraes FR, Fumian TM, Leite JPG, Gaspar AMC and**

**Miagostovich MP (2012).** Detection of enteric viruses in recreational waters of an urban lagoon in the city of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, 107(6), 778-784.

**Wani, D., Pandit, A.K., Kamili, A.N. (2013).** Microbial assessment and effect of seasonal

change on the removal efficiency of FAB based sewage treatment plant. *Journal of Environmental Engineering and Ecological Science* 2013, 2:1.  
dx.doi.org/10.7243/2050-1323-2-1

**World Health Organisation (WHO) (2005).** Water recreation and disease. Plausibility of

associated infections: acute effects, sequelae and mortality Pond K. Published by IWA Publishing, London, UK. ISBN: 1843390663.

**Xagorarakis, I., Kuo, D.H.W., Wong, K., Wong, M. and Rose, J.B. (2007).** Occurrence of

Human Adenoviruses at Two Recreational Beaches of the Great Lakes. *Applied Environmental Microbiology*, 73(24): 7874-7881.

**Xu, W., McDonough, M.C. and Erdman, D.D. (2000).** Species-specific identification of

human adenoviruses by a multiplex PCR assay. *Journal of Clinical Microbiology*, 38(11), 4114-4120.



# **CHAPTER SEVEN**

## **General Discussion and Conclusion**

## 7.1 Discussion

Freshwater is a finite and precious resource that is vital for the survival of life. Clean and safe water supplies, as well as adequate sanitation are essential for the protection of human health and have been underlined as basic human rights; ensuring the availability of these fundamental needs would contribute immensely to well-being and productivity for development. As demands for freshwater sources continue to grow due to rapid urbanisation population and economic growth, changing trade policies and climate change, many countries are beginning to experience water shortage along with its attendant problems. Degrading water quality also contributes to water shortage challenge in many countries and therefore, there is need to address both water quantity and water quality management issues together (Bjorklund, 2001; Abbarpour, 2011).

Currently, South Africa is experiencing a water crisis which has mainly been linked to decreased water flow and degrading water quality. Poor operational and maintenance of many of the existing wastewater treatment facilities has been noted as an important contributor to water quality degradation in the country (Mema, 2009; Okoh *et al.*, 2015; Adefisoye and Okoh, 2016). Discharged wastewater effluents may harbour potentially pathogenic organisms which can compromise human health; nutrients which can cause eutrophication in surface water; and other physicochemical substances that can adversely impact the environment (FAO, 1992; Naidoo and Olaniran, 2014). Assessment of municipal wastewater discharged effluents is very important in order to protect the

environment and safeguard public health. However, data on many of the existing wastewater treatment facilities in South Africa remain sketchy and poorly coordinated.

While water/wastewater might be erroneously adjudged clean and safe through the use of indicator organisms, studies continue to highlight the shortcomings of indicator organisms as proxy for the presence of pathogenic bacteria and viruses in water quality assessment (Ashbolt *et al.*, 2001; Payment *et al.*, 2003; Cabral, 2010; Levy *et al.*, 2012; Odonkor and Ampofo, 2013; Douterelo *et al.*, 2014).

*E. coli* is one of the bacteria that forms the normal gastro-intestinal microflora of human and other warm blooded animals, where it establishes a commensal (often beneficial) relationship with the host organism. But, some adapted strains of *E. coli* have acquired virulence capabilities and can cause pathological conditions both in the intestinal and extra-intestinal anatomical sites of humans (Katouli, 2010). *E. coli* is prominent bacterial cause of diverse forms of diarrhoea ranging from self-limiting to acute to chronic forms of the disease. While the extra-intestinal diseases caused by *E. coli* mainly include UTIs, neonatal meningitis and septicaemia (Kaper *et al.*, 2004).

Results from the current study confirmed that *E. coli* is capable of surviving the various wastewater treatment processes, including disinfection, and be released directly into surface water. The prevalence of *E. coli* in the final effluents analysed varied widely throughout the

twelve month sampling period of this study. The densities of presumptive *E. coli* in the final effluents generally ranged between 1 CFU/100ml and  $1.2 \times 10^5$  CFU/100ml. Even though the counts fell within the recommend limit of  $10^3$  CFU/100ml (faecal coliform standard in discharged effluents, DWAF, 2004) in some of the sampling months, it is worthy of note that the counts exceeding this limit were detected in about 37.5% (18/48) of the effluent samples analysed. As observed in the study, KWWTP (site 2) performed much better than SWWTP in terms of *E. coli* removal as only 29.2% (7/24) of the samples from KWWTP exceeded the faecal coliform set limit while 41.6% (10/24) samples from SWWTP had counts above the set limit. A similar result to our findings was recoded by Hendricks and Pool (2012) while studying the effectiveness of treatment processes on the removal of faecal pathogen (including *E. coli*) and antibiotic residues in wastewater samples from three sewage treatment plants in Western Cape, South Africa.

Molecular identification of the presumptive *E. coli* using the house keeping *uidA* gene yielded a positive reaction rate of 76.2% (381/500). Further characterisation of 223 of the confirmed *E. coli* isolates into the various pathotypes by means of virulence genes detection; using PCR, revealed 5 different pathotypes with detection as follows: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%), and UPEC (41.7%) while 26.9% (60/223) of the isolates were not positive for the tested pathotypes. As seen in the study, ExPEC (56.5%) strains of *E. coli* (NMEC and UPEC) were detected at higher frequencies than InPEC (16.6%) strains. Similar trends of detection of ExPEC at higher rates compared to InPEC in final effluents have been reported by other studies (Mokracka *et al.*, 2011; Frigon *et al.*,

2013) suggesting that factors in aquatic environment, including discharged wastewater effluents, may be more favourable for the survival of ExPEC strains of *E.coli* in relation to InPECs' survival. Our finding largely suggests that the discharged effluents from the understudied treatment plants may impact the receiving watershed by contributing to *E. coli* density of the rivers which in turn present a risk to the individual that may rely on these rivers for their domestic and agricultural usage.

*Vibrio cholerae* and other species of vibrio are now known as autochthonous resident of aquatic environment rather than human pathogens that transiently reside in the aquatic habitat (Lutz *et al.*, 2013). Vibrios have been detected in vast geographical regions ranging from the tropics to the temperate (Hug *et al.*, 2005; Collin and Rehnstam-Holm, 2011; de Magny *et al.*, 2011; Tall *et al.*, 2013). Several studies have also documented the presence of vibrios in discharged municipal effluents (Igbiosa *et al.*, 2009; Canigral *et al.*, 2010; Dungeni *et al.*, 2010). The findings of this current study revealed the survival of potentially pathogenic vibrio bacteria in all the 48 discharged effluent samples analysed, with concentrations varying from 4 CFU/100ml to  $1.4 \times 10^4$  CFU/100ml. Although there is no specific limit set for the densities of vibrio bacteria in the South Africa discharged effluent guidelines, nevertheless, using the faecal coliform limits of  $10^3$  CFU/100ml as basis for comparison, 23% (11/48) of the effluent samples had vibrio counts higher than the faecal coliform set limit. In contrast to our observation for *E. coli*, SWWTP showed better efficiency in terms of vibrio removal from the discharged effluents than KWWTP. Approximately 17% (4/24) of the effluent samples from SWWTP had vibrio counts higher than  $10^3$  CFU/100ml, while at

KWWTP about 29.2% (7/24) of the sample had vibrio counts more than the limit. The detection of *Vibrio* spp. throughout the sampling period indicates the ability of the bacteria to circumvent the treatment processes thus, their release into environmental water where they may be pathogenic to aquatic organisms, and may constitute health risk to human population upon exposure. Although the highest counts of both *E. coli* and *Virbio* species were generally observed in the spring months (September, October, November), there was no consistent seasonal trend in the distribution of these bacterial groups.

The emergence and spread of drug resistance among commensals and pathogenic bacteria has been a subject of major discussion to public health globally. Numerous studies have documented increasing trends in the emergence and spread of resistance to established drugs/antibiotics over the past decades (Charles *et al.*, 1985; Alonso *et al.*, 1999; Levy, 2002; White *et al.*, 2008; van Schaik, 2015). While much surveillance studies have been carried out on the use of antibiotics, as well as the emergence and proliferation of drug resistance among bacteria in the clinical settings, lesser attention has been paid towards the aquatic resistome. With much evidences pointing toward wastewater treatment plants as important reservoirs for wide spectrum of antibiotic resistance genes, it is important to assess discharged effluents for the presence of antibiotic resistant bacteria and their associated resistance determinants.

In this study, the antibiogram of 205 PCR-confirmed vibrio isolates against 18 antibiotics revealed antibiotic resistance frequencies ranging between 0.5% and 96.1%. Approximately

81% (166/205) of the vibrio isolates exhibited multidrug resistance (that is resistance against 3 or more antibiotics). The most common multiple antibiotic resistance phenotype observed among the vibrio isolates was AP-T-TM-SMX-PG-NI-PB which occurred in 8 of the isolates, while the multiple antibiotic indices (MARI) estimates at the two study side were 0.35 (SWWTP) and 0.33 (KWWTP). Also, the antibiogram profiles of the confirmed *E. coli* isolates in this study showed varying resistance frequencies ranging between 1.7% and 70.6% against 15 out of the 17 test antibiotics. About 32.7% of the test *E. coli* isolates exhibited multidrug resistance with the commonest multiple antibiotic resistance phenotype being AP-T-CFX; detected in 12 isolates. The multiple antibiotic resistance indices estimates for the confirmed *E. coli* isolates from the two treatment plants were 0.23 (SWWTP) and 0.24 (KWWTP). Even though the MARI estimates for the *E. coli* isolates were lower than the estimated values for *Vibrio* spp., all the calculated MARI were above the arbitrary set point of 0.2 (Krumperman, 1983), and thus implies the contamination of the effluent samples by antibiotics as identified in the isolates (Christopher *et al.*, 2013). The observed MARI values suggest the exposure of the isolates (both *E. coli* and *Vibrio* spp.) to antibiotic pressures in the wastewater treatment plants' environment.

The molecular analysis of antibiotic resistance determinants (ARDs) in the resistance-profiled *E. coli* isolates shows the presence of 11 different genes which may confer resistance against different classes of antibiotics. The mostly detected ARDs were those conferring resistance against tetracycline class of antibiotics, these including: *tetM* (68.6%), *tetD* (50%), *tetC* (42.2%), *tetA* (30.4%), *tetB* (28.4%) and *tetK* (11.8%). Two sets of resistance genes each

conferring resistance against aminoglycosides (*strA* and *aadA*) and penicols (*catI* and *cmlA1*) classes of antibiotics were detected in the isolates while *bla*TEM which confers resistance to  $\beta$ -Lactams was detected in 56.4% of the resistant the isolates. Findings similar to our observations have been reported elsewhere (Bailey *et al.*, 2010; Momtaz *et al.*, 2012); this suggesting the possible occurrence of selective pressure for the emergence and spread of antibiotic resistance genes among bacterial populations from the two study sites. This might also be a pointer to the potential occurrence of other resistance conferring genes and antibiotic-resistance strains of several other potentially pathogenic bacteria that were not assessed in this study

Human enteric viruses may be introduced into the environment on daily basis through the release of untreated or inadequately treated wastewater effluents. Discharged wastewater effluents may contain high concentrations of different types of viruses depending on the season, the types of viruses circulating among the population of a geographical area and virus inactivation during wastewater treatment processes (Paradise *et al.*, 2013). Typically, enteric viruses have been detected in effluents using cell cultures which are often expensive and laborious. Additionally, some epidemiologically important viruses do not have effective cell lines for their propagation making their detection difficult. Because of these shortcomings, the use of nucleic acid based techniques such as PCR and its various modified forms have being largely used to study the prevalence of enteric viruses in environmental samples including discharged wastewater effluents (Formiga *et al.*, 2005; Paradise *et al.*, 2013).



In this study, we utilised the real-time PCR (qPCR) technique to assess the incidence of some enteric viruses of public health importance in the discharge final effluents of two wastewater treatment plants over one year (monthly) sampling period. Human adenovirus (HAdV) was detected in 62.5% (30/48) of the effluent samples analysed with concentrations ranging between  $8.4 \times 10^1$  and  $1.0 \times 10^5$  genome copies/L while hepatitis A virus and rotaviruses were not detected in any of the samples. HAdV was detected in 16/24 of the samples collected from SWWTP with concentration ranging between  $8.4 \times 10^1$  and  $1.0 \times 10^5$  genome copies/L while 14/24 of the samples from KWWTP tested positive for HAdV with concentrations ranging from  $2.3 \times 10^2$  to  $6.6 \times 10^4$  genome copies/L. Even though HAdV was detected mostly in the winter months (June, July, August) during this study, it should be noted that there was no consistent seasonal trend in the occurrence of HAdV in the final effluents at both study sites. Also, there was lack of correlation between the occurrence of faecal indicator bacteria and HAdV in the samples, further confirming the unsuitability of using indicator bacteria as surrogate for assessing virological quality of effluents/water.

Species B (serotype Ad3) of HAdV was the most prevalent and was detected in 26/30 of the HAdV-positive samples while specie F (serotype Ad41) was the other serotype detected, and was detected in only 2 samples. HAdV species B and particularly, serotype 3 and 7 have been reported to be globally distributed and responsible for outbreaks of ocular and respiratory tract diseases among civilian populations, with clinical manifestation ranging from influenza-like fever to pneumonia and death (Marttila *et al.*, 2005; Metzgar *et al.*, 2005; 2007). Species F on the other hand have mainly been linked to gastrointestinal diseases mostly in children

(Wilhelmi *et al.*, 2003; Chmielewicz *et al.*, 2005). The detection of HAdV in the final effluents during the study suggests the possible circulation of this epidemiologically important enteric virus among the human population in the study area.

Monitoring of physicochemical characteristics of wastewater effluents is important in order to evaluate its quality, and identify possible impairment of the water quality of the receiving watersheds, and also to protect the environment and human health (Okoh *et al.*, 2007; Chigor and Okoh, 2012). In this study, twelve physicochemical parameters were monitored in the final of two treatment plants over twelve months. Some of these parameters were noted to be in compliance with set standards for discharged wastewater effluents in South Africa, these including: pH, total dissolved solids, temperature, electrical conductivity, nitrite and dissolved oxygen. Other parameters including: turbidity, biological oxygen demand, nitrate, phosphate, free chlorine and chemical oxygen demand fell short of recommended guidelines. The non-compliance of the effluents with set guidelines for nitrate and phosphate could be a source of concern in the receiving watershed as these two compounds are known to contribute to high nutrient loading or eutrophication at high concentrations. An important factor that might have contributed to the production of effluents that did not comply with set limits for nitrate and phosphate concentrations was the overworking of the treatment plants (particularly KWWTP) at levels beyond their designed capacities which sometimes leads to breakdown of some of the installations.

Turbidity is a measure of suspended organic and inorganic particles in water system. Microorganisms are usually associated with high concentrations of organic matters; therefore high turbidity may support the survival of organisms in discharged effluents. The turbidity measurements at both study plants varied widely across the sampling months. At SWWTP, the turbidity ranged between 1.5 NTU and 24.7 NTU while higher turbidity values ranging between 6.13 NTU and 65.70 NTU were recorded at KWWTP. KWWTP was particularly noted for excessive turbidity levels throughout the study period as the discharged effluents at this site appear “green” possibly as a result of cyanobacteria growth in the final effluent tanks. Although there is no set limit for turbidity in discharged effluent in South Africa, however, using the World health organisation recommended limit of <5 NTU (WHO, 2008), the effluents turbidity values at both plants were far above this limit. The high turbidity observed in this study might impact the receiving watershed negatively and further contribute to the survival of potentially pathogenic microorganisms in the watershed.

Chlorination remains the most widely used disinfection method in wastewater treatment in South Africa and the discharged effluent guidelines specify a value of 0.25 mg/L for free chlorine concentration in discharged effluents. The sole aim of chlorination is to inactivate pathogens from wastewater before discharging into the environment. To achieve this, the secondary treated effluent is exposed to sufficient chlorine concentration for optimum contact time which is about 30 min. The free chlorine concentration regimes observed in this study varied widely and generally ranged between 0.08 mg/L and 0.72 mg/L at both study sites. The concentrations of free chlorine ranged between 0.10 mg/L and 0.49 mg/L at SWWTP

and ranged between 0.08 mg/L and 0.68 mg mg/L at KWWTP. The free chlorine concentrations monitored in the study did not comply with the set guideline for most part of the sampling period, therefore making the effluent unfit for discharge into waterways. Also it should be noted that, while the free chlorine concentrations were high in some of the months, they failed to eliminate the bacterial pathogens in the effluent sufficiently. This phenomenon was particularly noted at KWWTP. Some studies (Obi *et al.*, 2008; Igbinsosa *et al.*, 2009) have also reported similar trends of survival of bacterial even in effluent with sufficient chlorine concentrations calling for more effecient methods of disinfection in other to inactivate all potentially pathogenic microorganisms that may have escaped previous wastewater treatment stages.

## **7.2 Conclusions**

The hypothesis for this study was that wastewater treatment plants are contributors to the microbiological and physicochemical pollution of fresh water sources in the Eastern Cape Province. Indeed, our findings revealed that effluent qualities of the two treatment plants understudied failed to meet most set guidelines for effluent discharged into freshwater environment in South Africa. This ultimately suggests the impairment of the quality of the receiving waterbodies, thus rendeing them unfit for immediate human use. The study also revealed the survival of potentially pathogenic *E. coli*, *Vibrio* and human adenoviruses (and possibly other pathogens that were not included in this study), suggesting the ability of these organisms to circumvent treatment processes and be discharged into freshwater environment where the may present risk to human and animal populations upon exposure. Also, the

detection of antibiotic resistance determinants may be a pointer to the presence of selective pressure for the emergence and spread of resistance among the bacterial communities in the receiving waterbodies thus presenting a public health concern. In view of the above findings, there is need for interventions and better management strategies for municipal effluents discharges as well as freshwater resources in order to protect the precious freshwater sources and also to forestall further environmental degradation caused by the discharge of poor quality effluents into waterways and also safeguard public health.

### **7.3 Recommendations**

The following could be suggested, based on the findings of this study:

1. There is need for efficient monitoring of existing wastewater treatment plants in order to ensure their compliance with the existing laws and regulations guiding their operations.
2. There should be periodical technical training programmes for persons handling the wastewater treatment processes to enhance their competence and to expose them to new innovations in water/ wastewater treatment technologies.
3. There is need for upgrading of some of the existing wastewater treatment plants as the rapid population growths has placed pressure on them, causing them to operate at levels above their designed capacities.
4. It also important that proper surveillance programmes are put in place to monitor the occurrence pathogenic organisms in the aquatic environment to forestall any possible

waterborne outbreaks that may result from the discharge of effluents into surface waters.

5. Future studies should incorporate cell infectivity assays in order to know the infectious capacity of the enteric viruses detected in this study.
6. Risk assessment studies must be done for exposure to the discharge effluents of wastewater treatment plants in the Eastern Cape Province and South Africa at large.

## References

- Abbaspour, S. (2011).** Water Quality in Developing Countries, South Asia, South Africa, Water Quality Management and Activities that Cause Water Pollution. International Conference on Environmental and Agriculture Engineering IPCBEE (15) IACSIT Press, Singapore.
- Adefisoye, M.A. and Okoh, A.I. (2016).** Identification and resistance prevalence of pathogenic *Escherichia coli* strained from treated wastewater effluents in Eastern Cape, South Africa. *MicrobiologyOpen* doi: 10.1002/mbo3.319. [Epub ahead of print]
- Alonso, A., Campanario, E. and Martinez, J.L. (1999).** Emergence of multidrug-resistant mutants is increased under antibiotic selective pressure in *Pseudomonas aeruginosa*. *Microbiology* 145, 2857-2862.
- Bailey, J.K., Pinyon, J.L., Anantham, S. and Hall, R.M. (2010).** Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. *Journal of Medical Microbiology* 59, 1331–1339. doi: 10.1099/jmm.0.022475-0.
- Bjorklund, G. (2001).** Water management in developing countries – policy and priorities for EU development cooperation. Stockholm International Water Institute, SIWI Report 12. Stockholm, Sweden.
- Cabral, J.P.S. (2010).** Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health* 7, 3657-3703.

- Canigral, I., Moreno, Y., Alonso, J.L., Gonzalez, A. and Ferrus, M.A. (2010).** Detection of *Vibrio vulnificus* in seafood, seawater and wastewater samples from a Mediterranean coastal area. *Microbiological Research* 165, 657-664.
- Charles, I.G., Harford, S., Brookfield, J.F. and Shaw, W.V. (1985).** Resistance to chloramphenicol in *Proteus mirabilis* by expression of a chromosomal gene for chloramphenicol acetyltransferase. *Journal of Bacteriology* 164, 114-122.
- Chmielewicz, B., Benzler, J., Pauli, G., Krause, G., Bergmann, F. and Schweiger, B. (2005).** Respiratory disease caused by a species B2 Adenovirus in a military camp in Turkey. *Journal of Medical Virology* 77 (2), 232-237.
- Christopher, A.F., Hora, S. and Ali, Z. (2013).** Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of *Escherichia coli* isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly-India. *Annals of Tropical Medicine and Public Health* 6, 285–289.
- Collin, B. and Rehnstam-Holm, A.S. (2011).** Occurrence and potential pathogenesis of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* on the South Coast of Sweden. *FEMS Microbiology Ecology* 78, 306-313.
- de Magny, G.C., Mozumder, P.K., Grim, C.J., Hasen, N.A., Naser, M.N., Alam, M., Sack, R.B., Hug, A. and Colwell, R.R. (2011).** Role of zooplankton diversity in *Vibrio cholerae* population dynamics and in the incidence of cholera in the Bangladesh Sundarbans. *Applied and Environmental Microbiology* 77, 6125-6132.



**Department of Water Affairs and Forestry Republic of South Africa (DWAF) (2004).**

Government Gazette No. 20526, 8th October 1999. Revision of General Authorisations in Terms of Section 39 of the National Water Act, 1998 (Act no. 36 of 1998). Available on: <http://www.ewisa.co.za/misc/WWManage/defaultStandards.htm>. (Accessed 26 January 2016).

**Douterelo, I., Boxall, J.B., Deines, P., Sekar, R., Fish, K.E. and Biggs, C.A. (2014).**

Methodological approaches for studying the microbial ecology of drinking water distribution systems. *Water Research* 65, 134-156.

**Dungeni, M., van der Merwe, R.R. and Momba, M.N.B. (2010).**

Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa. *Water SA* 36(5), 513-529.

**Food and Agriculture Organization of the United Nations (FAO). (1992).**

Wastewater treatment and use in agriculture – irrigation and drainage paper 47. Produced by Natural Resources Management and Environmental Department. URL: <http://www.fao.org/docrep/T0551E/t0551e00.htm>. (Accessed 26 January 2016).

**Formiga-Cruz M, Hundesa A, Clemente-Casares P, Albiñana-Gimenez N, Allard A.**

**and Girones, R. (2005).** Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage. *Journal of Virology Methods* 125: 111-118.

**Frigon, D., Biswal, B.K., Mazza, A., Masson, L. and Gehr, R. (2013).**

Biological and physicochemical wastewater treatment processes reduced the prevalence of virulent *Escherichia coli*. *Applied and Environmental Microbiology* 79(3):835-844.

- Hendricks, R. and Pool, E.J. (2012).** The effectiveness of sewage treatment processes to remove faecal pathogens and antibiotic residues. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*.
- Hug, A., Sack, R.B., Nizam, A., Longini, I.M., Nair, G.B., Ali, A., Morii, J.G. Jr., Khan, M.N., Siddique, A.K., Yunus, M., Albert, M.J., Sack, D.A. and Colwell, R.R. (2005).** Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. *Applied and Environmental Microbiology* 71, 4645-46.
- Igbinosa, E.O., Obi, C.L. and Okoh, A.I. (2009).** Occurrence of potentially pathogenic vibrios in the final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. *Research in Microbiology* 160, 531–537.
- Kaper, J.B., Nataro, J.P., Mobley, H.L. (2004).** Pathogenic *Escherichia coli*. *Nature Reviews Microbiology* 2, 123–140.
- Katouli, M. (2010).** Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections. *Iran Journal of Microbiology* 2, 59-72.
- Krumperman, P.H. (1983).** Multiple Antibiotic Resistance Indexing of *Escherichia coli* to identify high-risk of sources of faecal contamination of foods. *Applied and Environmental Microbiology* 46(1), 165–170.
- Levy, K., Nelson, K.L., Hubbard, A. and Eisenberg, J.N.S. (2012).** Rethinking indicators of microbial drinking water quality for health studies in tropical developing countries: case study in Northern Coastal Ecuador. *The American Journal of Medicine and Hygiene* 86, 499-507.

- Levy, S.B. (2002).** Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy* 49, 25-30.
- Lutz, C., Erken, M., Noorian, P., Sun, S. and McDougald, D. (2013).** Environmental reservoirs and mechanisms of persistence of *Vibrio cholerae*. *Frontiers in Microbiology* 4, 375. <http://doi.org/10.3389/fmicb.2013.00375>
- Marttila, M., Persson, D., Gustafsson, D., Liszewski, M.K., Atkinson, J.P., Wadell, G. and Arnberg, N. (2005).** CD46 is a cellular receptor for all species B adenoviruses except Types 3 and 7. *Journal of Virology*, 79(22), 14429–14436.
- Mema, V. (2009).** Impact of poorly maintained wastewater and sewage treatment plants: lessons from South Africa. Council for Scientific and Industrial Research (CSIR) Pretoria, South Africa, 2009.
- Metzgar, D., Osuna, M., Kajon, A.E., Hawksworth, A.W., Irvine, M. and Russell, K.L (2007).** Abrupt emergence of diverse species B adenovirus at US military recruit training centers. *Journal of Infectious Diseases* 196, 1465-1473.
- Metzgar, D., Osuna, M., Yingst, S., Rakha, M., Earhart, K., Elyan, D., Esmat, H., Saad, M.D, Kajon, A, Wu, J., Gray, G.C., Ryan, M.A. and Russell, K.L. (2005).** PCR analysis of Egyptian respiratory adenovirus isolates, including identification of species, serotypes, and coinfections. *Journal of Clinical Microbiology*, 43(11), 5743-5752.
- Mokracka, J., Koczura, R.; Jablonska, L., Kaznowski, L.A. (2011).** Phylogenetic groups, virulence genes and quinolone resistance of integron-bearing *Escherichia coli* strains

- isolated from a wastewater treatment plant. *Antonie Van Leeuwenhoek* 99, 817–824.
- Momtaz, H., Rahimi, E. and Moshkelani, S. (2012).** Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. *Veterinarni Medicina* 57(4), 193–197
- Naidoo, S. and Olaniran, A.O. (2014).** Treated wastewater effluent as a source of microbial pollution of surface water resources. *International Journal of Environmental Research and Public Health* 11(1), 249-270.
- Obi, C.L., Igumbor, J.O., Momba, M.B.N., and Samie, A. (2008).** Interplay of factors involving chlorine dose, turbidity flow capacity and pH on microbial quality of drinking water in small treatment plants. *Water SA* 34, 565-572.
- Odonkor, S.T. and Ampofo, J.K. (2013).** *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiology Research*, 4, e2.
- Okoh, A.I., Odjadjare, E.E., Igbinosa, E.O. and Osode, A.N. (2007).** Wastewater treatment plants as a source of microbial pathogens in the receiving watershed. *African Journal of Biotechnology* 6, 2932-2944.
- Okoh, A.I., Sibanda, T., Nongogo, V., Adefisoye, M., Olayemi, O.O. and Nontongana, N. (2015).** Prevalence and characterisation of non-cholerae *Vibrio* spp. in final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implication for public health. *Environmental Science and Pollution Research International* 22, 2008-2017.

- Parasidis TA, Konstantinidis TG, Alexandropoulou IG (2013).** Environmental Monitoring of Enteric Viruses in Wastewater. *Virology & Mycology* 2: e106. doi:10.4172/21610517.1000e106
- Payment, P., Waite M. and Dufour, A. (2003).** Introducing parameters for the assessment of drinking water quality. *In*, Assessing microbial safety of drinking water improving approaches and methods. WHO & OECD, IWA Publishing, London, UK. P 47-77.
- SouthAfrica.info (2015).** South Africa tackles water issues. Available on: <http://www.southafrica.info/> (Accessed 23 January 2016).
- Tall, A., Hervio-Heath, D., Teillon, A., Boisset-Helbert, C., Delesmont, R., Bodilis, J. and Touron-Bodilis, A. (2013).** Diversity of *Vibrio* spp. isolated at ambient environmental temperature in the Eastern English Channel as determined by pyrH sequencing. *Journal of Applied Microbiology* 114, 1713-1724.
- Van Schaik, W. (2015).** The human gut resistome. *Philosophical Transactions of the Royal Society B* 370, 20140087. <http://dx.doi.org/10.1098/rstb.2014.0087>
- White, W., Cuny, C., Klare, I., Nubel, U., Strommenger, B. and Wemer, G. (2008).** Emergence and spread of antibiotic-resistant Gram-positive bacterial pathogens. *International Journal of Medical Microbiology* 298, 365-377.
- Wilhelmi, I., Roman, E., Sanchez-Fauquier, A. (2003).** Viruses causing gastroenteritis. *Clinical Microbiology and Infection* 9, 247-262.

**World Health Organisation (WHO) (2008).** Guidelines for Drinking Water Quality. Third edition. Geneva, Switzerland.

# Appendix

## Appendix I: Photographs showing some of the sampling points



A. Collecting final effluent sample from SWWTP final effluent tanks



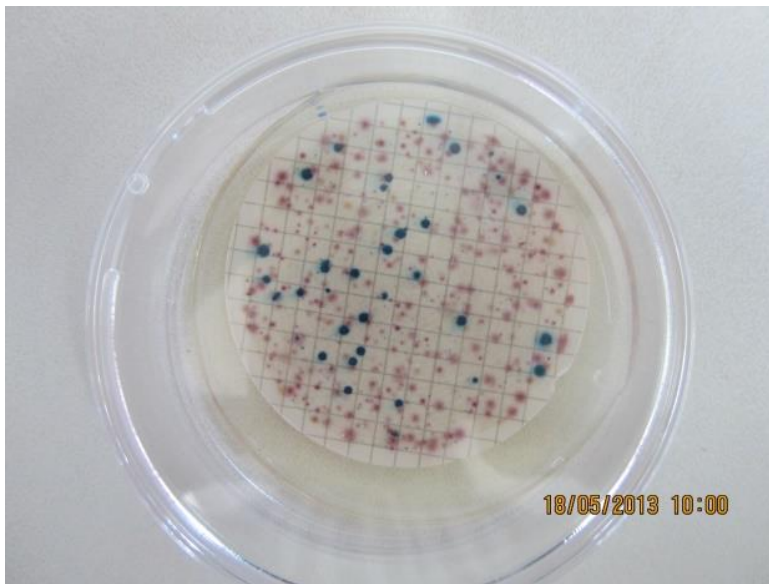
B. Students carrying out physicochemical analysis of final effluents



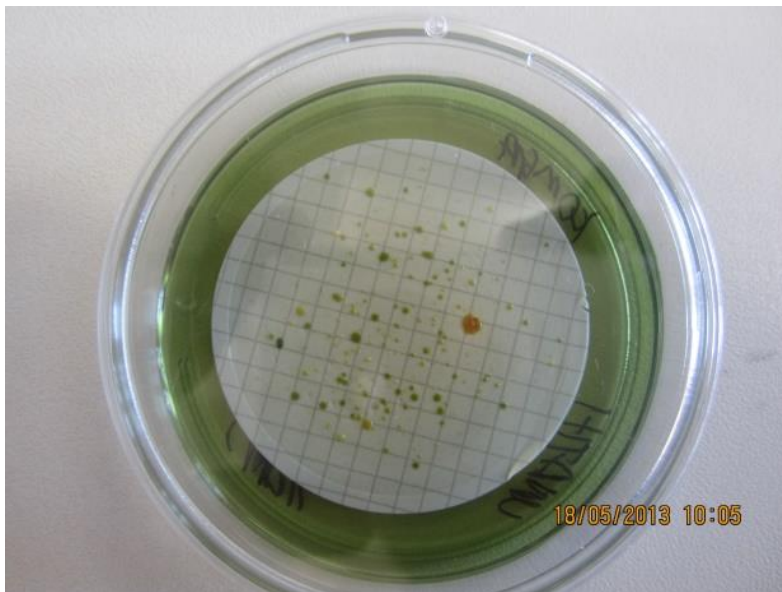
C. Student using membrane filtration set up for bacteriological analysis of effluent samples.



**Appendix II: Some Photographs of membrane filtration and antibiogram plates**

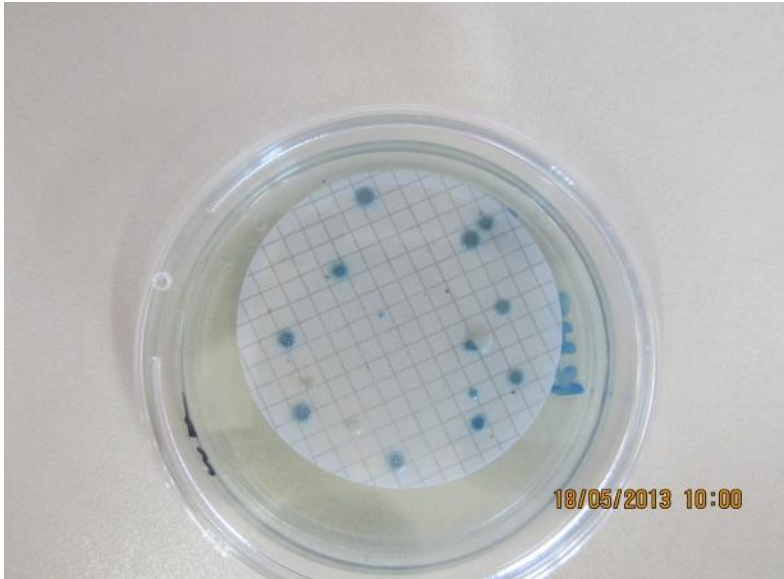


A. Presumptive *E. coli* (shade of blue) isolates as detection on *E. coli* chromogenic medium.



B. Presumptive *Vibrio* species as detected on TCBS agar



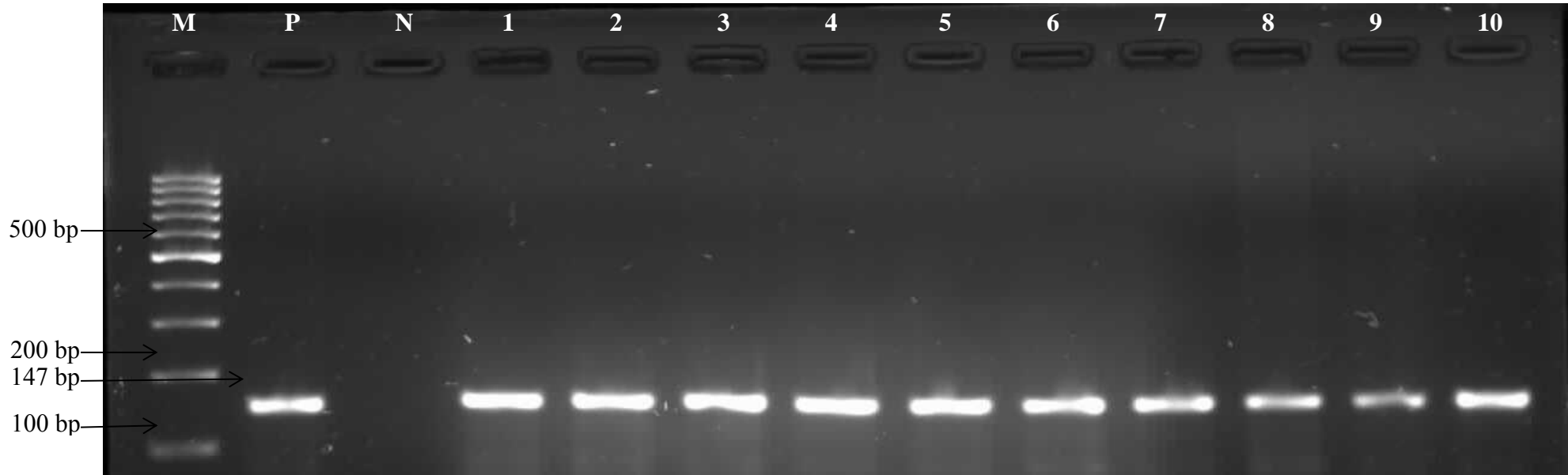


C. Faecal coliforms as detected on m-FC agar.

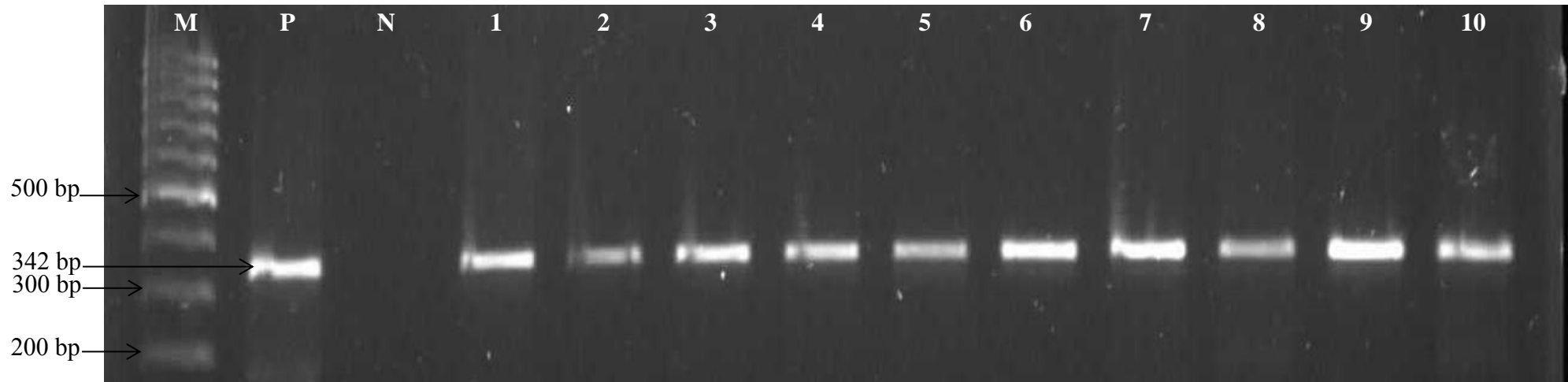


D. Presentative antibiotic susceptibility plates for some *E. coli* isolates tested.

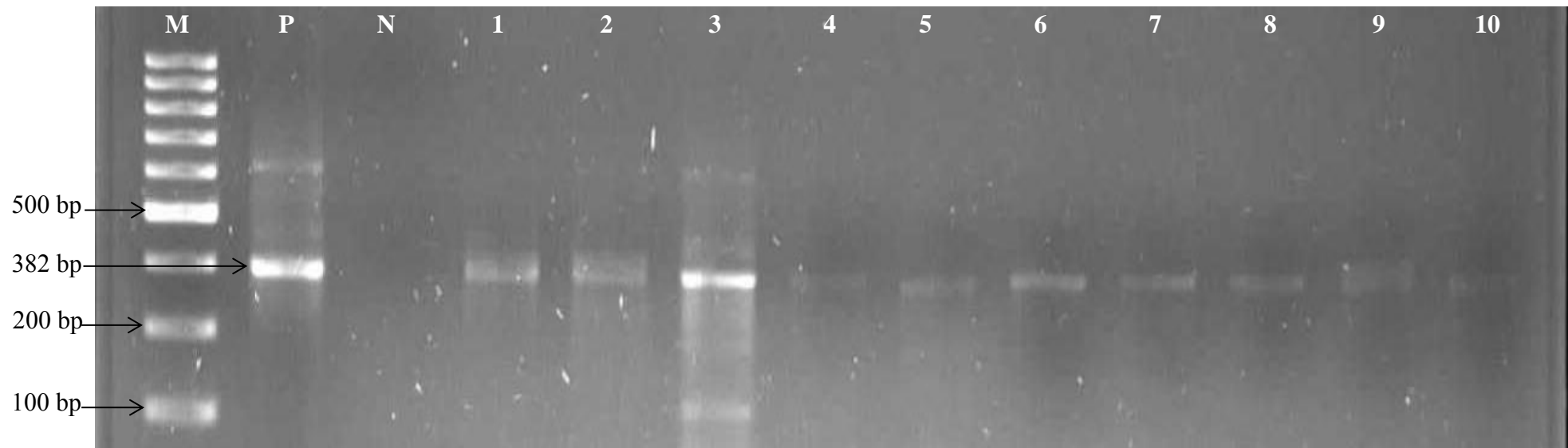
**Appendix III: Representative agarose gel electrophoresis images of some of the characterisation of *E. coli* isolates.**



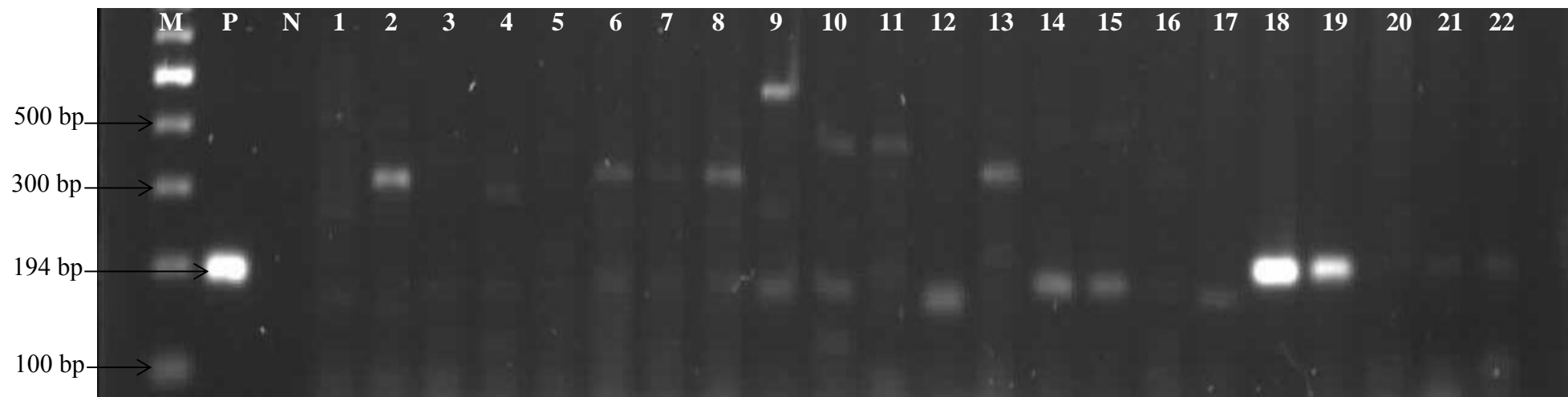
**Figure III a.** Molecular confirmation of *E. coli* isolates. Lane M: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* ATCC 25922 strain); lane N: negative control; lanes 1 to 10 *E. coli* isolates.



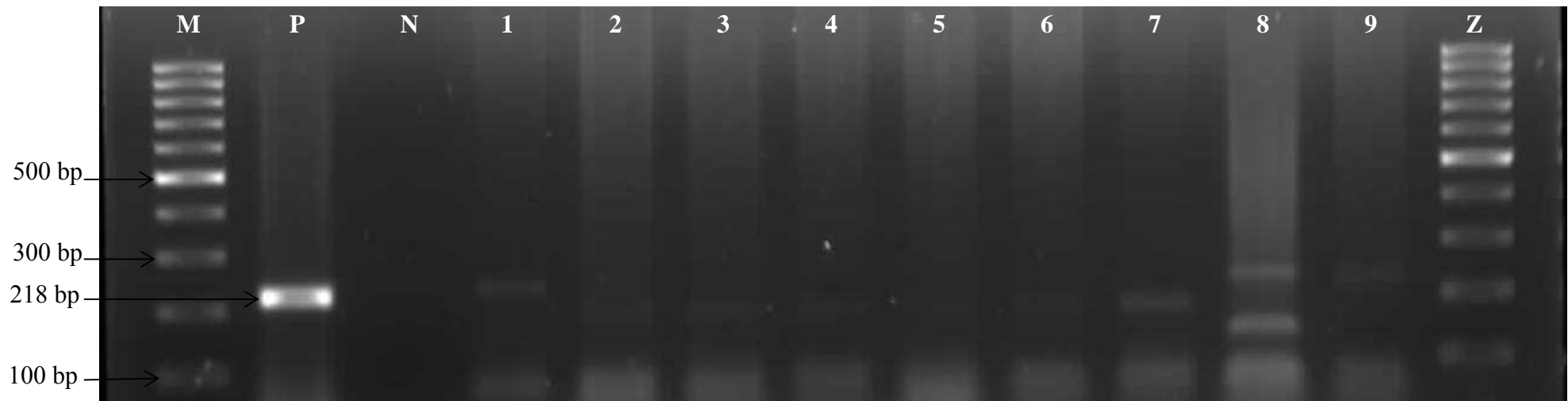
**Figure III b.** Molecular detection of NMEC pathotype by the amplification of *ibeA* gene (342 bp). Lane M: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* DSM 10819 strain); lane N: negative control; lanes 1 to 10 *E. coli* isolates.



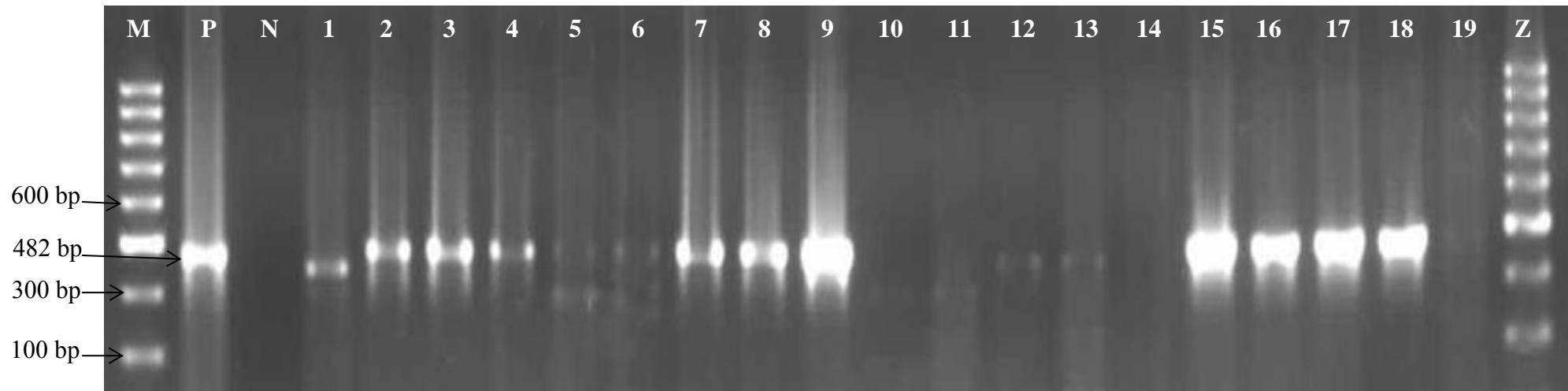
**Figure III c.** Molecular detection of UPEC pathotype by the amplification of *papC* gene (382 bp). Lane M: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* DSM 4816 strain); lane N: negative control; lanes 1 to 10 *E. coli* isolates.



**Figure III d.** Molecular detection of EAEC pathotype by the amplification of *eagg* gene (194 bp). Lane M: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* DSM 10974 strain); lane N: negative control; lanes 1 to 22 *E. coli* isolates.



**Figure III e.** Molecular detection of ETEC pathotype by the amplification of *lt* gene (218 bp). Lanes M & Z: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* DSM 10973 strain); lane N: negative control; lanes 1 to 9 *E. coli* isolates.



**Figure III f.** Molecular detection of EPEC pathotype by the amplification of *eae* gene (482 bp). Lanes M & Z: 100 bp molecular weight marker (Thermo Scientific Inc.); lane P: positive control (*E. coli* DSM 10973 strain); lane N: negative control; lanes 1 to 19 *E. coli* isolates.

## Appendix IV: Publications and conference presentations

### Publications:

**Martins Ajibade Adefisoye, Uchechukwu U. Nwodo, Ezekiel Green and Anthony**

**Ifeanyi Okoh (2016).** Quantitative PCR detection and characterisation of human adenovirus, rotavirus and hepatitis A virus in discharged effluents of two wastewater treatment facilities in the Eastern Cape, South Africa. *Food and Environmental Virology*. 2016 May 28. DOI 10.1007/s12560-016-9246-4. [Epub ahead of print].

**Adefisoye, M.A. and Okoh, A.I. (2016).** Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. *Microbiologyopen*. 2016 Jan 13. doi: 10.1002/mbo3.319. [Epub ahead of print].

**Martins Ajibade Adefisoye and Anthony Ifeanyi Okoh (2015).** Real-time detection of adenovirus and faecal indicator bacteria in the discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. *J Microb Biochem Technol* 2015, 5:4 <http://dx.doi.org/10.4172/1948-5948.S1.014>

**Okoh, A.I., Sibanda, T., Nongogo, V., Adefisoye, M.A., Olayemi, O.O. and Nontongana, N. (2015).** Prevalence and characterisation of non-cholerae *Vibrio* spp. in the final effluents of wastewater treatment facilities in two districts of the Eastern Cape Province of South Africa: implications for public health. *Environ Sci Pollut Res Int*. 22(3): 2008-2017.



### **Conference Presentations:**

**Adefisoye, M.A.,** Aghdasi, F., Green, E. and Okoh, A.I. (2013). Assessment of the prevalence of faecal coliforms and *Escherichia coli* O157:H7 in the final effluents of two wastewater treatment plants in Amathole District Municipality. A conference paper presented at the 4th Municipal Water Quality Conference, Sun City Hotel and Convention Center, North West Province, South Africa, 7 – 11 July 2013.

**Adefisoye, M.A.** and Okoh, A.I. (2013). Assessment of the prevalence of *Escherichia coli* and *Vibrio spp.* in the final effluent effluents of two wastewater treatment plants in Amathole District Municipality of the Eastern Cape Province of South Africa. A conference paper presentation at the 18th Biennial congress of the South African Society of Microbiology (SASM), Forever Resort Warmbaths Bela-Bela South Africa, 24 - 27 November 2013.

**Adefisoye, M.A.** and Okoh, A.I. (2014). An assessment of the performance indices of two wastewater treatment facilities in Amahlathi Local Municipality of the Eastern Cape Province, South Africa. A conference paper presented at WISA 2014 Biennial Conference, Mbombela Stadium, Mpumalanga, South Africa, 25 – 29 May 2014.

**Adefisoye, M.A.** and Okoh, A.I. (2014). Evaluation of the Occurrence of Faecal Coliforms and *Escherichia coli* O157:H7 in the Discharged Final Effluents of two Wastewater treatment Plants in Located in Amahlathi Local Municipality. Poster presented at University of Fort Hare Post Graduate Conference, Alice, Eastern Cape South Africa, 13 October 2014.

**Adefisoye, M.A.** and Okoh AI (2014). Gender and socioeconomic implications of polluted water sources: A case study of the performance indices of two wastewater treatment facilities in Amathole District Municipality of South Africa. A conference paper presented at the Gender, Water & Development: the untapped connection conference held at the International Conference Centre East London, South Africa, 7 – 11 November 2014.

**Martins Ajibade Adefisoye** and Anthony Ifeanyi Okoh (2015). Real-time detection of adenovirus and faecal indicator bacteria in the discharged final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa. A conference poster presented at the World Congress and Expo on Applied Microbiology held at Fleming's Conference Hotel Frankfurt Germany, 18 - 20 August 2015 (OMICs Group Inc.).

**Martins A. Adefisoye** and Anthony I. Okoh (2015). Occurrence and antibiogram of *E. coli* pathotypes in the final effluents of two wastewater treatment plants in the Eastern Cape Province. A conference paper presented at the South African Young Water Professional conference held at CSIR International Convention Center the Pretoria, South Africa, 7 – 11 November 2015.