DOCTOR OF PHILOSOPHY

Epidemiological gene carriage study of gram-negative extended spectrum -Lactamase-producing bacteria in urinary tract infection patients in North Wales: isolation of a novel CTXM-type enzyme

Edowik, Yasir

Award date:
2020

Awarding institution:
Bangor University

EPIDEMIOLOGICAL GENE CARRIAGE STUDY OF GRAM-NEGATIVE EXTENDED SPECTRUM β-LACTAMASE-PRODUCING BACTERIA IN URINARY TRACT INFECTION PATIENTS IN NORTH WALES; ISOLATION OF A NOVEL *CTXM-TYPE ENZYME*

Yasir Edowik

School of Medical Sciences
Bangor University

A thesis submitted for the degree of
Doctor of Philosophy
January 2020

Declaration and Consent

Details of the Work

I hereby agree to deposit the following item in the digital repository maintained by Bangor University and/or in any other repository authorized for use by Bangor University.

Author Name:

……………………………………………………………………………………………..

Title:

……………………………………………………………………………………..…………

…………….

Supervisor/Department:

....

Funding body (if any):

....

Qualification/Degree obtained:

……………………………………………………………………….

This item is a product of my own research endeavours and is covered by the agreement below in which the item is referred to as "the Work". It is identical in content to that deposited in the Library, subject to point 4 below.

Non-exclusive Rights

Rights granted to the digital repository through this agreement are entirely non-exclusive. I am free to publish the Work in its present version or future versions elsewhere.

I agree that Bangor University may electronically store, copy or translate the Work to any approved medium or format for the purpose of future preservation and accessibility. Bangor University is not under any obligation to reproduce or display the Work in the same formats or resolutions in which it was originally deposited.

Bangor University Digital Repository

I understand that work deposited in the digital repository will be accessible to a wide variety of people and institutions, including automated agents and search engines via the World Wide Web.

I understand that once the Work is deposited, the item and its metadata may be incorporated into public access catalogues or services, national databases of electronic theses and dissertations such as the British Library's EThOS or any service provided by the National Library of Wales.

Statement 1:

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless as agreed by the University for approved dual awards.

Signed ………………………………………….. (candidate)

Date ……………………………………………..

Statement 2:

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

All other sources are acknowledged by footnotes and/or a bibliography.

Signed …………………………………………. (candidate)

Date …………………………………………….

Statement 3:

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loan and for electronic repositories, and for the title and summary to be made available to outside organisations.

Signed …………………………………………. (candidate)

Date …………………………………………….

NB: Candidates on whose behalf a bar on access has been approved by the Academic Registry should use the following version of **Statement 3:**

Statement 3 (bar):

I hereby give consent for my thesis, if accepted, to be available for photocopying, for inter-library loans and for electronic repositories after expiry of a bar on access.

Signed …………………………………………… (candidate)

Date ………………………………………………

Statement 4:

Choose **one** of the following options

a) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University and where necessary have gained the required permissions for the use of third party material.	
b) I agree to deposit an electronic copy of my thesis (the Work) in the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University when the approved **bar on access** has been lifted.	
c) I agree to submit my thesis (the Work) electronically via Bangor University's e-submission system, however I **opt-out** of the electronic deposit to the Bangor University (BU) Institutional Digital Repository, the British Library ETHOS system, and/or in any other repository authorized for use by Bangor University, due to lack of permissions for use of third party material.	

Options B should only be used if a bar on access has been approved by the University.

In addition to the above I also agree to the following:

1. That I am the author or have the authority of the author(s) to make this agreement and do hereby give Bangor University the right to make available the Work in the way described above.
2. That the electronic copy of the Work deposited in the digital repository and covered by this agreement, is identical in content to the paper copy of the Work deposited in the Bangor University Library, subject to point 4 below.
3. That I have exercised reasonable care to ensure that the Work is original and, to the best of my knowledge, does not breach any laws – including those relating to defamation, libel and copyright.
4. That I have, in instances where the intellectual property of other authors or copyright holders is included in the Work, and where appropriate, gained explicit permission for the inclusion of that material in the Work, and in the electronic form of the Work as accessed through the open access digital repository, *or* that I have identified and removed that material for which adequate and appropriate permission has not been obtained and which will be inaccessible via the digital repository.
5. That Bangor University does not hold any obligation to take legal action on behalf of the Depositor, or other rights holders, in the event of a breach of intellectual property rights, or any other right, in the material deposited.
6. That I will indemnify and keep indemnified Bangor University and the National Library of Wales from and against any loss, liability, claim or damage, including without limitation any related legal fees and court costs (on a full indemnity bases), related to any breach by myself of any term of this agreement.

Signature: ………………………………………………………… Date : ………………………………………

Dedication

To the memory of my beloved parents, who would have been happy to see me

Follow in this step.

To my loving wife, a constant source of inspiration and motivation to me.

To my brothers and sisters.

I dedicate my work with wholehearted love and gratitude to you all.

Yasir

Acknowledgements

Many helped get me where I find myself today; most particularly and to whom I offer my sincerest gratitude is my supervisors Merfyn Williams and Thomas Caspari whose patience and support were well beyond the call of duty.

Also, my thanks and gratefulness go to research committee members, my internal examiner Dr. Prysor Williams and the Chairwomen Dr. Rachel Hallett ,all of whom gave me kind and valuable comments and advice.

Special thanks are given to my elder brother Dr. Ahmed Aldawek who made it possible for me to continue my studies in the UK after a long disruption.

Last but not least, it gives me great pleasure to acknowledge the help and support of my family. I am very grateful to my brothers and sisters for their unwavering belief in me and for supporting my dreams and my goals.

Abstract

Antibiotic resistance is considered a major public health concerns in the 21st century. Microbes that were once susceptible to antibiotics are becoming more and more difficult to treat as they acquire rapidly a wide range of resistance mechanisms including enzymes of the β-lactamase family. These proteins are typically plasmid-borne and inactivate beta-lactam ring containing antibiotics that are worldwide used to treat infections. This enzyme family with more than 2800 proteins is rapidly evolving through the spontaneous acquisition of missense mutations that enhance protein stability, increase catalytic turn-over and/or improve substrate specificity.

This study is an epidemiological snapshot of the antibiotic resistance conferred by CTX-M-type extended-spectrum β-lactamases (CTX-M type ESBL) in patients based in North Wales who were diagnosed with urinary tract infection (UTI).

Multiplex PCR amplification of β-lactamase genes of the *bla*CTX-M groups 1, 2, 9, and 8/25 followed by DNA sequencing of ESBL-producing isolates was performed with samples from three referral hospital in North Wales; Ysbyty Gwynedd, Glan Clwyd Hospital, and Wrexham Maelor Hospital

In line with previous studies, the β-lactamase CTX-M-15 was found to be the dominant CTX-M variant in the study region. The analysis of samples from Ysbyty Gwynedd hospital at Bangor revealed however an as yet unknown CTX-M variant that differed from CTX-M-14 by only three missense mutations. The amino acid substitutions A55T, A273P and R277C reside in the beta-strand domain opposite of the active site of the enzyme. CTX-M-15, CTX-M-14 and the novel variant were expressed in the periplasm of recombinant *E.coli* using the inducible vector pASK-IBA2C. Determination of the Minimum Inhibitory Concentration (MIC) of a series of clinical relevant antibiotics using the induced recombinant strains showed a significant increase in Nitrofurantoin resistance and a partial increase in Cefoxitin resistance that were both specifically linked with the three mutations. In vitro Kinetic studies using protein from periplasmic extracts of induced strains showed however that neither the affinity for Nitrofurantoin nor for Cefoxitin was increased by these substitutions. Taken together, the three missense mutations in CTX-M-14 may render UTI patients isolates more resistant to Nitrofurantoin because the three amino acid changes increase enzyme stability, its expression level in the resistant coliform bacteria or result in a higher turn-over rate of the mutated β-lactamase by facilitating conformational changes.

Abbreviations

APS	Ammonium persulfate
AMR	Amicrobial resistance
AMP	Ampicillin
AMC	Co-amoxiclav
AK	Amikacin
Amo	Amoxicillin
Aug	Augmentin
Ami	Amikacin
bp	Base pair
BLAST	Basic Local Alignment Search Tool
CTX	Cefotaxime
CAZ	`Ceftazidime
CA	Clavulanic acid
CL	Cephalexin
CIP	Ciprofloxacin
CPD	Cefpodoxime
CT	Cefotaxime
CFU	Colony Forming Unit CFU
C-terminal	Carboxy-terminal domain
CDC	Centres for Disease Control
CLSI	Clinical & Laboratory Standards Institute
DNA	Deoxyribonucleic acid
DDST	Double-disk synergy test
DMF	Dimethylformamide
ddH2O	distilled water
EUCAST	European Committee on Antimicrobial Susceptibility Testing
E.coli	*Escherichia coli*
EDTA	Ethylenediamine tetra-acetic acid
ESBLs	Extended-spectrum b –lactamases
ETP	Ertapenem
FX	Cefoxitin

GN	Gentamicin
GP	General Practice
HGT	Horizontal gene transfer
HCl	Hydrochloric Acid
IMP	Imipenem
IMI	Imidazole
IP	Inpatients
kD	Kilodalton
KPC	*Klebsiella pneumonia carbapenemase*
L	Litter
Mg	Milligram
MgCl2	Magnesium chloride
ml	Millilitre
mM	Millimolar
MHT	Modified Hodge test
MALDI	Matrix Assisted Laser Desorption Ionization-Time of Flight
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant S. aureus
MEM	Meropenem
MHB	Mueller Hinton Agar
µg	Microgram
µL	Microliter
NARMS	The national antimicrobial resistance monitoring system
NHS	National Health Service
NaCl	Sodium chloride
N-terminal	Amino-terminal domain
NaOH	Sodium hydroxide
Ni	Nitrofurantoin
oligo-dT	Oligodeoxythymidylic acid
OMPs	Outer membrane proteins
OD	Optical density
OP	Outpatients
PAGE	Polyacrylamide gel electrophoresis
PBPs	Penicillin-binding proteins

PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PCR	Polymerase chain reaction
PBP	Penicillin binding protein
Ptz	Piperacillin/tazobactam
Pi	Isoelectric point
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulfate-Polyacrylamide gel
SHV	Sulfhydryl variable
TBE	Tris-borate-EDTA
TEMED	Tetramethylethylenediamine
Tet	Tetracycline
TZP	Piperacillin/tazobactam
TZ	Cefotazidime
UV	Ultraviolet
UTI	Urinary Tract Infection
Vmax	Maximum Velocity
WB	Western blot

Contents

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 1 : INTRODUCTION

1.1. Antibiotics: The 'Miracle' Drugs

Infectious diseases were the main cause of human deaths worldwide in first half of the 20th century and the second leading cause of mortality worldwide in recent decades (Nathan & Cars, 2014). For millennia, medicine was in the dark ages, relying on plants, fungi, and lichen to treat infections (Bernardini et al, 2018), until research into microbial secondary metabolites led to the antibiotic era (Landecker, Hannah & Blackman, 2016). Antibiotics are one of the most important inventions in the pharmacological field and have been considered 'miracle' drugs since they were first introduced to the health care system in the 1940s (Brandt et al, 2014). The discovery of antibiotics revolutionized the medical sector, forever, changing the way medicine is practiced (Laxminarayan et al, 2013).

Many antibiotic agents have been developed to kill bacteria and inhibit their growth or reproduction (Yount, 2013). Antibiotics are categorised into three main groups according to the mechanism through which this action occurs. The first group are inhibitors of bacterial cell wall biosynthesis. These compounds cause the rupturing of the bacterial cell wall by inhibiting the action of transglycosidases, the enzymes responsible for the cross-linking of peptidoglycan (sugar polymers) molecules that give the cell wall strength (Kapoor et al, 2017). The second group includes the inhibitors of bacterial protein synthesis. These compounds target the 30S or 50S subunit of the bacterial ribosome (70S ribosome), which is responsible for the translation of mRNA into protein (Lin et al, 2018). The third group are the inhibitors of nucleic acid production. These are classified into *DNA inhibitors* and *RNA inhibitors*. DNA inhibitors, such as quinolones, act upon DNA gyrase as a topoisomerase inhibitor (Koo et al, 2017). RNA inhibitors, such as rifampin, acts upon DNA-dependent RNA polymerase (Harms et al, 2016). Researchers may also classify antibiotics into two groups according to their specific targets. *Broad spectrum* antibiotics affect a wide range of bacteria and *narrow spectrum* antibiotics target only specific types (Wright, 2007). Table 1.1 shows the most commonly used antibiotics and their target.

Table 1.1 The most commonly used antibiotics and their targets.

Antibiotic class	Example(s)	Target	Reference
β-Lactams	Penicillins (ampicillin), cephalosporins (cephamycin), penems (meropenem), monobactams (aztreonam)	Peptidoglycan biosynthesis	Sauvage et al, 2016
Aminoglycosides	Gentamicin, streptomycin, spectinomycin	Translation	Hong et al, 2014
Glycopeptides	Vancomycin, teicoplanin	Peptidoglycan	Binda et al, 2014
Tetracyclines	Minocycline, tigecycline	Translation	Goodman et al, 2016
Macrolides	Erythromycin, azithromycin	Translation	Jelić et al, 2016
Lincosamides	Clindamycin	Translation	Matzov et al, 2017
Streptogramins	Synercid	Translation	Noeske et al, 2014
Oxazolidinones	Linezolid	Translation	Belousoff et al, 2017
Phenicols	Chloramphenicol	Translation	Stavrianidi et al, 2018
Fluoroquinolone	Ciprofloxacin	DNA replication	Hangas et al, 2018
Pyrimidines	Trimethoprim	C1 metabolism	Holmes et al, 2016
Lipopeptides	Daptomycin	Cell membrane	Müller et al, 2016
Cationic peptides	Colistin	Cell membrane	O’Driscoll et al, 2018

The targets of the most commonly used antibiotic are peptidoglycan biosynthesis and bacterial ribosomes (70S ribosome). Peptidoglycan biosynthesis is affected through the inhibition of transglycosidases, the enzymes responsible for giving the cell wall strength through the cross-linking of peptidoglycan molecules; this results in the rupture of the bacterial cell wall. Antibiotics that target the bacterial 70S ribosome, the complex molecule responsible for the translation of mRNA into protein, act as the inhibitors of nucleic acids, which affects bacterial DNA replication.

For decades, the aforementioned antibiotics and other antimicrobial drugs have proven effective in reducing rates of illness and death from infectious diseases. However, the misuse and overuse of antibiotics has resulted in the emergence of pathogens possessing multi-antibiotic resistant genes (Van Boeckel et al, 2014), creating a significant health concern for modern medicine – antibiotic resistance.

1.2. Antibiotic Resistance: A Fast-Growing Global Health Problem

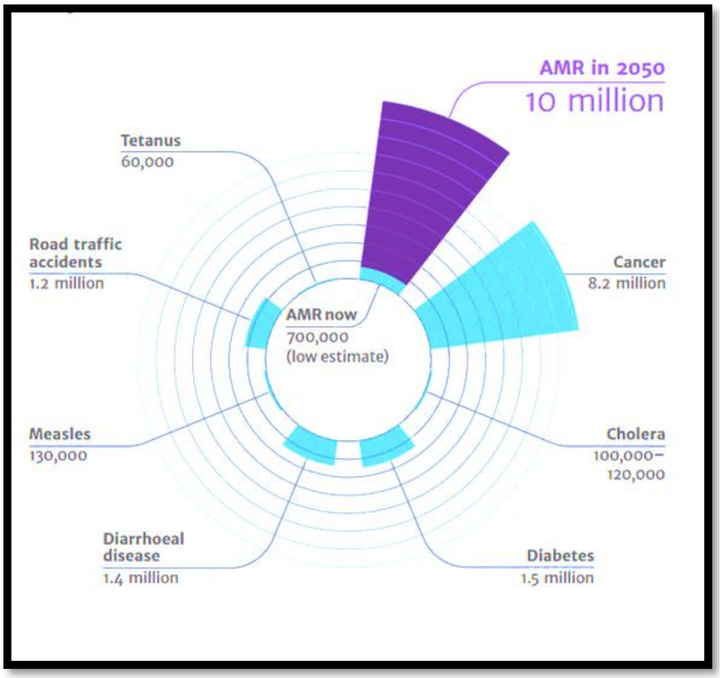
The successful use of any drug to treat infectious disease is determined by the potential for the bacteria to develop a tolerance or resistance to the drug from the time of the drug's introduction (Banin et al, 2017). This can be seen with antibiotics, which have shown continually decreasing efficacy since they were first discovered (Sabtu, 2015). Microbes that were once susceptible to antibiotics are becoming increasingly difficult to treat due to the development of a wide range of chemical and physiological mechanisms of resistance inside the bacterial cells (Davies & Davies, 2010).

Global efforts are necessary to prevent and control the development of further antibiotic resistance. In response to this urgent need, many research studies and reports have highlighted the severe threat of antibiotic resistance (Aslam et al, 2018). The Review on Antimicrobial Resistance (2014), commissioned by the UK Government and chaired by Jim O'Neill[1] reported that 50,000 lives are claimed each year by antimicrobial-resistant infection in Europe and the United States alone. In addition, it is expected that by 2050, antibiotic resistant pathogens will be the leading cause of mortality globally (Figure 1.1; Review on Antimicrobial Resistance, 2014).

---

[1] Jim O'Neill, a former chairman of Goldman Sachs Asset Management and a former UK Treasury Minister, is Chair of Chatham House.(https://www.project-syndicate.org)

Figure 1.1 The expected global deaths in 2050.



The predicted causes of global deaths in by 2050. The number of people expected to die because of antibiotic resistance is more than the number expected to die due to cancer (Review on Antimicrobial resistance chaired by Jim O' Neill, 2014)

By 2050, 300 million people are expected to die prematurely because of antibiotic resistance, resulting in a 2-3.5% decline in the gross world product (GWP), which equates to the loss of between 60 and 100 trillion USD worth of economic output if the problem of antibiotic resistance continues (Review on Antimicrobial Resistance, 2014). The threat of antimicrobial resistance should concern every country regardless of its economic level. For example, in 15 European countries, methicillin–resistant strains have been found to be the main cause of 10% of all blood stream *Staphylococcus aureus* infections, and the resistance rates in some of these countries is as high as 50% (EARS-NE, 2013).

1.2.1. Overuse and Misuse as the Main Cause of the Antibiotic Resistance

Overuse or over prescribing of antibiotics is considered one of two main causes of antibiotic resistance in many pathogens, particularly in outpatient settings such as clinics and emergency departments (Ventola, 2015), where in parts of Europe 80% to 90% of antibiotic prescriptions are written by general practitioners (Llor et al, 2014). Globally, the consumption of antibiotics has increased in parallel with the increase in antibiotic resistance (WHO, 2009). There is also high variation in the prevalence of antibiotic resistance between countries (WHO, 2018), which is likely caused by high variation in antibiotic consumption between countries. The WHO (2018), for example, reported antibiotic consumption rates across 65 countries varied between 1 and 2225 tonnes per year and the consumption of total antibiotics in Defined Daily Doses(DDD)[2] per 1000 inhabitants per day in these 65 countries ranged from 4.4 (in Burundi , Africa region) to 64.4 (in Mongolia ,Western Pacific Region) DDD per 1000 inhabitants per day.

Misuse of antibiotics, such as using inappropriate antibiotics to treat a particular type of infection, is considered the other main cause of antibiotics resistance (Viroj et al, 2018). The Centres for Disease Control and Prevention in the United States has reported that at least 30% of outpatient antibiotic prescriptions are unnecessary, meaning that they are prescribed for non-bacterial infections such as viral respiratory infections, or self-prescribed (CDC, 2016). Another type of antibiotic misuse is the prescribing of broad-spectrum antibiotics, which are classified as non-first line antibiotics, to treat infections that are susceptible to treatment by narrow-spectrum antibiotics. Typically, the prescripting of such broad-spectrum antibiotics occurs before confirming the specific type of bacterial infection (Llor et al, 2014).

1.2.2. The Evolution of Antibiotic Resistance

The resistance to certain antibiotics can exist naturally in some bacterial species. However, some susceptible bacteria can become resistant through either genetic mutations or through acquiring resistance genes from another bacterium (Lin et al, 2015).

Antibiotic resistance resulting from spontaneous genetic changes are rare and thought only to occur in about one in 1 million to one in 10 million bacterial cells (Long et al, 2016). Resistance can result from many different genetic mutations. Some mutations cause the target to which the antibiotic attaches to the bacterial cell to be revoked or cause the cell membrane pores through which the antibiotics enter the bacterial cell to close (Epand et al, 2016). Other mutations result in the production of enzymes that activate antibiotics compounds, such as β-lactamases (Palzkil et al, 2018). In addition, mutations can result in the production of a transport system that can export the antibiotic back outside of the bacterial cell, e.g. AmpC β-lactamase (Kohlmann et al, 2018).

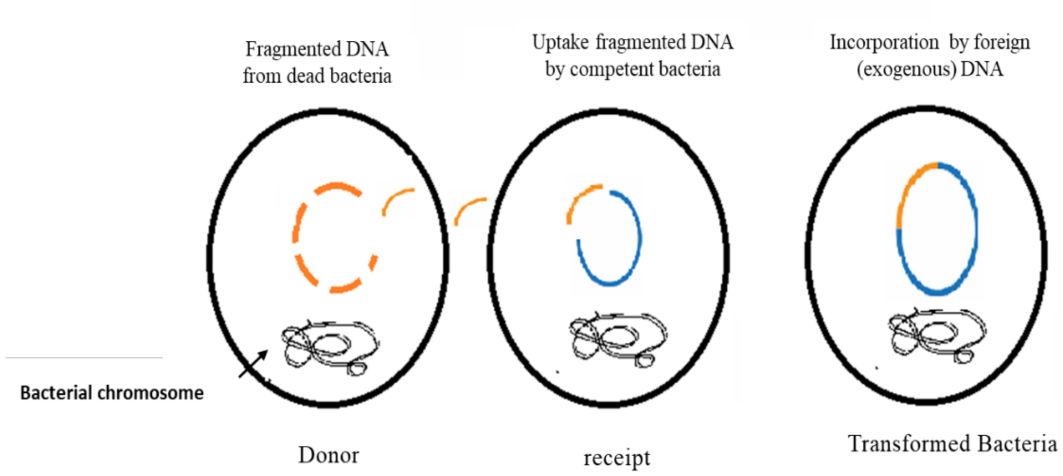
---

[2] Defined Daily Doses (DDD) is the assumed average maintenance dose per day for a drug used for its main indication in adults.

Antibiotic resistance can be acquired by the exchange of genetic material between resistant and susceptible bacteria in a process known as horizontal gene transfer (HGT) (Hong et al, 2018). Unlike vertical gene transfer, where genetic material is passed from parent to offspring, HGT involves genetic material being passed between bacterial cells of the same generation (Paquola et al, 2018). In this process, resistance genes are transferred in one of three vectors, either plasmids, transposons, or integrons, by one of three mechanisms, either transformation, conjugation, or transduction (Gyles & Boerlin, 2014).

Transformation is a process in which the genetic material carried by a bacteria (recipient) is altered by the incorporation of foreign (exogenous) DNA from another dead or degraded bacterium (Croucher et al, 2016; Figure 1.2). This process is considered a form of genetic recombination that occur predominantly between homologous DNA regions, which carry approximately the same nucleotide sequences (Johnston et al, 2014). The transformable or competent bacteria (recipient) is characterised by its ability to bind much more DNA than non-competent bacteria and to kill non-competent cells to release DNA for transformation (Blokesch & Melanie, 2016). *Streptococcus pneumoniae* and *Neisseria meningitides* are well-known of examples naturally competent bacteria (Bakkali, 2013).

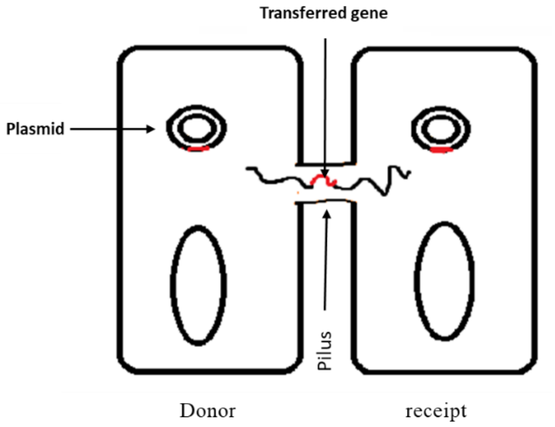
Figure 1.2 Bacterial transformation



Steps of Transformation; up taking of degraded DNA from dead bacterial cells by competent cell, followed by the incorporation of recipient DNA by foreign (exogenous) DNA.(modified from “conjugation by Adenosine [license; CC BY-SA 3.0])

The other mechanism of the Horizontal gene transfer (HGT) is conjugation. Here, the genetic material of chromosome from two sexually distinct types of cells are brought together through direct cell-to-cell contact (Grohmann et al, 2003). A partial gene from the donor bacteria moves indirectly through the pilus (Figure 1.3), which is produced by the donor bacteria and bridges the two cells, to complete gene transfer into the recipient bacterial cell (Shannon et al, 2015). This process is followed by the separation of the bacterial cells, after which the combined genetic material are altered further in the recipient cell (Vazquez et al, 2017). Some genetic material can be transferred in the reverse direction, from the recipient to donor bacteria, which is known as retro transfer (Sultan et al, 2018).

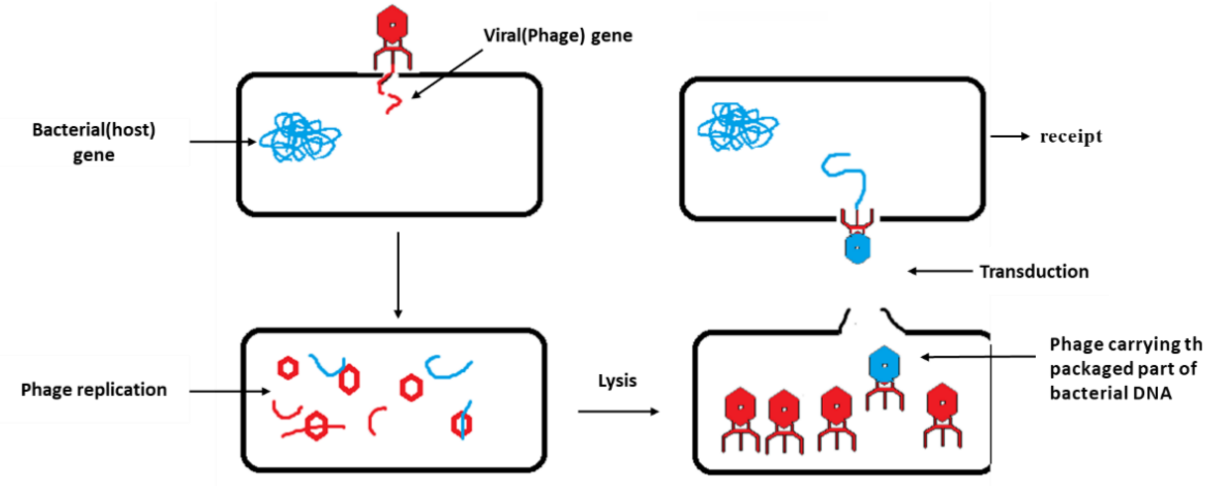
Figure 1.3 Bacteria conjugation.



Transferring of a partial gene from the donor bacteria to the recipient through pilus during the process of conjugation.(Modified from Zhang P et al,2015)

Bacterial genetic material, including resistance genes, can also be transferred horizontally by viral vector (bacteriophages), in a process known as transduction (De Sousa et al, 2018). In this process, bacteriophage bind to the bacterial cell and inject DNA into the cell through the cell membrane, whereupon small pieces of bacterial DNA can be packaged into the bacteriophage genome during the production of new phage particles (Casjens & Sherwood, 2003). The new phage particle is replicated instead of the bacterial genetic material, which is degraded by the phage during this process (Harada et al, 2018). The bacteriophage then lyse the bacterial cell releasing phage carrying the packaged part of bacterial DNA. This will be injected into another bacterium (recipient) as part of the phage replication cycle (Rodríguez-Rubio et al, 2013; Figure 1.4).

Figure 1.4 Bacterial Transduction.



The process of transduction which starts with the binding of the bacteriophage to the bacterial cell and the injection of its DNA into the bacterial cell. A small part of the bacterial DNA is packaged by the injected phage DNA, which then replicate inside bacteria instead the bacterial DNA, which is degraded by the phage. The host bacteria are then lysed and phage carrying the packaged part of bacterial DNA are released to bind the cell wall of other hosts (Modified from “conjugation by Adenosine.(modified from “conjugation by Adenosine [license; CC BY-SA 3.0]).

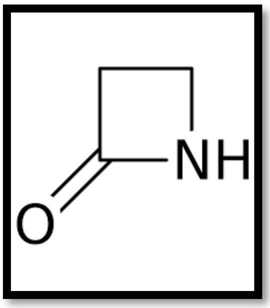
Transduction is classified into generalized transduction, in which any bacterial DNA can be transferred to another bacterium by a bacteriophage, and specialized transduction, in which a restricted set of bacterial genes is transferred to another bacterium (Chiang et al, 2019). The *mecA* gene[3] carried by methicillin-resistant *S. aureus* (MRSA) is an example of a resistance gene that is transferred horizontally by transduction (Paterson et al, 2014).

Aforementioned evolutions of antibiotic resistance grows with the increasing number of bacterial species becoming resistant to the most commonly used antibiotics. A group of these antibiotics that represents approximately over 65% of global antibiotic use (Poole, K,2004) and are considered the largest class of bacteria cell wall synthesis inhibitors are beta-lactam antibiotics (Livermore, 1998).

1.3. βeta-Lactam Antibiotics

β-lactam antibiotics are the most commonly used agents in the treatment of bacterial infections which are classified under the inhibitors of cell wall biosynthesis (Bush, 2018). They contain a four-atom ring (β-lactam) structure (Figure 1.5) and include penicillins, cephalosporins and carbapenems (Meletis, 2016).

Figure 1.5 β-Lactam ring.



β-Lactam ring is a four-membered lactam.

(Gilchrist T.1987).

---

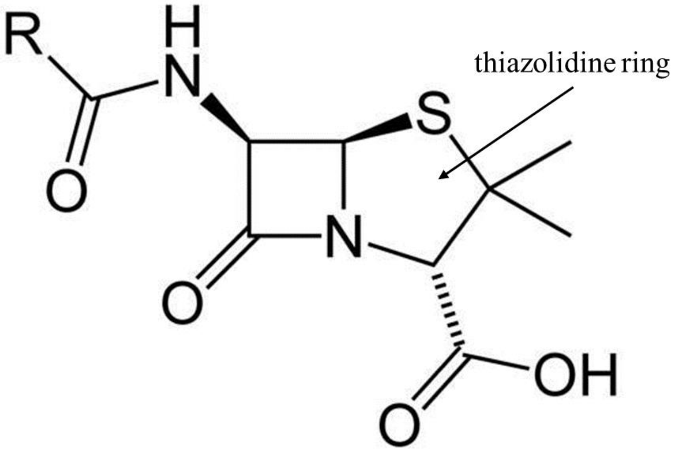
[3] *mecA is a gene found in bacterial cells that confers resistance to antibiotics such as methicillin, penicillin, and other penicillin-like antibiotics. mecA codes for PBP2a, which has a lower affinity for beta-lactams, which prevents cell death by maintaining the structural integrity of the cell wall.*

1.3.1. The Main Types of βeta-Lactam Antibiotics

1.3.1.1 Penicillin (penams)

Penicillin is considered the most commonly used β-lactam antibiotic and was the first discovered antimicrobial drug. It was derived originally from a fungus "moulds" and used to treat the bacterial infection caused by *streptococci* and *staphylococci* (Thom, 1945). Penicillin V (taken orally), Penicillin G (taken intravenously) and procaine penicillin are different forms of penicillin (Bush et al, 2016). These forms share the three basic chemical compounds; a thiazolidine ring, β-lactam ring, and a side chain (Lobanovska et al, 2017) (Figure 1.6).

Figure 1.6 Penicillin Structure.



The chemical structure of Penicillin. Note the β-lactam ring; thiazolidine and side chain. (Gonzalez-Estrada A et al.,2015)

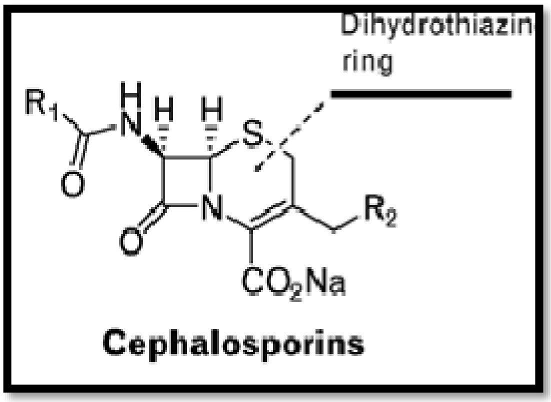
Based on chemical substitutions on the residue attached to the β-lactam ring, which confer different activities (Tahlan & Jensen, 2013), the penicillin family is categorised into five groups; natural penicillin, aminopenicillins, penicillinase-resistant penicillins, extended spectrum penicillins, and aminopenicillin/β-lactamase inhibitor combinations (Miller & Lane, 2002).

Despite the wide range of pathogens that can be combated by penicillin the first sign of antibiotic resistance has appeared soon after the discovery of penicillin when penicillinase producing *E. coli* was detected by Abraham and Chain in 1940 (Sillankorva at el, 2019).

1.3.1.2. Cephalosporins

Cephalosporin is considered the largest group of related β-lactam antibiotics, which are characterised by their broad spectrum of activity (Bush et al, 2016). Cephalosporins are utilised to treat many infections including meningitis, bacteraemia, and skin and soft tissue infections (Thønnings et al, 2016). Structurally, it differs from penicillin by having a dihydrothiazin ring (Figure 1.7).

Figure 1.7 Cephalosporin Structure.



The chemical structure of cephalosporin. Note the β-lactam ring, dihydrothiazin ring, and side chain.(Feng H et al.,2017)

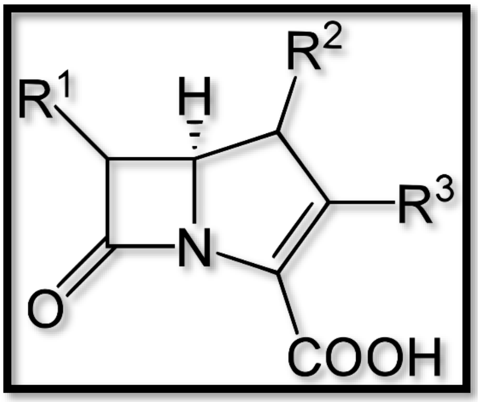
The large group of cephalosporins are categorised traditionally into five generations based on their antimicrobial spectrum and agents in the same generation are classified further according to their pharmacokinetics (Kim et al, 2014). First-generation cephalosporins exhibit higher activity with gram positive than that with the gram-negative bacteria, e.g. Cefazolin (Katzung et al, 2015). The second-generation cephalosporins, such as Cefoxitin, show the highest activity against gram-negative organisms and the lowest against gram-positive organisms (Bratzler et al, 2013).

The third-generation are the most prescribed cephalosporins and are the first generation to be considered as extended-spectrum cephalosporin. They are characterised by their greater resistance to the β-lactamase enzymes produced by gram-negative bacteria that are exhibited by first and second generations e.g. Cefotaxime and Ceftazidime which are considered the most commonly used antibiotics of this generation (Chaudhry et al, 2019). The fourth-generation retain the expanded activity of the third-generation against gram-negatives, in addition to an improved gram-positive spectrum, e.g. Cefpirome (Proticetal, 2016). The fifth-generation, which includes ceftaroline, is known as anti-methicillin-resistant *S. aureus* (MRSA) cephalosporins as it has high activity against gram-negative rods like MRSA and *Streptococcus* (Stevens et al, 2014)

1.3.1.3. Carbapenem

Carbapenems is the third main type of β-lactam antibiotics which is being used increasingly in recent years as a result of the rising the prevalence of cephalosporin resistant isolates (Hawkey et al, 2012). Among hundreds of β-lactam antibiotics, carbapenems are used as a last-line agent for the serious infections that cannot be treated by other β-lactams as it possess the broadest spectrum of activity and greatest potency against gram-positive and gram-negative bacteria (Papp-Wallace et al, 2011). The broad spectrum antibacterial activity of carbapenems is due to its a unique structure that is defined by a Carbapenem coupled to a β-lactam ring (Figure 1.8), which provides protection against most β-lactamases, such as metallo-β-lactamase (MBL), as well as extended spectrum β-lactamases (Codjoe et al, 2017).

Figure 1.8 Carbapenem Structure



The chemical structure of Carbapenem is defined as the 4:5 fused ring lactam of penicillin with double bond between C-2 and C3 but with the substitution of carbon for sulphur at C-1 (Papp-Wallace et al, 2011)

Based on their antibacterial activity, carbapenems are classified into three groups: Group 1, broad-spectrum carbapenems, such as Ertapenem, with limited activity against non-fermentative gram-negative bacilli; Group 2 broad-spectrum carbapenems, such as Imipenem, meropenem, and doripenem, with activity against non-fermentative gram-negative bacilli; and Group 3 which includes carbapenems that shows activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and are currently under development (El-Gamal et al, 2017).

In addition to the three main β-Lactams, βeta-lactamase inhibitors confer approximately the same antimicrobial activity with an expanded spectrum of activity (Toussaint et al, 2015). These expanded spectra promote the potency of the antibiotic and provide resistance against the hydrolytic activity of the β-lactamase enzyme (Frère et al, 2005). Clavulanic acid is the most commonly used and effective β-lactamase inhibitor, which was produced as a result of natural product screening and was used initially to inhibit the activity of most plasmid penicillinases (Bush, Karen & Bradford, 2016)

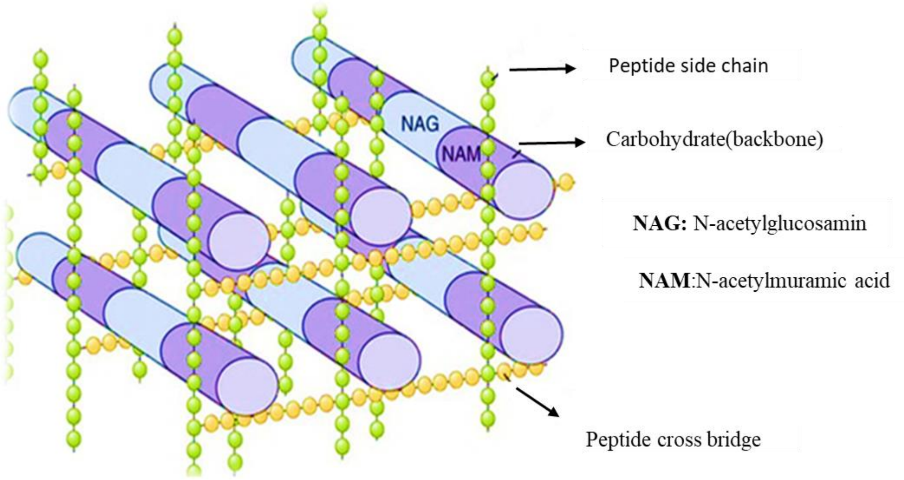
The efficiency of β-lactam antibiotics is continuously challenged by resistant bacterial strains. This is most often due to the resistant cells, particularly non-fastidious gram-negative bacteria, producing an enzyme known as β-lactamase (Bush & Jacoby, 2010). β-lactamase–based resistance cannot be understood without first knowing the mode of action of β-lactam antibiotics.

1.3.2. βeta-Lactam's Mode of Action

In all bacterial species a cell wall surrounds the bacterial cell, maintaining its shape and integrity, while also preventing cell lysis from high osmotic pressure (Yang et al, 2016). The cell wall is composed of a complex cross-linked peptidoglycan layer that contains repeated units of alternating disaccharides of N-acetyl glucosamine and N-acetylmuramic acids. A-5 amino-acid peptide is linked to N-acetylmuramic acid sugar and terminates in D-alanyl-D-alanine (Yadav et al, 2018).

In both gram-positive and gram-negative bacteria enzymes called penicillin-binding proteins (PBPs) act through a subgroup enzyme, transpeptidases (DD-transpeptidases), to remove the terminal alanine and catalyse the process of forming a cross-link with a nearby peptide (Rioseras, 2016; Figure 1.9). This cross-bridging mechanism is intimately intertwined with the discovery of the PBPs as it was concluded by some studies that the impact of penicillin on the synthesis of peptidoglycan in the transpeptidation reaction leads to the crosslinking of glycan chains is inhibited by B lactams (Penicillin) (Zapun et al, 2008).

Figure 1.9 Chemical structure of peptidoglycan.



Peptidoglycan structure; N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) chains linked by peptide cross-bridges (Modified from clinicalgate.com).

PBPs are commonly classified into three main groups based on their molecular weight and domain structure; high molecular weight PBPs, which include two families called class A and class B, and low molecular weight PBPs, which consists of class C (Sauvage et al, 2016). All PBPs are characterized by the presence of three conserved motifs in the active site: SxN, SxxK, and KTG, where x is any residue (Nicola et al, 2010). The penicillin-binding domain that catalyses transpeptidation (transpeptidase) is constituted in the C-terminal region of class A PBPs (Welsh et al, 2017).

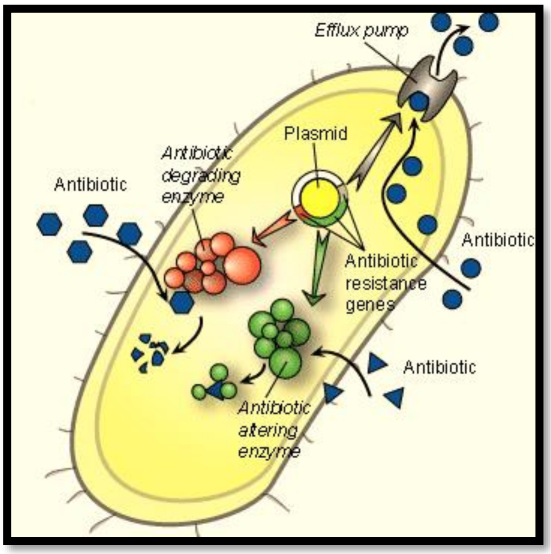
β-lactams inhibits the activity of transpeptidase by acting as a peptidoglycan precursor, which is targeted by the transpeptidases enzyme (Nikolaidis et al, 2014). The transpeptidation catalysed by the transpeptidase enzyme is completed in two steps. First, DD-trans peptidase interact with an acyl donor containing a pentapeptide stem ending in D-Ala-D-Ala, resulting in releasing of the second D-Ala, followed by an ester bond formation between the carbonyl of the first D-Ala and the catalytic serine residue of the enzymes. Second, the resulting acyl-enzyme is attacked nucleophilically by acyl acceptor generates the peptidoglycan cross-link (Edoo et al, 2017).

In the presence of β-lactam, the carbon carbonyl of β-lactams is attacked by the catalytic serine, similar to the first step of the transpeptidation reaction, which results in the breaking of the amide bond of the β-lactam ring and the inactivation of the D,D-transpeptidases (Vandavasi et al, 2017) preventing the formation of the cross-link and leading to the lysis of the bacterial cell (Tidwell, 2008).

1.3.3 Resistance to βeta-Lactams

The emergence of resistant bacteria has reduced the effectiveness of β-lactams. There are three main resistance mechanisms for β-lactams (Figure 1.10): (1) alteration of the target to which the β-lactam antibiotics binds, which in turn changes the penicillin-binding protein and makes the β-lactam less effective in disrupting cell wall synthesis (Kocaoglu et al, 2015); (2) altering the porins and efflux pump system to reduce the amount of β-lactam that reaches the target; specifically, efflux transport increases the amount of the drug pumped out and porin mutations reduce cell membrane permeability to decrease the amount of drugs entering the cell (Masi, 2017); and, the subject of this thesis, (3) enzyme degradation of β-lactams, totally or partially, by the β-lactamase family of enzymes (Bonomo et al, 2017).

Figure 1.10 Three mechanisms of antibiotic resistance against β-lactam.



Three main resistance mechanisms for β-lactam; alteration of the target, altering the porins and efflux pump system to reduce the amount of β-lactam and the enzymatic degradation (Todar, Ph.D., textbookofbacteriology.net).

1.3.3.1 β-Lactamases

β-lactamases are enzymes that hydrolyse the amide bond in β-lactam compounds and they are most frequently found in gram-negative bacterial isolates (Palzkill & Timothy, 2013). Abraham & Chain published the first paper identifying β-lactamase nearly 75 years ago. They documented the β-lactamase producing *E. coli*, but they were unable to extract the enzyme from these bacteria (Abraham & Chain, 1940). Four years later, Kirby successfully isolated the enzyme, which he called 'penicillin inactivator', and warned that its emergence could pose a significant clinical danger (Drawz & Bonomo, 2010).

The health concerns related to β-lactamase production has grown over the years as the number of unique, naturally occurring β-lactamases discovered has grown to more than 2,800 proteins (Bush, 2018). Treatments for many serious infections are still reliant on cephalosporins and carbapenems, which have continuously shown a declining efficiency over the years due to β-lactamase-based resistance (Shaikh et al, 2015).

1.3.3.1.1. β-Lactamase Classifications

Since the emergence of β-lactamase–based resistance, many attempts have been made to classify these enzymes. Richmond & Sykes (1960) proposed the first classification scheme. They based their classifications on whether the enzyme showed more hydrolytic activity against penicillin or cephaloridine, and whether it could be inhibited by P-chloromercuribenzoate and/or cloxacillin (Richmond & Sykes, 1973).

Jacoby & Bush (2010) have proposed another functional classification scheme in which the β-lactamases are categorised into four groups according to substrate and inhibitor profile: group 1 consists of cephalosporinase that are not inhibited well by clavulanic acid; group 2 is generally inhibited by active-directed β-lactamase inhibitors; group 3 is poorly inhibited by all classical β-lactamases inhibitors except EDTA and p-chloromercuribenzoate; and group 4 includes penicillinase that cannot be inhibited by clavulanic acid (Jacoby & Bush ,2010).

The most widely used classification is the sequence-based scheme, which was proposed by Ambler in 1980 and has been reviewed many times in the subsequent years. This classification divides β -lactamases into four classes (A, B, C, and D) based upon their amino acid sequences (Silveira et al, 2018). Ambler originally specified two classes: class A, whose activity depends on the presence of serine in the active site; and class B, which contain the metallo-β–lactamases that require zinc ($Zn+^2$) for their activity (Palzkill &Timothy, 2013).

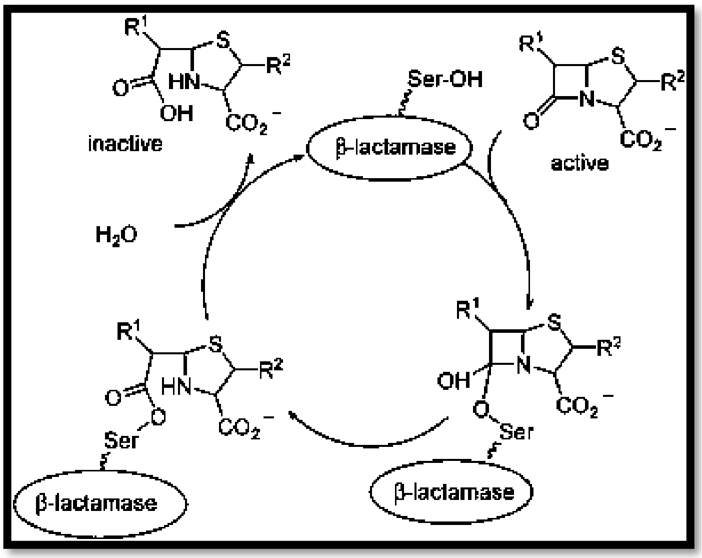
Class C, which is known as AmpC, and class D were found later and described as active-site serine β-lactamase but bore little sequence similarity to the serine class A enzyme (Öztürk et al, 2015).

A classification scheme based on functionality resulted in three major groups: group 1, cephalosporinase (class C); group 2, serine β-lactamase (class A and D); and group 3, metallo β-lactamase (class B) (White et al, 2017).

Group 1 Cephalosporinase: belonging to molecular class C, which are encoded predominantly on chromosome of *Enterobacteriaceae* (Ruppé et al, 2015). In terms of resistance, this group is more active on cephalosporins than benzylpenicillin and resistant to inhibition by clavulanic acid. Furthermore, this group shows a high affinity for aztreonam and a lack of activity on Cefoxitin (Bush & Jacoby, 2009).

Group 2 Serine β-lactamase: represents the largest group of β-lactamase and includes molecular classes A and D (Chandra & Seema, 2013). These enzymes are predominantly chromosomal, although some *staphylococcal* penicillinases are plasmid encoded (Livermore, 1995). The name of this group is derived from the role of serine in the active site of the enzyme's hydrolytic activity, which starts with the formation of a covalent Michael complex as a result of a non-covalent association between the enzyme and the β-lactam antibiotic (Brem et al, 2016). After this association, the active-site serine performs a nucleophilic attack on the carbonyl of the β-lactam, which results in a high-energy tetrahedral acylation intermediate (Lee et al, 2016). Next, as a result of the protonation of β-lactam nitrogen and the cleavage of the C-N bond, the tetrahedral acylation intermediate transitions into a lower-energy covalent acyl-enzyme, which is then attacked by activated water molecules and results in the formation of a high tetrahedral deacylation intermediate and hydrolysis (Meini et al, 2015). The bond between the oxygen of the nucleophilic serine and the carbonyl group of the β-lactam antibiotic regenerates the active enzyme and releases the inactive antibiotic. (Drawz & Bonomo, 2010) (Figure 1.11).

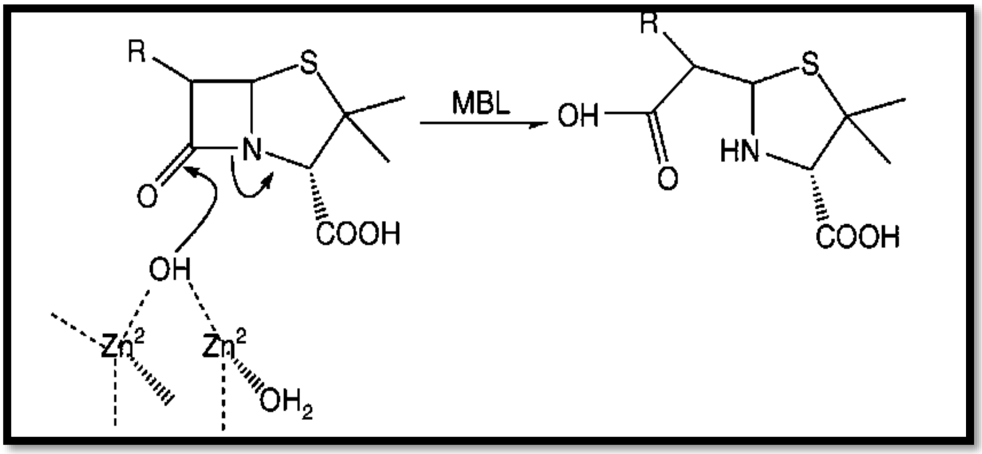
Figure 1.11 Serine β-lactamase.



General mechanism of hydrolysis of a β-lactam antibiotic by a serine β-lactamase (Touchet et al., 2011).

Group 3 MBLs: metallo- β-lactamases (MBLs) are chromosomal enzymes that are produced mainly from gram-positive bacteria and differ structurally from the other β-lactamases due to their requirement for zinc ions at the active site (Sun et al, 2018). Functionally, MBLs are characterised by their hydrolytic activity against carbapenems, which cannot be hydrolysed by serine β-lactamases (Lisa, 2017). Unlike serine β-lactamases, MBLs cannot be inhibited by clavulanic acid or tazobactam, but they are inhibited by metal ion chelators, e.g. EDTA (Patel & Bonomo, 2013). Furthermore, the class B β-lactamases utilises zinc to activate a water molecule and catalyse its direct addition to the β-lactam ring (Figure 1.12). This is unlike the class A, C, and D β-lactamases, which depend on opening the β-lactam ring via covalent acyl enzyme intermediates for their hydrolytic activity (Jeon et al, 2015).

Figure 1.12 Mechanism of hydrolysis by metallo-β-lactamases



The role of zinc ion in the mechanism of action of metallo-β-lactamases (Fernandes et al, 2013).

MBLs are classified structurally into three subclasses: B1, B2, and B3, which differ from each other in the consensus of amino acid at the zinc-active site (Table 1.2).

Table 1.2 MBL subclasses

β subclasses	Number of active site Zn^2 atoms	Consensus amino acids at active site		References
		Ligands to Zn^1	Ligands to Zn^2	
B1a	2	His116	Asp120	Garau & Getal, 2004
B1b	2	His118	Cys221	Young et al, 2009
		His196	His223	
		His116	Asp120	
B2	1	His118	Cys221	Ackerman et al, 2013
		His196	His263	
		Na	Asp120	
			Cys22	
			His263	
B3	2	His/Gln118	His121	Fre're et al, 2005
		His196	His263	

1.3.3.1.2. Main Types of β-Lactamase

There are three main types of β-lactamases that vary in their mechanism of action and in their antibiotic target (Madec et al, 2017), and each type is categorised under one of the aforementioned molecular classes. These types include:

(1) *AmpC* β-lactamase, which are classified as C class, are predominantly detected in Gram-negative organisms, e.g. *E. coli* and *K. pneumoniae* (Fenollar-Ferrer et al, 2008), and exhibit great hydrolytic activity against narrow and broad cephalosporinase and alpha-methoxy β-lactam, e.g. cefoxitin and aztreonam. In addition, they are poorly inhibited by clavulanic acid (Manoharan et al, 2012).

The *AmpC* gene is predominantly encoded on chromosomes, and here the production of the AmpC enzyme is known as inducible (as a result of exposure to inducible genes, e.g. penicillin, cefoxitin, and Carbapenem; Kaur et al, 2016), or it can be acquired by a transferable AmpC gene on a plasmid. This is termed plasmid-mediated AmpC β-lactamase (Coertze et al, 2017).

The resistance conferred by AmpC β-lactamase producing gram-negatives is enhanced by controlling the delivery of the β-lactam antibiotic to the periplasm. This is performed by the transport system in the outer membrane (Moreira et al, 2004), where the porin channels facilitate the penetration of β-lactam, and the porins and efflux pumps transport the β-lactam out of the bacterial cell (Pages et al, 2016; Figure 1.9).

The phenotypic detection of AmpC producing bacteria in clinical laboratories is always being improved as it can give a positive result with extended spectrum β-lactamase (ESBLs), the third main type of β-lactamases (Jacoby, 2009). The Three-Dimensional Test is still the most commonly used method to identify the AmpC producers. Here, extended-spectrum cephalosporin (Cefoxitin) is placed 3 mm from the inoculated slit with the test organism on a Mueller-Hinton agar. A positive result is indicated by the emergence of a distorted zone in the slit side (Gupta et al, 2014).

(2) Carbapenemases β-lactamases are characterised by their high hydrolytic activity against Carbapenem antibiotics, unlike the other β-lactamase types (Jeone et al, 2015). This ability is due to their distinct chemical structure: a 4:5 fused lactam ring with the presence of double bonds between C2 and C3 with the hydroxymethyl side (Nordmann & Poirel, 2002; Figure 8).

Based on the Ambler classification, carbapenemases can be categorised under three classes of β-lactamases; Class A and class D (Serine) carbapenemases β-lactamases and class B (zinc, metallo) carbapenemases β-lactamases (Codjoe et al, 2017). KPC, VIM, IMP, NDM and OXA-48 types are considered the most well know and effective carbapenemases (Poirel et al, 2012). Among the different carbapenemase-producing species, *Klebsiella pneumonia* (KPC) is among the most widespread carbapenemase producer and is one of the main causes of nosocomial infections (Van Duin et al, 2017).

The clinical identification of carbapenemase producers is based on the Modified Hodge Test (MHT) in which the tested isolates are streaked on lines away from Carbapenem disk (meropenem or Ertapenem) on Mueller Hinton agar, which has been inoculated with a Carbapenem-susceptible *E. coli* strain. A positive result in the MHT is indicated by a cloverleaf shaped growth of Carbapenem-susceptible *E. coli* around the streak line close to the disk of Carbapenem, which is permitted due to the decreasing concentration of the carbapenemase enzyme away from the disk (Tamma et al, 2018).

(3) The third main type of β-lactamases, which is also the most rapidly increasing type worldwide, is extended spectrum β-lactamases (ESBL). One of its subtypes is the subject of this study.

1.3.3.1.2.1. Extended Spectrum β-Lactamase (ESBL)

Extended spectrum β-lactamases are the third main type of β-lactamase and were first detected in Western Europe during the mid-1980s.Since then, their prevalence has been increasing steadily and has been associated with the widespread use of cephalosporins (Kim et al, 2017). The resulting mutation of the *TEM1, TEM2,* and *SHV* genes leads to the alteration of the amino acid configuration around the active site of the β-lactamase enzyme and, thus, ESBL production (Bajpai et al, 2017).

ESBL-producing bacteria exhibits resistance to most β-lactam antibiotics, including third and fourth generation cephalosporins (Rahmanetal, 2018), which severely limits the range of treatments available for infections caused by ESBLs (Blaak et al, 2014). However, these ESBL-producing strains can be inhibited by β-lactamase inhibitors, such as clavulanic acid, tazobactam, and sulbactam (Rawat & Nair, 2010).

The ESBL isolates were initially detected in hospital infections caused by *Klebsiella pneumonia,* but are now also associated with community acquired infections, particularly urinary tract infections caused by ESBL-producing *E. coli* (Koksal et al, 2019). In many parts of the world *E. coli* and *Klebsiella pneumoniae* represents 10-40% of the detected ESBL producers (Rupp & Fey, 2003).

ESBL enzymes are predominantly plasmid encoded, and are characterised by carrying genes encoding resistance to other antibiotics, such as aminoglycoside, which may result in further problems in the treatment of infections caused by ESBL-producing strains (Bush & Jacoby, 2010). This problem therefore requires the use of appropriate antibacterial agents, which could be unachievable due to misleading of phenotypic detections used in clinical laboratories (Poulou et al, 2014).

1.3.3.1.2.1.1. ESBL Types

Many classifications of ESBLs have been introduced since the first emergence of this enzyme. The most common classification is based on the type of β-lactam that is hydrolysed by the ESBL type. The most important types are: SHV, TEM, OXA, and CTX-M.

SHV ESBL

The name of this group is derived from the sulfhydryl variable, as it was thought that the active site of the enzyme can be bound with P-chloromercuribenzoate by sulfhydryl[4] to inhibit the activity of cephaloridine (Drawz et al, 2010). Since then, it has been reported that the active site of SHV type β-lactamase is serine hydroxyl sulfhydryl (Manageiro et al, 2012). Based on the study conducted by Chaves et al (2001), the most well-known SHV-producing bacteria are found in the *Enterobacteriaceae* family, which includes *Klebsiella pneumonia* where SHV-product is detected with a frequency of 80-90%.

Genetically, all SHV variants are derived from the *bla*SHV-1 gene, which is generally considered a plasmid-encoded enzyme with one to seven amino acid substitutions (Drieux et al, 2008). The premature protein consists of 286 amino acids (Thai et al, 2010), and the mature enzyme is yielded by removing the 21 amino acids at the N-terminus from the signal sequence (Kuzin et al, 1999). The *bla*SHV gene encoding has mutated rapidly and has transferred to other gram-negative bacteria in different geographical regions. Jacoby & Bush (2012) have described these as the 117 SHV variants.

---

[4] The sulfhydryl group is a family of organic compounds that contains an R group bound to a sulphur atom and a hydrogen atom that play an important role in biochemical processes and participate in enzymic reactions for the formation and transfer of acyl residues.

In resistance terms, initially SHV ESBL has conferred great hydrolytic activity against penicillin and first generation cephalosporins (Matthew et al, 1973). This hydrolytic activity has expanded over the years to inactivate extended spectrum cephalosporins and monobactam by some structural mutations in the *blaSHV-1* gene (Bush & Jacoby, 2010).

Based on functional properties and molecular characteristics SHV ESBL can be categorised in three main subgroups: subgroup 2b, which has ability to hydrolyse penicillin and only early cephalosporins, e.g. cephalothin and cephaloridine, and can be inhibited by clavulanic acid; subgroup 2br, which confers a broad-spectrum of activity against β-lactamase inhibitors including clavulanic acid; and subgroup 2be, which include SHV β-lactamase and includes enzymes that show hydrolytic activity towards oxyimino β-lactams; cefotaxime, Ceftazidime, and aztreonam (Liakopoulos et al, 2016).

TEM ESBL

TEM gene was first detected in 1965 in *E. coli* isolates (Bonardi et al, 2018). Over 100 TEMs have been discovered and the majority of these are ESBLs (Paterson & Bonomo, 2005). The most effective subtypes of this enzyme include TEM1, TEM2, which structurally substitute lysine for glutamine at position 39 (Grigorenko et al, 2018), and TEM3. TEM1 is capable of hydrolysis with penicillin and first generation cephalosporins, but it is unable to inactivate the oxyimino cephalosporin (Palzkill, 2018). Ninety percent of resistance against penicillin in *E. coli* is due to TEM1 production (Livermore, 1995).

TEM2, which is not categorised as an ESBL, has the same hydrolytic profile as TEM1, but it has a more active native promoter and differences in its isoelectric point (5.6 compared to 5.4; Jain et al, 2008). TEM3, which was originally named CTX-M-1 due to its activity against cefotaxime, is considered the first TEM variant with increased activity against extended spectrum cephalosporins (Rupp & Fey, 2003).

Some TEM derivatives confer negligible hydrolytic activity against extended spectrum cephalosporins and show low affinity for some β-lactamase inhibitors. These TEM derivatives are not classified as ESBL enzymes (Paterson & Bonomo, 2005). However, some recovered mutants in TEM β-lactamase, which are described as complex mutants, develop the hydrolytic activity of this enzyme against third generation cephalosporins and inhibitors, such as clavulanate (Ruppé et al, 2015).

OXA ESBL

The name of the OXA enzyme is derived from its oxacillin-hydrolysing abilities (Scriver et al, 1994), as it confers a hydrolysing rate for oxacillin and cloxacillin that is 50% greater than that of benzyl penicillin (Bush et al, 2010 .The most common OXA subtypes have been detected predominantly in *Pseudomonas aeruginosa* and *E. coli* (Antunes et al, 2014). Unlike other ESBL types, most OXA subtypes do not confer hydrolytic activity against extended spectrum cephalosporins to a significant degree (Santillana et al, 2007).

The different subtypes of the OXA enzyme are classified into two groups according to the hydrolytic activity against cefotaxime, ceftriaxone, and aztreonam. Group (1) includes OXA-11, OXA-14, OXA-16, OXA-17, OXA-19, OXA-15, OXA-18, OXA-28, OXA-31, OXA-32, OXA-35, and OXA-45, which all confer frank resistance against the three aforementioned antibiotics. Group (2) includes one member, OXA-10, which shows weak hydrolytic activity against the same antibiotics. However, it is described as a Carbapenem-hydrolysing enzyme (Toleman et al, 2003).

Most members of Group 1 OXA do not originate as ESBLs, but become ESBLs through mutations derived mainly from the OXA-10 ESBL (Bradford, 2001). The sequencing of any new OXA variant enzyme shows two amino acid changes from the OXA-10 enzyme, altering an arginine at amino acid 143 to serine and a glycine at position 157 to aspartate (Evans & Amyes, 2014). OXA-11 is considered the first example of an OXA enzyme that shows alteration from non-ESBL to ESBL characteristics as a result of OXA-10 mutations (Paterson & Bonomo, 2005).

The fourth type of ESBL β-lactamase enzymes, which has become the most common and rapidly growing health concern, is CTX-M β-lactamase. The epidemiology of different variants of this enzyme in North Wales and studying its activity characteristics are the main subjects of this study.

1.3.3.1.2.1.2. CTX-M Enzyme: The Most Rapidly Increasing Type of ESBLs

The name of the CTX-M enzyme is derived from cefotaxime antibiotics, which can be hydrolysed by CTX-M β-lactamase with a minimum inhibitory concentration (MIC) of less than 64 µg/mL (Baraniak, 2002). The CTX-M has become the predominant type of ESBL detected in many regions of the world (Zhao & Hu, 2013). Potz et al (2006) studied 16 British hospitals to determine the different ESBL types contributing to antibiotic resistance and found that out of 19,252 clinical isolates detected over a 12 week period, CTX-M-producing strains accounted for 1.7%, higher than the other ESBL-producing strains that were predominantly detected in *E. coli* and *Klebsiella* spp. The concern is that these enzymes have contributed to the increased detection of resistant *E. coli* in outpatients and patients admitted in hospitals for short periods of time, which suggests the community acquisition of these strains (Canton, 2006).

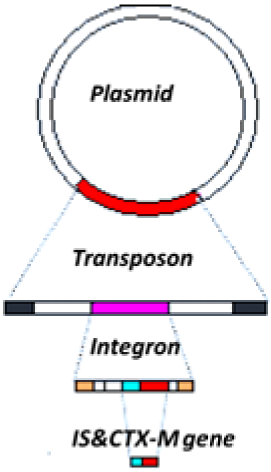
Functionally, based on Kinetic studies the CTX-M-type β-lactamases show higher hydrolytic activity against cephaloridine or cephalothin more than against benzyl penicillin, and they preferentially hydrolyse cefotaxime over Ceftazidime (Shaikh et al, 2015). The CTX-M enzyme's ability to inactivate cefotaxime is approximately 35 times greater than against Ceftazidime, which is inactivated with more efficiency by TEM and SHV cefotaxime (Bonnet, 2004). Furthermore, compared to TEM and SHV, CTX-M has the highest hydrolytic activity against the narrow-spectrum cephalosporins (Drieux et al, 2008). However, since the emergence of CTX-M, the most effective inhibitor for this enzyme has been the combination of tazobactam and penicillin (Bush & Bradford, 2016).

The *bla CTX-M* Gene and Hydrolytic Activity

Unlike other ESBL types, SHV, TEM and OXA, which are generated by substitutions of amino acids in their parent enzymes the different subtypes of CTX-M ESBL are acquired by a horizontal gene transfer between bacteria in which the CTXM gene is carried on either plasmids or transposons (Knudsen et al, 2018). In comparing the genes inside the cefotaxime-susceptible *Kluyvera spp* with the same genes inside the resistant one, it has been observed that the resistant species contain genes showing weak expression for β-lactamase, and they can be inhibited by cefotaxime. These genes, known as *bla*KLU, are found to acquire the CTX-M phenotypes via an insertion sequence (IS; Rodríguez et al, 2004).

ISs are the smallest elements that can transpose independently in the organism, and they can cause genome rearrangements and insertion mutations (Mahillon & Chandler, 1998). One of the main roles of the IS is to move IS mobilised genes among transposons, plasmids, integrons and chromosomes, and this increases the possibility of transferring the resistant determinant (Zhao & Hu, 2013). ISEcp1, which was detected in the plasmid pST01 carried by *E. coli*, is considered the most common insertion sequences and is characterised by having a high-level expression promoter for CTX-M (Bevan et al, 2017). These insertion sequences lie adjacent to the *blaCTX-M* gene and are present in transposon, which is integrated with a plasmid (Figure 1.13; Lartigue et al, 2004).

Figure 1.13 Plasmid-encoding extended-spectrum β-lactamase CTX-M.



blaKLU acquire the CTX-M phenotypes via an insertion sequence (ISEcp1), which is characterised by its high-level expression promoter for *CTX-M* gene (Modified from Cantón,et al.,2012)

The *blaCTX-M* gene is predominantly encoded on large plasmids that tend to carry additional resistance genes, such as those exhibiting resistance to fluoroquinolones and aminoglycosides. These plasmids range in size from 7 to 430 Kb (Cao et al, 2002). The *blaCTX-M* gene is mainly encoded on the incompatibility group FII plasmids, which are characterised by low copy numbers and are known as epidemic plasmids due to their potency to readily acquire and disseminate the resistance genes in *Enterobacteriaceae* members (Zhuo et al, 2013). The most well-known examples of these plasmids are the pandemic dissemination of CTX-M-15, favoured by IncFII plasmids (Irrgang et al, 2017). Unlike most other resistance-carrying plasmids, FII plasmids are thought to be widely distributed and adapted to their hosts even before exposure to antibiotics (Partridge et al, 2011).

In addition to FII plasmids, there are many other plasmids involved in the dissemination of different CTX-M types, including: the IncN plasmids that has been shown to contribute to the dissemination of CTX-3 in Poland (Baraniak et al, 2002); the F33:A-:B-type plasmids encoding CTX-M-65 were first detected in China (He et al, 2013); and the IncK plasmids were responsible for the dissemination of CTX-M-14 in the United Kingdom (Stokes et al, 2012).

Although *the blaCTX-M* gene is encoded predominantly in plasmids, the chromosomal *blaCTX-M* has been reported in some studies worldwide. In Korea, Song et al (2011) detected chromosomal *CTX-M* genes in 21 out of 25 *P. mirabilis* isolates. Furthermore, the chromosomal *blaCTX-M*-25 and *blaCTX-M*-41 have been detected in *P. mirabilis* isolates in Israel (Navon-Venezia et al, 2008).

The *blaCTX-M* gene encodes 291 amino acids and a single change of an amino acid constitutes a new CTX-M type (Giedraitienė et al, 2017). Unlike other ESBLs, this enzyme does not enlarge the active site to accommodate the antibiotics, but instead its hydrolytic activity depends on point substitutions (Taylor & Francis, 2015). The Ceftazidime antibiotic protects itself from the hydrolytic activity of the CTX-M enzyme by having a C7 β-amino thiazol-oxyimino-amide side chain compound, which can only be hydrolysed by Asp 240Gly CTX-M (Gwon et al, 2019). The resistance to Ceftazidime is frequently associated with mutation at amino acid 167 and 240 (Delmas et al, 2008).

CTX-M-Producing Isolates Causing UTI: Limitation of Phenotypic Detection

Urinary tract infections (UTIs) are considered one of the most infectious of diseases in both the healthcare setting and in the general community (Beahm et al, 2017). Every year 150 million people around the world are diagnosed with a UTI (Flores-Mireles et al, 2015) and approximately 40% of women worldwide develop an infection of urinary tract at least once in their lifetime (Micali et al, 2014). The estimated cost resulting from the eleven million people are diagnosed with UTI annually in US is five billion dollars (Foxman, 2014).

UTI are classified generally into two types; Complicated UTIs predominantly occur in elderly people, particularly among post-menopausal women and men with prostatic hypertrophy, and are associated with serious complications, including antibiotic resistance (Pallett & Hand, 2010). Uncomplicated UTIs typically occur across age groups of both sexes (Magliano et al, 2012) and are mostly associated with functional or structural abnormalities of the urinary tract such as obstructions, renal failure, and pregnancy (Durojaiye & Healy, 2015).

In addition to the dysfunction of urinary tract a high residual volume (RU) is considered another risk factor that can lead to the development of a UTI, even without any symptoms in the lower urinary tract (Cho & Kim, 2010). Adult male patients with residual urinary volume more than 180 ml are more likely to have bacteriuria (Jhang & Kuo, 2017). However, the role of RU in the development of UTIs in females is controversial (Truzzi et al, 2008). In addition, behavioural risk factors can contribute in recurrent UTIs among females, of which sexual intercourse is considered the main risk factors, particularly for young women (Mishra et al, 2016).

Uropathogenic *E. coli* (UPEC) as a Main Cause of UTIs

There are a broad range of pathogens that cause UTIs, but uropathogenic *E. coli* (UPEC) remains the most common, representing 80-90% of pathogens associated with community acquired UTIs (Noormandi & Dabaghzadeh, 2015). UPEC is continuously developing resistance against the most commonly used antibiotics (Flores et al, 2015). UPEC is classified into four main phylogroups (A, B1, B2 and D) based on the occurrences of genomic pathogenicity islands (PAI) and the expression of virulence factor acquired by *E coli* to cause infections of urinary tract, such as toxins, adhesins, iron-acquisition system and surface polysaccharides (Hannan et al, 2012).

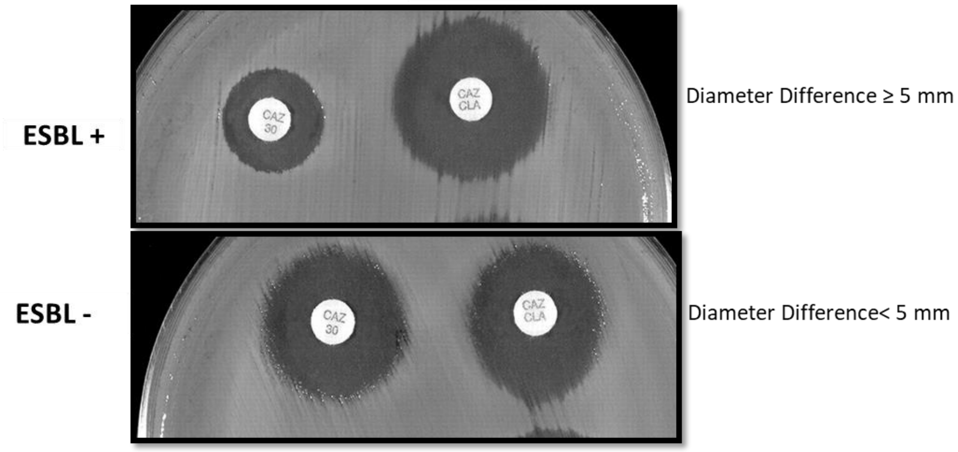
UTIs can be caused by other pathogens, although less frequently than UPEC, including *K. pneumonia*, *P. mirabilis*, *Enterococcus faecalis*, *Streptococcus bovis*, and *Pseudomonas aeruginosa*, in addition to the fungus Candida (Palou et al, 2013).

Role of CTX-M Type ESBL-Producing *E. coli* in UTI

The detection of resistant *E. coli* in UTI patients has increased mainly due to the spread of ESBL-producing bacteria (Laupland et al, 2008). Before 2003 these were TEM and SHV penicillinase-producing bacteria, however they are now being replaced by CTX-M-producing *E. coli*, which is the strain most often acquired in hospitals (Livermore, 2005).

In clinical diagnostic laboratories, the detection of ESBL is primarily based on phenotypic testing, e.g. a double-disc synergy test (Figure 15), which is considered the crucial step in the appropriate management of the patient. This test is highly cost-effective and easy to use (Woodford & Sundsfjord, 2005), but phenotypic methods are unable to distinguish between the different types of ESBLs or to determine the gene responsible for the production of each enzyme. This results in treatment failures in many cases due to the use of inappropriate antibiotics, and to the outbreak of multi-resistant gram-negative pathogens that require expensive control efforts (Tenover, 2007).

Figure 1.14 Combination disc method.



Combined disc diffusion test used to identify ESBL producing bacteria. CAZ = Ceftazidime and CLA = clavulanic acid. Note the difference in the diameter between the two discs, which is the determinant if the test is positive (≥ 5 mm) or negative (Modified from Kumar et al, 2012).

This study attempts to respond to this need by using molecular-based techniques for ESBL detection with the additional ability to detect low-level genetic resistance. With these techniques, it would be possible to determine the contribution of the most rapidly increasing type of ESBL (CTX-M) to the resistance conferred by the causative pathogens of urinary tract infections. In addition, this study will create an epidemiological snapshot of the antibiotic resistance pattern based on CTX-M enzyme production in UTI patients in North Wales.

The samples of the study are ESBL-producing bacteria isolated from 300 urine samples of UTI patients in three referral hospitals in North Wales: Ysbyty Gwynedd, Glan Clwyd Hospital and Wrexham Maelor Hospital.

The research question of this study is to determine to what extent CTX-M-type ESBLs contribute to the resistance phenomenon detected in isolates from UTI patients in North Wales, and whether these CTX-M-producing bacterial isolates carry the same or different CTX-M genes.

1.4. Objectives

This research aims to study the antibiotic resistance of bacterial species isolated from urine of urinary tract infection (UTI) patients at three referral hospitals in North Wales in order to:

(1) Determine the role of *CTX-M-type ESBL* β-lactamases in the antibiotic resistance of UTI patients in North Wales.

(2) Search for novel CTX-M-type gene variants.

(3) In vitro characterisation of recombinant CTX-M-type β-lactamases with novel mutations, including determination of posttranslational modifications (e.g. phosphorylation status) and enzyme kinetics.

Chapter 2 : MATERIALS AND METHODS

Overview

The aims of the study were reached through five main steps. First, samples and data were collected, including patient ages, gender as well as phenotypic resistance to various antibiotics of clinical samples. Second, genes of interest were screened using multiplex PCR amplification of β-lactamase genes *blaCTX-M 1, 2, 9* and *8/25* followed by gel electrophoresis and sequencing. Third, the mutant *CTXM* gene was cloned into an *E.coli* expression plasmid allowing for the purification of a C-terminally strep-tagged recombinant protein. Fourth, protein expression and protein purity were verified using western blotting technique and fifth the minimum inhibitory concentration (MIC) and kinetic constants of induced strep-tagged recombinant proteins were determined against selected antibiotics.

2.1 Microbiology—Cultivation

2.1.1 Sample collection

The samples of the study were Extended Spectrum Beta Lactamase (ESBL)-producing bacteria which were isolated from patients with urinary tract infections and whose specimens were submitted to three referral hospitals in North Wales: Ysbyty Gwynedd, Glan Clwyd Hospital and Wrexham Maelor Hospital (Figure 2.1). One hundred clinical samples of ESBLs were obtained from each hospital when the study started in 2014. The collected data of the study included age, gender and phenotypic resistance to various antibiotics.

Figure 2.1 Location of the three referral hospitals of North Wales.

Map created by geography department, Bangor University, shows the location of the three-referral hospital in North Wales, UK. Ysbyty Gwynedd, Glan Clwyd Hospital and Wrexham Maelor Hospital.

2.1.2 Sub culturing of bacterial isolates

The isolates were all sub cultured from their agar slopes on the primary UTI Agar. In order to identify *Enterococcus spp.*, *Escherichia coli,* and *coliform* bacteria, samples were cultured on the primary UTI Agar (#2421517).

The UTI Agar (formerly Chromogenic UTI Agar) is a chromogenic medium for the presumptive identification and differentiation of all the main micro-organisms that cause urinary tract infections (UTIs) and contain a chromogenic substrate that is hydrolysed by enzymes produced by these bacteria. The β-D-glucosidase produced by *E. coli* hydrolyses the chromogenic substrate to produce pink-red colonies, and the β-glucosidase from coliform bacteria hydrolyses the chromogenic substrate to produce blue colonies (Oxoid Ltd, 2008).

The isolates were sub cultured using a plastic loop for the streak dilution method to achieve single colonies, which ensures the purity of the isolates to carry out the multiplex PCR assay. The plates were then incubated at 37°C for 18–24 h, and the growth of the colonies was recorded. Two types of colonies were observed in the cultured samples: pink colonies (indicating the presence of *E. coli*) and blue colonies (*coliform bacteria*).

2.1.3 Identification of isolates using the Bruker Daltonik MALDI Biotyper technique

Principle: Bacterial identification by matrix-assisted laser desorption/ionisation (MALDI) is based on the use of mass spectrometry to measure the protein content of bacteria, using the mass-to-charge ratio of the proteins. The bacteria are exposed to an ion source to ionise and separate their proteins based on their mass-to-charge ratio, and the mass spectrum generated is compared against a database of mass spectra. Of note, the proteins extracted by the lysis solvantin acidic conditions are mostly basic cytoplasmic proteins, specifically mitochondrial, ribosomal and RNA binding proteins, which all have a range of 4000 to 15000 DA (Ryzhov V. & Fenselau C, 2001).

Sample preparation: First, the bacteria are extracted to generate sufficient spectra. The isolate is dissolved in 50 µL of 70% ethanol, placed in a micro centrifuge and centrifuged at 20,800 rpm for 10 min. The obtained supernatant is discarded, and the cell pellet is dried and mixed with acetonitrile and 50 µL of 70% formic acid before being centrifuged for 2 min at 20,800 rpm. Then, 1 µl of the obtained supernatant is placed in the target plate, dried and loaded with 2 µl of the matrix. Pre-treatment is beneficial because it inactivates the organisms and enhances detection of biomarkers above 15 KD.

Once the samples are loaded, they are introduced into a high-vacuum environment in which they are ionised by a precise laser release of a cloud of protein that is accelerated by an electric charge. The speed at which the proteins unravel is based on their weight: the heavier the protein, the slower it travels. The time of flight (TOF) is recorded, and when the proteins reach the end of the path, a mass spectrum is created for each sample by comparing its obtained spectrum against a library of mass spectra for different bacteria that can be identified at three levels: species, genus and family.

2.1.4 Microbanking the isolates

All isolates were maintained in Microbank cryovials for long-term storage and retrieval (Figure 2.2). Each Microbank™ vial contains approximately 25 sterile coloured beads (single colour) and specially formulated cryopreservative. The specially treated beads are of a porous nature which allows microorganisms to readily adhere onto the bead surface. After inoculation, the Microbank™ vials are kept at -80°C for extended storage. When a fresh culture is required, a single bead is easily removed from the Microbank™ vial and is used to directly inoculate a suitable culture medium (White & Sand., 1985).

2.2. Molecular Analysis

Genotype detection was used to determine the dominant *bla*CTX-M genes responsible for CTX-M-type-ESBL production among *bla*CTX-M groups 1, 2, 9 and 8/25, which were detected between UTI patients. CTX-M-type-ESBL genotypic detection was conducted using the following three procedures: preparing cell-free lysate, amplifying the PCR of *bla*CTX-M genes with oligonucleotide primers and screening these genes using gel electrophoresis and visualization of the gel strips using Quantity One software followed by sequencing.

2.2.1 Preparing cell-free lysates

The isolates of interest were lysed to extract their genomic DNA, which was used as the DNA template. The lysis solution was prepared by making a solution of 0.125 g of 25% sodium dodecyl sulphate (SDS) and 1 g of 0.05% sodium hydroxide (NaOH) dissolved in distilled water to make a volume of 50 ml. A small amount of sub-cultured bacteria from each plate was scraped with a sterile pipette tip, which was dipped into a PCR tube containing 20 µL of lysis buffer and labelled before loading into the PCR machine.

The following protocol was used: the samples were heated at 95°C for 15 min, after which they were maintained at 10°C until the machine was turned on. Once the samples were unloaded from the PCR machine, 180 µL of sterile water was added to each sample, and each was stored at 20°C until it was subject to PCR amplification.

2.2.2. The primer designs

The primers used in this study for blaCTX-M groups 1, 2, 9 and 8/25 were designed using the following specifications to produce accurate results: length of (19–22) nucleotide, base composition of 50–55% (G-C) and average melting temperature of 52–60°C. Computer software was used for the primer design, such as OLIGO 6, which guarantees minimizing the probability of stable primer-dimer formation (Table 2-1).

2.2.3. Primer preparation

The primers were purchased from the Eurofins MWG Operon Company. All primers were diluted to a concentration of 100 pmol/µL, as indicated in the manufacturer’s instructions. The working solution was prepared by the further dilution of 100 pmol/µL solution to 10 pmol/µL (1:10 dilution).

2.2.4. Multiplex PCR amplification of β-lactamase genes *bla*CTX-M 1, 2, 9 and 8/25

The β-lactamase genes *bla*CTX-M 1, 2, 9 and 8/25 were amplified using a multiplex PCR. A total volume of 20 µL of the PCR reaction mixture was prepared for each cell lysate sample. The master mix for the multiplex PCR included 10 µL of Biomex red PCR, 2 µL of forward primers (0.5 µL of the specific primer for each of the groups 1, 2, 9 and 8/25); 2 µL of reverse primers (0.5 µL of the specific primer for each of the groups 1, 2, 9 and 8/25); and 5 µL of water to create a final volume of 19 µL, which was added to 1 µL of bacterial cell lysate. The 3 published primer was used in the multiplex PCR of *bla*CTX-M genes, as shown in Table 2.

Table 2.1 Primer sets used in the PCR amplification of blaCTX-M genes

β-lactamase gene target	**Primer sets**	**Sequences (5′-3′)**	**Amplicon (bp)**	**Reference**
CTX-M group 1	MultiCTXMGp1- for MultiCTXMGp1-2 Orev	TTAGGAARTGTGCCGCTGYA[b]/ CGATATCGTTGGTGGTRCCAT[b]	688	Dallenn et al, 2010
CTX-M group 2	MultiCTXMGp2-for MultiCTXMGp1-2 Orev	CGTTAACGGCACGATGAC[b]/ CGATATCGTTGGTGGTRCCAT[b]	404	Dallenn et al, 2010
CTX-M group 9	MultiCTXMGp2-for MultiCTXMGp1-2 Orev	TCAAGCCTGCCGATCTGGT/ TGATTCTCGCCGCTGAAG	561	Dallenn et al, 2010
CTX-M group 8/25	CTX-Mg8/25 for CTX-M8/25 rev	AACRCRCAGACGCTCTAC[b]/ TCGAGCCGGAASGTGTYAT[b]	326	Dallenn et al, 2010

The positive control (*E. coli* ESBL ATCC51446) was included in the assay. All the labelled PCR tubes were mixed briefly and centrifuged at 13,000 rpm for 30 s before they were arranged in the PCR thermo cycler, which was set in the BLAM program. The conditions of the multiplex PCR were set according to the method published by Dallennet al. (2010) as shown in Table 2.2.

Table 2.2 The multiplex PCR program

Heated lid	**105 °C**	**1 min**
Denaturation	94 °C	40 sec
Annealing	60 °C	40 sec
Extension	72 °C	1 min
Final extension	72 °C	7 min
Number of cycles	30	-

The amplified PCR products were run on agarose gels.

2.2.5 Agarose gel preparation

A 2 % agarose gel was prepared as follows: 100 mL of TBE buffer (1X) was added to 900 mL of dH2O to achieve a final volume of 1000 mL of diluted buffer. Then, 3 g of agarose powder (#9012-36-6) was weighed in a clean flask and 150 mL of diluted buffer was added. The dissolved mixture was then boiled in a microwave for 3–5 min and cooled to 45°C before 15 µL of nucleic acid dye (ethidium bromide) was added; the solution was poured into a gel mould once the gel was solidified. The set gel was placed in the BIO-RAD tank and filled with 500 mL of 1x TBE (Tris-borate-EDTA) buffer.

2.2.6 Samples loading on agarose gel

The first lane of the gel was loaded with 5 µL aliquot of 100 bp ladder (Promega) 5 µL of PCR products. And positive were loaded into the appropriate wells of the gel. After 45 min of electrophoresis, the PCR products were visualised using the Doc200 machine (Bio-Rad) and Quantity One 1-D analysis software (Bio-Rad).

2.2.7 DNA purification

Separating PCR products on agarose gel, the bands of interest were cut under UV light and transferred into an Eppendorf tube in order to purify its DNA using ISOLATE II PCR and Gel Kit (Bioline) (#IS494-BO37210) based on the manufacturer's instructions.

2.2.8 Sequencing the gene-of-interest using the pJET-1.2 forward primer

Samples containing successful recombinant plasmids were sequenced using the pJet-1.2 forward primer, one of Thermo Scientific's sequencing primers that has single-stranded oligonucleotides with 5'-hydroxyl and 3'-hydroxyl ends. The pJET1.2 sequencing primers flanked the Eco32I site in the eco47IR gene of positive selection cloning vector pJET1.2. All primers were supplied as 10 µM aqueous solutions (Thermo Scientific, n.d.).

Samples containing successful recombinant plasmids were sequenced using a pJET plasmid primed with Thermo Scientific sequencing primers that have single-stranded oligonucleotide with 5'-hydroxyl group and 3'-hydroxyl ends. All primers were supplied as 10 µM genes of positively cloned victors. Flank the *E.coli* site.

2.2.9 Cloning the gene of interest into an *E.coli* expression pASK-IBA2C plasmid

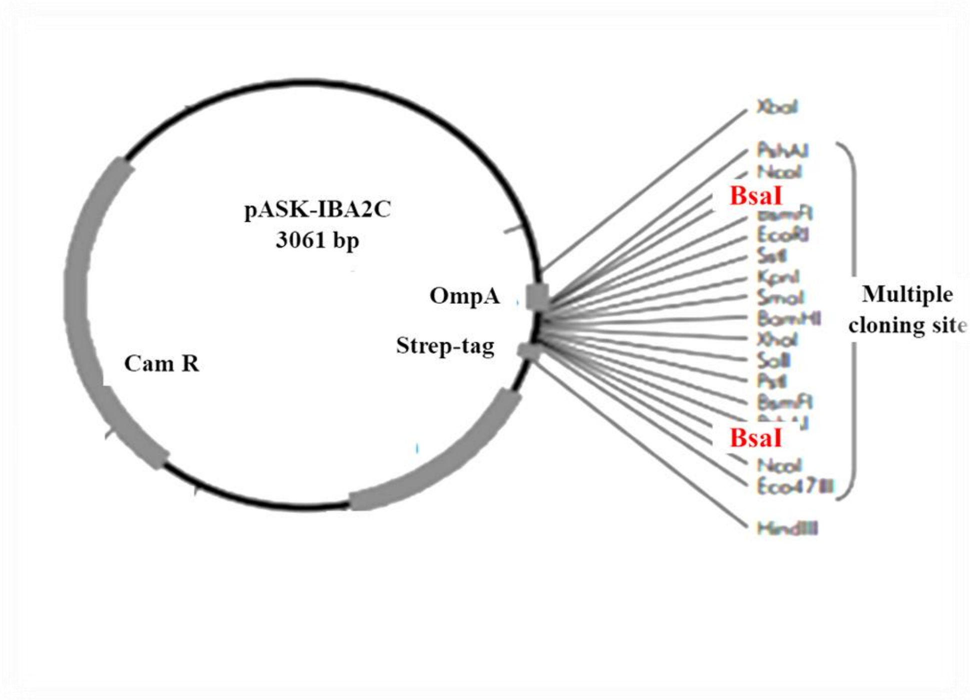
Genes of interest were further analysed upon cloning of the mutant gene into an *E.coli* expression plasmid, pASK-IBA2C, which allows for the purification of strip-tagged recombinant proteins.

To clone genes of interest, full-length genes were amplified from the corresponding samples with the forward and reverse primers (Table 2.3). Both primers contain the BsaI restriction site, which is located in the multiple cloning site of the plasmid (Figure 2.2). The trick is that the enzyme generates an asymmetric cut with long sticky ends (GGTCTCN1 on the forward strand and CCAGAGN5 on the reverse strand). Since the enzymes cut five nucleotides away from the recognition site and because these sequences are different at either site of the two BsaI cuts in the plasmid, the plasmid cannot re-ligate, and only the cloned fragments should give colonies.

Table 2.3 Primer sets

Primer sets	**Sequences (5′-3′)**
CTX-M-14 like-F	atggcataat**ggtctc**a**ggcc**ATGGTGACAAAGAGAGTGCAACGG
CTX-M-15-F	atggcataat**ggtctc**a**ggcc**ATGGTTAAAAAATCACTGCGCCAG
CTX-M-14-like-R	atggcataat**ggtctc**a**gcgct**CAGCCCTTCGGCGATGATTC
CTX-M-15-R	atggcataat**ggtctc**a**gcgct**CAAACCGTCGGTGACGATTTTAG

Figure 2.2 Features of pASK -IBA2C plasmid.



pASK-IBA2C plasmid features: Promoter (*37 to 72 bp*) ,tet-repressor (*1893 to 2516 bp*) ,OmpA signal sequence (*139 to 201 bp*),multiple cloning site (*232 to 318 bp*),Strep-tag® from (*202 to 231bp*), forward primer binding site (*57 to 76 bp*), reverse primer binding site (*407 to 423bp*), CamR resistance gene from (*1023 to 1883 bp*) (Modified from IBA Data Sheet ,2010)

2.2.10. Ligation and transformation of competent *E.coli* with pASK-IBA2C plasmid carrying the gene of interest

The ligation of the expression plasmid with the gene of interest was performed by the T4 DNA ligase enzyme (Promega). This enzyme catalyses the joining of DNA strands between the 5´-phosphate and the 3´-hydroxyl groups of adjacent nucleotides in either the blunt-ended or the cohesive-ended configuration. The pASK-IBA2C plasmid was used as victor to be inserted within the gene of interest as well as the T4 DNA Ligase Buffer (10X), as seen in Table 2.4.

Table 2.4 Ligation mixture

T4 DNA Ligase Buffer (10X)	**2 µL**
Pask-IBA2C plasmid	2 µL
Insert DNA	5 µL
T4 DNA Ligase	1 µL
Nuclease-free water	Up to 20 µL

The ligation mixture was then incubated at 16°C overnight to be transformed into competent cells (competent *E.coli*).

2.2.11 Transformation of plasmid DNA into *E. coli* using the heat shock method

In order to replicate the plasmid carrying the gene of interest, the plasmid was transformed into chemically competent *E.coli* (BIOLINE) (#Bio-85025) using the heat shock method. Then, 5 µL of ligation mixture was added to 50 µL of competent E coli and placed on ice before it was incubated at 42°C for 30 min (heat shock) and then placed back in the ice; 1mL of LB broth media (Table 2.5) was then added to the transformed cells before they were incubated at 37°C for 30 min with agitation.

Table 2.5 500 mL LB broth preparation

Trypton	**5 g**
NaCl	5 g
Yeast Extract	2.5 g
ddH2O	Up to 500 mL & Autoclave (121 C, 20 minutes)

2.2.12 Plating of transformed cells on LB Chloramphenicol agar

As pASK-IBA2C plasmid carries Chloramphenicol-resistant genes, the transformed cells with the plasmid were plated on 25 µg/mL Chloramphenicol LB agar (Table 2.6) in order to isolate the cells containing recombinant plasmid from those that did not. The plated transformed cells were then incubated at 37°C overnight.

After overnight incubation, the growth colonies were incubated overnight in LB broth at 37°C with agitation.

Table 2.6 LB Chloramphenicol agar preparation

Trypton (#D23821)	**5 g**
NaCl (#38011-38036)	5 g
Yeast Extract (#12/MFM/1102)	2.5 g
Agar (#9002-18-0)	6 g
ddH2O	Up to 500 mL & Autoclave (121 C, 20 minutes)
chloramphenicol	50 µL from (50mg/ml Chloramphenicol Stock)

2.2.13 Purification of recombinant plasmids from the transformed *E. coli*.

In order to confirm the presence of the gene of interest in the cloning site of pASK-IBA2C plasmid, the recombinant plasmid from the transformed *E.coli* was purified using ISOLATE II PLASMID KIT (Bioline) (#IS493-BO27120) according to the manufacturer's instructions.

The purified recombinant plasmid was then digested by the restriction enzymes HindIII (Promega) and Xbal (Promega) (Table 2.7). Agarose gel was used to analyse the restricted recombinant plasmid.

Table 2.7 Restriction reaction mix

Recombinant plasmid	**5 µL**
HindIII(Promega)	1 µL
Xbal(Promega)	1 µL
10x NEBuffer 2 (BioLabs)	2 µL
Nuclease-free water	Up to 20 µL

The restriction mixture was then incubated at 37°C for 2h before it was analysed by 1% agarose gel.

2.2.14 Cell growth, gene expression and preparation of bacterial protein extract

2.2.14.1 Host strains

Chemically competent *E. coli* (Bioline) was used as a host strain for periplasmic secretion from recombinant gene expression with the promoter system. The expression of a Strep-Tag fusion protein was induced by the addition of an anhydrotetracycline to logarithmically growing cells. The protein was then exported to the periplasmic space of *E. coli* strain by the ompA leader sequence. The leader sequence was removed when the protein reached the periplasmic space (Plfickthun & Skerra, 1989).

2.2.14.2 Culture, cell harvest and periplasmic extract

To produce the protein, 5 mL of LB/chloramphenicol was incubated with fresh colonies of *E.coli* strains harbouring expression plasmids and was left overnight at 37°C (200 rpm). The preculture was then transferred to 20 mL LB/chloramphenicol in 50-mL Falcon tubes; it was incubated again with agitation (200 rpm) at 25°C for periplasmic extract. For this extract, the optical density (OD) of the culture was monitored at 550 nm, and when an OD550 reached 0.5 gene expression, it was induced by adding 10µL of an anhydrotetracycline solution (aTe; 2 mg/mL in dimethylformamide, DMF), and shaking continued for 3 hr. The induced *E. coli* samples were then harvested by centrifugation (4200g, 12 min, 4°C).

The supernatant was discarded, and the cell pellets were suspended in 200–1000 µl of cold buffer P (Table 2.8) before they were incubated on ice for 30 min and centrifuged for 5 min at 14k rpm. The supernatant, which consisted of recombinant proteins released from periplasmic space, was carefully pipetted as the periplasmic extract and transferred to an Eppendorf tube. This protein solution was directly readied for Strep-Tactin affinity chromatography, and the cell pellet, which consisted of recombinant proteins left in the cytoplasm, was resuspended in the same volume that was used in the buffer P. in 1× SDS sample buffer to be tested with the periplasmic extract on SDS-PAGE.

Table 2.8 Preparation of buffer P

Tris (pH 8.0)	**100 mM**
Sucrose	500 mM
Na2EDTA]	1 Mm

2.2.15 Protein verification by the Western blotting technique (SDS-PAGE analysis)

The aim of the western blotting technique is to identify a desired protein by using a specific antibody which can only bind to the antigen of this protein in complex mixture of proteins. These proteins have been fractionated by a polyacrylamide gel and transferred into blotting membrane to be identified based on their molecular weight in the polypeptide chain.

The immunoblotting can be done in six main steps: denaturing of the extracted protein; separating the proteins of the sample using sodium dodecyl sulfatepolyacrylamide (SDS-PAGE) gel; transferring the separated polypeptides to a blotting membrane; blocking nonspecific binding sites on the membrane; incubating the blocking membrane with the specific antibodies; and detecting and visualising the desired protein based on molecular weight.

2.2.15.1 Protein denaturation and sample preparation

Before loading the protein samples for SDS-PAGE gel electrophoresis, 10 µL from the soluble extract was added to 10 µL of 4 × SDS sample buffer proteins and heated for 3 min at 95°C in order to denature the proteins. This enables the separation of the proteins based on their sizes and prevents proteases enzymes from degrading the samples.

2.2.15.2 Normal SDS-PAGE preparation

Two gels were prepared to analyse the protein extraction samples: lower separation gel and upper stacking gel. The samples were loaded onto different concentrations of lower SDS-PAGE gels according to their size, as they were analysed using 10% gel and then 12%. The four SDS-PAGE concentrations used in this study are shown in Table 2.9.

Table 2.9 Preparation of lower separation gel

Concentration	**8%**	**10%**	**12%**	**15%**
Number of gels	4	4	4	4
1 M Tris-HCl pH = 8.8	7.5 mL	7.5 mL	7.5 mL	7.5 Ml
40% Acrylamide/Bis 37.5:1	4mL	5 mL	6 mL	7.5 mL
10% *APS	100 µL	100 µL	100 µL	100 µL
20% *SDS	150 µL	150 µL	150 µL	150 µL
ddH2O	8.5 mL	7.5 mL	6.5 mL	5 mL
TEMED	30 µL	30 µL	30 µL	30 µL
Total	20 mL	20 mL	20 mL	20 mL

*SDS: Sodium dodecyl persulfate.
*APS: Ammonium Persulfate. *TEMED: Tetramethylethylenediamine

The gel mixture was poured out between Bio-Rad Blotting System glass plates, and the bubbles at the top of the gels were removed by adding drops of isopropanol. The gels were left to set at room temperature for 30 min, and then the isopropanol was washed by dH2O.

Once the lower separation gel was set, the upper stacking gels were prepared, as shown in Table 2.10. Then, the mixture of stacking gels was placed on top of the prepared separation gel layers, and combs were inserted immediately to form the loading. The gels were then left to set at room temperature for 15 min.

Table 2.10 Preparation of upper stacking gel

Concentration of gel	**4%**
Number of gels	4
40% Acrylamide/Bis 37.5:1	1 mL
1 M Tris-HCl pH = 6.8	1.5 mL
10% APS	100 µL
20% SDS	50 µL
dH2O	7.5 mL
TEMED	10 µL
Total	20 mL

2.2.15.3 Running samples on SDS-PAGE

The glass plates carrying the gels were placed in Bio-Rad protein electrophoresis tanks filled with running buffer 1x SDS (Table 2.11). The combes were removed, and the first well of the gel was loaded with 5 µL of the protein standard ladder (Thermo Fisher Scientific), which is a commercially available mixture of proteins of known molecular weights, and the other wells were loaded with the protein samples.

Table 2.11 Preparation of 1x SDS buffer

Glycine(#4037241194)	**28.8 g**
Tris base(#26841)	6.04 g
SDS(#3634737/88)	2 g
dH2O	up to 2 L

Once the samples were loaded, the electrophoresis gels were run at 100 V for 1.45 hr. When the electrophoresis was finished, the gels were removed from the tanks and transferred onto blotting membranes.

2.2.15.4 Membrane transfer

The aim of this step was to make the proteins accessible to antibody detection by transferring the protein from the gel to the blotting membrane, PVDF, or to the nitrocellulose membrane (#115197535710) (GE Health Life Sciences). This was done using an electric current that can pull the negatively charged proteins on the gel towards the membrane on the positively charged anode. The PVDF and nitrocellulose membranes were chosen for their non-specific protein binding properties (i.e., it binds all proteins equally well).

The transfer sandwich was set up in the following order: the black cassette was on the bottom, whereas the pre-wet sponge, filter paper (GE Health Life Sciences), equilibrated gel, PVDF or nitrocellulose membrane, filter paper, pre-wet sponge and red cassette were on top. The transfer sandwiches were then placed in the transfer tank filled with 1x transfer buffer (Table 2.12), and the transfer was run at 65 V for 2.5 hr or at 10 V overnight.

Table 2.12 Preparation of 1x transfer buffer.

Glycine	**28.8 g**
Tris base	6.04 g
Methanol	200 mL
dH2O	2 L

2.2.15.5 Membrane blocking, antibody staining

In order to prevent the interaction between the membrane and the antibodies used to detect the target protein, the membrane was placed in a 3% milk blocking buffer (3% milk powder in 1x PBS, 0.05% Tween 20) for 1 hr on the rocking platform. This enabled the milk buffer to attach to the membrane in all the places where the target proteins had not attached so that the added antibodies could bind to only the target protein.

Once the membrane was blocked, the diluted primary antibody (Table 2.13) was added to the membranes in sealed plastic bags and incubated at -20°C overnight. The primary antibodies were then removed by using the rocking platform and washing them with 1x washing buffer (1x PBS with 0.05% Tween 20) for three times (10 min each) at room temperature.

The washed membranes were then incubated with the diluted secondary antibody in the blocking buffer (Table 2.13) in sealed plastic bags for 1 hr at room temperature using the rocking platform. The membranes were then washed three times (10 min each) by 1x washing buffer to remove any non-binding secondary antibodies.

Table 2.13 Antibodies used in this study

Name of Antibody	Type	Company	Dilution	Product number
Anti-step-TagII	Primary antibody	Bioscience	1:1000	193988
Anti-Rabbit	Secondary antibodies	Millipore	1:10000	401315

2.2.15.6 Detection and visualization

Once the unbounded secondary antibodies were washed, the bounded proteins were detected using X-ray film. The washed membranes were covered completely with white (0.5 mL) and brown (0.5 mL) Western Lightning Plus-ECL solution (PerkinElmer), which is a non-radioactive light-emitting system designed to detect proteins immobilized on a membrane (perkinelmer website).

The membranes were then placed in plastic wallets. These were then put in a dark developing room, under an X-ray film in the cassette of exposure. After few min of exposure (10–20 min), the film was then developed using the OptiMax X-ray Processor machine.

The protein of interest then was identified based on its size by comparing the obtained bands on the X-ray film with the marker or ladder loaded during electrophoresis.

2.2.16 Purification of Strep-Tag® fusion proteins using Strep-Tactin® Spin Columns

The produced recombinant protein was tagged with a short peptide Strep-Tag® II (8 amino acids, WSHPQFEK), which can be genetically fused upstream or downstream to the reading frame of any gene and can be expressed as fusion peptide and has a high selectivity to Strep-Tactin®.

The tagged protein can be purified by the binding affinity between Strep-Tag II and Strep-Tactin using prepacked chromatography columns (iba #18000069), which allows for a gravity flow purification of the Strep-Tag fusion proteins. Table 2.13 shows the buffers needed for the purification.

Table 2.14 Buffers used in the protein purification

Buffers	**concentrations**
Buffer W	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA
Buffer BE (elution buffer)	100 mM Tris/HCl, pH 8.0 150 mM NaCl 1 mM EDTA 2 mM D-biotin

Before the extracts were loaded to the columns, they were equilibrated three times. They were filled with 500 µL Buffer W with centrifuging at each step for 30 seconds at 700 x g (approx. 2000 rpm), which rehydrates the dried Strep-Tactin® resin for subsequent use. The lysate was also centrifuged at 13.000 rpm, for 5 min, and at 4°C in order to remove any aggregates that could clog the column. The Tactin® Spin Column.

The preequilibrated Strep-Tactin® spin column was then loaded with up to 500 µL supernatant of cleared lysate and centrifuged for 30 seconds at 700 x g (approx. 2000 rpm), and 2 µL of collected flow through were analysed with SDS-PAGE. The column was then washed 4 times by 100 µL Buffer W with centrifuging for 30 seconds at 13.000 rpm; 2 µL of the first washing fraction and 20 µL of each subsequent fraction were taken to be analysed with SDS-PAGE.

The recombinant protein in the washed Strep-Tactin® spin column was eluted by placing the column into a fresh 1.5 mL reaction tube and by adding 150µL Buffer BE (Biotin-Elution-Buffer) three times to the column with centrifuging for 30 seconds at 700 x g (approx. 2000 rpm) in the first time and 15 seconds at maximum speed in the subsequent centrifuging. The purified protein was then analysed with SDS-PAGE.

2.2.17 Protein concentrating using Vivaspin 500 Centrifugal Concentrators

In order to maximise the concentration the protein yield, the elute was placed in Vivaspin® 500 centrifugal concentrators (#1703013), which is an ultrafiltration device that offers a simple, one step procedure for sample concentration with starting volumes of up to 500 µL.

2.2.18 Using a Q5® Site-Directed Mutagenesis Kit for the mutagenesis of the gene of interest

The gene of interest was mutated to create substitutions using a Q5® Site-Directed Mutagenesis Kit (#0071605). This was selected because, unlike other Mutagenesis Kits used to mutate genomic DNA (e.g. CRISPR-CAS9), it is capable of mutating plasmid DNA. Mutagenesis was carried out in three steps: (1) exponential PCR amplification using mutagenic primers (Table 2.15, 2.16) and a Q5 Hot Start High-Fidelity DNA Polymerase; (2) the circularisation of amplified material; and (3) the transformation at room temperature into competent *E. coli* (BioLabs website).

Table 2.15 Mutagenic primers sets used in the exponential PCR amplification

Primer sets	**Sequences (5′-3′)**
CTXM-14-P273A C277R F	CCGCAACAGAACGCAGAGAGCCGCAGAGATGTGCTGGC
CTXM-14-P273A C277R R	CTGGGTAAAATAGGTCACCAG
CTXM-14-A55TR	ACGCCCAGCCGCCCTCCGTGC
CTXM-14-A55TR	AGAGATGTGCTGGCCTGGGT

Table 2.16 Mutagenesis PCR reaction mix

Q5 Hot Start High-Fidelity 2X Master Mix	**12.5 µL**
10 µM Forward Primer	1.25 µL
10 µM Reverse Primer	1.25 µL
Template DNA	1 µL
Nuclease-free water	Up to 25 µL

The PCR reaction mixture was run using the PCR program, as shown in Table 2.16.

Table 2.17 PCR program of the PCR Amplification

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
25 Cycles	98°C	10 seconds
	50–72°C	10–30 seconds
	72°C	20–30 seconds
Final Extension	72°C	2 minutes
Hold	4–10°C	

The amplified material was circularized by adding Kinase-Ligase-DpnI (KLD) enzyme mix, as shown in Table 2.18.

Table 2.18 KLD Reaction

PCR Product	1 µL
2X KLD Reaction Buffer	5 µL
10X KLD Enzyme Mix	1 µL
Nuclease-free Water	Up to 10 µL

After 5 µL of KLD mix was added to 50 µL of competent *E coli* and placed on ice, it was incubated at 42°C for 30 min (heat shock) and then placed back in the ice; 1mL of LB broth media (Table 2.6) was then added to the transformed cells before they were incubated at 37°C for 30 min with agitation. The transformed cells with the plasmid were plated on 25 µg/mL Chloramphenicol LB agar (Table 2.7). The plated transformed cells were then incubated at 37°C overnight. After overnight incubation, the growth colonies were incubated in LB broth at 37°C overnight with agitation.

The recombinant plasmid carrying the gene with mutation was purified from the transformed *E. coli* using the ISOLATE II PLASMID KIT and prepared for sequencing. After sequencing, the mutated gene was induced, purified and concentrated (see sections 2.2.15.5, 2.2.15.6 and 2.2.15.7).

2.2.19 Biochemical characterization

Biochemical characterization involved studying the phosphorylation status of B-lactamase proteins, which was completed by two methods:

2.2.19.1 Isoelectric-focused analysis of the CTX-M proteins

The isoelectric (2 D) gel electrophoresis technique is a molecular technique in which proteins are distributed across the 2 D gel profile; they are first separated by their isoelectric point (pI) in isoelectric focusing and then are further separated by molecular weight using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). This technique expands the number of proteins that can be identified, and it efficiently provides detailed data for proteomics analysis (Florian Weiland et al., 2014).

In the 2D analysis, 15 μL of protein extract was further diluted in 110 μL of IEF buffer, and the total 125 μL was loaded onto the 2D tray. The immobilized pH gradient (IPG) strips (#163-2000) nonlinear (pH 3–10, Bio-Rad) were then put over the protein solution, and the strips were then overlaid with 2 mL of mineral oil, and the 2D program was set, as shown in Table 2.19.

Table 2.19 Programme of 2D analysis

Running	**Active dehydration at 50 V for 12 hr**
Focusing	Rapid method, 10,000 V, hold to 500 V

After the run was completed, the 2D strips were washed in 2D tray using the rocking platform with 2D solution: solution I (6 M urea, 0.375 M Tris-HCl [pH = 8.8], 2% SDS, 20% glycerol, 2% [w/v] Dithiothreitol [DTT]) for 10 min; and solution II (Tris-HCl pH = 8.8, 2% SDS, 20% glycerol, 2.5% [w/v] iodoacetamide) for another 10 min.

The 2D strips were run on a 10% normal SDS-PAGE gel with only the lower separation layer (Table 2.9). The separation gels were cast right to the top of the glass plates, leaving approximately 5 mm space to apply the strip. The electrophoresis gels were run at 100 V for

1.5 hr and the transfer, blocking and visualizing were performed, as mentioned in sections 2.2.15.5 and 2.2.15.6.

2.2.19.2 Phos-tag gels

In order to analyse the phosphorylation status of the proteins, the manganese (II) Phos-tag SDS-PAGE was used, which specifically delays the mobility of the phosphorylated proteins at a neutral pH. The lower Phos-tag SDS was prepared in the way shown in Table 2.20.

Table 2.20 Preparation of phos-tag gel

Acrylamide/Bis 29:1	**1.4 mL**
1 M Tris-HCl pH = 8	1.8 mL
5 mM Phos-tag	70 µL
10 mM MnCl2	70 µL
20% SDS	27 µL
TEMED	15 µL
10% APS	70 µL

The gel mixture was poured out between the Bio-Rad Blotting System glass plates, and the bubbles at the top of the gels were removed by adding drops of isopropanol. The gels were left to set at room temperature for 30 min, and then the isopropanol was washed by dH2O.

Once the lower separation gel was set, the upper stacking gels were prepared, as shown in (Table 2.10) then, the mixture of stacking gels was placed on top of the prepared separation gel layers, and combs were inserted immediately to form the loading. The gels were left to set at room temperature for 15 min.

The gels were then run with electrophoresis as described in section 2.2.15.3. Once the run was finished, the gels were washed three times in 1x transfer buffer with 20 mM EDTA to remove the Mn++ ions, as they interfere with the transfer. The transfer, blocking and visualizing were performed as mentioned in sections 2.2.15.5 and 2.2.15.6.

2.2.20 Determining the MIC of induced strep-tagged recombinant genes using the E-Test

The determination of minimum inhibitory concentrations (MICs) of induced strep-tagged recombinant genes using the E-Test method reveals how active the β-lactamases are against a range of different antibiotics. The E-Test method is a well-established assay for testing antimicrobial resistance by applying a predefined gradient of antibiotic concentrations on a plastic strip onto a layer of bacterial cells (Biomerieux website).

Materials needed for E-Test are, Agar plate with selected media for susceptibility test, inculcation suspension media, swabs used for inclusion of suspended strains on agar plate, 0.5 McFarland turbidity standards, and antibiotic strips.

To determine the MIC of the recombinant genes, the fresh colonies of the *E.coli* strains that harboured expression plasmids with the gene of interest were induced before they were plated with E-Test strips of selected antibiotic (Table 2.21). Plates were incubated at 35 °C. MIC detection was repeated in biological triplicates for each antibiotic.

Table 2.21 E-test strip of selected antibiotics

Name of Antibiotic strip	Company	Product number
Nitrofurantoin	BIOMÉRIEUX	10047676160
Ceftazidime	BIOMÉRIEUX	1004719296
Cefoxitin	BIOMÉRIEUX	1004857420
Cefotaxime	BIOMÉRIEUX	100495820
Imipenem	BIOMÉRIEUX	100412373

2.2.21 Determination of kinetic constants

The kinetic constants of the protein of interest was measured by mixing a limited amount of purified β-lactamase proteins with different concentrations of selected lactam antibiotics at 25°C in the assay buffer (50 mM phosphate buffer, pH 7.0) in a 1-cm quartz cuvette using a spectrometer for 12 min.

The absorbance was measured in triplicate with each concentration and then a nonlinear regression of the Michaelis-Menten equation was applied to the data in order to determine the Km (He, DD et al., 2015).

The protein concentration in the tested samples at absorbance (at λ 280nm) was determined using Protein Concentration Calculator web (www.aatbio.com/tools/calculate-protein-concentration). The determination of the protein in solution was based on substituting of the molecular weight of the protein, extinction coefficient, and λmax (280nm), which was measured by spectrophotometer, into a derived form of the Beer-Lambert law.

Chapter 3 : URINARY TRACT INFECTIONS DATA ANALYSIS

3.1 Introduction

Urinary tract infections (UTIs) are considered one of the most infectious diseases occurring both in the healthcare setting and in the general community (Rowe & Juthani-Mehta, 2013). UTIs can often be diagnosed quickly and treated effectively with antibiotics (Schmiemann et al., 2010). However, some UTI patients cannot be treated with commonly-used antibiotics, a situation thought to be caused by resistant species, of which producers of Extended-spectrum beta-lactamases (ESBLs) are the most common and detectable pathogens in UTI patients (Al Yousef et al., 2016).

ESBLs are enzymes produced mainly by gram-negative bacteria (*E. coli*, coliforms, *Acinetobacter spp.* and others), and they can exhibit a resistance to most beta-lactam antibiotics, including third- and fourth-generation cephalosporins, which severely limits the applicable treatments for infections caused by ESBLs (Blaak H et al., 2013). However, these ESBL-producing strains may be inhibited by beta-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam (Engler et al., 2017). The spread of ESBL-producing bacteria has become a major global health concern over the past generation (Kuralayanapalya et al., 2019).

Despite the high number of patients referred for urinalysis due to infection of the urinary tract in North Wales health care settings, as it is shown in table 3.1, between 2011 and 2013 from the total number of 193142 cases 68% were referred from their local GP to the hospital, few epidemiological studies have looked for a pattern of antibiotic resistance resulting from ESBL producers.

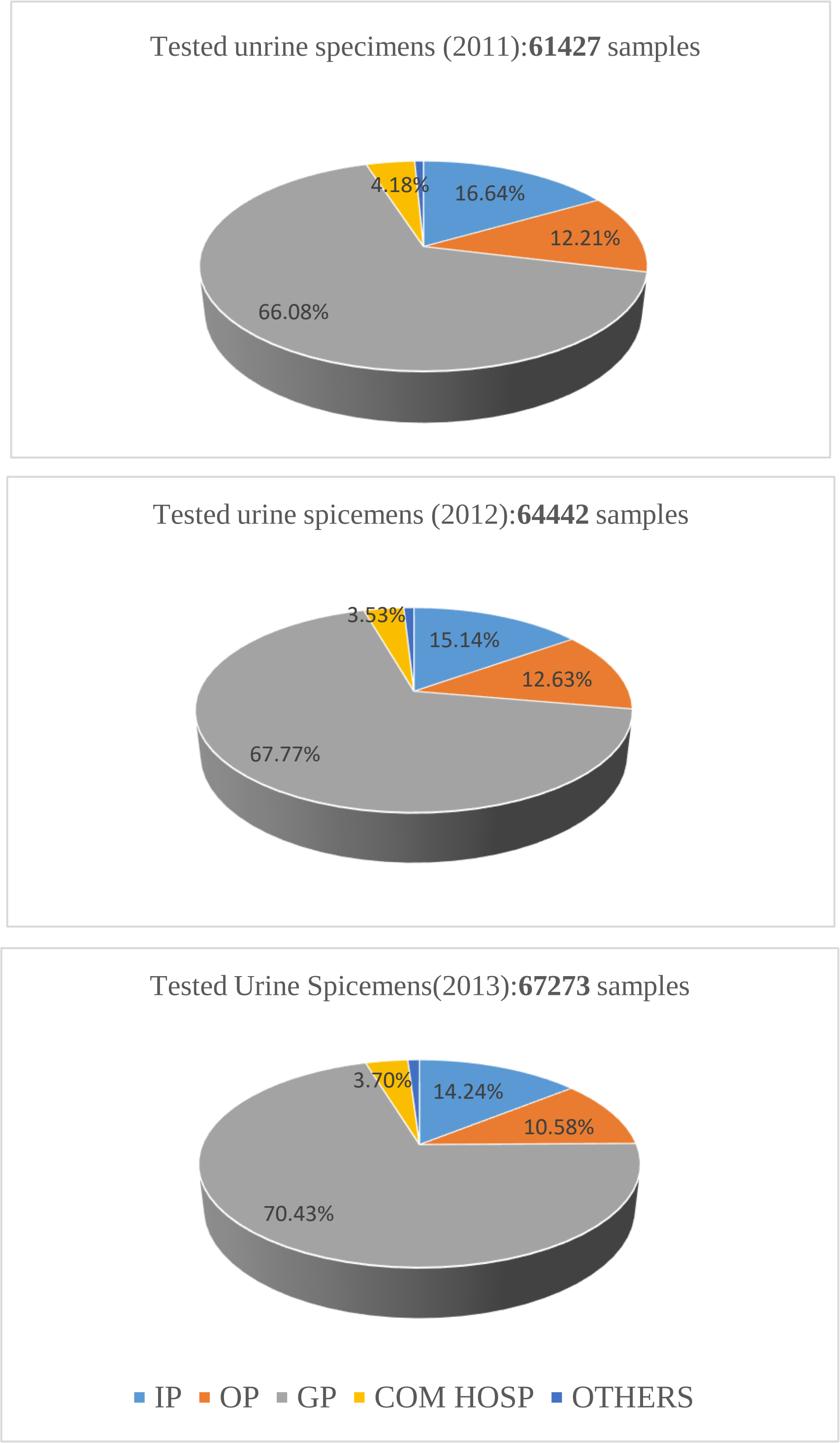
This part of the study attempts to respond to this research need by analysing the data of UTI patients in a North Wales tertiary care hospital, Ysbyty Gwynedd Hospital, to identify the risk factors associated with isolations of *E. coli* carrying ESBL in urine samples from hospitalised patients and outpatients.

Table 3.1 Urine specimens tested from 2011 to 2013 in Ysbyty Gwynedd Hospital

Source/Years	**2011**	**2012**	**2013**
IP	10227	9761	9584
OP	7503	8145	7120
GP	40658	43677	47386
COM HOSP	2570	2278	2493
OTHERS	469	581	691
Total	61427	64442	67273

Data taken from Ysbyty Gwynedd Hospital for patients referred for urine tests over three years (2011-2013); the groups are IP (Inpatients), OP (Outpatients), GP (General practice), com hospital (Community hospital: Dolgellau , Alltwen Hospital in Porthmadog and Stanley Hospital in Holyhead), and other sources.

Figure 3.1 The number of urine specimens submitted to Ysbyty Gwynedd Hospital over the three years (2011-2013)



Number of patients referred for urinalysis due to symptoms of the urinary tract infection to one of the three main referral hospital in in North Wales. It is noticeable that the number of tested urine samples has increased from 61427 in 2011 to 67273 in 2013. It is noticeable also that GP samples represents more than two thirds (68%) from the total number of tested samples over the three years which indicates to increasing in community acquired infection.

Research ethics

The patient specimens were routine diagnostic requests sent to the Microbiology laboratory for testing from primary and secondary care settings. Once in the laboratory the data collected included source, age and gender of patients, phenotypic antibiotic screening and causative organisms. Each data set was anonymised and no patient identifiable information was recorded.

3.2 Results

3.2.1 Data collection

Isolates from Ysbyty Gwynedd Hospital were screened and tested phenotypically and genetically to detect the cause of CTXM-type ESBL–producing bacteria in the antibiotic-resistance pattern among UTI patients in North Wales. Despite there being 300 such isolates at the three main referral hospitals in North Wales, access to only 100 samples and medical data was available from Ysbyty Gwynedd Hospital. These 100 isolates were statistically analysed in terms of frequency of UTI among ages and genders, detected ESBLs and the distribution of phenotypic resistance against various antibiotics. Table 3.2 shows the medical data of 100 samples (No 201–300) collected from Ysbyty Gwynedd Hospital in 2014.

Table 3.2 Characterisation of isolates from Ysbyty Gwynedd Hospital (201-300)

				Phenotypic antibiotic															
Lab #	**Isolate #**	**Age**	**Sex**	AMO	CPD	AUG	NIT	TRI	CTX	CAZ	GEN	AMI	IMI	MER	PTZ	CIP	CPD/CV	**ID**	**GP/Hosp**
27697	201	77	F	R	R	S	S	R	R	R	S	S	S	S	S	S	+	*Colif*	GP
G28282	202	55	M	R	R	S	S	R	R	S	S	S	S	S	S	R	+	*Colif*	GP
27855	203	56	M	S	S	S	S	R	S	S	S	S	S	S	S	S	+	*E. coli*	GP
28469	204	88	M	R	R	S	R	R	R	R	S	S	S	S	S	S	+	*E. coli*	Hosp
25696	205	72	M	R	R	S	S	S	R	R	R	S	S	S	S	S	+	*E. coli*	Hosp
23484	206	71	F	R	R	R	R	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
23623	207	69	F	R	R	S	S	S	R	R	S	S	S	S	S	S	+	*E. coli*	GP
23414	208	100	F	R	R	R	S	R	R	R	S	S	S	S	S	R	+	*E. coli*	Hosp
23842	209	77	M	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
12030	210	75	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
12294	211	92	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
12675	212	93	F	R	R	S	R	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
12640	213	90	F	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
13095	214	68	M	R	R	R	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
15309	215	57	F	R	R	S	S	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
15227	216	59	M	R	R	R	R	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
20057	217	83	F	R	R	R	S	R	R	S	R	S	S	S	R	R	+	*E. coli*	GP
27700	218	93	M	R	R	S	R	R	R	R	R	S	S	S	S	S	+	*Prote*	GP
28287	219	90	M	R	R	S	S	R	R	R	R	S	S	S	S	R	+	*Colif*	GP
11773	220	80	M	R	R	S	R	R	R	R	R	S	S	S	S	S	+	*Colif*	Hosp
28316	221	92	F	R	R	S	R	R	R	R	S	S	S	S	S	S	+	*Colif*	GP
23635	222	68	M	R	R	R	S	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
23456	223	92	F	R	R	S	S	R	R	R	S	S	S	S	S	R	+	*E. coli*	GP
23670	224	85	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
23362	225	81	M	R	R	S	R	R	R	R	S	S	S	S	S	R	+	*E. coli*	Hosp
12472	226	81	F	R	R	S	S	R	R	R	S	S	S	S	S	R	+	*E. coli*	Hosp
12564	227	62	F	R	R	S	S	R	R	R	R	S	S	S	S	R	+	*Colif*	Hosp
12672	228	52	M	R	R	S	R	R	R	R	S	S	S	S	S	S	+	*Colif*	GP

				Phenotypic antibiotic															
Lab #	**Isolate #**	**Age**	**Sex**	AMO	CPD	AUG	NIT	TRI	CTX	CAZ	GEN	AMI	IMI	MER	PTZ	CIP	CPD/CV	**ID**	**GP/Hosp**
12447	229	70	M	R	R	R	R	R	R	R	S	S	S	S	S	S	+	*Colif*	GP
13428	230	62	F	R	R	R	S	R	R	R	R	S	S	S	S	R	+	*Colif*	GP
15581	231	97	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	Hosp
15217	232	40	F	R	R	R	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
18069	233	84	M	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*Colif*	Hosp
27717	234	96	F	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
15306	235	79	F	R	R	S	S	S	R	R	S	S	S	S	S	R	+	*E. coli*	GP
15468	236	73	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	Hosp
13373	237	82	M	R	R	S	S	R	R	S	S	S	S	S	S	R	+	*E. coli*	GP
13388	238	86	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
13232	239	74	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
13291	240	88	F	R	R	R	R	R	R	R	S	S	S	S	S	R	+	*Colif*	GP
13369	241	77	F	R	R	R	R	R	R	R	S	S	S	S	S	S	+	*Colif*	Hosp
12405	242	66	F	R	R	S	S	R	R	R	S	S	S	S	S	S	+	*E. coli*	GP
23471	243	93	F	R	R	R	S	R	R	R	S	S	S	S	S	S	+	*E. coli*	Hosp
23604	244	25	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*Colif*	Hosp
23631	245	74	M	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
28223	246	87	F	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
28096	247	96	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*E. coli*	GP
28194	248	25	F	R	R	R	R	R	R	R	R	S	S	S	S	R	+	*Colif*	GP
27651*	249	91	F	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*Colif*	GP
27551*	250	92	F	R	R	S	R	R	R	R	R	S	S	S	S	R	+	*Protu*	GP
27717	251	96	F	R	R	S	R	R	R	R	S	S	S	S	R	R	+	*E. coli*	GP
23946	252	74	M	R	S	S	R	R	R	S	S	S	S	S	S	R	+	*Steno*	Hosp
27301	253	94	F	R	R	R	S	S	R	S	R	S	S	S	R	S	+	*E. coli*	GP
27297	254	86	F	R	R	S	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
27181*	255	73	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
27181*	256	73	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*Prote*	Hosp
26083	257	91	F	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*Colif*	GP
26988	258	71	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
26548	259	86	F	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp

				Phenotypic antibiotic															
Lab #	**Isolate #**	**Age**	**Sex**	AMO	CPD	AUG	NIT	TRI	CTX	CAZ	GEN	AMI	IMI	MER	PTZ	CIP	CPD/CV	**ID**	**GP/Hosp**
25942	260	64	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
25673	261	73	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
23781	262	91	F	R	R	S	R	R	R	R	R	S	S	S	R	R	+	*Colif*	GP
24954	263	75	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
24543	264	88	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
24623	265	73	F	R	R	R	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
23096	266	80	F	R	R	R	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
25706	267	84	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
23759	268	89	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
24026	269	90	F	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
24526	270	73	M	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
23760	271	22	F	R	R	R	R	S	R	R	R	S	S	S	R	R	+	*E. coli*	GP
23787	272	3	F	R	R	S	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
23789	273	94	F	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
23975	274	20	F	R	R	R	S	R	R	R	R	S	S	S	S	S	+	*E. coli*	GP
24542	275	86	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
24654	276	86	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
25006	277	88	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*Colif*	GP
25065	278	95	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
25864	279	89	M	R	R	R	R	R	R	R	R	S	S	S	S	S	+	*Colif*	GP
24785	280	80	M	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
25792	281	90	M	R	R	S	S	R	R	R	R	S	S	S	R	R	+	*Colif*	Hosp
26477	282	80	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*Colif*	GP
25691	283	90	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
25898	284	49	F	R	R	S	S	R	R	R	S	S	S	S	R	R	+	*E. coli*	GP
26523	285	100	F	R	R	R	R	R	R	R	S	S	S	S	R	R	+	*E. coli*	GP
26592	286	80	F	R	R	S	S	R	R	R	S	S	S	S	R	R	+	*E. coli*	Hosp
27083	287	80	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
26602	288	20	F	R	R	R	S	R	R	R	S	S	S	S	S	S	+	*E. coli*	GP
26852	289	86	F	R	R	S	S	R	R	S	S	S	S	S	R	R	+	*E. coli*	Hosp
26822	290	93	F	R	R	R	S	R	R	R	S	S	S	S	R	R	+	*E. coli*	Hosp

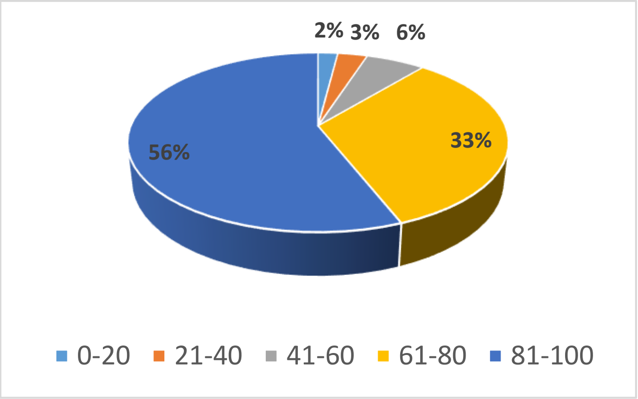
				Phenotypic antibiotic															
Lab #	**Isolate #**	**Age**	**Sex**	AMO	CPD	AUG	NIT	TRI	CTX	CAZ	GEN	AMI	IMI	MER	PTZ	CIP	CPD/CV	**ID**	**GP/Hosp**
26675	291	50	F	R	R	S	S	R	R	R	S	S	S	S	R	R	+	*E. coli*	GP
27449	292	79	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
27554	293	90	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
27442	294	79	M	R	R	S	S	R	R	S	S	S	S	S	R	R	+	*E. coli*	GP
27611	295	76	M	R	R	R	R	R	R	R	S	S	S	S	R	R	+	*E. coli*	GP
27184	296	59	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	Hosp
27303	297	93	F	R	R	S	S	R	R	S	S	S	S	S	R	S	+	*E. coli*	Hosp
27231	298	69	M	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
27383	299	80	F	R	R	R	R	R	R	R	R	S	S	S	R	R	+	*E. coli*	GP
27112	300	79	F	R	R	R	R	R	R	R	S	S	S	S	R	R	+	*E. coli*	Hosp

F: female, M: male, R: resistant, S, Sensitive, Amo: Amoxicillin, CPD: Cefpodoxime, CIP: ciprofloxacin, Aug: Augmentin, Ni: Nitrofurantoin, CTX: cefotaxime, CAZ: Ceftazidime, Gen: Gentamicin, Ami: amikacin, IMI: Imidazole, Mer: Meropenem, Ptz: piperacillin/tazobactam, GP: general practice

3.2.2 Frequency of UTI according to patient age and gender

The data collected in the present study (Table 3.2) show that the prevalence of UTI may be affected by age. People aged 0–20 represents the lowest frequency of UTI problems (3%) while those aged 61–100 are most susceptible to UTIs (89%), followed by those aged 41–60 (8%) and 21–40 (4%).

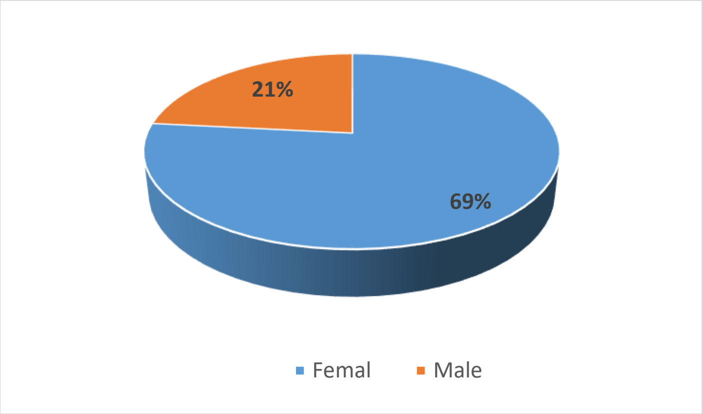
Figure 3.2 UTI prevalence based on patient age



The cut-off value used to group the prevalence (i.e. percentage) of each age group from 100 UTI patients (>20, 21–40, 41–60, and 81–100) based on the patient ages provided in Table 3.2 (Medical data collected from Ysbyty Gwynedd Hospital in 2014.

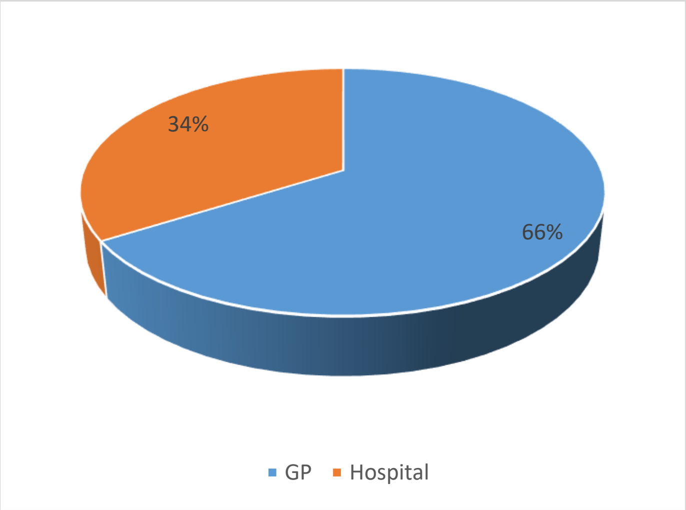
The data also shows that 69% of diagnosed UTIs occur in females, which is thought to be due to anatomical differences between the sexes (Figure 3.3). Moreover, most of the infections were community-acquired, with 66% of the infection cases detected by general practitioners (GPs; Figure 3.4).

Figure 3.3 UTI prevalence based on patient gender



Susceptibility for urinary tract infections according to gender. % of females to males from 100 UTI patients (Medical data collected from Ysbyty Gwynedd Hospital in 2014.

Figure 3.4 Hospital and community acquired UTI



Percentage of community acquired to hospital acquired urinary tract infections in 100 UTI patients (Medical data collected from Ysbyty Gwynedd Hospital in 2014). * GP (General practice).

3.2.3 ESBL-producing isolates as a causative organism for UTI

The screening of extended-spectrum beta-lactamase was carried out in the microbiology department of Ysbety Gwynedd by the Combination Disc (diffusion method) in accordance with CLSI (Clinical and Laboratory Standards institution) guidelines which propose diffusion methods for 21 antibiotics susceptibility testing used in secreting of ESBL-producing isolates (CLSI,2016).

In the combination Disc method two discs containing antibiotics are positioned on the agar plates: one Cefpodoxime disc (CPD) and one Cefpodoxime with clavulanic acid (CPD/CV) disc. Following incubation with the same bacterial isolate, the diameter of the inhibition zone around the two discs is measured, and the test is considered positive (ESBL-producer) if the difference in diameter between the two discs is ≥ 5 mm (Carter et al., 2000).

Medical data from Ysbyty Gwynedd Hospital on UTI patients shows that 99% of the detected isolates among UTI patients display a resistance to CPD which are susceptible to the combination of Cefpodoxime and clavulanic acid. The resistant to Cefpodoxime indicates the expression of an Extended-spectrum beta-lactamases (ESBL) (Figure 3.5). Only one isolate in the study exhibited sensitivity to CPD disc (isolate #203).

The high prevalence of ESBLs in the study samples is emphasised by the ESBL data from Ysbyty Gwynedd Hospital (Tables 3.3, 3.4 and 3.5), which show that the total number of detected CPD-resistant isolates among UTI patients from different areas in North Wales has increased over three years (2011–2013) from 831 to 1345, while the detection of ESBL-producing isolates-which represent two thirds of detected CPD-resistant isolates (Figure 3.6)-has grown accordingly from 569 to 963 over the same period.

Table 3.3 Total Number of detected *E.coli/coliforms* amongst UTI patients from 2011-2013 in YG hospital.

Source/Years	**2011**	**2012**	**2013**
IP	1424	1570	1964
OP	872	1195	1252
GP	7793	9970	11654
COM HOSP	414	462	624
OTHERS	52	50	56
Total	10555	13247	**15549**

Table 3.4 Detected CPD-resistant isolates in *E.coli/coliforms* between 2011 and 2013 at Ysbyty Gwynedd Hospital

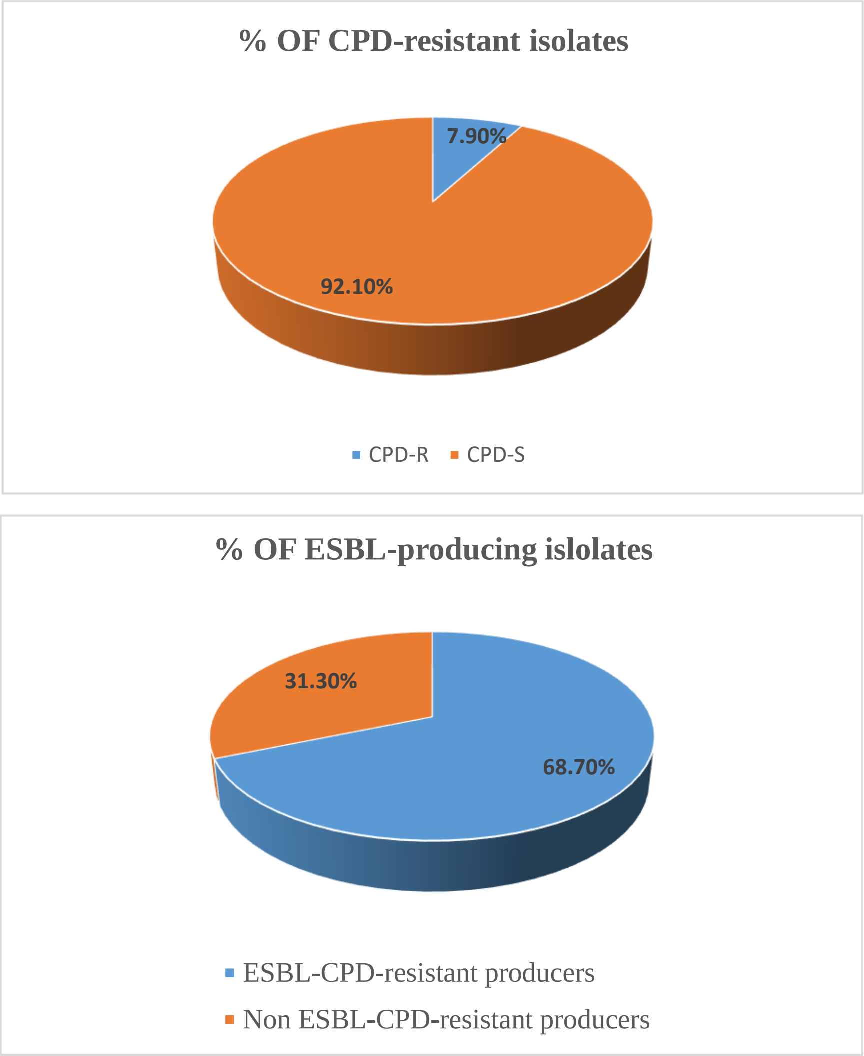
Source/Years	**2011**	**2012**	**2013**
IP	155	163	274
OP	67	59	94
GP	552	654	864
COM HOSP	51	57	109
OTHERS	6	5	4
Total	831	938	**1345**

Table 3.5 Number of detected extended-spectrum beta-lactamase (ESBL)–positive isolates from CPD-resistant isolates between 2011 and 2013 at Ysbyty Gwynedd Hospital

Source/Years	**2011**	**2012**	**2013**
IP	116	113	211
OP	40	35	65
GP	371	410	590
COM HOSP	38	46	94
OTHERS	4	4	3
Total	569	608	**963**

Data taken from Ysbyty Gwynedd Hospital on patients referred for urine tests over three years (2011–2013): **(Table 3.3.)** shows the total number of *E. coli* and coliform, which increased from 10,555 to 15,549 over the three years; **(Table.3.4)** shows the total number of CPD-resistant isolates (Cefpodoxime is one of the antimicrobial agents recommended by the National Committee for Clinical Laboratory Standards for screening isolates of *Klebsiella spp.* and *Escherichia coli* for extended-spectrum β-lactamase (ESBL) production), which rose from 831 to 1,345 over the same three years); **(Table.3.5)** shows the number of detected ESBL-producing isolates, which approximately doubled between 2011 and 2013.* IP (Inpatients) *OP (Outpatients),* GP (General practice),* com hospital (Community hospital: Dolgellau , alltwen hospital in porthmadog and stanley hospital in Holyhead)

Figure 3.5 ESBL among CPD-resistant isolates over three years (2011-2013)



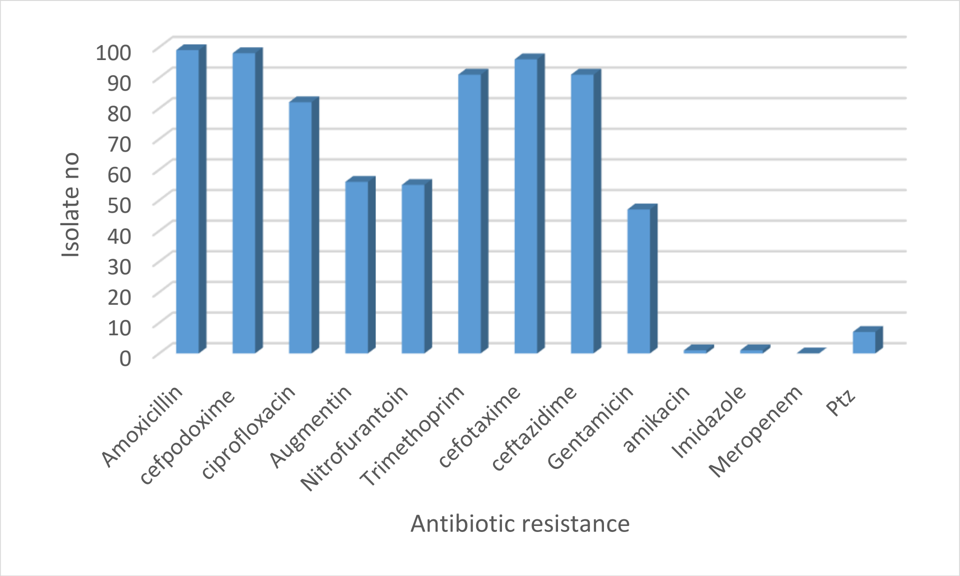
The percentage of CPD-resistant isolates among 39351 *Ecoli/Coliforms* detected in urine samples tested over three years 2011-2013:**7.9%** (3114). Among 3114 CPD-resistant *Ecoli/Coliforms* more than two thirds (68.70%) of isolates show ESBL production-based resistance.

3.2.4 Antibiotic resistance profile

Antibiotic profile includes agreed first line antibiotic usage for treatment of UTIs in primary and secondary care with diagnostic protocol following HPA (Health Protection Agency) and UCAST guideline.

It can be observed from Table 3.2 that the isolates displayed the highest resistance to amoxicillin (99%), Cefpodoxime (98%), cefotaxime (96%), Ceftazidime (91%) and trimethoprim (91%), while the isolates were almost completely susceptible to amikacin, imidazole and meropenem (Figure 3.6).

Figure 3.6 Phenotypic resistance to various antibiotics.



Charts shows the resistance of ESBL producing isolates to agreed first line antibiotic usage for treatment of UTIs in primary and secondary care with diagnostic protocol following HPA (Health Protection Agency) and UCAST guideline.

3.2. Discussion

According to the data from Ysbyty Gwynedd Hospital (Table 3.1), 67,273 patients were referred to the hospital between January and December 2013 for urinalysis after reporting symptoms of the urinary tract infections. This is a high incidence rate, representing approximately 34.5% of population of the Gwynedd and Anglesey study area (population of 194,780; Welsh Government. statistics and research department, 2019).

One possible explanation is that North Wales is a rural area, with approximately 80% land managed for farming (National Assembly for Wales, 2017). The consumption of antibiotics by livestock, which is considered a major risk factor for the emergence of antibiotic resistance, has increased significantly in recent years according to the review on antimicrobial in agriculture and the environment published in 2015 (Review on Antimicrobial Resistance, 2015). However, it should be remembered that AB usage is much reduced on the beef and sheep farms typical of North Wales (UK Veterinary Antibiotic Resistance and Sales Surveillance Report, 2019), making this less likely to be the main explanatory factor for our results.

The present study confirms previous reports of a significantly higher prevalence of UTIs among females. Based on data by Ysbyty Gwynedd Hospital, women represent around two thirds of infected patients. Proposed reasons for this cite anatomic differences including shorter urethral length and the moist periurethral environment in women (Hickling et al., 2015). However, unlike other studies including that by Sobel (2014) which conclude that young, sexually-active women 18–24 years of age have the highest incidence of UTIs, the present study shows that the majority of women with UTIs in North Wales are over 40; only 7.2% are aged under 25.

As can be seen in Table 3.2, a large majority of patients (88%) were over 60 years of age, which supports the conclusion of many studies that increasing age is itself a risk factor for UTIs. There are many possible reasons for the susceptibility of elderly people to UTIs, including: urinary incontinence and urinary retention, hospitalisations and accompanying urinary catheterisations, long-term medical institutionalisation, immunity senescence as well as other factors such as anatomic abnormalities of the urinary tract, particularly those which produce incontinence or urinary retention (e.g., prostatic hyperplasia) (Wang et al.,2017). The high UTI incidence in North Wales can be explained by the high percentage of old people in the population of North Wales Which, according to Welsh Government services and information, is expected to rise from 18% (as determined in 2008) to almost 26 % of the Welsh population by 2033.

Regarding community and nosocomial (hospital) acquisition of UTIs, the data support previous research finding that community-acquired (uncomplicated) UTI infections are of a higher prevalence than those acquired in hospitals. According to many reviews (e.g. Kucheria et al. 2005), *E. coli* causes less than 50% of nosocomial UTIs but causes more than 80% of community-acquired infections. However, the present study shows that 27 out of 34 (79%) hospital-acquired UTIs are caused by *E. coli*. This may indicate an increase in the spread of *E. coli* in hospitals as an uropathogen.

Analysis of ESBL data in this study can be added to other studies that show an alarming increase in the threat posed by antibiotic resistance conferred by ESBLs due to the two-fold increase in the number of ESBLs detected over the three years of this study, 2011-2013 (Table 3.5).The antibiotic resistance profiles of the samples analysed in this study showed that 68.70% of the CPD (Cefpodoxime) resistant isolates tested over the three years (2011-2013) belong to ESBL positive class. The resistance in the remaining isolates may be caused by other β-lactamase enzymes that show hydrolytic activity against cephalosporins, including CPD. AmpC β-lactamase could be responsible for CPD resistance in these isolates.

Lewis et al. (2015) studied the prevalence of AmpC promoter mutations in groups of ESBL-negative uropathogenic *E.coli* strains that confer CPD resistance. They detected AmpC-carrying plasmids in only 10 isolates from 50 clinical samples, while the analysis of the chromosomal AmpC promoter regions in the remaining strains revealed mutations at 16 different positions and they found that AmpC mediated resistance in uropathogenic *E.coli* to Cefpodoxime can result from three point mutations in chromosome.

One of the possible reasons behind the rapid growth of ESBL-based resistance is the inaccurate phenotypic methods used for ESBL screening in clinical laboratories (e.g. the double-disc synergy test; Bajpai et al., 2017). These methods are unable to distinguish between the approximately 200 types of ESBLs identified to date (Ghafourian et al., 2015) and this is likely to lead to treatment failures due to the use of inappropriate antibiotic and outbreaks of ESBL-based antibiotic resistance.

To highlight the inadequacies of the routine screening methods for ESBLs and to detect the responsibility of the most rapidly growing ESBL types, the CTXM-type-ESBL gene, which confers resistance in the isolates of the study to most antibiotics, were screened further genetically, using PCR followed by sequencing (Chapter 4).

Chapter 4 : DETECTION OF GENES ENCODING *CTX-M-TYPE-ESBL* IN ISOLATES FROM NORTH WALES UTI PATIENTS

4.1 Introduction

The detection of resistant isolates in diagnostic laboratories is primarily based on phenotypic testing, which is characterised by cost-effectiveness and ease of use (Ayefoumi et al., 2019). However, the limitations of the phenotypic methods result in their inability to determine the gene responsible for antibiotic resistance, which often results in treatment failures due to the use of inappropriate antibiotics, in addition to the outbreak of multi-resistant, gram-negative pathogens (Srivastava et al., 2018). Such outbreaks could be prevented by the correct molecular analysis, which enables the identification of the genetic determinants of pathogenicity and antibiotic resistance (Hiltunen et al., 2017).

Genes that code for multiple types of β-lactamases (*bla* genes), predominately located on plasmids, are considered the most common antibiotic resistance genes (Chen et al., 2014). In a significant part, it refers to extended-spectrum β-lactamases (ESBLs), which inactivate most beta-lactam antibiotics, such as penicillins, cephalosporins (except for cephamycins) and monobactams, in addition to third- and fourth-generation cephalosporins, by hydrolysis of the β-lactam ring (Bachiri et al., 2017). This severely limits the possibilities of treatment for infections caused by ESBLs. In urinary tract infections (UTIs), *E. coli* remains the most common producer of ESBLs (Damus-Białek et al., 2018).

Many classifications of ESBLs have been introduced since the first emergence of this group of broad substrate enzymes. The most common classification is based on the type of beta-lactam that is hydrolysed by this ESBL type more than the other type (Rahman et al., 2018). The most important types are SHV-type-ESBL, which efficiently hydrolyses penicillin and first-generation cephalosporins, TEM-type-ESBL, which hydrolyses ampicillin at a greater rate than carbenicillin, oxacillin or cephalothin, OXA-type-ESBLs which are so named because of their oxacillin-hydrolysing abilities, and CTX-M-type-ESBLs' name reflects their potent hydrolytic activity against cefotaxime in addition to rare ESBL types e.g. VEB-1 and BES-1 (Taylor & Francis, 2015).

CTX-M has become the predominant type of ESBL enzymes detected in many regions of the world (Chaudhary et al., 2016). It has been reported that among ESBLs producing *E. coli* detected in clinical laboratories, the most commonly identified are enzymes from the family

CTX-M (mostly CTX-M-1 lineage), followed by SHVs and recently the less frequent TEMs (Maria et al., 2018).

The *blaCTX-M* gene is predominantly encoded on large plasmids that tend to carry additional resistance genes, which explains the increasing detection of resistant *E. coli* in outpatients and patients admitted to hospitals for short periods. This suggests that this plasmids spread in bacterial communities outside of hospitals for example in farming environments (Riccobono et al., 2015).

The *blaCTX-M* gene encodes 291 amino acids, and a single change of an amino acid constitutes a new CTX-M type (Giedraitienė et al., 2017). Unlike other ESBLs, this enzyme does not enlarge the active site to accommodate the antibiotics, instead its hydrolytic activity depends on point substitutions (Taylor & Francis, 2015).

Since the emergence of the CTX-M enzyme there have been at least 128 CTX-M types that have been described (Po et al., 2017). The phylogenetic tree of CTX-M-type ESBLs has been constructed by using amino acid sequences in which the CTX-M enzyme is divided into 5 groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 (Figure 4.1). They are each named after the archetypal enzymes of their individual groups:

CTX-M-1 cluster; CTX-M-1, 3, 10, 12, 15, 22, 23,

CTX-M-2 cluster; CTX-M-2, 4, 5, 6, 7, 20, 76, 77,

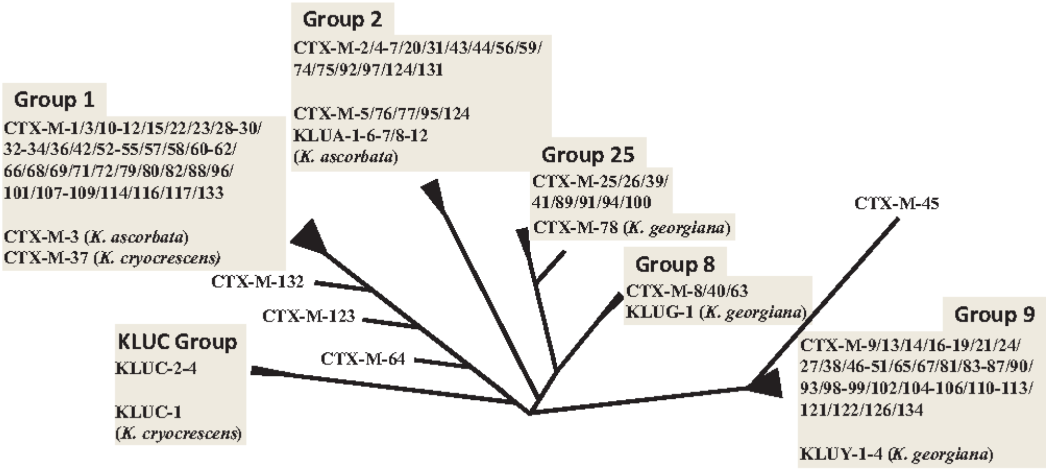
CTX-M-8 cluster; CTX-M-8, 40, 63,

CTX-M-9 cluster; CTX-M-9, 14, 15, 16, 17, 18, 19,

CTX-M-25 cluster; and CTX-M-25, 26, 39, 41, 91.

The 5 groups differ from each other by ≥ 10 amino acid residues, while each group includes a number of minor allelic variants that differ from each other by ≤ 5 amino acid residues (Bevan et al., 2017). Furthermore, there are at least 4 *CTX-M* variants that show a hybrid structure: CTX-M-45, which is a hybrid of CTX-M-14 with a protein of unknown origin; CTX-M-64; CTX-M-123; and CTX-M-132 variants, which are hybrids of CTX-M-15 with different segments of CTX-M-14. Most of these variants are categorised under the CTX-M groups 1 and 9 (Canton et al., 2012).

Figure 4.1 The phylogenetic tree of *CTX-M-type ESBLs*



Tree diagram showing the similarity among the enzymes of the CTX-M lineage and the clustering of members of different CTX-M groups. The tree was constructed with the TREEVIEW program on the basis of the amino acid sequence alignment of available sequences of CTX-M and cognate proteins from the Kluyvera spp. Available at the Lahey Clinic website (http://ww.lahey.org/Studies/).

Clinically, each CTX-M type can be inhibited by a specific antibiotic and the inability of phenotypic methods to distinguish between the different types of CTX-M or to determine the gene responsible for the production of each enzyme, which results in treatment failures due to the inappropriate use of antibiotics, is a major problem. Using molecular-based techniques would help to guide the use of appropriate antibiotics, while have the additional advantage of being able to detect low-level genetic resistance. Genotyping method could provide a fast surveillance tool for detecting resistant strains.

In order to create an epidemiological snapshot of the antibiotic resistance pattern in UTI patients in North Wales, and to determine to what extent CTX-M-type ESBLs contribute to the resistance phenomenon detected in isolates from UTI patients in North Wales and whether these CTX-M-producing bacterial isolates carry the same or different *CTX-M* genes, 300 ESBL-producing isolates from 3 referral hospitals in North Wales (Ysbyty Gwynedd, Glan Clwyd Hospital and Wrexham Maelor Hospital) were sub cultured on UTI agar, from which I was able to identify *Enterococcus spp.*, *Escherichia coli* and coliform bacteria before they were tested by multiplex PCR using primers for genes' phylogenetic groups (Gp) (Table 2.1):

CTX-MGp1 (688bp), Gp2 (404bp), Gp9 (561bp) and Gp8/25 (326bp) based on percentages of similarity followed by gel electrophoresis (Dallenne et al., 2012).

Sequencing analysis of the amplicons was performed on approximately 50% of the samples that had bands with lengths corresponding with 1 of the CTX-M groups from each hospital in order to identify *blaCTX-M* genes. Sequences were then compared with known b-lactamase gene sequences via multiple-sequence alignments using the BLAST programme.

4.2 Results

4.2.1 ESBL-producing isolates from Wrexham Maelor Hospital (1-100)

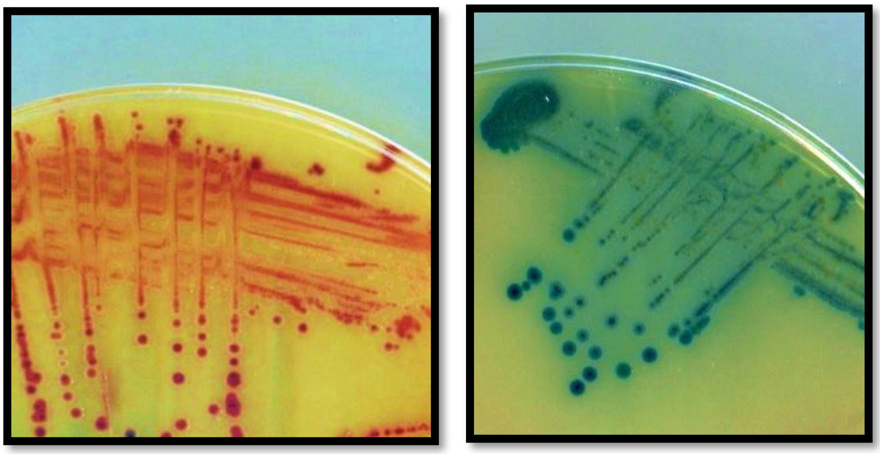
The screening of ESBL-producing isolates for the CTX-M groups was done in 3 main steps: sub culturing of preserved isolates from labelled bijoux tubes of nutrient agar slop, testing of extracted DNA for *blaCTX-M* 1, 2, 9 and 8/25 using multiplex PCR followed by electrophoresis and DNA sequencing analysis of amplicon PCR products.

4.2.1.1 Bacterial culturing on primary UTI agar

In order to achieve single colonies that would ensure the purity of the isolates to carry out the multiplex PCR identification of *Enterococcus spp.*, *Escherichia coli* (*E. coli*) and coliform bacteria, the preserved samples were cultured on primary UTI agar. Two types of colonies were observed in the 100 cultured samples (Figure 4.2): pink colonies (indicative of the presence of *E. coli*) and blue colonies (coliform bacteria). The growth of the colonies was recorded (Table 4.1) with their hospital lab numbers.

Figure 4.2 Sub culture isolates on UTI agar

(A) **(B)**



Sub cultured isolates from UTI patients on primary UTI agar; (A) *E. coli* with pink colonies (sample 44); (B) *coliform* bacteria with blue colonies (sample 55). The agar contains a chromogenic substrate that is hydrolysed by enzymes produced by these bacteria; β-D-glucosidase produced by *E. coli* hydrolyses the chromogenic substrate to produce pink-red colonies, and β-glucosidase from *coliform* bacteria hydrolyses the chromogenic substrate to produce blue colonies (Oxoid Ltd., 2008).

Table 4.1 Bacterial identification based on colony characteristics for samples 1-100

Isolate number	Lab number	Date of subculture	Indicative bacteria based on colony colour
1	19152	7/5/2015	*E. coli*
2	18620	7/5/2015	*E. coli*
3	18257	7/5/2015	*E. coli*
4	18570	7/5/2015	*E. coli*
5	17893	7/5/2015	*E. coli*
6	19166	7/5/2015	*E. coli*
7	18322	7/5/2015	*E. coli*
8	18144	7/5/2015	*E. coli*
9	16548	7/5/2015	*E. coli*
10	17046	7/5/2015	*E. coli*
11	17464	7/5/2015	*E. coli*
12	17578	7/5/2015	*E. coli*
13	16920	7/5/2015	*E. coli*
14	17571	7/5/2015	*Coliform*
15	17084	7/5/2015	*E. coli*
16	17392	7/5/2015	*E. coli*
17	17212	7/5/2015	*E. coli*
18	16785	7/5/2015	*E. coli*
19	16497	7/5/2015	*E. coli*
20	16632	7/5/2015	*E. coli*
21	17580	18/5/2015	*Coliform*
22	14379	18/5/2015	*Coliform*
23	13843	18/5/2015	*Coliform*
24	12456	18/5/2015	*E. coli*
25	13808	18/5/2015	*Coliform*
26	14098	18/5/2015	*E. coli*
27	13651	18/5/2015	*E. coli*
28	13830	18/5/2015	*E. coli*
29	14122	18/5/2015	*E. coli*
30	14386	18/5/2015	*E. coli*
31	14152	18/5/2015	*E. coli*
32	13756	18/5/2015	*E. coli*
33	13738	18/5/2015	*E. coli*

34	13700	18/5/2015	*E. coli*
35	14384	18/5/2015	*E. coli*
36	13856	18/5/2015	*E. coli*
37	13745	18/5/2015	*E. coli*
38	13656	18/5/2015	*E. coli*
40	14758	18/5/2015	*E. coli*
41	15314	2/6/2015	*E. coli*
42	15378	2/6/2015	*E. coli*
43	15029	2/6/2015	*E. coli*
44	15340	2/6/2015	*E. coli*
45	14822	2/6/2015	*E. coli*
46	15194	2/6/2015	*E. coli*
47	15211	2/6/2015	*E. coli*
48	15060	2/6/2015	*Coliform*
49	24341	2/6/2015	*E. coli*
50	19677	2/6/2015	*E. coli*
51	19630	2/6/2015	*E. coli*
52	19658	2/6/2015	*E. coli*
53	19349	2/6/2015	*E. coli*
54	19274	2/6/2015	*E. coli*
55	20650	2/6/2015	*Coliform*
56	21176	16/6/2015	*E. coli*
57	21184	16/6/2015	*E. coli*
58	21087	16/6/2015	*Coliform*
59	20050	16/6/2015	*E. coli*
60	14773	16/6/2015	*E. coli*
61	15638	16/6/2015	*E. coli*
62	15643	16/6/2015	*E. coli*
63	15571	16/6/2015	*E. coli*
64	15393	16/6/2015	*E. coli*
65	15395	16/6/2015	*E. coli*
66	16013	16/6/2015	*E. coli*
67	16092	16/6/2015	*E. coli*
68	16368	16/6/2015	*E. coli*
69	15419	16/6/2015	*E. coli*
70	15457	16/6/2015	*E. coli*
71	15979	30/6/2015	*E. coli*
72	15881	30/6/2015	*E. coli*
73	15787	30/6/2015	*E. coli*

74	15920	30/6/2015	*E. coli*
75	14819	30/6/2015	*E. coli*
76	14838	30/6/2015	*E. coli*
77	15278	30/6/2015	*E. coli*
78	15190	30/6/2015	*E. coli*
79	14620	30/6/2015	*E. coli*
80	14437	30/6/2015	*Coliform*
81	14465	30/6/2015	*E. coli*
82	14479	30/6/2015	*E. coli*
83	14611	30/6/2015	*Coliform*
84	15051	30/6/2015	*E. coli*
85	14575	30/6/2015	*E. coli*
86	14506	30/6/2015	*E. coli*
87	15039	20/7/2015	*E. coli*
88	14648	20/7/2015	*E. coli*
89	15248	20/7/2015	*E. coli*
90	23810	20/7/2015	*E. coli*
91	23631	20/7/2015	*E. coli*
92	23955	20/7/2015	*E. coli*
93	22860	20/7/2015	*E. coli*
94	24147	20/7/2015	*E. coli*
95	23999	20/7/2015	*Coliform*
96	22802	20/7/2015	*E. coli*
97	23733	20/7/2015	*E. coli*
98	24236	20/7/2015	*E. coli*
99	24337	20/7/2015	*E. coli*
100	12986	20/7/2015	*E. coli*

4.2.1.2 Multiplex PCR amplification of β-lactamase genes ***blaCTX-M*** 1, 2, 9 and 8/25 followed by gel electrophoresis of samples 1-100

The isolates of interest were lysed to extract their DNA, which was used as the DNA template for PCR amplification. The total volume of 20 µL of the PCR reaction mixture was prepared for each cell lysate sample. After 2 h of amplification, the amplified PCR products were run on agarose gels. Around 91% of the samples had bands located at the length of 1 of *CTX-M* groups.

4.2.1.2.1 PCR gel analysis of the ***blaCTX-M*** genes (Isolates 1-14)

As shown in Figure 4.3, which depicts the gel analysis of isolates 1-14, 12 samples had bands with lengths that corresponded to 2 groups of CTX-M genes: *CTX-MGp1* (688bp) samples 1 (*E. coli*), 4 (*E .coli*), 5 (*E. coli*), 6 (*E. coli*), 7 (*E. coli*), 8 (*E. coli*), 9 (*E. coli*), 10 (*E. coli*), 11 (*E. coli*) and 12 (*E. coli*). Two samples had bands with lengths of *CTX-MGp2* (404bp), samples 2 (*E. coli*) and 3 (*E. coli*).

4.2.1.2.2 PCR gel analysis of the ***blaCTX-M*** genes (Isolates 15-28)

Gel analysis of samples 15-28 shows that 13 samples had bands with lengths that corresponded to *CTX-MGp1* (688bp): samples 15, 16, 17, 18, 19, 20, 24 and 26 (all *E. coli*) and 21, 22, 23 and 25 (all coliforms). Sample 17 had 2 bands, 1 with a length corresponding to *CTX-MGp1* (688bp) and another corresponding to *CTX-MGp9* (561bp) (Figure 4.4).

4.2.1.2.3 PCR gel analysis of the ***blaCTX-M*** genes (Isolates 29-42)

Fourteen samples (29-42) displayed bands located at the lengths of 3 groups of *CTX-M* genes on gel analysis: *CTX-MGp1* (688bp) samples 29, 30, 31, 32, 34, 35, 38 and 42 (all *E. coli*), CTX-MGp2 (404bp) samples 36, 40 and 41 (all *E. coli*) and *CTX-MGp9* samples 37 and 39 (all *E. coli*) (Figure 4.5).

4.2.1.2.4 PCR gel analysis of the ***blaCTX-M*** genes (Isolates 43-56)

This group of samples showed bands with lengths that corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp) samples 43, 44, 45, 46, 49, 50, 51, 52, 53 and 56 (all *E. coli*) and 48 and 55 (all coliforms) and *CTX-MGp9* (561bp) sample 47. Sample 54 had 2 bands; the first corresponded to *CTX-MGp1* and the other corresponded to CTX-MGp9 (Figure 4.6).

4.2.1.2.5 PCR gel analysis of the *blaCTX-M* genes (Isolates 57-70)

All samples of this group had bands with lengths that corresponded to *CTX-MGp1* (688bp): samples 57,59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 and 70 (all *E. coli*) and 58 (coliform) (Figure 4.7).

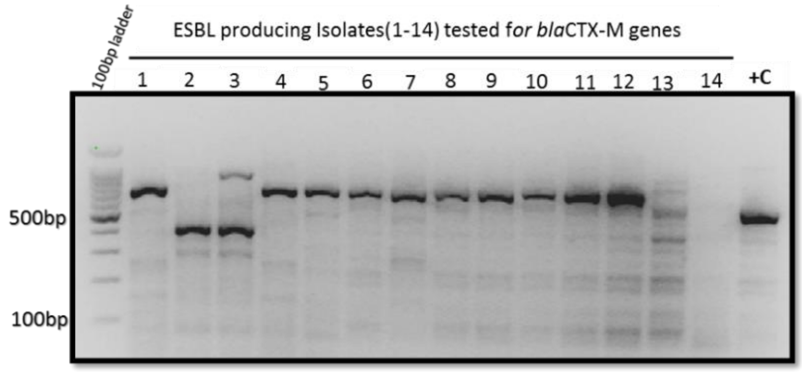
4.2.1.2.6 PCR gel analysis of the *blaCTX-M* genes (Isolates 71-84)

As shown in Figure 4.8, 11 samples displayed bands located at lengths of 2 groups of CTX-M genes: *CTX-MGp1* (688bp), which includes samples 72, 75, 76, 77, 79, 81, 82 and 84 (all *E. coli*), and *CTX-MGp9* (561bp), which includes samples 71 *(E. coli*) and 80 (coliform). Sample 73 had 2 bands; the first corresponded to *CTX-MGp1* and the other corresponded *to CTX-MGp9.*

4.2.1.2.7 PCR gel analysis of the *blaCTX-M* genes (Isolates 85-100)

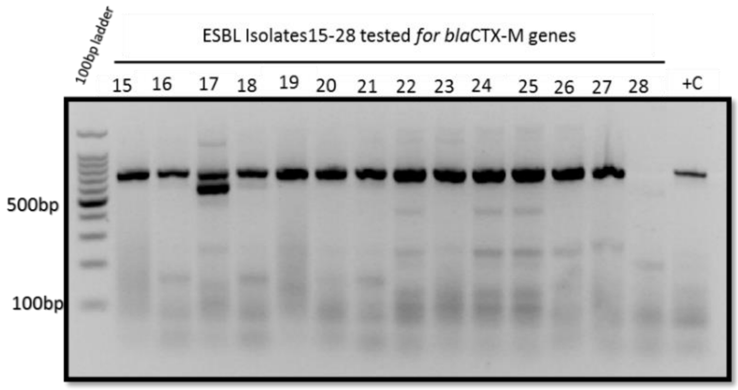
Based on gel analysis, 2 types of *CTX-M* genes seem to be carried by 15 isolates from this group: *CTX-MGp1* (688bp), which includes samples 86, 87, 88, 89, 90, 92, 93, 94, 96, 98 and 99 (all *E. coli*) and sample 95 (coliform); and *CTX-MGp9* (561bp), which includes samples 91, 97 and 100 (all *E.coli*) (Figure 4.9).

Figure 4.3 Multiplex PCR assay for the *blaCTX-M* genes, samples 1-14



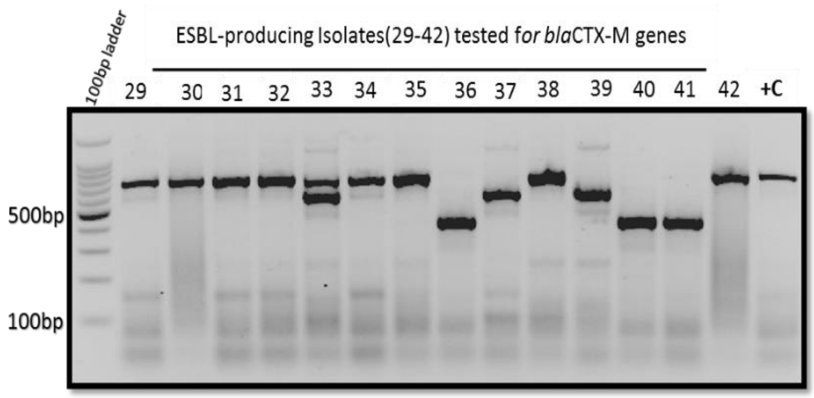
Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1: the 100-bp ladder; lanes 2–15: the 14 ESBL samples. Twelve samples had bands with lengths that corresponded to two groups of *CTXM* genes; *CTXMGp1* (688 bp) samples (1, 4, 5, 6, 7,8,9,10,11 and 12) and *CTXMGp2* (404 bp) samples 2&3.Lane 16: positive control.

Figure 4.4 Multiplex PCR assay for the *blaCTX-M* genes, samples 15-28



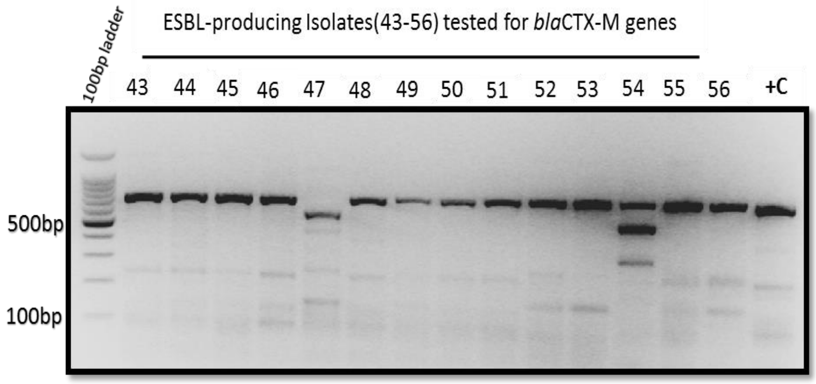
Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1: 100-bp ladder. Lanes 2–15: 14 ESBL samples. Thirteen samples had bands with lengths that corresponded to *CTXMGp1* (688 bp); samples (15-27) and sample (3) had two bands one with length corresponded to CTXMGp1 (688 bp) and another with *CTXMGp 9* (561bp) .Lane 16: positive control.

Figure 4.5 Multiplex PCR assay for the *blaCTX-M* genes, samples 29-42



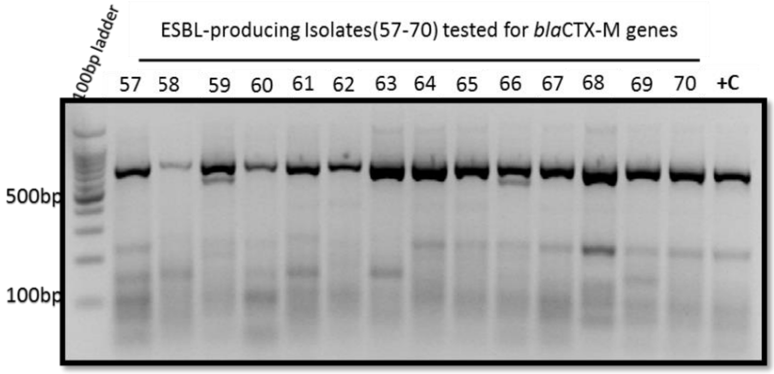
Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1: 100-bp ladder. Lanes 2–15:14 ESBL samples. Fourteen samples had bands with lengths that corresponded to three groups of *CTXM* genes ; *CTXMGp1* (688 bp) samples (29,30,31,32,34,35,38,42*), CTXMGp2* (404bp)samples(36,40 and 41) *CTXMGp*9(37&39).

Figure 4.6 Multiplex PCR assay for the *blaCTX-M* genes, samples 43-56



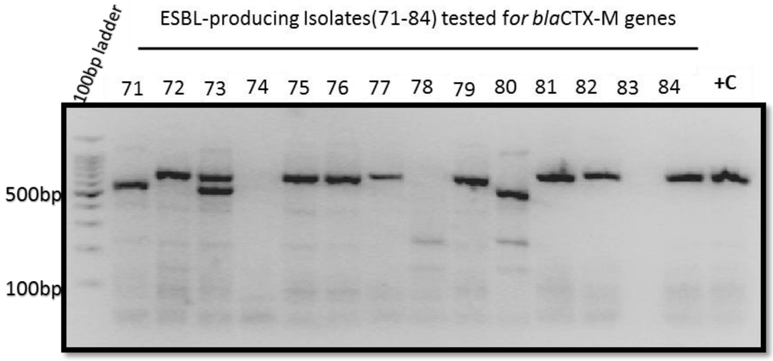
Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1: the 100-bp ladder. Lanes 2–15: 14 ESBL samples. samples had bands with lengths that corresponded to two groups of *CTX-M* genes; CTXMGp1 (688 bp) samples (43,44,45,46,48,49,50,51,52,53,55,56) , *CTXMGp9* (561 bp) sample(47),sample 54 had two bands one corresponded to *CTXMGp1* and another to *CTXMGp9*. Lane 16:positive control.

Figure 4.7 Multiplex PCR assay for the *blaCTX-M* genes, samples 57-70



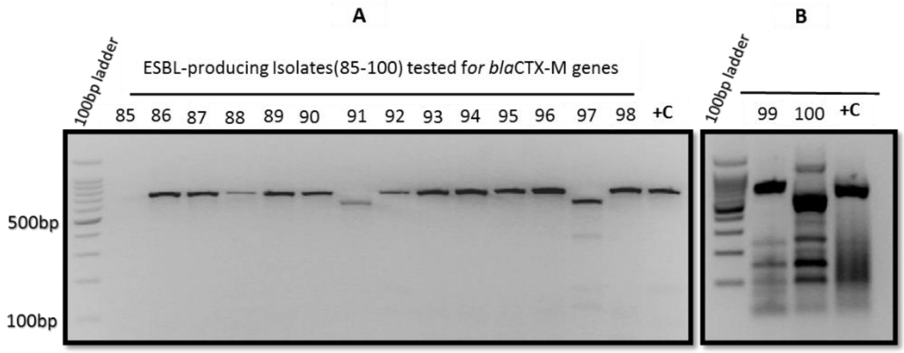
Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100-bp ladder. Lanes 2–15: 14 ESBL samples. Fourteen samples had bands with lengths that corresponded to *CTXMGp1* (688 bp) samples (57-70). Lane 16: positive control.

Figure 4.8 Multiplex PCR assay for the *blaCTX-M* genes, samples 71-84



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100-bp ladder. Lanes 2–15: 14 ESBL samples. Eleven samples had bands with lengths that corresponded to two groups of *CTXM* genes; *CTXMGp1* (688bp) samples (72,75,76,77,79,81,82 and 84) ,*CTXMGp9* (561bp) sample (71&80),sample (73) had two bands one corresponded to *CTXMGp1* and another to *CTXMGp9*. Lane 16:positive control.

Figure 4.9 Multiplex PCR assay for the *blaCTX-M* genes, samples 85-100



A) Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–15: 14 ESBL samples. Thirteen samples had bands with lengths that corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp) samples (86, 87, 88, 89, 90, 92, 93, 94, 95, 96 and 98) and CTX-MGp9 (561bp) samples 91 and 97. Lane 16: positive control. (**B**) Gel analysis of *bla*CTX-M amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–3: 2 ESBL samples. Two samples had bands with lengths that corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp) sample 99 and *CTX-MGp9* (561bp) sample 100. Lane 16: positive control.

The screening of ESBL-producing isolates from Wrexham Maelor Hospital (1–100) for the CTX-M groups using multiplex PCR followed by gel analysis shows, based on the amplicon size, that the predominant CTX-M group is group 1 (Table 4.2).

Table 4.2 PCR amplicon sizes of the *blaCTX-M* genes, samples 1-100

CTX-M group	Amplicon size (bp)	Sample No
CTX-M group 1	688	1, 4 ,5, 6, 7, 8, 9, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 29, 30, 31, 32, 34, 35, 38, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 72, 73, 75, 76, 77, 79, 81, 82 ,84, 86, 87, 88, 89, 90, 92, 93, 94, 95, 96, 98 and 99.
CTX-M group 2	404	2, 3, 36, 40, 41, 91, 97 and 100.
CTX-M group 9	561	17, 37, 39, 47, 71, 80.
CTX-M group 8/25	326	

4.2.1.3 Sequencing analysis of multiplex PCR products (1-100)

The DNA of amplified PCR was purified using the ISOLATE II PCR kit for sequencing in order to identify the *CTX-M* genes detected in the multiplex PCR assays. The sequencing analysis was performed on 47 (50%) samples that displayed bands with sizes corresponding to *CTX-M* groups. The sequenced samples were compared with DNA sequences of known b-lactamase genes using the BLAST programme. Table 4.3 shows the BLAST output of the sequenced samples.

Table 4.3 Sequencing analysis of positive samples from 1-100

Sample No	Blast output	Accession number	CTX-M group				DNA sequence
			Gp1	Gp2	Gp9	Gp8/25	
1	*CTX-M-15* gene	KP325146.1	✓				Appendix I
3	*CTX-M-59* gene	MH661248.1		✓			Appendix I
5	*CTX-M-3* gene	CP034325.1	✓				Appendix I
7	*CTX-M-15* gene	KR338941.1	✓				Appendix I
9	*CTX-M-15* gene	KY640551.1	✓				Appendix I
11	*CTX-M-66* gene	NG_049017.1	✓				Appendix I
15	*Klebsiella pneumoniae complete genome*	CP011985.1					Appendix I
17 (lower band)	*CTX-M-108* gene	JF274245.1			✓		Appendix I
19	*CTX-M-15* gene	MK113957.1	✓				Appendix I
21	*CTX-M-15* gene	KY640536.1	✓				Appendix I
23	*CTX-M-32* gene	MH900527.1	✓				Appendix I
25	*CTX-M-15* gene	MH900522.1	✓				Appendix I
27	*CTX-M-15* gene	KY640528.1	✓				Appendix I
29	*CTX-M-55* gene	MH900523.1	✓				Appendix I
31	*E coli strain MNCRE44, complete genome*	CP010876.1					Appendix I
33(lower band)	*CTX-M-9* gene	MF797877.1			✓		Appendix I
35	*CTX-M-15* gene	MK113960.1	✓				Appendix I
36	*CTX-M-27* gene	MH900525.1			✓		Appendix I
41	*CTX-M-2* gene	MH661245.1			✓		Appendix I
43	*CTX-M-15* gene	MH900522.1	✓				Appendix I
45	*CTX-M-15* gene	MK113960.1	✓				Appendix I
47	CTX-M-9 gene	KT459753.1			✓		Appendix I
49	*CTX-M-15* gene	MK405591	✓				Appendix I
51	*CTX-M-15* gene	LT628520	✓				Appendix I
54(lower band)	*CTX-M-27* gene	MH900525			✓		Appendix I
57	*CTX-M-15* gene	CP040398	✓				Appendix I
59	*CTX-M-1* gene	MH037035	✓				Appendix I

61	*CTX-M-15* gene	KY640536	✓				Appendix I
63	*E.coli4928STDY7071340* genome assembly	LR607331					Appendix I
65	*CTX-M-55* gene	MF958462	✓				Appendix I
67	*CTX-M-15* gene	KP325147	✓				Appendix I
69	*CTX-M-163* gene	KP681698	✓				Appendix I
71	*CTX-M-15* gene	MK113957	✓				Appendix I
73	*CTX-M-16* gene	AY029068			✓		Appendix I
75	*CTX-M-15* gene	KP325146	✓				Appendix I
77	*CTX-M-15* gene	KY640536	✓				Appendix I
80	*CTX-M-9* gene	MF797877			✓		Appendix I
82	*CTX-M-160* gene	NG_048945	✓				Appendix I
84	*CTX-M-15* gene	LT628516	✓				Appendix I
86	*CTX-M-15* gene	KY640534	✓				Appendix I
88	*CTX-M-225* gene	NG_064720.	✓				Appendix I
90	*CTX-M-1* gene	MG255315	✓				Appendix I
91	*Ecoli strain VRES hospital6495320 genome assembly*	LR607054					Appendix I
93	*CTX-M-172* gene	NG_048957	✓				Appendix I
95	*CTX-M-15* gene	LT628516.	✓				Appendix I
97	*CTX-M-9* gene	MF797877			✓		Appendix I
98	*CTX-M-15* gene	MK113957	✓				Appendix I
100	*CTX-M-15* gene	MH900525			✓		Appendix I

The BLAST output shows that the most common (dominant) *CTX-M* in Wrexham Maelor Hospital isolates is *CTX-M-15*, which is classified under group 1 as 23 (48.9%) shows high identity with *CTX-M-15*, followed by *CTX-M-9*; 4 (8.5%), *CTX-M-27, CTX-M-2 gene* and *CTX-M-55*; 2 (4.2%), *CTX-M-59*; 1 (2.1%), *CTX-M-3*; 1 (2.1%), *CTX-M-172* gene; 1 (2.1) *CTX-M-66*; 1 (2.1%), *CTX-M-108*; 1 (2.1%), *CTX-M-32*; 1 (2.1%), *CTX-M-1*; 1 (2.1%), *CTX-M-160*; 1 (2.1%), CTX-M-163; 1 (2.1%) and *CTX-M-225*; 1 (2.1%).

4.2.2 ESBL-producing isolates from Glan Clwyd Hospital (101-200)

The 3 steps described in section 4.2.1 were carried out in order to screen the Glan Clwyd Hospital isolates for CTX-M genes.

4.2.2.1 Bacterial culturing on primary UTI agar

Isolates from Glan Clwyd Hospital were cultured on primary UTI agar as prescribed in section 4.2.1.1. The growth of the colonies was recorded (Table 4.4) according to the hospital lab numbers.

Table 4.4 Bacterial identification based on colony characteristics for samples 101-200

Isolate number	Lab number	Date of subculture	Indicative bacteria based on colony colour
101	12457	17/9/2015	*E. coli*
102	107166	17/9/2015	*E. coli*
103	12249	17/9/2015	*E. coli*
104	12261	17/9/2015	*E. coli*
105	12823	17/9/2015	*E. coli*
106	12906	17/9/2015	*E. coli*
107	112575	17/9/2015	*Coliform*
108	12808	17/9/2015	*E. coli*
109	12847	17/9/2015	*E. coli*
110	12167	17/9/2015	*E. coli*
111	12609	17/9/2015	*E. coli*
112	11512	17/9/2015	*E. coli*
113	13108	17/9/2015	*E. coli*
114	10604	17/9/2015	*Coliform*
115	12734	17/9/2015	*E. coli*
116	11471	17/9/2015	*E. coli*
117	12472	17/9/2015	*E. coli*
118	12722	17/5/2015	*E. coli*
119	13138	17/5/2015	*E. coli*
120	107156	17/9/2015	*E. coli*
121	19800	17/9/2015	*E. coli*
122	22522	17/9/2015	*E. coli*
123	225512	17/9/2015	*E. coli*
124	22903	17/9/2015	*E. coli*
125	22867	17/9/2015	*E. coli*
126	22664	18/9/2015	*E. coli*
127	22941	18/9/2015	*E. coli*
128	22877	18/9/2015	*E. coli*
129	22803	18/9/2015	*E. coli*
30	22847	18/9/2015	*E. coli*
131	22429	18/9/2015	*E. coli*
132	22426	18/9/2015	*E. coli*
133	22658	5/10/2015	*E. coli*
134	22753	5/10/2015	*Coliform*
135	22662	5/10/2015	*E. coli*

136	22655	5/10/2015	*E. coli*
137	22804	5/10/2015	*E. coli*
138	23621	5/10/2015	*E. coli*
139	24056	5/10/2015	*E. coli*
140	23789	5/10/2015	*E. coli*
141	23870	5/10/2015	*E. coli*
142	23641	5/10/2015	*E. coli*
143	23799	5/10/2015	*E. coli*
144	28433	5/10/2015	*E. coli*
145	20012	5/10/2015	*E. coli*
146	18481	5/10/2015	*E. coli*
147	19702	5/10/2015	*E. coli*
148	18431	5/10/2015	*Coliform*
149	18720	5/10/2015	*E. coli*
150	18694	5/10/2015	*E. coli*
151	19037	5/10/2015	*E. coli*
152	18514	5/10/2015	*E. coli*
153	18251	2/11/2015	*E. coli*
154	19107	2/11/2015	*E. coli*
155	19927	2/11/2015	*E. coli*
156	18601	2/11/2015	*E. coli*
157	18543	2/11/2015	*E. coli*
158	18477	2/11/2015	*E. coli*
159	21810	2/11/2015	*E. coli*
160	21677	2/11/2015	*E. coli*
161	21640	2/11/2015	*E. coli*
162	20782	2/11/2015	*Coliform*
163	21649	2/11/2015	*E. coli*
164	21793	2/11/2015	*E. coli*
165	22041	2/11/2015	*E. coli*
166	21761	2/11/2015	*E. coli*
167	21988	2/11/2015	*E. coli*
168	22192	2/11/2015	*E. coli*
169	20541	2/11/2015	*E. coli*
170	20510	2/11/2015	*E. coli*
171	20665	2/11/2015	*E. coli*
172	21862	2/11/2015	*E. coli*
173	20853	2/11/2015	*Coliform*
174	22238	2/11/2015	*E. coli*

175	21361	2/11/2015	*E. coli*
176	25798	2/11/2015	*E. coli*
177	26377	2/11/2015	*E. coli*
178	25705	2/11/2015	*E. coli*
179	28162	2/11/2015	*E. coli*
180	25927	2/11/2015	*E. coli*
181	28381	6/1/2016	*E. coli*
182	25784	6/1/2016	*E. coli*
183	25886	6/1/2016	*E. coli*
184	26672	6/1/2016	*E. coli*
185	28022	6/1/2016	*E. coli*
186	28211	6/1/2016	*E. coli*
187	26280	6/1/2016	*E. coli*
188	28374	6/1/2016	*E. coli*
189	28006	6/1/2016	*E. coli*
190	25956	6/1/2016	*E. coli*
191	28333	6/1/2016	*E. coli*
192	28502	6/1/2016	*E. coli*
193	28511	6/1/2016	*E. coli*
194	28457	6/1/2016	*E. coli*
195	26668	6/1/2016	*E. coli*
196	28648	6/1/2016	*E. coli*
197	26576	6/1/2016	*E. coli*
198	26894	6/1/2016	*E. coli*
199	26864	6/1/2016	*Coliform*
200	28084	6/1/2016	*E. coli*

4.2.2.2 Multiplex PCR amplification of β-lactamase genes *blaCTX-M* 1, 2, 9 and 8/25, followed by gel electrophoresis of samples 101-200

The extracted genomic DNA of each isolate was amplified using multiple PCR as explained in section 4.2.1.2. Around 73% of the samples had bands located at the length of 1 of the *CTX-M* groups.

4.2.2.2.1 PCR gel analysis of the *blaCTX-M* genes (Isolates 101-114)

Thirteen samples from this group displayed bands located that corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp), which includes samples 101, 103, 104, 105, 109, 110, 112 and 113 (all *E. coli*) in addition to samples 107 and 114 (all coliforms), and *CTX-MGp2* (404bp), which includes samples 106, 108 and 111 (all *E. coli*) (Figure 4.10).

4.2.2.2.2 PCR gel analysis of the *blaCTX-M* genes (Isolates 115-128)

As shown in Figure 4.11, 7 samples had bands with lengths that corresponded to *CTX-MGp1* (688 bp): samples 115, 118, 120, 122, 124 and 125 (all *E. coli*).

4.2.2.2.3 PCR gel analysis of the *blaCTX-M* genes (Isolates 129-142)

The gel analysis of these samples shows that 2 groups of CTX-M seem to be carried by the isolates: *CTX-MGp1* (688bp), which includes samples 132, 133, 134, 135, 136, 137, 138, 140, 141 and 142 (all *E. coli*), and *CTX-MGp2* (404bp), which includes sample 129 (Figure 4.12).

4.2.2.2.4 PCR gel analysis of the *blaCTX-M* genes (Isolates 143-156)

Bands with lengths corresponding to *CTX-MGp1* (688bp) were displayed in samples 143, 144, 145, 146, 147, 150 and 151 (all *E. coli*) in addition to sample 48 (coliform) (Figure 4.13).

4.2.2.2.5 PCR gel analysis of the *blaCTX-M* genes (Isolates 157-170)

Samples from this group displayed bands located at the lengths of 3 *CTX-M* groups: *CTX-MGp1* (688bp), which includes samples 157, 160, 161, 164, 165, 166, 167 and 168 (all *E. coli*); CTX-MGp2 (404bp), which includes sample 158 (*E. coli*); and *CTX-MGp9*, which includes sample 159 (*E. coli*) (Figure 4.14).

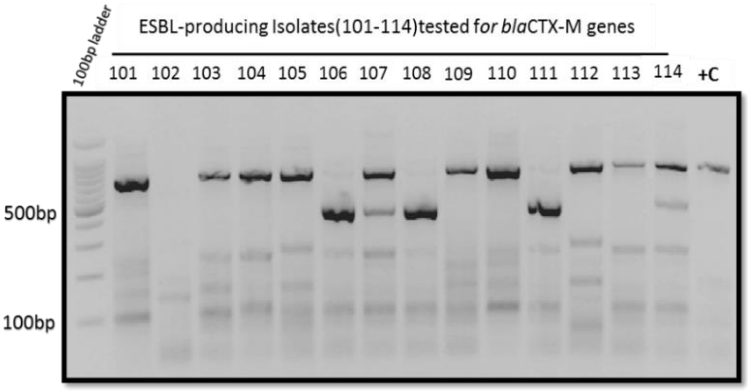
4.2.2.2.6 PCR gel analysis of the *blaCTX-M* genes (Isolates 171-184)

As shown in Figure 4.15, 12 samples—172, 174, 175, 176, 177, 178, 179, 180, 181, 182 and 184 (all *E. coli*) in addition to sample 173 (coliform)—had bands with sizes corresponding to *CTX-MGp1* (688bp).

4.2.2.2.7 PCR gel analysis of the *blaCTX-M* genes (Isolates 185-200)

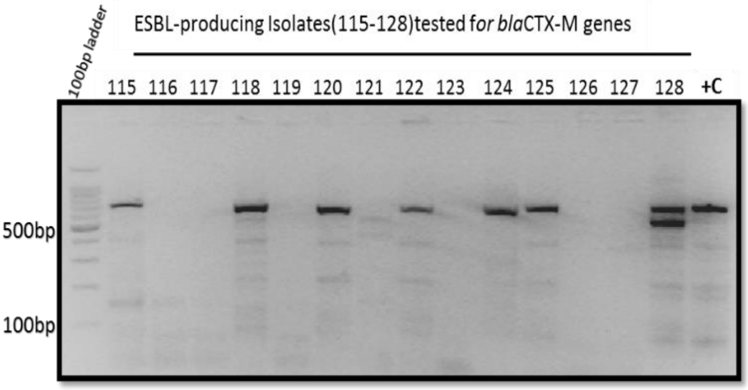
Twelve samples from this group displayed bands located at the lengths of *CTX-MGp1* (688bp): 185, 186, 187, 189, 190, 192, 193, 194, 195, 196, 198 and 200 (*all E.coli*) (Figure 4.16).

Figure 4.10 Multiplex PCR assay for the *blaCTX-M* genes, samples 101-114



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Thirteen samples had bands with lengths that corresponded to two groups of *CTX-M* genes;*CTX-MGp1*(688bp) samples (101,103,104,105,107,109,110,112,113,114),CTXMGp2 (404bp) sample (106,108 and 111). Lane 16: positive control.

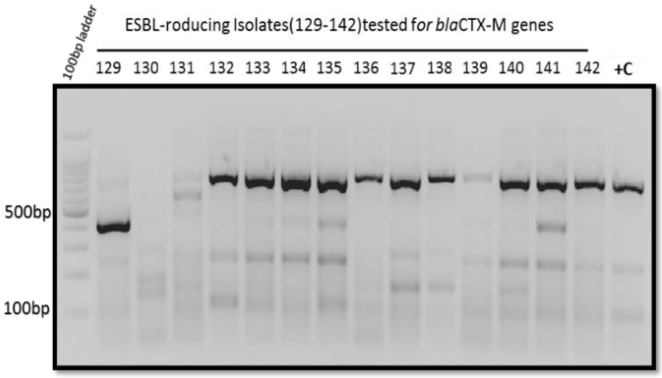
Figure 4.11 Multiplex PCR assay for the *blaCTX-M* genes, samples 115-128



Analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1: the 100bp ladder; lanes 2–15: 14 ESBL samples. Seven samples had bands with lengths that corresponded to *CTXMGp*1 (688 bp) samples (115,118,120,122,124,125), sample (128) had two bands one corresponded to CTXMGp1 and another to *CTXMGp9*. Lane 16: positive control.

.

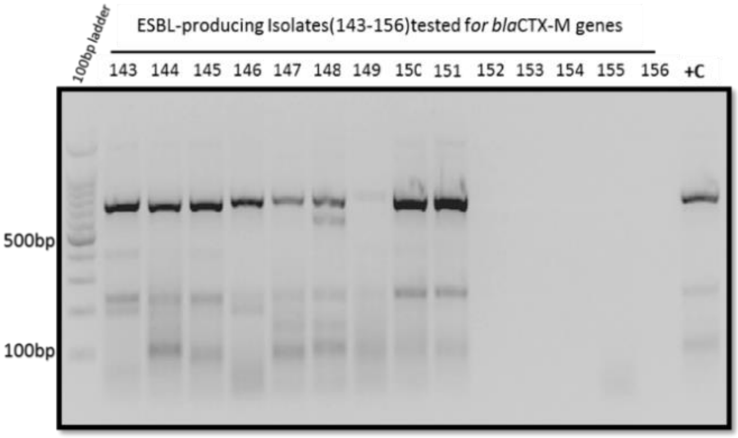
Figure 4.12 Multiplex PCR assay for the *blaCTX-M* genes, samples 129-242



Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Eleven samples had bands with lengths that corresponded to two groups of CTXM genes; CTXMGp1 (688bp) samples (132,133,134,135,136,137,138,140,141,142), CTXMGp2 (404bp) sample (129). Lane 16: positive control.

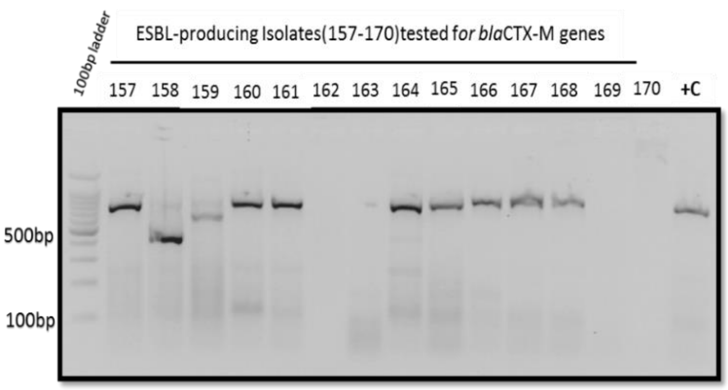
.

Figure 4.13 Multiplex PCR assay for the *blaCTX-M* genes, samples 143-156



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Eleven samples had bands with lengths that corresponded to CTXMGp1 (688bp) samples (143,144,145,146,147,148,150,151). Lane 16: positive control.

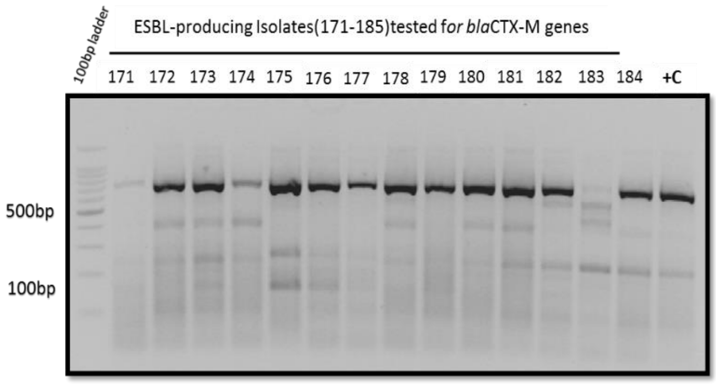
Figure 4.14 Multiplex PCR assay for the *blaCTX-M* genes, samples 157-170



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Ten samples had bands with lengths that corresponded to two groups of CTXM genes; CTXMGp1 (688bp) samples (157,160,161,164,165,166,167,168), CTXMGp2 (404bp) sample (158), CTXMGp9 (sample159).Lane 16: positive control.

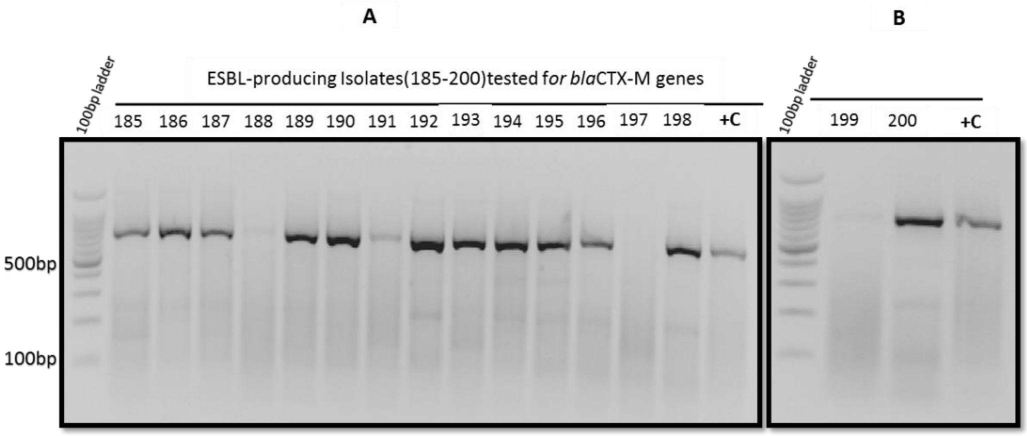
.

Figure 4.15 Multiplex PCR assay for the *blaCTX-M* genes, samples 171-184



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Twelve samples had bands with lengths that corresponded to *CTXMGp1* (688bp) samples (172,173,174,175,176,177,178,179,180,181,182 and 184). Lane 16: positive control.

Figure 4.16 Multiplex PCR assay for the *blaCTX-M* genes, samples 185-200



(**A**) Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–15: 14 ESBL samples. Eleven samples had bands with lengths that corresponded to *CTX-MGp1* (688bp) samples 185, 186, 187, 189, 190, 192, 193, 194, 195, 196 and 198. Lane16: positive control. **(B)** Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–3: 2 ESBL samples. One sample had bands with lengths that corresponded to *CTX-MGp1* (688bp) sample 200. Lane 16: positive control.

The screening of ESBL-producing isolates from Wrexham Maelor Hospital (101–200) for the CTX-M groups using multiplex PCR followed by gel analysis shows, based on the amplicon size, that the predominant CTX-M group is group 1 (Table 4.5).

Table 4.5 PCR amplicon sizes of the *blaCTX-M genes*, samples 101-200

CTX-M group	Amplicon size (bp)	Sample No
CTX-M group 1	688	101, 103, 104, 105, 107, 109, 110, 112, 113, 114, 115, 118, 120, 122, 124,125, 132, 133, 134, 135, 136, 137, 138, 140, 141, 142, 143, 144, 145, 146, 147, 148, 150, 151, 157, 160, 161, 164, 165, 166, 167 168, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 184, 185, 186, 187, 189, 190, 192, 193, 194, 195, 196, 198 and 200.
CTX-M group 2	404	106, 108, 111, 129, 158
CTX-M group 9	561	159
CTX-M group 8/25	326	

4.2.2.3 Sequencing analysis of multiplex PCR products (201-200)

The sequencing analysis was performed on 46 (60%) samples that displayed bands with sizes that corresponded with 1 of the CTX-M groups. The BLAST programme was then used to compare each sequence with DNA sequences of known b-lactamase genes. Table 4.6 shows the BLAST output of the sequenced samples.

Table 4.6 Sequencing analysis of positive samples from 101-200

Sample No	Blast output	Accession number	CTX-M group				DNA sequence
			Gp1	Gp2	Gp9	Gp8/25	
101	*CTX-M-15* gene	KP325147.1	✓				Appendix II
103	*CTX-M-15* gene	MH900522.1	✓				Appendix II
106	*CTX-M-59* gene	MH661247.1		✓			Appendix II
108	*CTX-M family*	MK896928.1					Appendix II
109	*CTX-M-15* gene	MK113960.1	✓				Appendix II
111	*Escherichia coli O1:H42 strain CLSC36 chromosome, complete genome.Sequence*	CP041300.1					Appendix II
113	*CTX-M-15* gene	MH900522.1	✓				Appendix II
115	*CTX-M-15* gene	MK405591.1	✓				Appendix II
118	*CTX-M-15* gene	MH900522.1	✓				Appendix II
120	*CTX-M-15* gene	KY640536.1	✓				Appendix II
122	*CTX-M-203* gene	NG_055269.1					Appendix II
124	*CTX-M-66* gene	NG_049017.1	✓				Appendix II
128(lower band)	*Escherichia coli strain 4928STDY7071340 genome assembly*	LR607331.1					Appendix II
129	*CTX-M-9* gene	MF797877.1			✓		Appendix II
132	*CTX-M-15* gene	MH523447.1	✓				Appendix II
135	*CTX-M-32* gene	MH900527.1	✓				Appendix II
136	*CTX-M-15* gene	MH900522.1	✓				Appendix II
138	*CTX-M-15* gene	KY640528.1	✓				Appendix II
141	*CTX-M-15* gene	CP040398.1	✓				Appendix II
142	*CTX-M-1*gene	MH037035.1	✓				Appendix II
143	*CTX-M-108* gene	JF274245.1	✓				Appendix II
145	*CTX-M-15* gene	MK113958.1	✓				Appendix II
147	*CTX-M-15* gene	MH900522.1	✓				Appendix II
150	*CTX-M-15* gene	MH900522.1	✓				Appendix II
151	CTX-M-15 gene	MH900522.1	✓				Appendix II
157	*CTX-M-65* gene	KX495605.1	✓				Appendix II

158	*CTX-M-51* gene	NG_049002.1			✓		Appendix II
159	*Escherichia coli strain MNCRE44, complete genome*	CP010876.1|					Appendix II
161	*CTX-M-15* gene	KP325147.1|	✓				Appendix II
164	*CTX-M-15* gene	MH900522.1	✓				Appendix II
166	*CTX-M-15* gene	KY640528.1	✓				Appendix II
168	*CTX-M-59* gene	MH661247.1		✓			Appendix II
172	*CTX-M-15* gene	CP040398.1	✓				Appendix II
174	*CTX-M-172* gene	NG_048957.1					Appendix II
175	*CTX-M-15* gene	MH900522	✓				Appendix II
177	*CTX-M-15* gene	MK113956.1	✓				Appendix II
179	*CTX-M-3* gene	CP034325.1					Appendix II
181	*CTX-M-15* gene	KY640536.1	✓				Appendix II
184	*CTX-M-15* gene	NG_048947.1	✓				Appendix II
185	*CTX-M-15* gene	KP325147.1|	✓				Appendix II
187	*CTX-M-15* gene	MH523447.1					Appendix II
190	*CTX-M-15* gene	LT628518.1	✓				Appendix II
193	*CTX-M-55* gene	MH900523.1	✓				Appendix II
195	*CTX-M-2* gene	MH661246.1		✓			Appendix II
198	*CTX-M-27* gene	MH900525.1			✓		Appendix II
200	*CTX-M-15* gene	MH891569.1	✓				Appendix II

The sequence analysis using the BLAST tool shows that 27 (58%) of Glan Clwyd Hospital isolates had a high identity with *CTX-M-15*, followed by *CTX-M-59*: 2 (4%), *CTX-M-66*: 1 (2%), *CTX-M-203*: 1 (2%), *CTX-M-9*: 1 (2%), *CTX-M-32*: 1 (2%), *CTX-M-1*: 1 (2%), *CTX-M-108:* 1 (2%), *CTX-M-65*: 1 (2%), *CTX-M-51*: 1 (2%), *CTX-M-55:* 1 (2%), *CTX-M-172*: 1 (2%), *CTX-M-3*: 1 (2%), *CTX-M-2*: 1 (2%), *CTX-M family:*1(2%) and *CTX-M-27*: 1 (2%).

4.2.3 ESBL-producing isolates from Ysbyty Gwynedd (YG) Hospital (201-300)

The 3 steps described in section 4.2.1 were carried out in order to screen the YG Hospital isolates for CTX-M genes.

4.2.3.1 Bacterial culturing on primary UTI agar

Isolates from YG Hospital were cultured on primary UTI agar as prescribed in section 4.2.1.1. The growth of the colonies was recorded in Table 4.5 according to the hospital lab number.

Table 4.7 Bacterial identification based on colony characteristics for samples 201-300

Isolate number	Lab number	Date of subculture	Indicative bacteria based on colony colour
201	27697	12/05/2016	*Coliform*
202	28282	12/05/2016	*Coliform*
203	27855	12/05/2016	*Coliform*
204	28469	12/05/2016	*E. coli*
205	25696	12/05/2016	*E. coli*
206	23484	12/05/2016	*E. coli*
207	23623	12/05/2016	*E. coli*
208	23414	12/05/2016	*E. coli*
209	23842	12/05/2016	*E. coli*
210	12030	12/05/2016	*E. coli*
211	12294	12/05/2016	*E. coli*
212	12675	12/05/2016	*E. coli*
213	12640	12/05/2016	*E. coli*
214	13096	12/05/2016	*E. coli*
215	15309	12/05/2016	*E. coli*
216	15227	12/05/2016	*E. coli*
217	20057	12/05/2016	*E. coli*
218	27700	10/08/2016	*Coliform*
219	28287	10/08/2016	*Coliform*
220	11773	10/08/2016	*Coliform*
221	28316	10/08/2016	*Coliform*
222	23635	10/08/2016	*E. coli*
223	23456	10/08/2016	*E. coli*
224	23670	10/08/2016	*E. coli*
225	23362	10/08/2016	*E. coli*
226	12472	10/08/2016	*E. coli*
227	12564	10/08/2016	*Coliform*
228	12672	10/08/2016	*Coliform*
229	12447	10/08/2016	*Coliform*
230	13428	10/08/2016	*Coliform*
231	15581	10/08/2016	*E. coli*
232	15217	10/08/2016	*E. coli*
233	18069	10/08/2016	*Coliform*
234	27717	10/08/2016	*E. coli*
235	15306	10/08/2016	*E. coli*

236	15468	10/08/2016	*E. coli*
237	13373	10/08/2016	*E. coli*
238	13388	10/08/2016	*E. coli*
239	13232	10/08/2016	*E. coli*
240	13291	10/08/2016	*E. coli*
241	13396	10/08/2016	*Coliform*
242	12405	10/08/2016	*E. coli*
243	23471	10/08/2016	*E. coli*
244	23640	10/08/2016	*Coliform*
245	23631	10/08/2016	*E. coli*
246	28223	10/08/2016	*E. coli*
247	28096	10/08/2016	*E. coli*
248	28194	08/02/2017	*Coliform*
249	27651*	08/02/2017	*Coliform*
250	27651*	08/02/2017	*E. coli*
251	27717	08/02/2017	*E. coli*
252	23946	08/02/2017	*E. coli*
253	27301	08/02/2017	*E. coli*
254	27297	08/02/2017	*E. coli*
255	27181*	08/02/2017	*E. coli*
256	27181*	08/02/2017	*Coliform*
257	26083	08/02/2017	*Coliform*
258	26988	08/02/2017	*E. coli*
259	26548	08/02/2017	*E. coli*
260	25942	08/02/2017	*E. coli*
261	25673	08/02/2017	*E. coli*
262	23781	08/02/2017	*Coliform*
263	24954	08/02/2017	*E. coli*
264	24543	08/02/2017	*E. coli*
265	24623	08/02/2017	*E. coli*
266	23096	08/02/2017	*E. coli*
267	25706	08/02/2017	*E. coli*
278	23759	08/02/2017	*E. coli*
279	24026	08/02/2017	*E. coli*
270	24526	08/02/2017	*E. coli*
271	23760	08/02/2017	*E. coli*
272	23787	11/04/2017	*E. coli*
273	23789	11/04/2017	*E. coli*
274	23975	11/04/2017	*Coliform*

275	24542	11/04/2017	*E. coli*
276	24654	11/04/2017	*E. coli*
277	25006	11/04/2017	*E. coli*
278	25065	11/04/2017	*E. coli*
279	25864	11/04/2017	*Coliform*
280	24785	11/04/2017	*E. coli*
281	25792	11/04/2017	*Coliform*
282	26477	11/04/2017	*Coliform*
283	25691	11/04/2017	*E. coli*
284	25898	11/04/2017	*E. coli*
285	26523	11/04/2017	*E. coli*
286	26592	11/04/2017	*E. coli*
287	27083	11/04/2017	*E. coli*
288	26602	11/04/2017	*E. coli*
289	26852	11/04/2017	*E. coli*
290	26822	11/04/2017	*E. coli*
291	26675	11/04/2017	*E. coli*
292	27449	11/04/2017	*E. coli*
293	27554	11/04/2017	*E. coli*
294	27442	11/04/2017	*E. coli*
295	27611	11/04/2017	*E. coli*
296	27184	11/04/2017	*E. coli*
297	27303	11/04/2017	*E. coli*
398	27231	11/04/2017	*E. coli*
399	27383	11/04/2017	*E. coli*
300	27121	11/04/2017	*E. coli*

4.2.3.2 Multiplex PCR amplification of β-lactamase genes *blaCTX-M* 1, 2, 9 and 8/25 followed by gel electrophoresis of samples 201-300

The extracted genomic DNA of each isolate was amplified using multiple PCR as explained in section 4.2.1.2. Around 89% of the samples had bands located at the length of 1 of the *CTX-M* groups.

4.2.3.2.1 PCR gel analysis of the *blaCTX-M* genes (Isolates 201-214)

Based on the sizes of the displayed bands, these isolates seem to carry *CTX-M* genes: *CTX-*MGp1 (688bp) samples 201 (coliforms) and 204, 205, 206, 207, 208, 209, 210, 211 and 212 (all *E. coli*), in addition to *CTX-MGp2* (404bp) samples 202 (*E. coli*) and 203 (coliform) (Figure 4.17).

4.2.3.2.2 PCR gel analysis of the *blaCTX-M* genes (Isolates 215-228)

As shown in Figure 4.18, 13 samples from these isolates corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp), which includes samples 215, 216, 217, 218, 221, 222, 223 and 225 (all *E. coli*) and 219, 220 and 227 (all coliforms), in addition to CTX-MGp9 (561bp) samples 226 (*E. coli*) and 228 (coliform).

4.2.3.2.3 PCR gel analysis of the *blaCTX-M* genes (Isolates 229-242)

Nine isolates from this group—229, 231, 236, 237, 238, 239 and 242 (all *E. coli*) in addition to 240 and 241 (all coliforms)—displayed with lengths that corresponded to *CTX-MGp1* (688bp) (Figure 4.19).

4.2.3.2.4 PCR gel analysis of the *blaCTX-M* genes (Isolates 243-256)

Three *CTX-M* groups seem to be carried by these isolates based on the displayed bands: *CTX-MGp1* (688bp) in samples 244 (coliform), 245 (*E. coli),* 247 (*E .coli),* 248 (coliform), 249 (coliform), 250 *(E. coli*), 251 *(E. coli*), 253 (*E. coli*), 255 (*E. coli*) and 256 (coliform); *CTX-MGp2* (404bp) in samples 252 and 254 (all *E. coli*); and *CTX-MGp9* (561bp) in sample 246 (*E.coli*) (Figure 4.20).

4.2.3.2.5 PCR gel analysis of the *blaCTX-M* genes (Isolates 257-270)

Only 1 type, *CTX-MGp1* (688bp), of the CTX-M genes seemed to be detected in11 isolates of this group, which includes samples 258, 259, 260, 263, 264, 265, 266 and 267 (all *E.coli*), in addition to samples 268, 257 and 262 (all coliforms) (Figure 4.21).

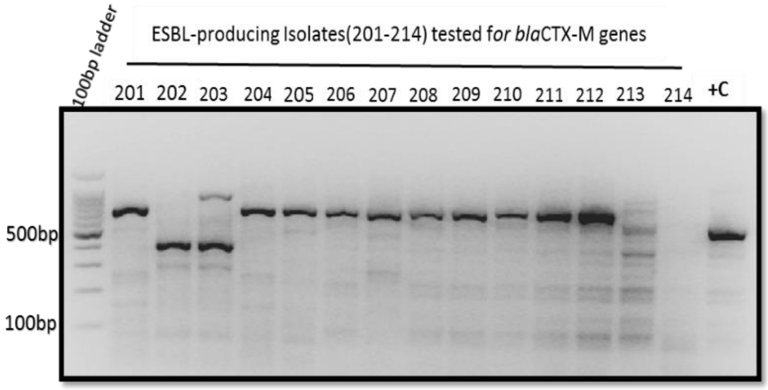
4.2.3.2.6 PCR gel analysis of the *blaCTX-M* genes (Isolates 271-284)

Gel analysis of this group shows that the 14 samples had bands with lengths that corresponded to *CTX-MGp1* (688 bp): 271, 272, 273, 274, 275, 276, 276, 277 and 278 (all *E. coli*) in addition to 279, 280 and 281 (all coliforms) (Figure 4.22).

4.2.3.2.7 PCR gel analysis of the *blaCTX-M* genes (Isolates 285-300)

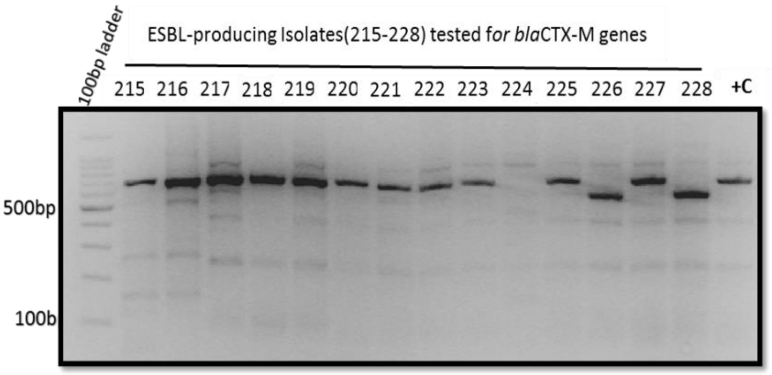
The displayed bands of the gel analysis show that 2 types of CTX-M genes seem to be carried by this group of isolates: *CTX-MGp1* (688bp) samples, which include samples 285, 286, 287, 288, 289, 290, 291, 292, 295, 296, 297, 298, 299 and 300 (all *E.coli*) and *CTX-MGp9* (561bp) samples 293 and 294 (both *E.coli*) (Figure 4.23).

Figure 4.17 Multiplex PCR assay for the *blaCTX-M* genes, samples 201-214



Gel analysis of *bla*CTXM amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Twelve samples had bands with lengths that corresponded to two groups of CTXM genes; CTXMGp1 (688bp) samples (201,204,205,206,207,208,209,210,211,212), CTXMGp2 (404bp) sample (202&203). Lane 16: positive control.

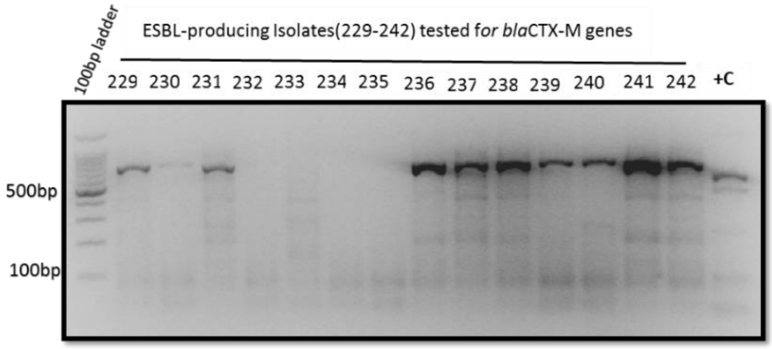
Figure 4.18 Multiplex PCR assay for the *blaCTX-M* genes, samples 215-228



Gel analysis of blaCTX-M amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Fourteen samples had bands with lengths that corresponded to two groups of CTX-M genes; CTXMGp1 (688bp) samples (244,245,247,248,249,250,251,253,255,256), CTXMGp2 (404bp) sample (252&2254),CTXMGp9 (561bp) sample (246). Lane 16: positive control.

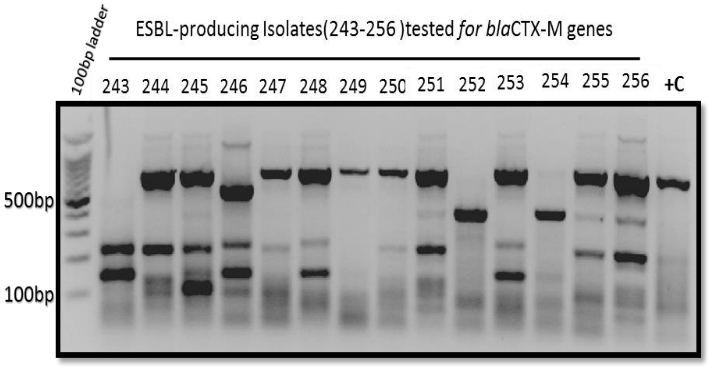
.

Figure 4.19 Multiplex PCR assay for the *blaCTX-M* genes, samples 229-242



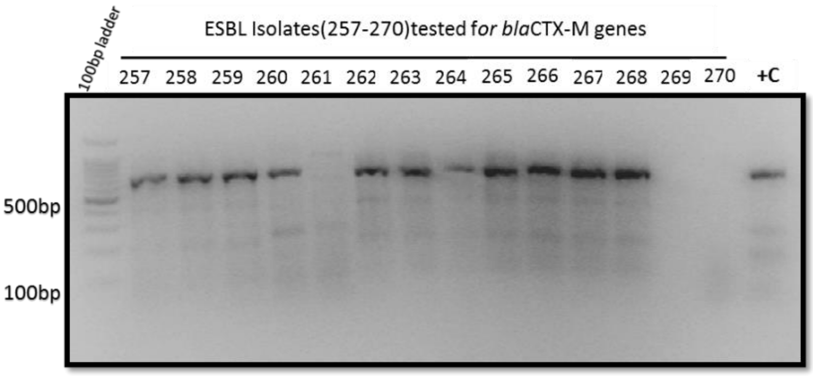
Gel analysis of blaCTX-M amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Nine samples had bands with lengths that corresponded to CTXMGp1 (688bp) samples (229,231,236,237,238,239,240,241,242). Lane 16: positive control.

Figure 4.20 Multiplex PCR assay for the *blaCTX-M* genes, samples 243-256



Gel analysis of blaCTX-M amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Fourteen samples had bands with lengths that corresponded to two groups of CTX-M genes; CTXMGp1 (688bp) samples (244,245,247,248,249,250,251,253,255,256), CTXMGp2 (404bp) sample (252&2254), CTXMGp9 (561bp) sample (246). Lane 16: positive control.

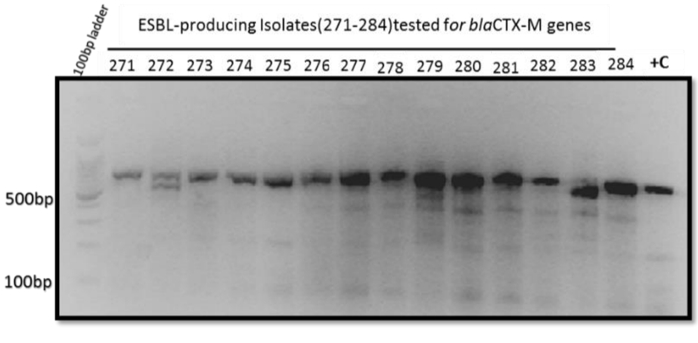
Figure 4.21 Multiplex PCR assay for the *blaCTX-M* genes, samples 257-270



Gel analysis of blaCTX-M amplicon on 2% agarose gel. Lane 1:100bp ladder. Lanes 2–15: 14 ESBL samples. Eleven samples had bands with lengths that corresponded to CTXMGp1 (688bp) samples (257,258,259,260,262,263,264,265,265,266,267,268). Lane 16: positive control.

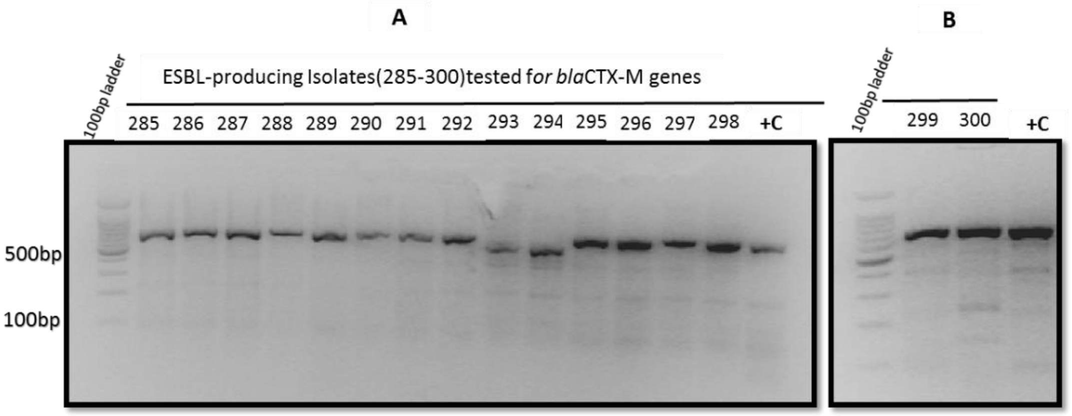
.

Figure 4.22 Multiplex PCR assay for the *blaCTX-M* genes, samples 271-284



Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1: 100bp ladder; lanes 2–15: 14 ESBL samples. Fourteen samples had bands with lengths that corresponded to *CTX-MGp1* (688 bp) samples (271-284). Lane 16: positive control.

Figure 4.23 Multiplex PCR assay for the *blaCTX-M* genes, samples 285-300



(**A**) Gel analysis of *bla*CTX-M amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–15: 14 ESBL samples. Fourteen samples had bands with lengths that corresponded to 2 groups of *CTX-M* genes: *CTX-MGp1* (688bp) (samples 285, 286, 287, 288, 289, 290, 291, 292, 295, 296, 297 and 298) and *CTX-MGp9* (561bp) (samples 293 and 294). Lane 16: positive control. **(B)** Gel analysis of *blaCTX-M* amplicon on 2% agarose gel. Lane 1: the 100bp ladder. Lanes 2–3: 2 ESBL samples. Two samples had bands with lengths that corresponded to *CTX-MGp1* (688bp) samples (299 and 300). Lane 16: positive control.

The screening of ESBL-producing isolates from Wrexham Maelor Hospital (201–300) for the CTX-M groups using multiplex PCR followed by gel analysis shows, based on the amplicon size, that the predominant CTX-M group is group 1 (Table 4.8).

Table 4.8 PCR amplicon sizes of the *blaCTX-M genes*, samples 201-300

CTX-M group	Amplicon size (bp)	Sample No
CTX-M group 1	688	201, 204, 205, 206, 207, 208, 209, 210, 211, 212, 215, 216, 217, 218, 219, 220, 221, 222, 223, 225, 219, 227, 229, 231, 236, 237, 238, 239, 240, 241, 242, 244, 245, 247, 248, 249, 250, 251, 253, 255, 256, 258, 259, 260, 263, 264, 265, 266, 267, 268, 257, 262, 271, 272, 273, 274, 275, 276, 276, 277, 278, 279, 280, 281, 285, 286, 287, 288, 289, 290, 291, 292, 295, 296, 297, 298, 299, 300.
CTX-M group 2	404	202, 203, 246.
CTX-M group 9	561	293 and 294.
CTX-M group 8/25	326	

4.2.3.3 Sequencing analysis of multiplex PCR products (201-300)

Forty-five (50%) of samples that displayed bands with sizes that corresponded with 1 of the CTX-M groups were sequenced and then compared with DNA sequences of known b-lactamase genes using the BLAST programme Table 4.9 shows sequencing analysis of positive samples from 201-300.

Table 4.9 Sequencing analysis of positive samples from 201-300

Sample No	Blast output	Accession number	CTX-M group				DNA sequence
			Gp1	Gp2	Gp9	Gp8/25	
201	*CTX-M-15* gene	MH900522	✓				Appendix III
202	*CTX-M-2* gene	LC229068		✓			Appendix III
205	*CTX-M-15* gene	MF346615	✓				Appendix III
207	*CTX-M-108* gene	JF274245	✓				Appendix III
209	*Escherichia coli strain 3385 chromosome, complete genome*	CP029420					Appendix III
211	*CTX-M-15* gene	MF346615	✓				Appendix III
215	*CTX-M-172* gene	NG_048957.1	✓				Appendix III
217	*CTX-M-15* gene	CP040398	✓				Appendix III
219	*CTX-M-15* gene	MK405591	✓				Appendix III
223	*CTX-M-15* gene	LT628518	✓				Appendix III
226	*CTX-M-14(like)*	CP032888			✓		Appendix III
228	*CTX-M-27* gene	MfH900525			✓		Appendix III
229	*CTX-M-15* gene	LT628518.1	✓				Appendix III
231	*CTX-M-15* gene	MK113959	✓				Appendix III
238	*CTX-M-15* gene	LT628520	✓				Appendix III
241	*CTX-M-32* gene	MH900527	✓				Appendix III
242	*CTX-M-15* gene	LT628518	✓				Appendix III
244	*CTX-M-15* gene	MK234851.1	✓				Appendix III
246	*CTX-M-14(like)*	CP032888			✓		Appendix III
248	*CTX-M-15* gene	MH900522	✓				Appendix III
250	*CTX-M-15* gene	MK405591	✓				Appendix III
252	*CTX-M-2* gene	MH900526.1		✓			Appendix III
254	*Escherichia coli strain 4928STDY7071340 genome assembly.*	LR607331.1					Appendix III
257	*CTX-M-14(like)*	CP032888			✓		Appendix III
259	*CTX-M-15* gene	LT628516	✓				Appendix III
262	*CTX-M-15* gene	MK113960	✓				Appendix III

264	*CTX-M-15* gene	MK405591	✓				Appendix III
266	*CTX-M-15* gene	MF977517.1	✓				Appendix III
268	*CTX-M-15* gene	MK405591.1	✓				Appendix III
271	*CTX-M-15* gene	KY640534	✓				Appendix III
272(lower band)	*Escherichia coli strain 4928STDY7071340 genome assembly*	LR607331.1					Appendix III
275	*CTX-M-90* gene	MF418175.1			✓		Appendix III
277	*CTX-M-163* gene	NG_048948.1	✓				Appendix III
279	*CTX-M-15* gene	KR338941	✓				Appendix III
281	*CTX-M-15* gene	MK113960	✓				Appendix III
283	*CTX-M-14(like)*	CP032888			✓		Appendix III
286	*CTX-M-15* gene	MH891569	✓				Appendix III
288	*CTX-M-15* gene	KY640534	✓				Appendix III
290	*CTX-M-15* gene	MK113957	✓				Appendix III
292	*CTX-M-15* gene	KR338941	✓				Appendix III
293	*CTX-M-9* gene	CP031724.1			✓		Appendix III
294	*CTX-M-14(like)*	CP032888			✓		Appendix III
296	*CTX-M-15* gene	KY640536.1	✓				Appendix III
298	*CTX-M-1* gene	MH037035.1	✓				Appendix III
300	*CTX-M-15* gene	MH900522.1	✓				Appendix III

The BLAST output of the sequenced YG Hospital sample shows that the *CTX-M-15* gene is the most prevalent type of *CTX-M*; 27(60%) followed by *CTX-M-14* (like); 5 (11%), *CTX-M-2*: 2 (4%),*CTX-M-108*: 1 (2.2%), *CTX-M-172*: 1 (2.2%), *CTX-M-27*: 1 (2.2%), *CTX-M-32*: 1 (2.2%), *CTX-M-90*: 1 (2.2%), *CTX-M-163*: 1 (2.2%), *CTX-M-9*: 1 (2.2%).

Sequencing of ≈ 50% of ESBL-producing isolates, from all three hospitals, that had bands on gel analysis correspond to one of the CTX-M genes showed that 55.73% were CTX-M-15 producers. Table 4.10 summarises the percentage of each detected CTX-M type among the sequenced samples.

Table 4.10 Table 4.10 CTX-M genes identified at the three hospital sites

Blast out	CTX-M group	Number (%)
CTX-M-15	Gp1	77 (55.7%)
CTX-M-14 (like)	Gp9	5 (3.62%)
CTX-M-9	Gp9	5 (3.62%)
CTX-M-27	Gp9	4 (2.8%)
CTX-M-59	Gp2	4 (2.8%)
CTX-M-2	Gp2	4 (2.8%)
CTX-M-108	Gp9	3 (2.1%)
CTX-M-32	Gp1	3 (2.1%)
CTX-M-1	Gp1	3 (2.1%)
CTX-M-172	Gp1	3 (2.1%)
CTX-M-55	Gp1	3 (2.1%)
CTX-M-3	Gp1	2 (1.4%)
CTX-M-66	Gp1	2 (1.4%)
CTX-M-163	Gp1	2 (1.4%)
CTX-M-160	Gp1	1 (0.72%)
CTX-M-225	Gp1	1 (0.72%)
CTX-M-203	Gp1	1 (0.72%)
CTX-M-65	Gp1	1 (0.72%)
CTX-M-51	Gp9	1 (0.72%)
CTX-M-90	Gp9	1 (0.72%)
CTX-M family		1 (0.72%)
Others e.g,Escherichia coli strain MNCRE44, complete genome		11 (7.9%)

4.3 Discussion

4.3.1 Primers competition in Multiplex PCR

In multiplex PCR, more than one target sequence can be amplified by including multiple pairs of primers in the reaction, which increases the detection of resistance genes in a single test, and results in a considerable saving of time and effort (Elnifro et al., 200). Limitations of multiplex PCR include a lack of optimization and false negatives. The latter is caused by primer competition for reagents, which could favour stronger over weaker binding primers, and increases the chance of obtaining spurious amplification products, due primarily to the formation of primer dimers (Brownie, 1997). However, this competition for reagents is not so crucial because it mainly reduces the yield, and in this study the problem could be overcome by using a published primer in which the design parameters had been selected to account for the homology of primers with their target nucleic acid sequences, their length, the GC content, and their concentration.

4.3.2 CTX-M-15 is the dominant CTX-M-type ESBL in North Wales

CTX-Ms have become the most rapidly growing type of ESBL since they were first identified in Germany, France and South America in 2000. Many studies have been established to draw the global picture of *CTX-M* variants, and it has been concluded that *bla*CTX-M-15 has increased over time in most countries and is considered the dominant *E.coli* resistant gene in most regions (Edward et al., 2017). Interestingly, the exception from this rule are regions like South America, where *blaCTX-M-2* is the most detectable CTX-M gene, and Spain, South-East Asia, China, Japan and South Korea, where the group 9 variants are the most prevalent CTX-Ms (Woerther et al., 2013).

The United Kingdom is classified as a region with a high prevalence of *blaCTX-M-15*. However, the previous studies by Mushtaq et al. (2003), who conducted a study at 26 hospitals in the UK and Ireland where the researchers collected up to 200 consecutive, clinically significant isolates from separate inpatients during a multi-centre survey of inpatient isolates concluded that among 122 cephalosporin-resistant isolates of *Enterobacteriaceae*, there were only 4 *CTX-M-15*-producing isolates that were collected at hospitals in London (2 isolates), Newcastle-upon-Tyne and Belfast. The region of this study, North Wales, was part of Mushtaqs et al' study.

The present study shows that the prevalence of *CTX-M-15* has reached North Wales as among the 138 (50% of samples had bands on gel analysis corresponded with of CTX-M genes) sequenced ESBL-producing isolates collected from 3 main referral hospitals in North Wales, 77 (55.73%) have been detected as *CTX-M-15* producers. This is considered a remarkable increase in the UK epidemiology of *CTX-M-15* and presents huge challenges to healthcare as it restricts options to treat infections caused by CTX-M-producing bacteria.

In terms of antibiotic resistance, it has been reported that *CTX-M-15* possesses a high activity against Ceftazidime (Malik et al., 2018). The YG Hospital data (Table 3.2) indicates that the isolates that were identified genotypically as *CTX-M-15* producers conferred resistance to other antibiotic profiles, such as amoxicillin and ciprofloxacin, suggesting the presence of other mechanisms in addition to *CTX-M-15* or novel mutations in the *CTX-M-15* gene that may develop as a result of misuse of antibiotics due to the limitations of ESBL phenotypic dictions in clinical laboratories (Rahman et al., 2018). An alternative explanation may be provided by the rural environment of the North Wales hospitals which serve a large farming community. Given the presence of antibiotic resistant *Enterobacteriaceae* in food producing animals like lambs (Gozi kS et al., 2019), the resistance conferring plasmids may be transmitted to humans.

It has been suggested (e.g. Carattoli, 2013; Orhue et al., 2017) that the high global spread of *CTX-M 15* is promoted by its location on the conjugative plasmid *IncF*, which facilitates the horizontal transfer of the antibiotic resistance plasmid by the conjugation in *Enterobacteriaceae*. This singles *CTX-M-15* out as other *blaCTX-M* genes like *blaCTX-M-14*, the second dominant CTX-M type is carried on a variety of plasmid types, including IncF, like the IncK plasmid that is prevalent in the Far East, (Ang et al., 2015). Moreover, the main host of the IncF plasmid is *Enterobacteriaceae* (Fortini et al., 2015), which explains the high percentage of *E.coli* that carries the *CTX-M-15* gene in compression with coliforms among the isolates of this study.

4.3.3 The novel beta-Lactamase, *CTX-M-14-LIKE*

Sequencing of the YG Hospital samples revealed that, in addition to *CTX-M-15* as the dominant CTX-M type, some isolates ;226 (*E. coli*), 246 (*E. coli*), 257 (coliform), 283 (*E. coli*) and 296 (*E. coli*), which represent 11% of the sequenced samples, revealed a new β-lactamase gene that is closely related to *CTX-M-14* at the protein level as it substitutes only 3 amino acids when compared to the known CTX-M-14 protein. The three amino acids are T55A, A273P and R277C (Figures 4.24). Because of its close similarity to CTX-M-14, the novel gene was named *CTX-M-14-Like*.

Figure 4.24 Sequence alignment of CTX-M14&CTX-M14 LIKE proteins

```
Query  1     MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDAADNTQ  60

             MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALID ADNTQ
Sbjct  1     MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQ  60

Query  61    VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120
             VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM
Sbjct  61    VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120

Query  121   TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180
             TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP
Sbjct  121   TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180

Query  181   RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240
             RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS
Sbjct  181   RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240

Query  241  GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNPESRCDVLASAARIIAEGL  291

             GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQN ESR DVLASAARIIAEGL
Sbjct  241   GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGL  291
```

The sequence alignment of CTX-M-14 (query sequence 1) and CTX-M-14 LIKE (query sequence 2) proteins using the Clustal Omega tool, as indicated by the 3 highlighted differences between CTX-M-14 and CTX-M-14 LIKE, is T55A, A273P and R277C.
BLAST output CTX-M-14 [Enterobacteriaceae], Sequence ID: ref|WP_001617865.1|.

The two CTX-M genes, *CTX-M-15* as the dominant CTX-M in North Wales and the novel *CTX-M-14-LIKE* β-lactamase gene were then further analysed upon cloning into an *E.coli* expression plasmid (pASK-IBA2), which allowed for the purification of a C-terminally strep-tagged recombinant protein. The activity of the purified, recombinant enzymes was then tested with the most commonly used antibiotic for UTI patients in the clinical laboratories of North Wales hospitals. This work is detailed in Chapter 5 and 6 respectively.

Chapter 5 : OVEREXPRESSION AND PURIFICATION OF RECOMBINANT CTX-M ENZYMES

5.1. Introduction

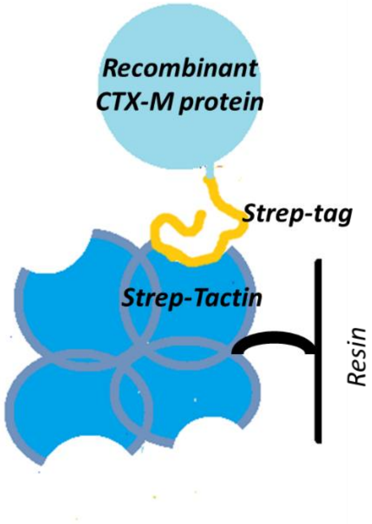
As the study of a protein's properties requires a large number of proteins to study (and these proteins usually cannot be produced by native hosts), protein overexpression was developed to increase protein production by an appropriate host and to facilitate the purification of proteins (Tripathi & Nagesh, 2016). Three main elements are required for protein overexpression: a gene, a vector (an expression plasmid) carrying the gene of interest and an expression host, which maximises the amount and quality of the protein produced by the vector–gene combination (Goh et al., 2017).

An expression plasmid is generally engineered to carry regulatory sequences, that is, the enhancer and promoter regions responsible for the transcription of the inserted gene in the expression plasmid (Singha et al., 2017). An appropriate vector should be selected precisely to ensure the efficient production of proteins. Thus, the expression plasmid chosen in this study to obtain an abundant amount of proteins from *CTX-M* genes was the *pASK-IBA2C* plasmid. *pASK-IBA2C* is an expression plasmid that is under the transcriptional control of the tetracycline promoter/operator and that is characterised by its ability to export the expressed recombinant protein into the periplasm of *Escherichia coli* to facilitate protein harvesting and purification (Schmidt & Skerra, 2015). Chemically competent *E. coli* cells, which is considered one of the most popular hosts for protein expression, was used as an organism for the overexpression of recombinant CTX-M proteins.

The purification of the produced recombinant protein can be achieved using a tag protein affinity system, in which the protein is genetically tagged with a small amino acid peptide sequence. This is a predominantly viral protein sequence that can be attached to the N-terminus or C-terminus of the recombinant proteins based on the needs of the experiment and the properties of the protein of interest (Kosobokova et al., 2016). In the *pASK-IBA2C* plasmid, the included tag sequence is the Strep-tag, which constitutes the nine amino acid peptide sequence Ala-Trp-Arg-His-Pro-Gln-Phe-Gly-Gly. This small amino acid sequence was engineered to reversibly bind to the protein Streptavidin and is in this sense distinct from other commonly used affinity peptides like HA or Myc that are peptides from natural proteins (Chromatogr, 1994).

The reversible binding of the Strep-tag sequence to a genetically modified Streptavidin protein known as Strep-Tactin®, allows for a one-step protein purification (Yeliseev et al., 2017). Transfer of the recombinant protein is facilitated by the OmpA leader peptide that is later removed during the transport of the recombinant protein by a membrane-bound protease inside the periplasmic space (Inouye& Halegoua, 1980). The OmpA leader peptide is encoded by the *pASK-IBA2C* plasmid and sits at the N-terminus of the recombinant protein. Figure 5.1 shows the Strep-Tactin®XT purification system used to purify the recombinant Strep-tagged CTX-M proteins.

Figure 5.1 Protein purification using Strep-Tactin®



Purification of Strep-tagged CTX-M proteins using the Strep-Tactin® purification system, which relies on binding the Strep-Tag II® and Strep-Tactin® included in prepacked chromatography columns (Modified from IBA Data Sheet, 2017).

The objectives of this experiment were to obtain an abundant amount of pure CTX-M-14, CTX-M-14-LIKE and CTX-M-15 proteins to measure the enzymatic activity of these beta-lactamases against commonly used antibiotics for UTI patients and to compare the catalytic activity of the novel B-lactamase, CTX-M-14-LIKE, with the known CTX-M-14 enzyme. CTX-M protein expression was completed in four steps: the full-length gene encoding *CTX-M* was inserted into the plasmid *pASK-IBA2C*, the transformation of *pASK-IBA2C* into carrying *CTX-M* in *E. coli*, production of the protein of interest by the expression host followed by lysing and purification from the periplasmic space and, finally, the verification of the protein expression level using the Western blot technique.

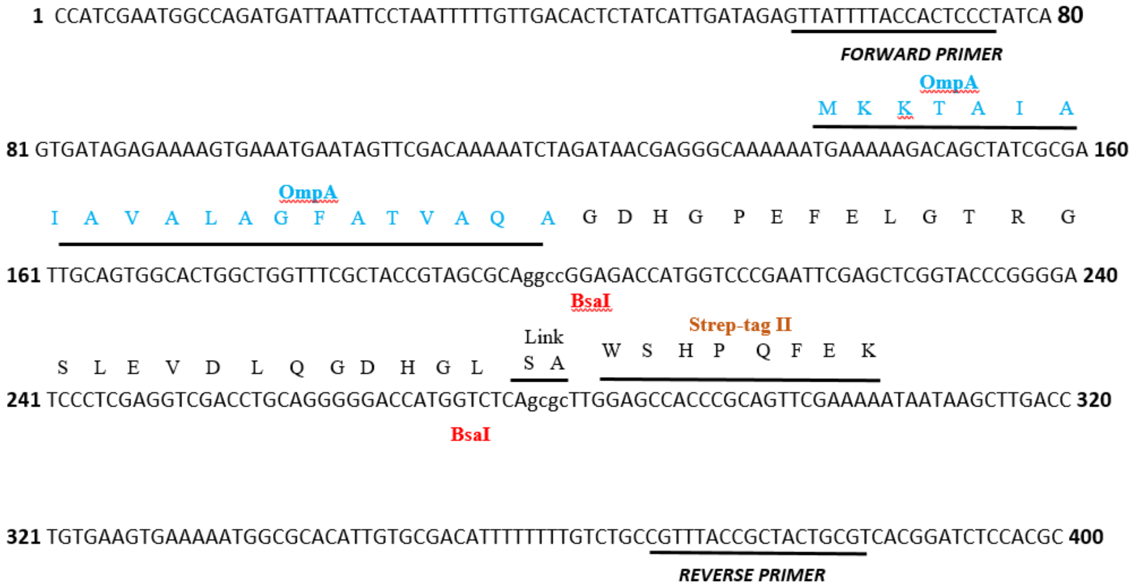
5.2. Results

5.2.1. Cloning of a mutant *CTX-M* gene into a *pASK-IBA2C* plasmid expressing *E. coli*

The two *CTX-M* genes, the novel *CTX-M-14-LIKE* and *CTX-M-15*, were cloned into a *pASK-IBA2C* plasmid expressing *E. coli* to allow for the purification of a Strep-tagged recombinant protein. This vector carries a *Tet* promoter, which was developed to produce C-terminal fusion proteins and the Strep-tag II®. The produced recombinant protein is transported into the periplasm of *E. coli* via the vector-encoded OmpA signal sequence.

The full-length *CTX-M* genes were amplified from the corresponding samples using forward and reverse primers (Section 2.2.9, Table 2.3). Both primers contained BsaI restriction sites at multiple cloning sites in the plasmid (Figure 5.2).

Figure 5.2 Multiple Cloning Site of pASK-IBA2C

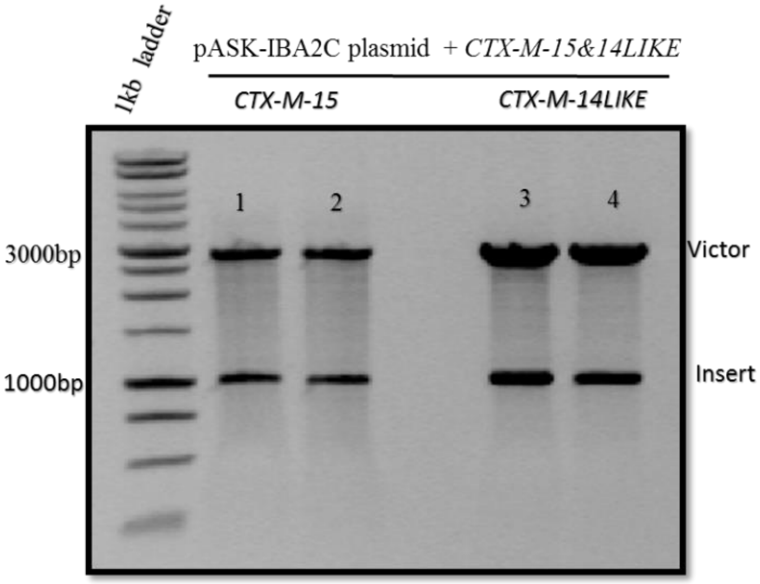


Features of multiple cloning sites of *pASK-IBA2C*, which are responsible for transferring the protein produced in the *E. coli* cytoplasm to the periplasmic space, Strep-tag II® and the restriction sites of BsaI (Modified from IBA Data Sheet, 2010).

The ligation of the *pASK-IBA2C* plasmid vector carrying the *CTX-M-14-LIKE* and *CTX-M-15* genes was performed using the T4 DNA ligase enzyme. To replicate the plasmid carrying the gene of interest, the plasmid was transformed into chemically competent *E. coli* using the heat shock method (Section 2.2.11).

The cells transformed with the plasmid were plated on 25 µg/mL of LB agar with chloramphenicol (Section 2.2.12). After overnight incubation, the growth colonies were incubated overnight in LB/chloramphenicol at 37 °C, and the recombinant plasmid was agitated. The transformed *E. coli* was purified using an ISOLATE II Plasmid Kit. The purified recombinant plasmid was then digested by BsaI enzyme (Section 2.2.13). In order to verify the insertion, agarose gel was used to analyse the restricted recombinant plasmid, where two bands can be seen in the lane of the digestion reaction of the *pASK-IBA2C* plasmid vector and *CTX-M* genes; the vector had a length of 3,000 bp and an insert of 1,000 bp (Figure 5.3).

Figure 5.3 Gel analysis of pASK-IBA2C, *CTX-M-14-LIKE* and *CTX-M-15*



Plasmid mapping analysis on 0.8% agarose gel. Lane 1: 6 µL of the DNA ladder (1 kp of the ladder). Lanes 2 and 3: 10 µL of the digestion reaction of the *pASK-IBA2C* plasmid vector and *CTX-M-15* (plasmids 1 and 2). Lanes 6 and 7: 10 µL of the digestion reaction of the *pASK-IBA2C* plasmid vector and *CTX-M-14-LIKE* (plasmids 3 and 4). The vector had a length of 3,000 bp and an insert of 1,000 bp.

5.2.2 Sequencing analysis of a cloned pASK-IBA2C plasmid with *CTX-M-14-LIKE* and *CTX-M-15*

The purified *pASK-IBA2C* plasmid carrying *CTX-M-14-LIKE* and *CTX-M-15* was sequenced using *pASK-IBA2C* forward primer in order to verify the insertion of the *CTX-M* genes into the expression plasmid. Sequencing analysis was performed on one plasmid from each *CTX-M* gene (plasmids 1 and 3). The sequenced samples were compared with the DNA sequences of known β-lactamase genes using the BLAST programme. Table 5.1 shows the BLAST output of the sequenced samples.

Table 5.1 Sequencing analysis of the pASK-IBA2C plasmid carrying CTX-M-14-LIKE and CTX-M-15

Plasmid Number	BLAST Output	Accession Number
1	*Escherichia coli* plasmid pryc110.1 insertion sequence ISCR1 putative recombinase gene, partial cds and beta-lactamase CTX-M-14 (blaCTX-M-14b) gene, complete cds	GQ892051.1
3	*Klebsiella pneumoniae* strain KPTR7239-17 insertion sequence ISEcp1, partial sequence and beta-lactamase CTX-M-15 (blaCTX-M) gene (blaCTX-M-15) allele, complete cds	MH544748.1

5.2.3 Translation and sequence alignments of the recombinant CTX-Ms

The plasmids that shared a high sequence identity with the *CTX-M-14-LIKE* and *CTX-M-15* genes were translated to protein sequences using ExPASy (Figures; 5.4(A) &5.5(B)), which allows for the translation of a nucleotide sequence to a protein sequence. After, the sequences were aligned using Clustal Omega, which arranges the protein sequences to identify regions of similarity that may be a consequence of functional, structural or evolutionary relationships between the sequences. Figure 5.5(B) shows the three substitutions between *CTX-M-14-LIKE* and *CTX-M-14*: T55A, A273P and R277

Figure 5.4 Protein Sequence of plasmid (1); pASK-IBA2C plasmid carrying *CTX-M-15*

(A)

MKKTAIAIAVALAGFATVAQAMVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELER
QSGGRLGVALINTADNSQILYRADERFAMCSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLV
NYNPIAEKHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEP
TLNTAIPGDPRDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWV
VGDKTGSGGYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAAKS

OmpA Leader (incomplete sequence)CTX-M-15Strep Tag

(B)

```
Query  1    MVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTADNSQ  60
            MVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTADNSQ
Sbjct  1    MVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTADNSQ  60

Query  61   ILYRADERFAMCSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTM  120
            ILYRADERFAMCSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTM
Sbjct  61   ILYRADERFAMCSTSKVMAAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAEKHVNGTM  120

Query  121  SLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDP  180
            SLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDP
Sbjct  121  SLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTEPTLNTAIPGDP  180

Query  181  RDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGS  240
            RDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGS
Sbjct  181  RDTTSPRAMAQTLRNLTLGKALGDSQRAQLVTWMKGNTTGAASIQAGLPASWVVGDKTGS  240

Query  241  GGYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAAK  285
            GGYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAAK
Sbjct  241  GGYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLASAAK  285
```

(A) Forward protein Translation of ***CTX-M-15***gene using Expasy tool **.**The three elements needed for protein overexpression (OmpA lead, inserted gene and strep tag are highlighted **(B)** Protein alignments created Using Clustal Omega tool. Protein Blast output: class A extended-spectrum beta-lactamase *CTX-M-15*.
Sequence ID: WP_000239590.1

Figure 5.5 Protein Sequence of plasmid (3); pASK-IBA2C plasmid carrying *CTX-M-14LIKE*

(A)

ASALAGFATVAQAMVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDAA
DNTQVLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTMTLAELS
AAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDPRDTTTPRAMAQTLRQLT
LGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGSGDYGTTNDIAVIWPQGRAPLVLVTYFT
QPQQNPESRCDVLASAARIIAEGLSAWSHPQFEK

OmpA Leader (incomplete sequence)CTX-M-14 like Strep Tag

(B)

```
Query  1    MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDAADNTQ  60
            MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALID ADNTQ
Sbjct  1    MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQ  60

Query  61   VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120
            VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM
Sbjct  61   VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120

Query  121  TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180
            TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP
Sbjct  121  TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180

Query  181  RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240
            RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS
Sbjct  181  RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240
Query  241  GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNPESRCDVLASAARIIAEGL  291
            GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQN ESR DVLASAARIIAEGL
Sbjct  241  GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGL  291
```

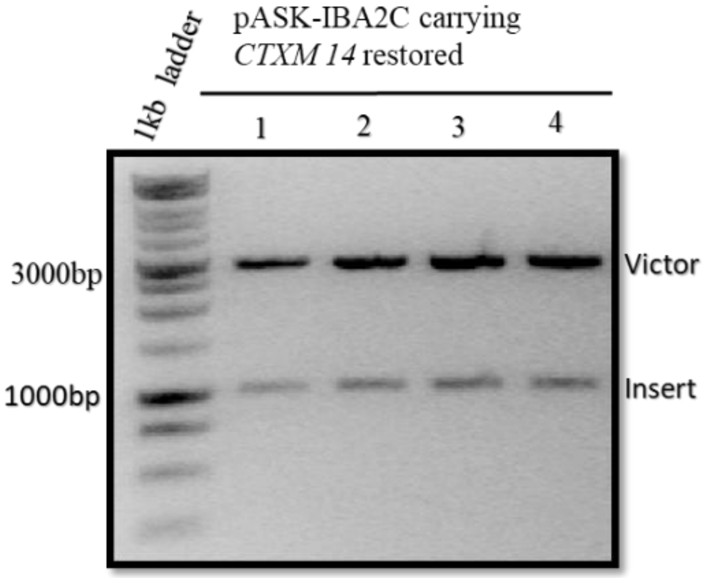
(A) Forward protein Translation of ***CTX-M-14LIKE*** gene using Expasy tool**.** The three elements needed for protein overexpression (OmpA lead, inserted gene and strep tag are highlighted**. (B)** Protein alignments created Using Clustal Omega tool. Protein Blast output: class A extended-spectrum beta-lactamase ***CTX-M-14***.
Sequence ID: WP_001617865.1.As indicated the three highlighted differences between CTXM-14 & CTXM-14 – Like are: T55A, A273P and R277C.

5.2.4 Using a Q5® Site-Directed Mutagenesis Kit for the reversion of the three *CTX-M-14-LIKE* changes

In order to compare CTX-M-14 and CTX-M-14-LIKE enzymes in terms of their enzymatic activity against commonly used antibiotics for UTI patients, T55A, A273P and R277C were mutated back to the CTX-M-14 wild-type sequence using the Q5® Site-Directed Mutagenesis Kit (Section 2.2.18). After the triple mutagenesis of the *CTX-M-14-LIKE* gene, the *pASK-IBA2C* plasmid carrying the restored *CTX-M-14* gene was transformed into *E. coli*, plated on LB agar with chloramphenicol, incubated overnight in LB broth, purified using an ISOLATE II Plasmid Kit and restricted using BsaI before sequencing.

Figure 5.6 shows the gel analysis of the restricted and purified *pASK-IBA2C* plasmid carrying the restored *CTX-M-14* in which two bands can be seen in the lane of the digestion reaction: the vector at length of 3,000 bp and an insert of 1,000 bp.

Figure 5.6 Gel analysis of pASK-IBA2C and the triple reverted CTX-M-14



Plasmid mapping analysis on 0.8% agarose gel. Lane 1: 6 µL of the DNA ladder (1 kp of the ladder). Lanes 2, 3 and 4: 10 µL of the digestion reaction of the pASK-IBA2C plasmid vector and *CTX-M-14* restored (T55A, A273P and R277C). The vector had a length of 3,000 bp and an insert of 1,000 bp.

The *pASK-IBA2C* plasmid carrying triple reversion of *CTX-M-14-LIKE* (A55T, P273A and C277R) was sequenced using the *pASK-IBA2C* forward primer in order to verify that *CTX-M-14-LIKE* was mutated back to *CTX-M14*. The sequencing analysis was performed on plasmids 1 and 2. The sequenced samples were compared with the DNA sequences of known β-lactamase genes using the BLAST programme and then translated and aligned using ExPASy and Clustal Omega, respectively (Figure 5.7).

Figure 5.7 Protein Sequence of plasmid (1); pASK-IBA2C plasmid carrying the triple reversion of *CTXM 14-LIKE*

(A)

MKKTAIAIAVALAGFATVAQAMVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEK
SSGGRLGVALIDTADNTQVLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLV
NYNPIAEKHVNGTMTLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEP
TLNTAIPGDPRDTTTPRAMAQLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVG
DKTGSGDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGLSAWSHPQFE
K

OmpA Leader (incomplete sequence) CTX-M-14 likeStrep Tag

(B)

```
Query  1    MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQ  60
            MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQ
Sbjct  1    MVTKRVQRMMFAAAACIPLLLGSAPLYAQTSAVQQKLAALEKSSGGRLGVALIDTADNTQ  60

Query  61   VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120
            VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM
Sbjct  61   VLYRGDERFPMCSTSKVMAAAAVLKQSETQKQLLNQPVEIKPADLVNYNPIAEKHVNGTM  120

Query  121  TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180
            TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP
Sbjct  121  TLAELSAAALQYSDNTAMNKLIAQLGGPGGVTAFARAIGDETFRLDRTEPTLNTAIPGDP  180

Query  181  RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240
            RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS
Sbjct  181  RDTTTPRAMAQTLRQLTLGHALGETQRAQLVTWLKGNTTGAASIRAGLPTSWTVGDKTGS  240

Query  241  GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGL  291
            GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGL
Sbjct  241  GDYGTTNDIAVIWPQGRAPLVLVTYFTQPQQNAESRRDVLASAARIIAEGL  291
```

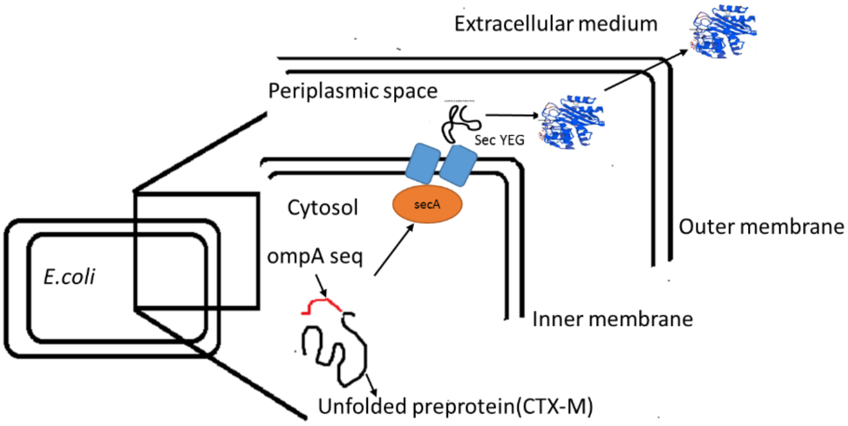
(A) Forward protein Translation of ***triple reversion of CTXM 14-LIKe*** gene using Expasy tool**.** The three elements needed for protein overexpression (OmpA lead, inserted gene and strep tag are highlighted **(B)** Protein alignments created Using Clustal Omega tool. Protein Blast output : class A extended-spectrum beta-lactamase ***CTX-M-14*** [*Enterobacterales*] Sequence ID : WP_001617865.1

Length: 291 .As indicated the three highlighted changes of ***CTX-M-14LIKE***: T55A, A273P and R277C were mutated back to ***CTX-M-14.***

5.2.5 Producing Strep-tag fusion proteins for CTX-M-14, CTX-M-14-LIKE and CTX-M-15

The production of the Strep-Tag fusion proteins for CTX-M-14, CTX-M-14-LIKE and CTX-M-15 was performed in three main steps: growth of host strains harbouring the expression plasmid using the three CTX-Ms, gene expression of the Strep-Tag fusion proteins induced by the addition of 10 µL of an anhydrotetracycline solution (aTe;2 mg/mL in dimethylformamide, DMF) to logarithmically growing cells (Section 2.2.14.2) and protein extraction (Sections 2.2.14.1 and 2.2.14.2). Upon induction, the protein produced in the cytoplasm of the host cell was exported to the periplasmic space by the OmpA leader sequence (Figure 5.8), which was removed while the protein was transported through the inner membrane (Plfickthun & Skerra, 1989).

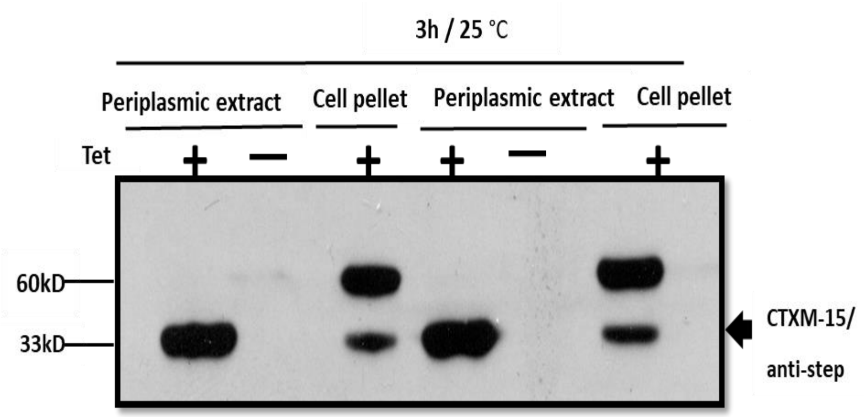
Figure 5.8 Periplasmic Expression of recombinant CTX-M enzymes



Periplasmic secretion strategy for production of recombinant CTX-M proteins in *E.coli.* The exportation of the produced unfolded recombinant protein to the periplasmic space is led by OmpA leader sequence.

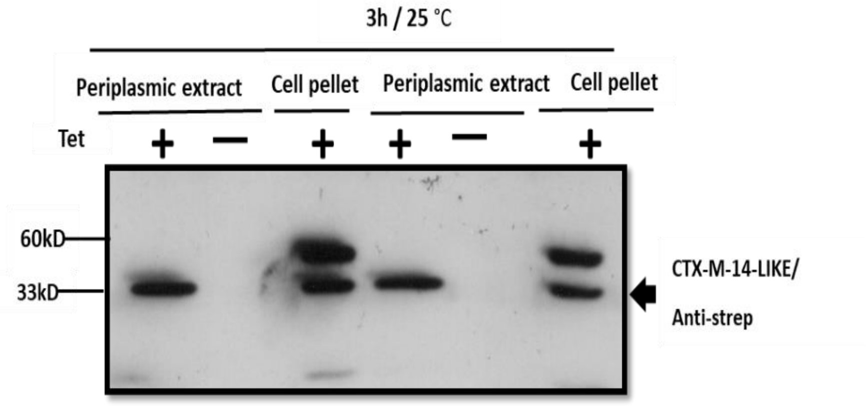
The expression level of the extracted protein was verified using the Western blot technique (Section 2.2.15). Figures 5.9, 5.10, and 5.11 show the Western blot analysis of the extracted periplasmic CTX-M proteins; CTX-M-15, CTX-M-14-LIKE and CTX-M-14, the supernatant of centrifuged, which had bands with sizes corresponding with the expected sizes of the CTX-M proteins (33 KD), induced and uninduced strains, as well as the remaining cell pellet. The cell pellet consisted of the recombinant proteins left in the cytoplasm to check the efficiency of the export from the cytoplasm to the periplasmic space. Retention in the cytoplasm is frequently caused by the formation of inclusion bodies made up of mis-folded, recombinant protein.

Figure 5.9 Western blot analysis of Induced strep-tagged recombinant *CTX-M-15*



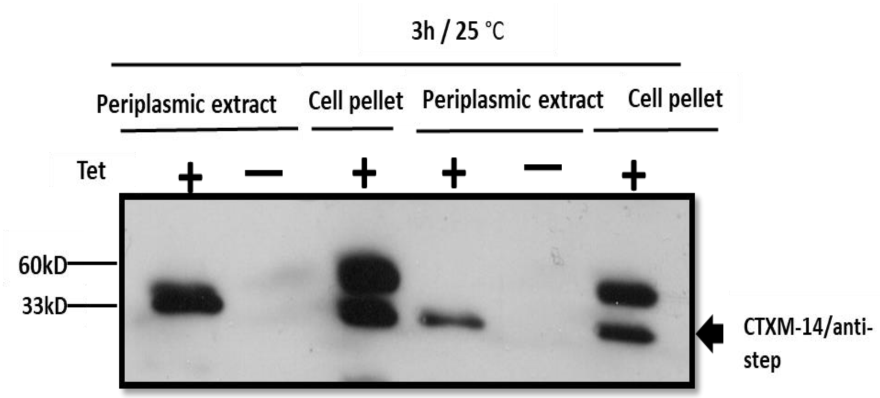
Western blot analysis after 3h induction at 25°C with anhydrotetracycline of strep-tagged recombinant CTX-M-15.(+) = induced strains with anhydrotetracycline; (-) = Uninduced strains (negative control). Tested induced strains cell pellet (i.e., Cytoplasmic produced recombinant protein), show the bands' sizes corresponding with the expected sizes of the CTX-M proteins (33 KD), which indicates that some of protein hasn't been exported despite the high yielded of recombinant CTX-M extracted from the periplasmic space, in addition to a band at approximately 60 KD, could be dimeric 60KD Which is insoluble and therefore in the pellet.

Figure 5.10 Western blot analysis of Induced strep-tagged recombinant *CTX-M-14LIKE*



Western blot analysis after 3h induction at 25°C with anhydrotetracycline of strep-tagged recombinan.t *CTX-M-14LIKE*. (+) = induced strains with anhydrotetracycline; (-) = Uninduced strains (negative control). Tested induced strains cell pellet (i.e., Cytoplasmic produced recombinant protein), show the bands’ sizes corresponding with the expected sizes of the CTX-M proteins (33 KD), which indicates that some of protein hasn’t been exported despite the high yielded of recombinant CTX-M extracted from the periplasmic space, in addition to a band at approximately 60 KD, could be dimeric 60KD Which is insoluble and therefore in the pellet.

Figure 5.11 Western blot analysis of Induced strep-tagged recombinant *CTX-M-14*



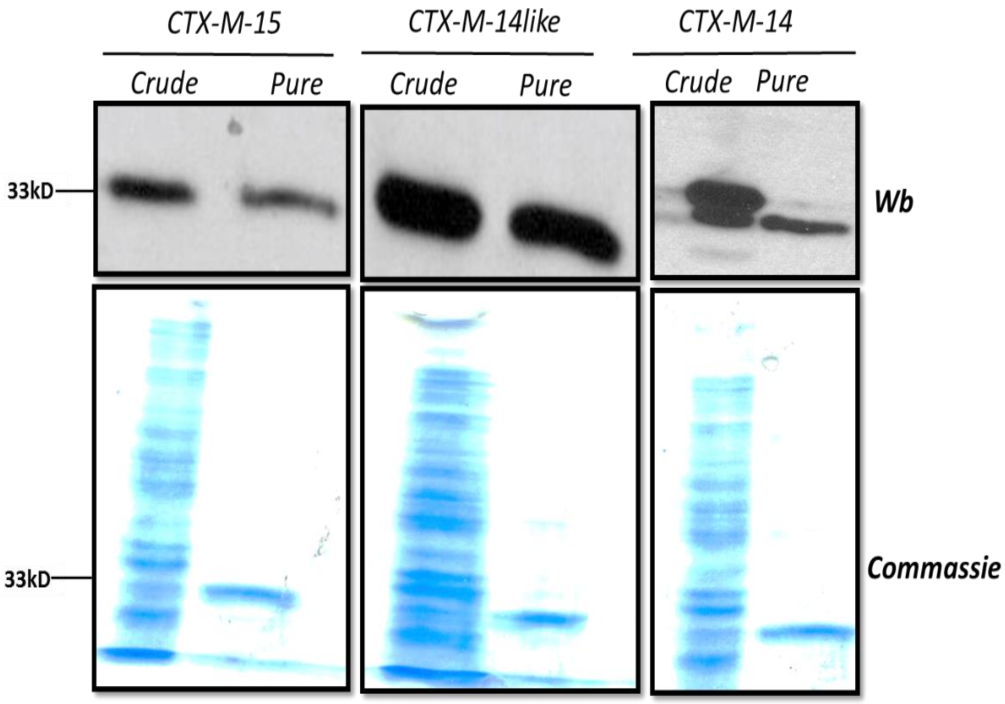
Western blot analysis after 3h induction at 25°C with anhydrotetracycline of strep-tagged recombinan.t *CTX-M-14.* (+) = induced strains with anhydrotetracycline; (-) = Uninduced strains (negative control).Tested induced strains cell pellet (i.e., Cytoplasmic produced recombinant protein), show the bands' sizes corresponding with the expected sizes of the CTX-M proteins (33 KD), which indicates that some of protein hasn't been exported despite the high yielded of recombinant CTX-M extracted from the periplasmic space, in addition to a band at approximately 60 KD, could be dimeric 60KD which is insoluble and therefore in the pellet.

5.2.6 Protein purification using Strep-Tactin®XT followed by protein concentration using VIVASPIN® 500

The recombinant protein was C-terminally tagged with the short peptide Strep-Tag II®, and has a high selectivity to Strep-Tactin®. The tagged recombinant CTX-M proteins with a Strep-tag can be purified from the crude extract using the binding affinity between the Strep-Tag II® and Strep-Tactin® included in prepacked chromatography columns, which allows for the gravity-flow purification of the Strep-tagged fusion proteins (Section 2.2.16). The purified CTX-M proteins were then concentrated using VIVASPIN® 500 to increase the concentration of the protein yield.

To check the purity of the CTX-M-14, CTX-M-14-like and CTX-M-15 proteins, the three enzymes were analysed using Coomassie stains, which are a family of dyes commonly used to stain proteins in SDS-PAGE gels. The gels were soaked in dye, and the excess stain was eluted with a solvent. This treatment allows the proteins to be visualised as blue bands on a clear background. Figure 5.14 shows the verification of the three purified CTX-M proteins using Coomassie stains by comparing the crude extract with the eluate of the affinity columns (purified proteins).

Figure 5.12 Checking the purity of the three CTX-Ms using Coomassie stains



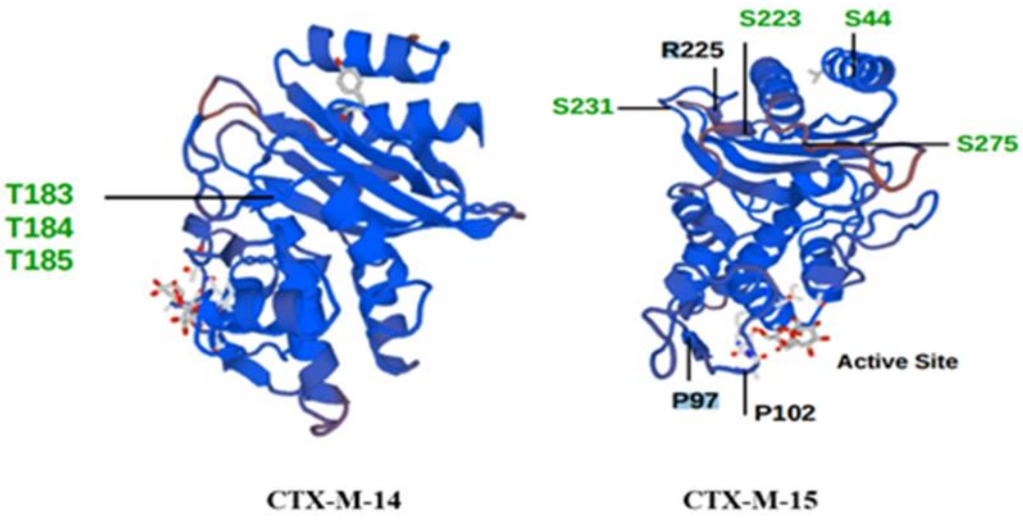
Coomassie stain analysis of the purified CTX-M proteins. The expression level of the three produced proteins were verified with Western blot analysis followed by purity verification Using Coomassie stains by comparing the crude extract with the eluate of the affinity columns (purified proteins).

5.2.7 Biochemical characterisation of the CTX-M proteins

One important biochemical characterisation that should be taken into account when studying protein properties is the protein phosphorylation status, which is responsible for a variety of important protein functions, including subcellular localisation, protein degradation and stabilisation and biochemical activities (Manning Getal, 2002). This study sought to determine how dephosphorylating affects the activity of CTX-M enzymes against commonly used antibiotics.

NetPhos predicts the serine, threonine and tyrosine phosphorylation sites of proteins. It showed that CTX-M-14 and CTX-M-15 possess both some potential phosphorylation sites. Figure 5.15 shows the three-dimensional structures of the two CTX-Ms as well as the predicted phosphorylated amino acids.

Figure 5.13 Three-dimensional CTX-M-14 and CTX-M-15 structures



Three-dimensional structures of CTX-M-15 and CTX-M-14. The green highlighted amino acids indicate the expected phosphorylation sites using NetPhos. The active site is indicated with a beta-lactam antibiotic. The structure was generated using the SWISS-MODEL (99.62% identity).

The phosphorylation sites of the CTX-M proteins were analysed using two techniques: isoelectric gel electrophoresis and Phos-tag gel analysis.

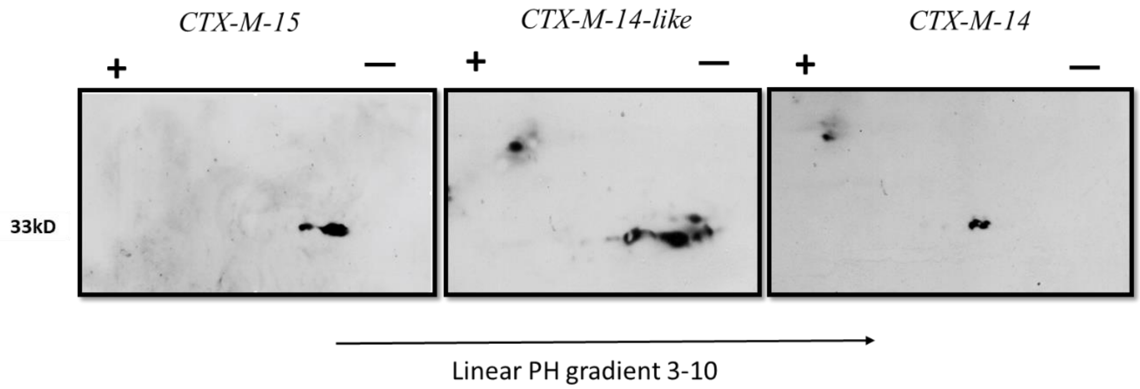
5.2.7.1 Isoelectric-focused analysis of the CTX-M-14, CTX-M-14-LIKE and CTX-M-15 proteins

The three CTX-M proteins were analysed in two-dimensional gels in order to check for posttranslational modifications that either add or remove protein charges (e.g. phosphorylation, acetylation) and to find out how homogenous the purified CTX-M protein samples are. This was achieved by separating the protein first based on its isoelectric point (pI) and then by molecular weight using normal SDS-PAGE (Section 2.2.19.1). Figure 5.16 shows the Western blot analysis of the distributed proteins in the two-dimensional gel. At least two isoforms with the expected MW of 33kD were detected in the three protein extracts. This indicates phosphorylation or another charge changing post-translational modification.

5.2.7.2 Phos-tag gel analysis of the CTX-M-14, CTX-M-14-LIKE and CTX-M-15 proteins

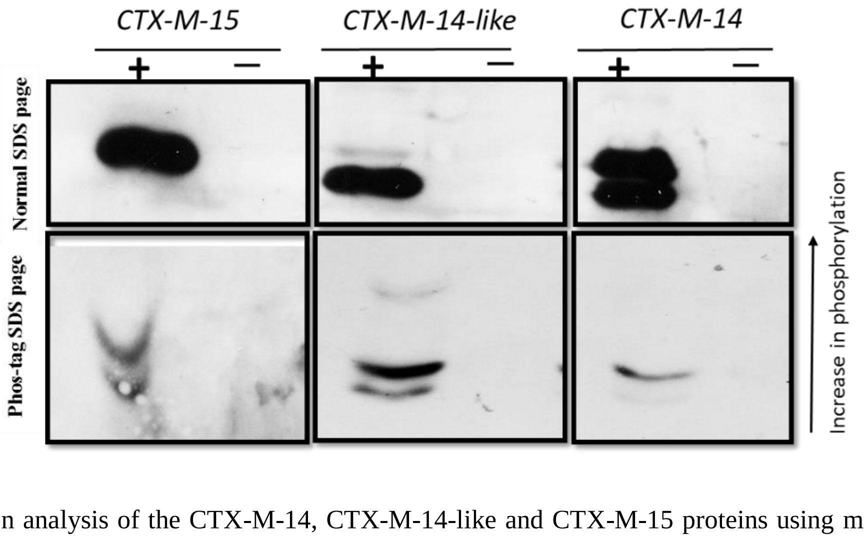
Manganese(II)-Phos-tag SDS-PAGE (Table 2.20) was used to verify whether the observed changes in the isoelectric values are due to phosphorylation as the Phos-tag reagent reacts specifically with phosphorylared protein and slows down their mobility on SDS page (Horinouchi et al,2016) (Section 2.2.19.2). Phos-tag can bind the phosphate groups of the phosphorylated proteins, which delays their electrophoretic mobility so that only phosphorylated proteins can be seen in the Phos-tag gel. As shown in Figure 5-17, at least two bands were observed for all three CTX-M proteins in the presence of Phos-tag. While the band intensity was comparable for CTX-M-15, the slower migration, phosphorylated band was more intense in the case of CTX-M-14 and CTX-M-14-LIKE. Especially, the intensity of the faster migrating band of CTX-M-14-like was very low. CTX-M-14-like displayed a third, strongly retarded band that might indicate a higher phosphorylation level. It was however interesting to observe that even in the absence of the Phostag reagent CTX-M-14-like showed a slower migrating band. The latter finding is not inconsistent with phosphorylation as some phosphorylated proteins migrate slower in normal SDS page (Caspari T, et al, 200).

Figure 5.14 Two-dimensional gel analysis of the CTX-M-14, CTX-M-14-LIKE and CTX-M-15 proteins



Two-dimensional gel analysis showed that the 33 kDa protein exists in two forms (CTX-M-14 and CTX-M-15) and four forms (CTX-M-14-like) with different pI, indicating phosphorylation. CTX-M-14 and CTX-M-14-like exist in heavier molecular weight forms at a higher acidity (90 kDa).

Figure 5.15 Phos-tag gel analysis of the Strep-tagged recombinant CTX-M-15, CTX-M-14-like and CTX-M-14 proteins



Phosphorylation analysis of the CTX-M-14, CTX-M-14-like and CTX-M-15 proteins using manganese (II)-Phos-tag SDS-PAGE. At least one shift band can be seen in the Phos-tag gel analysis of the three CTX-M proteins, which indicates phosphorylation.

5.3 Discussion

5.3.1 Possibility of the *pASK-IBA2C* plasmid's self-ligation with the BsaI restriction enzyme

The BsaI restriction enzyme has restriction sites located at multiple cloning sites of the plasmid and should be selected based on its ability to generate asymmetric cuts with long sticky ends (GGTCTCN1 on the forward strand and CCAGAGN5 on the reverse strand). It should therefore be unable to re-ligate as BsaI cuts five nucleotides away from the recognition site. In addition, these sequences are different at either site of the two BsaI cuts in the plasmid, meaning that only the cloned fragments should provide colonies after the transformation of the pASK-IBA2C plasmid carrying the *CTX-M* genes in *E. coli* strains (Schmidt & Skerra, 2015).

5.3.2 Periplasmic expression as a suitable strategy for producing CTX-M proteins

There are two strategies for the secretion of proteins from cloned genes in the *pASK-IBA2C* plasmid: cytosolic expression and periplasmic expression. Periplasmic expression was selected in the production of CTX-M proteins due to its advantages. First, it is able to separate the produced recombinant proteins from host cell proteases, which are mostly resident in the bacterial cytosol. Second, it is easy to extract molecules from the periplasmic space by osmotic shock which results in an already highly enriched protein extract (Van Den Berg et al., 2015). Third, unlike cytosolic secretion, periplasmic expression allows for efficient disulphide bond formation, which prevents the aggregation or degradation of unfolded polypeptides (Gąciarz et al., 2017). However, the aggregate formation in the periplasmic space and the production of non-secretory proteins are considered disadvantages of periplasmic expression (Baumgarten., 2018).

5.3.3 Efficiency of the OmpA sequence in exporting proteins to the periplasmic space

Despite the high yield of recombinant proteins produced in the *E. coli* expression system, they are mainly expressed as insoluble and inactive inclusion bodies, which must be refolded (Costa et al., 2014). This problem has been overcome by two main strategies. The first is the use of fusion tags, which enables the purification of the tagged protein based on an affinity system. The second involves exporting the recombinant proteins to the periplasmic space to enhance their solubility and function by fusing the N-terminus of the protein to a signal peptide, which acts as the leader in exploring the heterologous protein from the cytoplasm into the periplasmic space (Rosano et al., 2014).

However, It was reported by some studies (e.g., Zhang et al., 2018) that the presence of a signal peptide does not guarantee the efficient exporting of the protein to the periplasmic space. This conclusion can be emphasised by the Western blot analysis conducted in this study on the induced strains cell pellet (i.e., Cytoplasmic produced recombinant protein), figures 5.10, 5.11 and 5.12,which show the bands' sizes corresponding with the expected sizes of the CTX-M proteins (33 KD), which indicates that some of protein hasn't been exported despite the high yielded of recombinant CTX-M extracted from the periplasmic space, in addition to a band at approximately 60 KD, could be dimeric 60KD which is insoluble and therefore in the pellet.

5.3.4 Analysis of phosphorylation statues

Phosphorylation status analysis of the purified CTX-M proteins using Phos-tag gel indicate phosphorylation or another post-translational modification of the three CTX-M proteins (CTX-M-15, CTX-M-14 and CTX-M-14-LIKE). This is supported by the detection of at least two isoforms with the expected size of CTX-M proteins (33KD) using western blot analysis of the distributed proteins in 2D gel. The 2D and Phos-tag data support the conclusion that all the CTX-M proteins are phosphorylated when recombinant expressed in *E.coli*. CTX-M-14-LIKE might even be hyper-phosphorylated. However, phosphorylation status of these protein could be a consequence of the overexpression in *E. coli*, which could be further investigated by testing the same proteins from the parental strains under endogenous conditions followed by protein sequencing.

Determination of any marked differences in MIC (minimum inhibitory concentration) and the kinetics of hydrolysis between the purified, recombinant CTX-M proteins with antibiotics commonly used to treat UTI patients is detailed in Chapter 6.

.

Chapter 6 : ANTIMICROBIAL SUSCEPTIBILITY OF DOMINANT CTX-M PRODUCING BACTERIA IN NORTH WALES

6.1 Introduction

Antimicrobial susceptibility testing (AST) is a critical procedure that aims to identify the antimicrobial agents that are effective against a particular infectious disease (Lagier et al, 2015). National programs for control and prevention of infectious diseases, as well as hospitals and clinics, rely on the information provided through AST to maintain effective treatment regimens (Bayot & Bragg, 2019). AST aims to determine the lowest concentration of antibiotic required to inhibit visible growth of bacteria over a defined time period, which is known as the minimum inhibitory concentration (MIC)(Lowman, 2018).

The antibiotic concentrations associated with the classification of bacteria as sensitive (S), intermediate (I), and resistant (R) are defined based on these the AST results (Ersoy et al, 2017). The minimum inhibitory concentration (MIC) breakpoints (S, I, R) have been defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). A classification of sensitive (S) indicates that the infection should be treated using the recommended antimicrobial dosage for the species and site of infection. A classification of intermediate (I) indicates that the normal dosing of the antimicrobial agent should be increased to achieve a clinical response. A classification of resistant (R) indicates that the microorganism cannot be inhibited with normal dosing of the antimicrobial agent (EUCAST, 2018). These definitions are adopted in this study.

There are many techniques for determining the MIC values of antimicrobial drugs, but the most commonly used method is the broth dilution test. In this test a standardised bacterial suspension (1.5×10^5 CFU)[5] is inoculated in tubes with two-fold dilutions of antibiotic (e.g., 1, 2, 4, 8 µg/mL) followed by incubation at 35°C for 24 hours (Schumacher et al, 2018). The lowest concentration that prevents bacterial growth (MIC) is determined by examining the turbidity of the antibiotic suspension (Jorgensen & Ferraro, 2009). Despite the method's popularity there are considerable short-comings to this procedure, including the excessive amount of reagents and space required, and the high potential for errors to occur during the preparation of antibiotic solutions (Rennie et al, 2012). The latter challenge has been overcome by replacing the broth

---

[5] Colony Forming Unit (CFU) unit used to estimate the number of viable bacteria (bacteria which is able to multiply via fission under the controlled conditions) in a sample.

dilution procedure with the antimicrobial gradient diffusion method in which an antimicrobial concentration gradient is established to test susceptibility of cultured isolates on an agar medium known as Epsilometer test or E-test (Sader & Pignatari, 1994).

The E-test procedure consists of a plastic strip that carries a concentration gradient of dried and stabilised antimicrobial drugs (Leigue et al, 2016). The MIC is determination by placing the graduated antibiotic strips on inoculated agar medium with adjusted bacterial suspension followed by incubation. Due to an immediate release of antibiotics from the E-test strip to the agar surface during the incubation a symmetrical inhibition ellipse is formed along the strip and the MIC value in µg/mL is read from where the ellipse edge intersects the strip (Khan et al, 2019). This procedure is used for antibiotic susceptibly testing in this study. The results of antibiotic susceptibility testing in this study was further confirmed by the determination of the kinetic parameters of enzyme produced by the tested isolates (β-lactamase enzymes), which can give addition information about the interaction between the antimicrobial agents and enzyme-based resistances conferred by some bacterial species (Zygmunt et al, 1992).

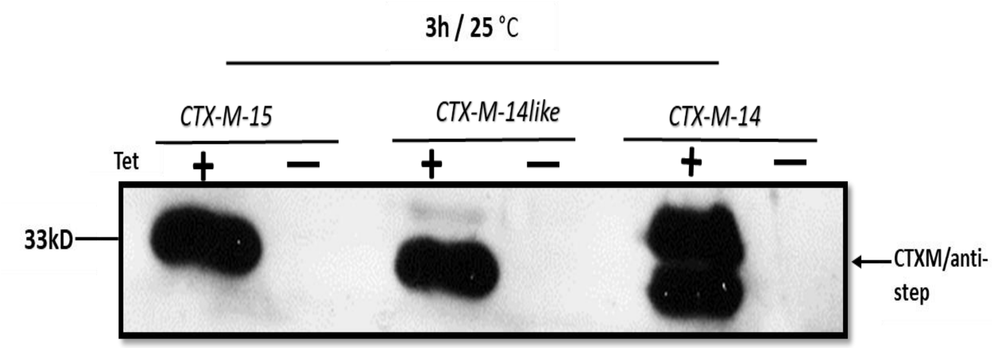
The main objective of this part of the study is to determine any marked differences in MIC and the kinetics of hydrolysis between CTX-M-14 and CTX-M-14-LIKE with antibiotics commonly used to treat UTI patients, i.e. to determine whether the three detected mutations (T55A, A273P, and R277C) change the substrate specificity of CTX-M-14 β-lactamase in addition to the characterisation of CTX-M 15. The latter enzyme has been identified as a dominant CTX-M β-lactamase in North Wales that may in part explain why some UTIs fail to respond to β-lactam antibiotics. To achieve this aim, three recombinant *CTX-Ms*, (*CTXM-15, CTXM-14* and *CTX-M-14 LIKE*) were recombinantly expressed in *E.coli* and purified (sections 5.2.5 & 5.2.6) before for their antibiotic susceptibility and enzymatic activity were tested using the E-test method and in vitro assays, respectively.

6.2 Results

6.2.1 Overexpression of step-tagged recombinant *CTX-M* genes.

Freshly cultured chemically competent *E. coli* carrying the plasmid *pASK-IBA2-CTX-M* were induced with a 10µL of anhydrotetracycline solution (2mg/mL dimethylformamide, DMF) at a cell density of 0.5 measured at 550nm (OD550), and incubated with shaking for 3 hr at 25°C. The induced bacteria were then plated on MHB medium with chloramphenicol and E-test strips of five commonly used antibiotics were applied to the plates. The expression level of the three proteins, CTXM-15, CTXM-14 and CTX-M14 LIKE was verified using the Western blot technique (Figure 6.1).

Figure 6.1 Expression level verification of CTX-M proteins using western blot.



Western blot analysis after 3h induction at 25°C with 10µl of anhydrotetracycline of strep-tagged recombinant *CTX-M-15, CTX-M-14like and CTX-M-14*. Periplasmic extraction of the three proteins can be seen in the expected size of CTX-M protein (33KD).

6.2.2 Determination of the minimum inhibitory concentration (MIC) of induced strep-tagged recombinant CTX-M genes using E-test

The minimum inhibitory concentrations (MICs) of *E.coli* carrying *pASK-IBA2-CTX-M* were measured for five β-lactams commonly used as a first line treatment for UTIs (Imipenem [IP], Cefoxitin [FX], Nitrofurantoin [NI], Cefotazidime [TZ], and Cefotaxime [CT]) using the E-test protocol (Section 2.2.20) following the MIC break points guidelines of European Committee on Antimicrobial Susceptibility Testing (EUCAST). MIC detection was repeated in biological triplicates for each antibiotic.

6.2.2.1 MIC determination of Imipenem with CTXM-15, CTXM-14 and CTX-M-14-LIKE

An E-test strip with the Imipenem antibiotic was positioned on Mueller Hinton Agar (MHB) with chloramphenicol agar inoculated with suspended (in MHB medium with chloramphenicol) induced or uninduced *E.coli* carrying *pASK-IBA2-CTX-M-15, CTXM-14* and *CTXM-14-LIK* with 10 µL anhydrotetracycline solution followed by incubation at 37°C for 18-24 hrs. The approximate number of the suspended bacteria was standardized before the inoculation by McFarland Standards in which the inoculation suspension was diluted 1:1000 and then compared and adjusted with the turbidity of 0.5 McFarland standard, which gives approximately 1.5×10^8 bacterial cells per mL.

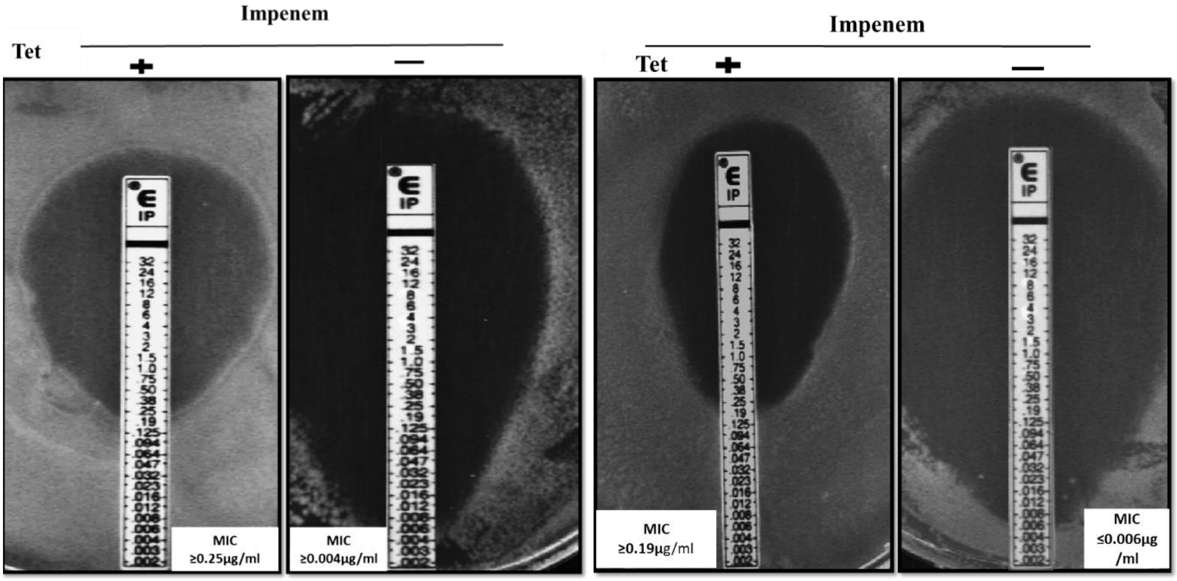
As it is shown in Figure 6.2, the MIC of Imipenem with the induced strains carrying *CTXM-15, CTX-M-14,* and *CTXM-14-like* are ≥0.25 µg/mL (intermediate), ≥0.19 µg/mL (susceptible), and 0.064 µg/mL (susceptible), respectively. Test for the uninduced strains show MIC < 0.003 µg/mL with Imipenem.

6.2.2.2 MIC determination of Cefoxitin with CTX-M-15, CTX-M-14 and CTX-M-14LIKE

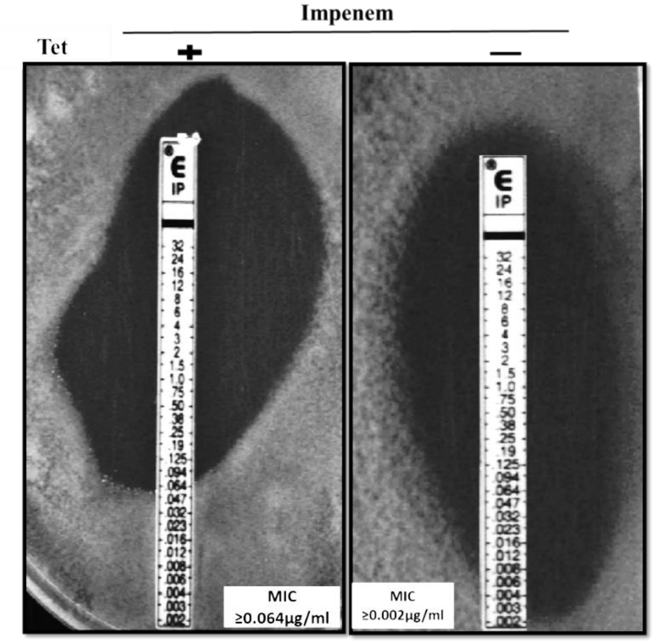
Induced *E. coli* strains carrying *pASK-IBA2-CTX-M-15*, *CTXM-14,* and *CTXM-14-LIK* with 10µl an anhydrotetracycline solution (Section 6.2) was inoculated on Mueller Hinton Agar (MHB) with an E-strep strip containing the Cefoxitin antibiotic after standardising using the 0.5 McFarland standard (1.5×10^8 approximate bacterial cells per mL) followed by incubation at 37°C for 18-24 hrs.

The highest MIC of Cefoxitin was observed with strains carrying pASK-IBA2- CTXM-14-lik (3 µg/ml) (intermediate resistance), while the MIC value of the strains carrying *pASK-IBA2-CTX-M-15 or pASK-IBA2-CTXM-14* with Cefoxitin 2 µg/mL and 1 µg/mL respectively indicated susceptibility (Figure 6.3).

Figure 6.2 MIC of Imipenem against *E. coli* carrying pASK-IBA2-CTX-M.



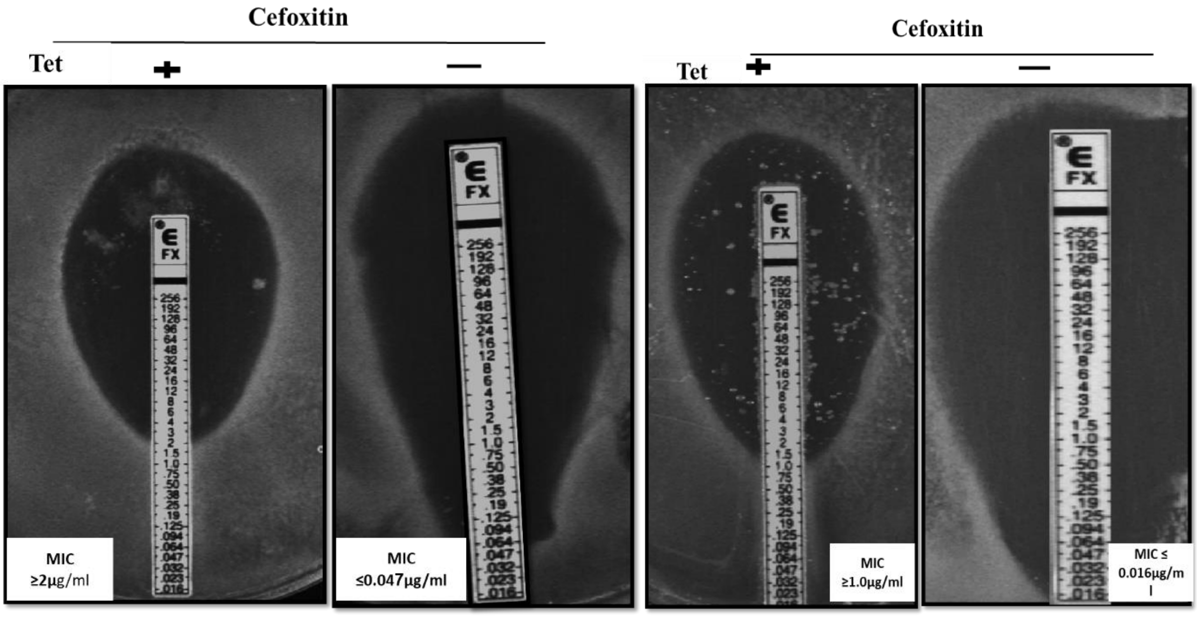
CTXM-15 ***CTXM-14***



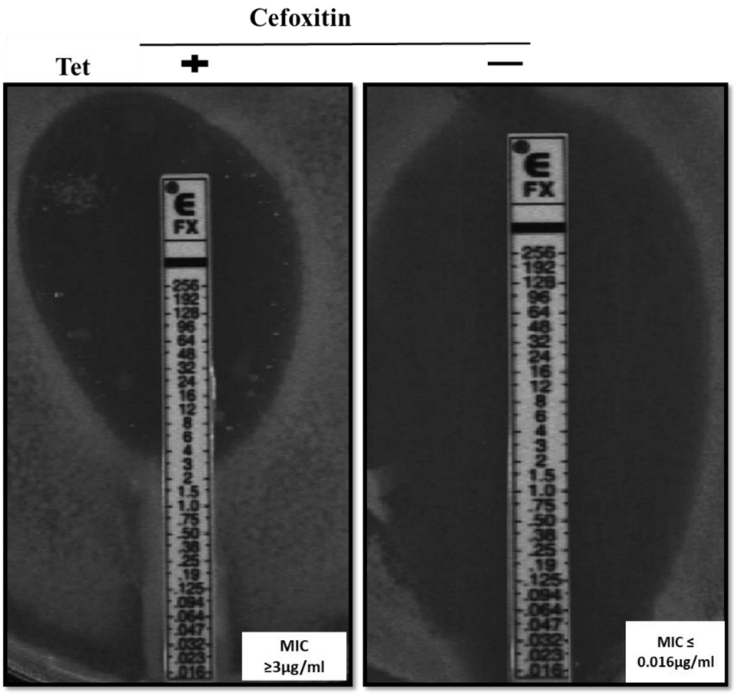
CTXM-14-LIKE

MIC of Imipenem against *E. coli* strains carrying CTX-M genes (CTXM-15, CTX-M-14 and CTX-M-14-LIKE), measured using E-test strips. The highest MIC of Imipenem can be seen with CTX-M-15 (≥0.25 µg/mL) which is considered a value of resistance based on EUCAST guideline followed by CTX-M-14(≥0.19 µg/mL) and CTX-M-14LIKE (≥0.064 µg/mL) which are corresponding with susceptible range. (+) = Induced strain with anhydrotetracycline, **(-)** = uninduced strain.

Figure 6.3 MIC of Cefoxitin against *E. coli* carrying pASK-IBA2-CTX-M.



CTX-M-15 ***CTX-M-14***



CTX-M-14-LIE

MIC of Cefoxitin against *E. coli* strains carrying CTX-M genes (CTX-M-15, CTX-M-14 and CTX-M-14-LIKE) measured using E-test strips. Cefoxitin has the highest MIC with CTX-M-14-like (≥3 µg/mL) followed by CTX-M-15 (≥2 µg/mL) and CTX-M-14(≥1 µg/mL). (+) = Induced strain with anhydrotetracycline, (-) = uninduced strain (negative control).

6.2.2.3 MIC determination of Nitrofurantoin with CTX-M-15, CTX-M-14, and CTX-M-14-LIKE

The MIC of Nitrofurantoin for induced strains carrying *pASK-IBA2-CTX-M15, CTX-M-14* or *CTX-M-14LIKE* was determined by placing a Nitrofurantoin E-test strip on Mueller Hinton Agar (MHB) cultured with the induced or uninduced strains (Section 6.2) and incubated at 37°C for 24 hrs. The suspended strains were adjusted by dilution to 0.5 McFarland standard (1.5×10^8 approximate bacterial cells per mL) before culturing. MIC of an uninduced strain was determined using the same conditions.

The MIC values of Nitrofurantoin with the three tested strains are illustrated in Figure 6.4. This shows that the highest MIC of Nitrofurantoin is with the strain carrying *pASK-IBA2-CTX-M-14LIKE* (≥512 µg/mL), which is categorised as a value of Nitrofurantoin resistance (> 64 µg/ml), while the strains expressing CTX-M-15 and CTX-M-14 show a susceptibility against Nitrofurantoin (≤ 64 µl/mL); ≥4 µg/mL and ≥6 µg/mL, respectively.

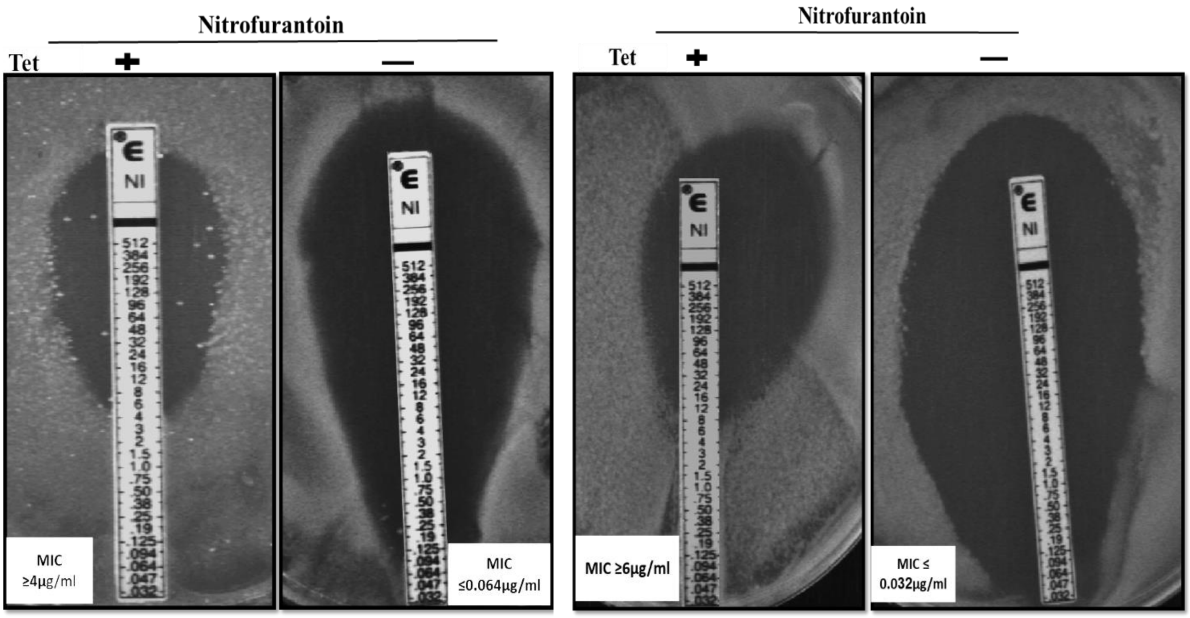
6.2.2.4 Determination of Ceftazidime MIC with CTX-M-15, CTX-M-14, and CTX-M-14 like

In order to determine MIC of Ceftazidime with strains expressing CTX-M14, CTX-M-14LIKE or CTX-M-15 genes, the strains were induced using 10µl anhydrotetracycline (section 6.2) before they were adjusted with 0.5 McFarland standard (1.5×10^8 approximate bacterial suspension per mL) and cultured on Mueller Hinton with chloramphenicol Agar with Ceftazidime E-test strips. These were then incubated 37°C for 24 hrs. Figure 6.4 shows that the three strains are susceptible to Ceftazidime (≤1 µg/mL); ≤1 µg/mL, ≤1µg/mL, and ≤0.75 µg/mL respectively.

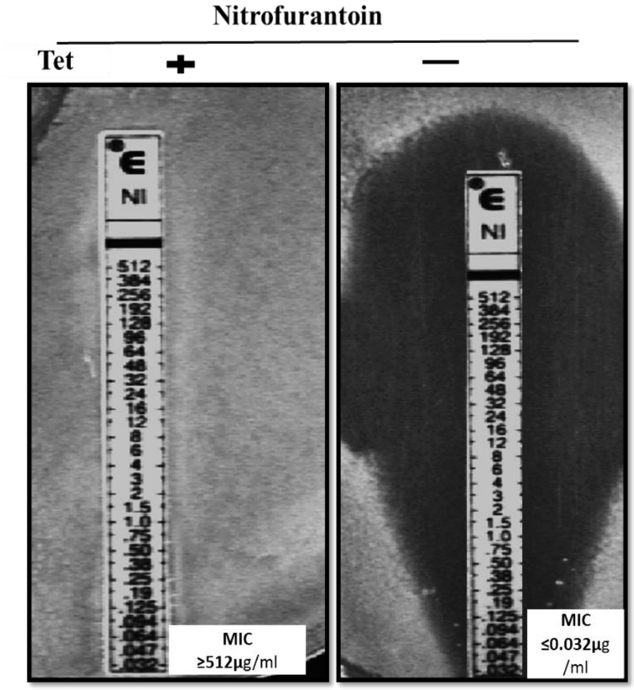
6.2.2.5 Cefotaxime MIC Determination with CTX-M-15, CTX-M-14, and CTX-M-14 like

Cefotaxime E-test strips were used to determine the MIC of Cefotaxime with strains carrying *pASK-IBA2-CTX-M-15, CTX-M-14* or *CTX-M-14LIKE.* This was conducted in three steps: (1) induction of strains by anhydrotetracycline (section 6.3) adjusting the suspended strains with 0.5 McFarland standard (1.5×10^8 approximate bacterial cell suspension per mL); (2) culturing the induced strains on Mueller Hinton with chloramphenicol Agar with E-test strips of Ceftazidime; and (3) incubation at 37°C for 24 hrs. The strains carrying pASK-IBA2-CTX-M-15 are the only strains to have a value of MIC corresponded to a Cefotaxime resistance (32 µg/mL), while the MIC of cells harbouring CTX-M-14 (1.5 µg/mL) or CTXM-14-LIKE (0.5 µg/mL) indicate a susceptibility of these strains to Cefotaxime (≤1 µg/mL).

Figure 6.4 MIC of Nitrofurantoin against *E. coli* carrying pASK-IBA2-CTX-M.



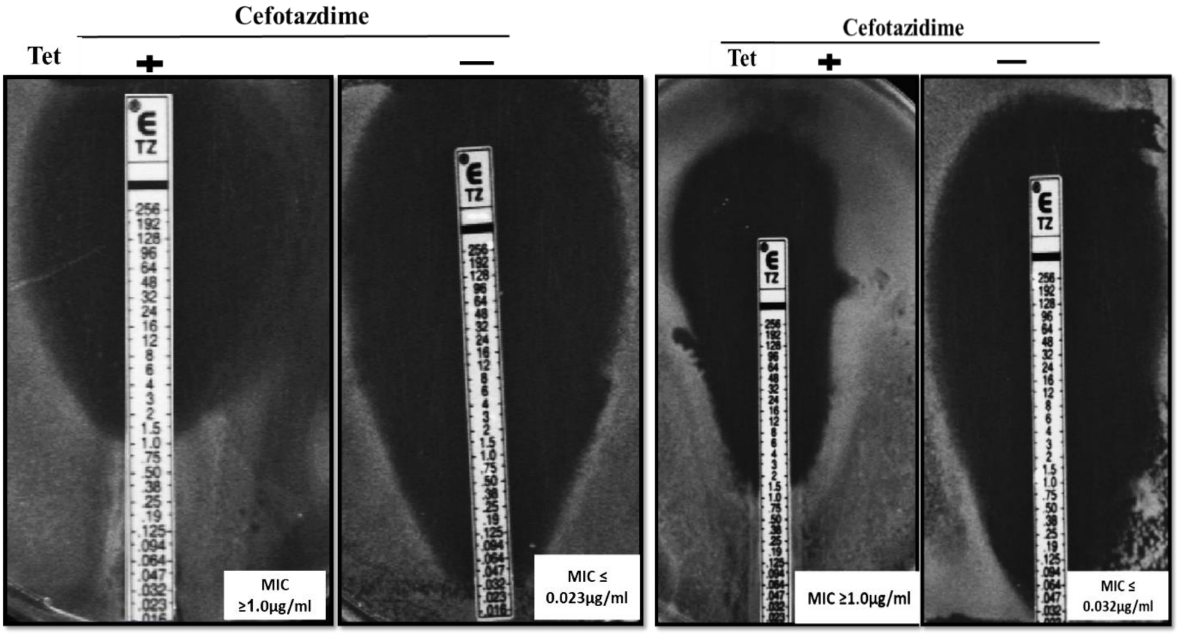
CTX-M-15 ***CTX-M-14***



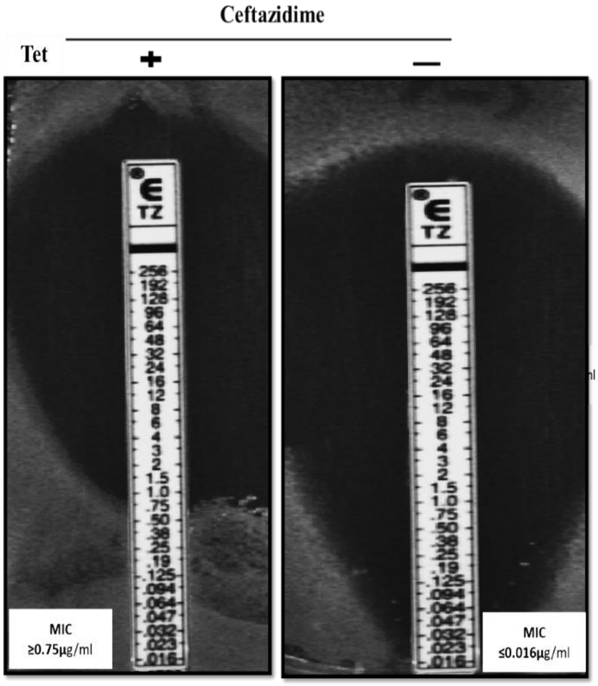
CTX-M-14-LIKE

MIC of Nitrofurantoin against *E. coli* strains carrying CTX-M genes (CTXM-15, CTX-M-14 and CTX-M-14-LIKE), measured using E-test strips. Noticeably, strains carrying *CTX-M-14-LIKE* show high MIC for Nitrofurantoin (≥ 512 µg/mL) in comparison with the MIC for Nitrofurantoin shown in strains carrying CTX-M-15 (≥ 4 µg/mL) and CTX-M-14 (≥ 6 µg/mL). (+) = induced strains with anhydrotetracycline; (-) = Uninduced strains (negative control).

Figure 6.5 MIC of Ceftazidime against *E. coli* carrying pASK-IBA2-CTX-M.



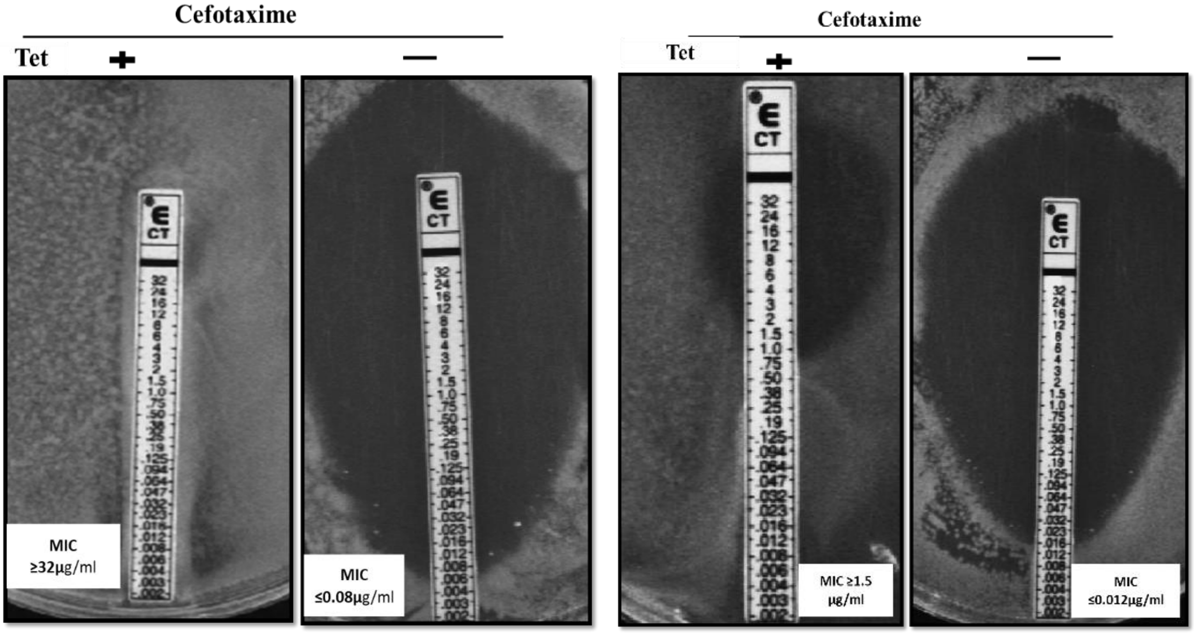
CTX-M-15 ***CTX-M-14***



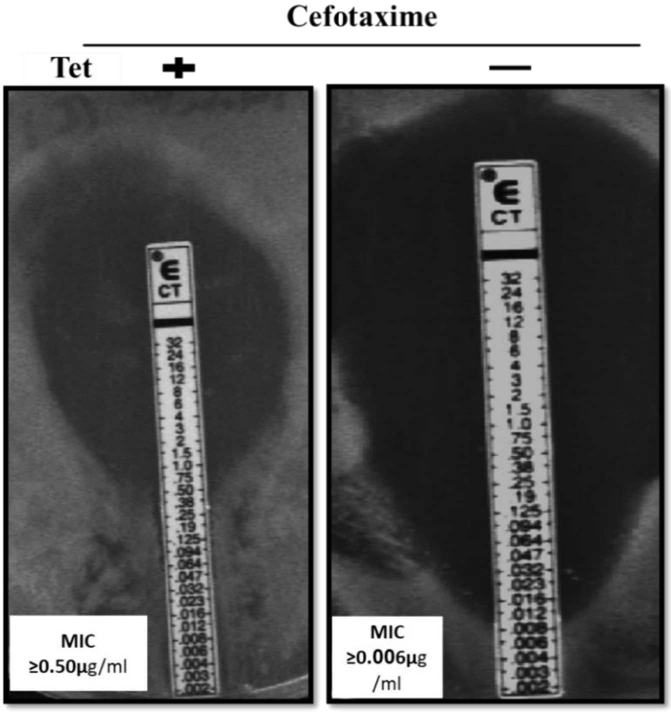
CTX-M-14-LIKE

MIC of Ceftazidime against *E.coli* strains carrying CTX-M genes (CTX-M-15, CTX-M-14 and CTX-M-14-LIKE), measured using E-test strips. The MIC value for the three proteins is approximately the same (0.75-1.0 µg/mL). (+) = induced strain with anhydrotetracycline; (-) = negative control (uninduced strain)

Figure 6.6 MIC of Cefotaxime against *E.coli* carrying pASK-IBA2-CTX-M



CTX-M-15 ***CTX-M-14***



CTX-M-14-LIKE

MIC of Cefotaxime against *E. coli* strains carrying CTX-M genes (CTX-M-15, CTX-M-14 and CTX-M-14-LIKE), measured using E-test strips. Strains carrying *CTX-M-15* show high MIC for Cefotaxime (≥ 32 µg/mL) in comparison with strains carrying CTX-M-14 (≥1.5 µg/mL) and CTX-M-14-LIKE (≥ 0.5 µg/mL). (+) = induced strains with anhydrotetracycline; (-) = uninduced strains (negative control).

Comparison of the MIC breakpoints of all E-Test experiments (Table 6.1) shows that two selected antibiotics, Nitrofurantoin and Cefoxitin, have a higher MIC indicating an enhanced resistance with strains carrying *CTX-M-14-LIKE* than those carrying *CTX-M-14*. This indicates that the three amino acid changes (T55A, A273P, and R277C) in CTX-M-14-Like could enhance the recognition and/or turn-over of Nitrofurantoin and Cefoxitin.

Table 6.1 MICs of *E. coli* isolates harbouring *blaCTX-M, 15, 14 or 14-LIKE* towards selected antibiotics.

	MIC(µg/mL)									
	IP		**FX**		**NI**		**TZ**		**CT**	
***bla*CTX-M**	+	-	+	-	+	-	+	-	+	-
M-15	≥0.25	≤0.004	≥2	≤0.047	≥4	≤0.064	≥1.5	≤0.023	≥32	≤0.08
	≥0.19	≤0.004	≥1.5	≤0.047	≥4	≤0.064	≥1	≤0.023	≥32	≤0.06
	≥0.25	≤0.004	≥2	≤0.047	≥3	≤0.064	≥1	≤0.023	≥32	≤0.08
SD	*0.03*	*0*	*0.2*	*0*	*0.5*	*0*	*0.2*	*0*	*0*	*0.01*
M-14	≥0.19	≤0.006	**≥1**	≤0.016	**≥6**	≤0.032	≥1	≤0.047	≥1.5	≤0.012
	≥0.19	≤0.006	**≥1**	≤0.016	**≥8**	≤0.032	≥1	≤0.032	≥1.5	≤0.012
	≥0.19	≤0.006	**≥1**	≤0.016	**≥6**	≤0.032	≥1	≤0.032	≥1	≤0.012
SD	*0*	*0*	*0*	*0*	*1.15*	*0*	*0*	*0.008*	*0.2*	*0*
M-14-LIKE	≥0.064	≤0.002	**≥3**	≤0.016	**≥512**	≤0.032	≥0.75	≤0.016	≥0.75	≤0.06
	≥0.064	≤0.002	**≥3**	≤0.016	**≥512**	≤0.032	≥0.75	≤0.016	≥0.75	≤0.06
	≥0.064	≤0.002	**≥3**	≤0.016	**≥512**	≤0.047	≥1	≤0.016	≥0.75	≤0.06
SD	*0*	*0*	*0*	*0*	*0*	*0.008*	*0.14*	*0*	*0*	*0*

MIC breakpoints of selected antibiotics with *E. coli* strains carrying CTX-M-15, CTX-M-14 and CTX-M-14-LIKE; (+) = induced strains; (-) = uninduced strains; IP = Imipenem; FX = Cefoxitin; NI = Nitrofurantoin; TZ = Ceftazidime; CT = Cefotaxime; SD = standard deviation. It is noticeable that two antibiotics out of the selected antibiotics show higher MIC with CTX-M-14-LIKE carrying strains than those with strains harbouring CTX-M-14; Nitrofurantoin (≥ 512 µg/mL with CTX-M-14-LIKE and 6 µg/mL with CTX-M-14; significantly higher) and Cefoxitin (3 µg/mL and 1 µg/mL towards CTX-M-14-LIKE and CTX-M-14; not significantly higher). MIC detection was repeated in biological triplicates for each antibiotic.

The MIC values of Nitrofurantoin and Cefoxitin indicated by the E-test assay were subsequently confirmed by determining the affinity (Km) constants of these antibiotics with CTX-M-14 and CTX-M-14-LIKE in order to determine whether the three revealed changes (T55A, A273P, and R277C) could affect the CTX-M-14 enzyme specificity and render cells resistant to Nitrofurantoin and less susceptible to Cefoxitin.

6.2.3 Determination of kinetic constants

The kinetic parameters of the purified CTX-M-14 and CTX-M-14-LIKE proteins, in addition to CTX-M-15, were determined with antibiotics that shows higher MIC with CTX-M-14-LIKE than with CTX-M-14 (see section 2.2.21). These were Nitrofurantoin and Cefoxitin, plus Ceftazidime, which shows low MIC with the three CTX-Ms.

6.2.3.1 Km of CTX-M-14, CTX-M-14-LIKE, and CTX-M-15 towards Nitrofurantoin

The velocity of hydrolysis of Nitrofurantoin by CTX-M-15, CTX-M-14 or CTX-M-14-LIKE were measured by monitoring the changes of absorbance at $\lambda = 266$ nm in a Quartz cuvette in a spectrophotometer over 12 min (Tables: 6.2, 6.3, 6.4, 6.5, 6.6, and 6.7, respectively). The assay was set up such that the amount of the purified protein was not rate limiting and the linear velocities of antibiotic turnover could be monitored by the decline in absorbance, a measure for the hydrolysis of the tested antibiotic. Figures 6.7, 6.8 and 6.9 show the Km value of CTX-M-15, CTX-M-14, and CTX-M-14-LIKE respectively.

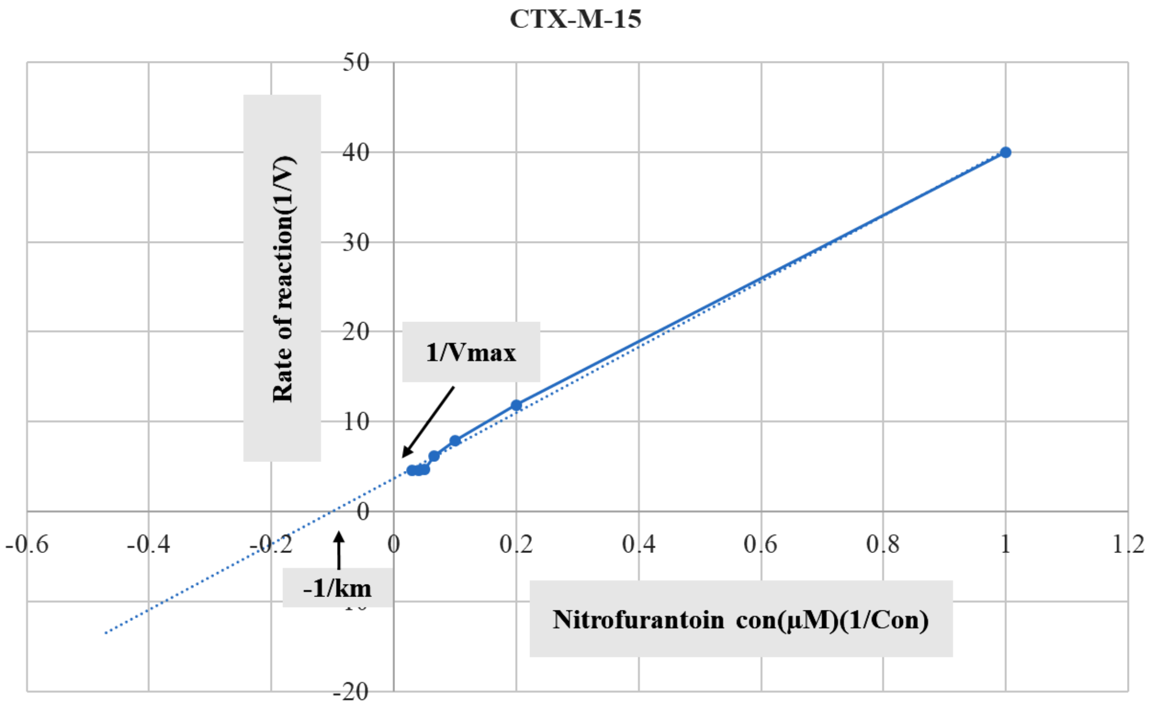
Table 6.2 OD of Nitrofurantoin + CTX-M-15 protein mixture

Time/min	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0.02	0.031	0.182	0.309	0.436	0.604	0.784	0.879
1	0.02	0.023	0.17	0.285	0.418	0.585	0.747	0.843
2	0.02	0.012	0.157	0.274	0.397	0.562	0.711	0.807
3	0.02	0	0.143	0.256	0.378	0.538	0.675	0.77
4	0.02	0	0.129	*0.238*	0.352	0.514	0.639	0.732
5	0.02	0	0.113	0.211	0.296	0.484	0.604	0.698
6	0.02	0	0.098	0.189	0.26	0.45	0.568	0.658
7	0.02	0	0.077	0.167	0.22	0.401	0.531	0.628
8	0.02	0	0.054	0.144	0.169	0.343	0.498	0.592
9	0.02	0	0.03	0.114	0.117	0.272	0.464	0.554
10	0.02	0	0.006	0.088	0.057	0.19	0.424	0.515
11	0.02	0	0	0.06	0.003	0.105	0.389	0.481
12	0	0	0	0.037	0	0.004	0.353	0.447

Table 6.3 Velocity Values (decline OD/min) of Nitrofurantoin + CTX-M-15

Δ/min								
T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0	0	0	0	0	0	0	0
1	0	0.008	0.012	0.024	0.018	0.019	0.037	0.036
2	0	0.019	0.025	0.035	0.039	0.042	0.073	0.072
3	0	0.031	0.039	0.053	0.058	0.066	0.109	0.109
4	0	0.031	0.053	0.071	0.084	0.09	0.145	0.147
5	0	0.031	0.069	0.098	0.14	0.12	0.18	0.181
6	0	0.031	0.084	0.12	0176	0.154	0.216	0.221
7	0	0.031	0.105	0.139	0.0216	0.203	0.253	0.252
8	0	0.031	0.128	0.165	0.267	0.261	0.286	0.287
9	0	0.031	0.152	0.195	0.319	0.332	0.32	0.325
10	0	0.031	0.176	0.221	0.379	0.412	0.36	0.364
11	0	0.031	0.182	0.249	0.433	0.499	0.395	0.398
12	0	0.031	0.182	0.269	0.436	0.6	0.431	0.432
Average	***0***	***0.025***	***0.092***	***0.126***	***0.197***	***0.215***	***0.216***	***0.217***

Figure 6.7 Km determination of CTX-M-15 towards Nitrofurantoin.



Determination of hydrolytic activity of CTX-M-15 towards the Nitrofurantoin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 266 nm over 12 mins. The concentration of Nitrofurantoin that corresponded to ½Vamx (1/0.217≈4.60), solid line, indicates a Km value (1/0.11 ≈9 µM).

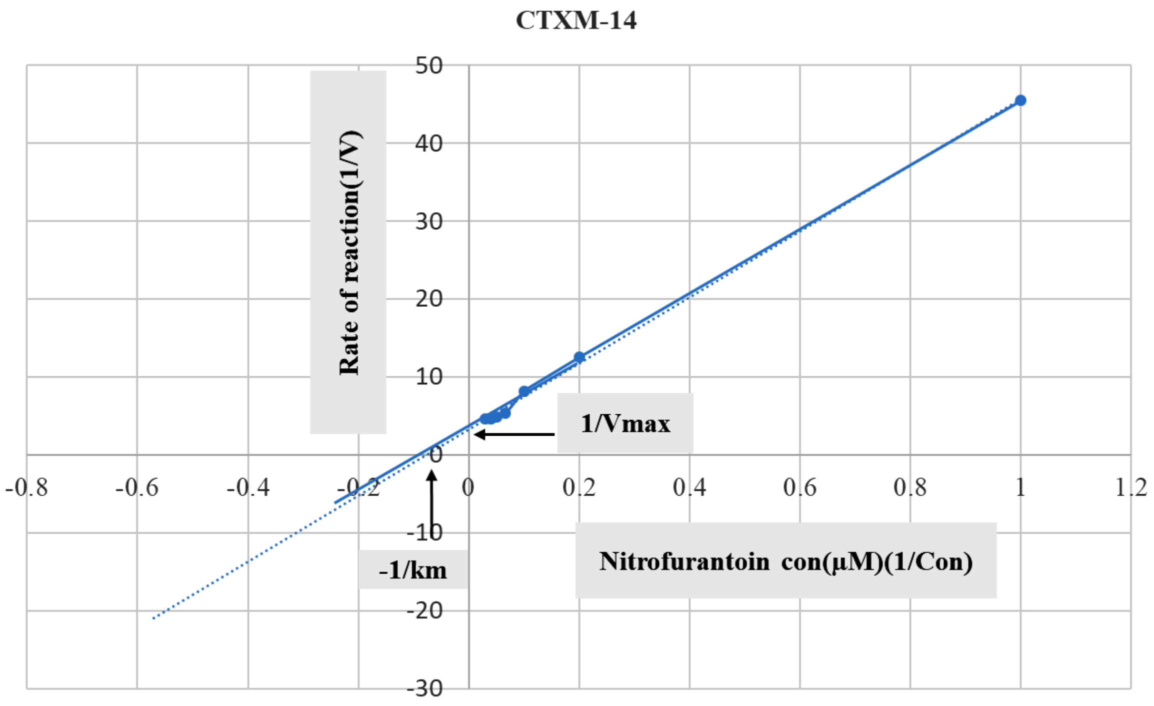
Table 6.4 OD of Nitrofurantoin+ CTX-M-14 protein mixture

Time/min	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.02	0.027	0.141	0.24	0.38	0.522	0.693	0.798
1	0.02	0.02	0.13	0.217	0.362	0.504	0.657	0.762
2	0.02	0.011	0.117	0.206	0.342	0.481	0.621	0.727
3	0.02	0.005	0.105	0.189	0.323	0.458	0.584	0.691
4	0.02	0	0.091	0.171	0.298	0.433	0.549	0.652
5	0.02	0	0.076	0.147	0.243	0.405	0.514	0.618
6	0.02	0	0.061	0.123	0208	0.37	0.478	0.582
7	0.02	0	0.044	0.102	0.168	0.322	0.443	0.553
8	0.02	0	0.024	0.079	0.118	0.263	0.407	0.516
9	0.02	0	0.001	0.05	0.058	0.193	0.373	0.478
10	0.02	0	0	0.024	0	0.111	0.34	0.441
11	0.02	0	0	0	0	0.013	0.301	0.408
12	0	0	0	0	0	0	0.266	0.374

Table 6.5 Velocity Values (decline OD/min) of Nitrofurantoin+CTX-M-14 mixture

Δ/min								
T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0	0	0	0	0	0	0	0
1	0	0..09	0.011	0.019	0.018	0.018	0.036	0.037
2	0	0.019	0.024	0.031	0.038	0.041	0.072	0.071
3	0	0.025	0.036	0.047	0.057	0.064	0.109	0.108
4	0	0.027	0.05	0.066	0.082	0.089	0.144	0.146
5	0	0.027	0.065	0.089	0.137	0.117	0.179	0.18
6	0	0.027	0.08	0.108	0.172	0.152	0.215	0.216
7	0	0.027	0.097	0.132	0.212	0.2	0.25	0.245
8	0	0.027	0.117	0.158	0.262	0.259	0.286	0.282
9	0	0.027	0.14	0.188	0.322	0.329	0.32	0.32
10	0	0.027	0.141	0219	0.38	0.411	0.353	0.357
11	0	0.027	0.141	0.24	0.38	0.509	0.392	0.39
12	0	0.027	0.141	0.24	0.38	0.522	0.451	0.424
Average	***0***	***0.022***	***0.08***	***0.122***	***0.187***	***0.208***	***0.215***	***0.214***

Figure 6.8 Km determination of CTX-M-14 towards Nitrofurantoin



Determination of hydrolytic activity of CTX-M-14 towards the Nitrofurantoin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 266 nm over 12 mins. The concentration of Nitrofurantoin that corresponded to ½ Vmax (1/0.214≈4.67), solid line, indicates a Km value (1/0.1 ≈ 10 μM).

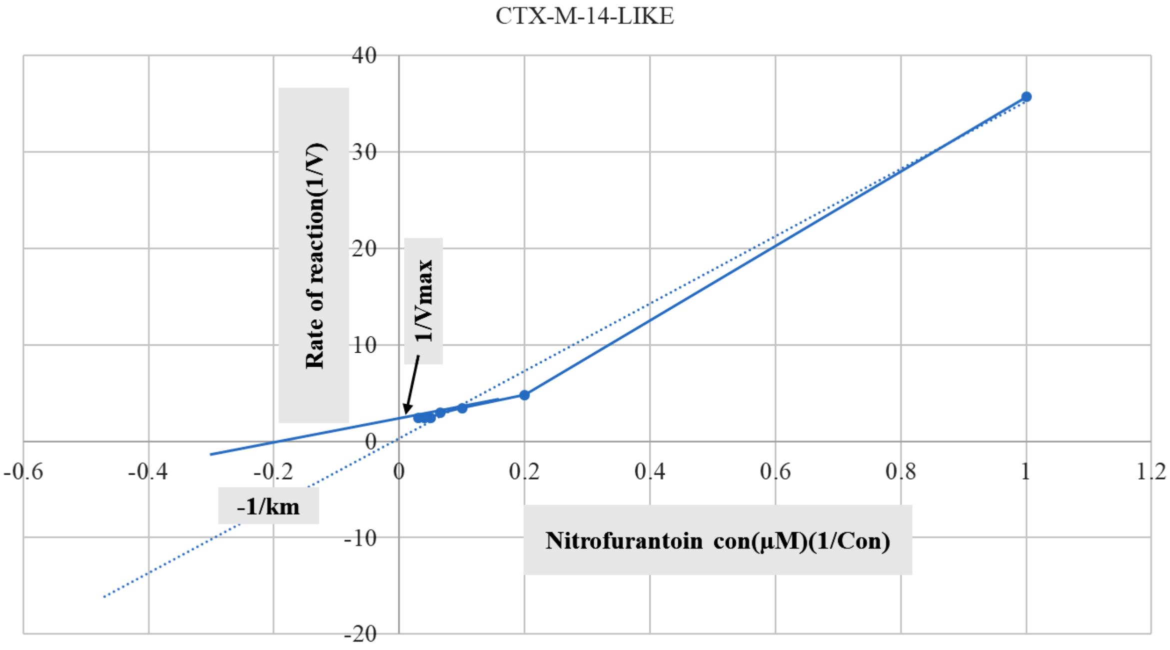
Table 6.6 OD of Nitrofurantoin+ CTXM-14-LIKE protein mixture

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.03	0.032	0.24	0.358	0.511	0.693	0.797	0.931
1	0.03	0.016	0.138	0.299	0.463	0.662	0.767	0.898
2	0.03	0.004	0.031	0.218	0.405	0.616	0.722	0.852
3	0	0	0	0.117	0.335	0.565	0.672	0.802
4	0.02	0	0	0	0.259	0.428	0.606	0.736
5	0.02	0	0	0	0.167	0.34	0.533	0.662
6	0.02	0	0	0	0.057	0.237	0.445	0.574
7	0.02	0	0	0	0	0.117	0.326	0.471
8	0.02	0	0	0	0	0	0.189	0.351
9	0.02	0	0	0	0	0	0.038	0.213
10	0.02	0	0	0	0	0	0	0.074
11	0.02	0	0	0	0	0	0	0
12	0.02	0	0	0	0	0	0	0

Table 6.7 Velocity Values (Decline OD/min) of Nitrofurantoin+CTX-M-14-LIKE protein mixture

				∆/min				
T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0	0	0	0	0	0	0	0
1	0	0.016	0.102	0.059	0.048	0.031	0.033	0.033
2	0	0.028	0.209	0.14	0.106	0.077	0.078	0.078
3	0	0.032	0.24	0.241	0.176	0.128	0.132	0.129
4	0	0.032	0.24	0.358	0.252	0.265	0.206	0.195
5	0	0.032	0.24	0.358	0.344	0.353	0.294	0.269
6	0	0.032	0.24	0.358	0.454	0.456	0.415	0.357
7	0	0.032	0.24	0.358	0.511	0.576	0.552	0.46
8	0	0.032	0.24	0.358	0.511	0.693	0.687	0.58
9	0	0.032	0.24	0.358	0.511	0.693	0.789	0.718
10	0	0.032	0.24	0.358	0.511	0.693	0.798	0.857
11	0	0.032	0.24	0.358	0.511	0.693	0.798	0.931
12	0	0.032	0.24	0.358	0.511	0.693	0.798	0.931
Average	***0***	***0.028***	***0.208***	***0.29***	***0.34***	***0.41***	***0.42***	***0.46***

Figure 6.9 Km determination of Nitrofurantoin towards CTX-M-14-LIKE.



Determination of hydrolytic activity of CTX-M-14-LIKE towards the Nitrofurantoin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 266 nm over 12 mins. The concentration of Nitrofurantoin that corresponded to ½ Vmax (1/0.46 ≈2.17) , solid line, indicates a Km value (1/0.2 ≈5 µM)

6.2.3.2 Km of CTX-M-15, CTX-M-14 and CTX-M-14-like towards Cefoxitin

Absorbance changes (at λ = 235 nm) were monitored (section 6.2.3.1) to measure the hydrolysis velocity of Cefoxitin by the three CTX-M proteins (Tables 6.8, 6.9, 6.10, 6.11, 6.12 and 6.13 respectively). Km values of CTX-M-15, CTX-M-14, CTX-M-14-LIKE, CTX-M-14-are shown in Figures 6.10, 6.11 and 6.13 respectively.

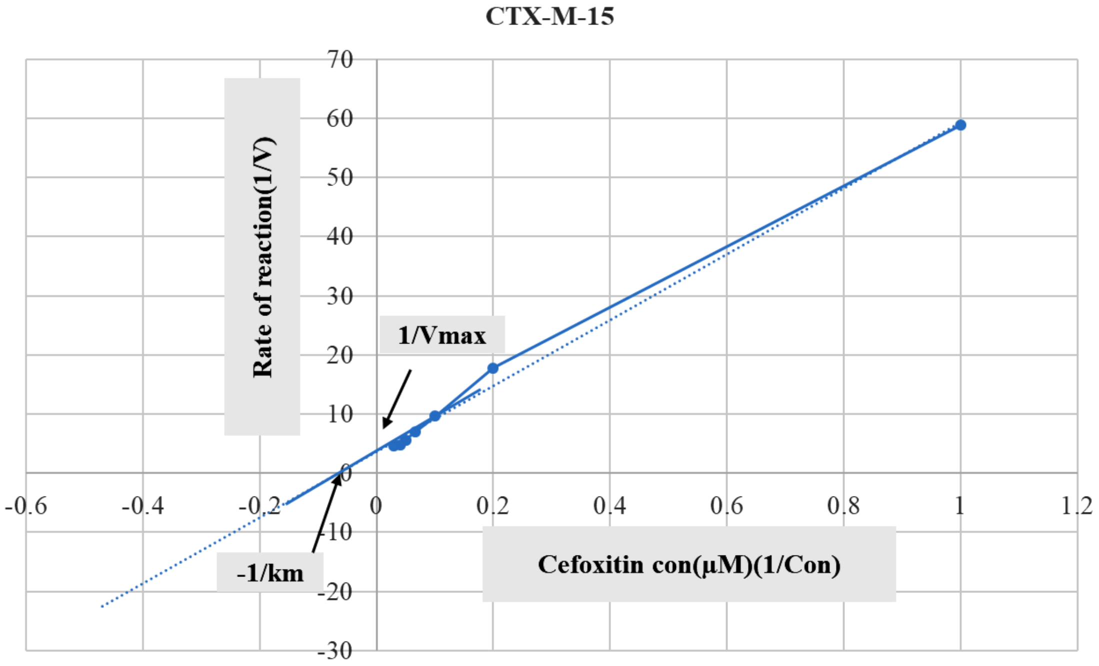
Table 6.8 Cefoxitin+ CTX-M-15 protein mixture

Time(min)/con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.008	0.019	0.078	0.171	0.227	0.356	0.403	0.492
1	0.008	0.006	0.066	0.148	0.209	0.338	0.367	0.456
2	0.008	0	0.055	0.136	0.189	0.315	0.33	0.421
3	0.008	0	0.042	0.124	0.17	0.292	0.292	0.385
4	0.008	0	0.028	0.105	0.146	0.267	0.258	0.346
5	0.008	0	0.013	0.08	0.091	0.239	0.223	0.312
6	0.008	0	0	0.056	0.036	0.204	0.187	0.276
7	0.008	0	0	0.034	0.001	0.156	0.152	0.24
8	0.008	0	0	0.011	0	0.097	0.116	0.211
9	0.008	0	0	0	0	0.027	0.082	0.174
10	0.008	0	0	0	0	0	0.049	0.136
11	0.008	0	0	0	0	0	0.01	0.097
12	0.008	0	0	0	0	0	0	0.066

Table 6.9 Velocity Values (decline OD/min) of Cefoxitin+CTX-M-15

			∆/min					
T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0	0	0	0	0	0	0	0
1	0	0.013	0.012	0.023	0.018	0.018	0.036	0.036
2	0	0.019	0.023	0.035	0.038	0.041	0.073	0.071
3	0	0.019	0.036	0.047	0.057	0.064	0.111	0.107
4	0	0.019	0.05	0.066	0.081	0.089	0.145	0.146
5	0	0.019	0.065	0.091	0.136	0.117	0.18	0.18
6	0	0.019	0.078	0.115	0.191	0.152	0.216	0.216
7	0	0.019	0.078	0.137	0.226	0.2	0.251	0.252
8	0	0.019	0.078	0.16	0.227	0.259	0.287	0.281
9	0	0.019	0.078	0.171	0.227	0.329	0.321	0.318
10	0	0.019	0.078	0.171	0.227	0.356	0.354	0.356
11	0	0.019	0.078	0.171	0.227	0.356	0.393	0.395
12	0	0.019	0.078	0.171	0.227	0.356	0.403	0.426
Average	***0***	***0.017***	***0.056***	***0.104***	***0.144***	***0.179***	***0.213***	***0.214***

Figure 6.10 Km determination of Cefoxitin towards CTX-M-15.



Determination of hydrolytic activity of CTX-M-15 enzyme towards the Cefoxitin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 235 nm over 12 mins. The concentration of Cefoxitin that corresponded to ½ Vmax (1/0.214≈4.67), solid line, indicates a Km value (1/0.066≈ 15.1 µM).

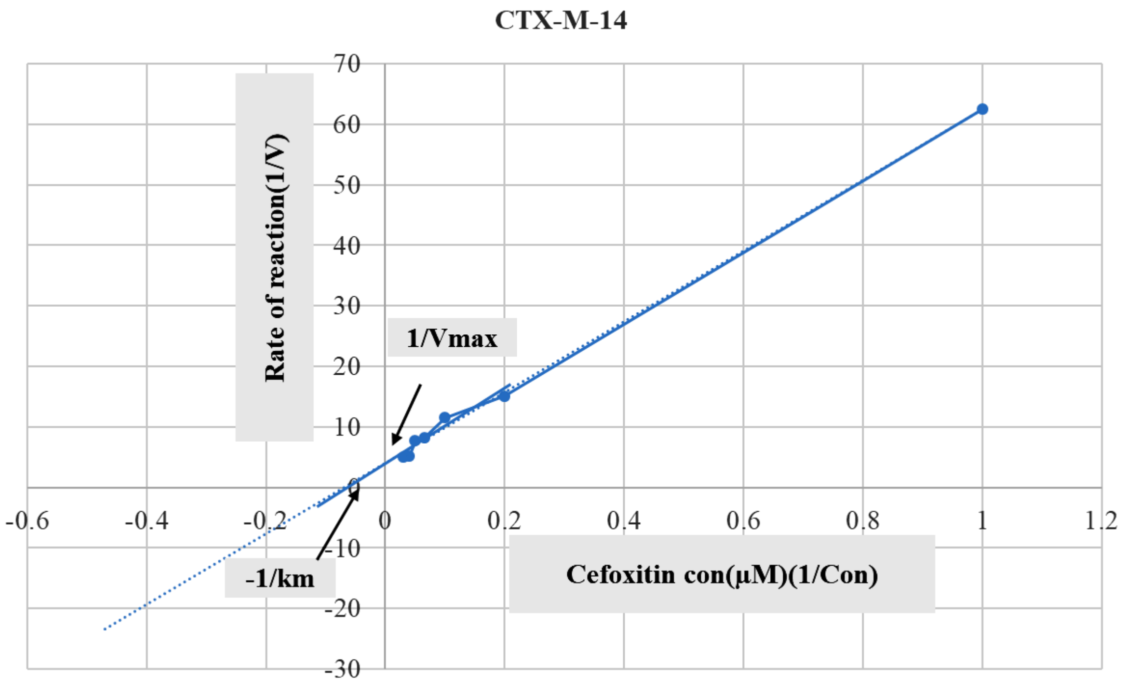
Table 6.10 OD of Cefoxitin+CTX-M-14 protein mixture

T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0.011	0.018	0.098	0.13	0.18	0.209	0.297	0.34
1	0.011	0.01	0.087	0.108	0.162	0.189	0.261	0.304
2	0.011	0.002	0.075	0.098	0.143	0.171	0.223	0.27
3	0.011	0	0.061	0.082	0.125	0.149	0.189	0.235
4	0.011	0	0.046	0.066	0.101	0.126	0.157	0.198
5	0.011	0	0.031	0.044	0.048	0.098	0.124	0.165
6	0.011	0	0.014	0.021	0.013	0.065	0.091	0.132
7	0.011	0	0	0.002	0	0.018	0.056	0.102
8	0.011	0	0	0	0	0	0.021	0.066
9	0.011	0	0	0	0	0	0	0.029
10	0.011	0	0	0	0	0	0	0.004
11	0.011	0	0	0	0	0	0	0
12	0.011	0	0	0	0	0	0	0

Table 6.11 Velocity Values (decline OD/min) of Cefoxitin+CTX-M-14 protein mixture

Δ/min								
T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0	0	0	0	0	0	0	0
1	0	0.008	0.011	0.022	0.018	0.02	0.036	0.036
2	0	0.016	0.023	0.032	0.037	0.038	0.074	0.07
3	0	0.018	0.037	0.048	0.055	0.06	0.187	0.105
4	0	0.018	0.052	0.064	0.079	0.083	0.14	0.142
5	0	0.018	0.067	0.086	0.132	0.111	0.173	0.175
6	0	0.018	0.084	0.109	0.167	0.144	0.206	0.208
7	0	0.018	0.098	0.128	0.18	0.191	0.241	0.238
8	0	0.018	0.098	0.13	0.18	0.209	0.276	0.274
9	0	0.018	0.098	0.13	0.18	0.209	0.297	0.311
10	0	0.018	0.098	0.13	0.18	0.209	0.297	0.336
11	0	0.018	0.098	0.13	0.18	0.209	0.297	0.34
12	0	0.018	0.098	0.13	0.18	0.209	0.297	0.34
Average	***0***	***0.015***	***0.066***	***0.087***	***0.12***	***0.13***	***0.193***	***0.198***

Figure 6.11 Km determination of Cefoxitin towards CTX-M-14.



Determination of hydrolytic activity of CTX-M-14 towards the Cefoxitin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 235 nm over 12 mins. The concentration of Cefoxitin that corresponded to ½ Vmax (1/0.198≈5.05), solid line, indicates a Km value (1/0.055 ≈ 18 µM).

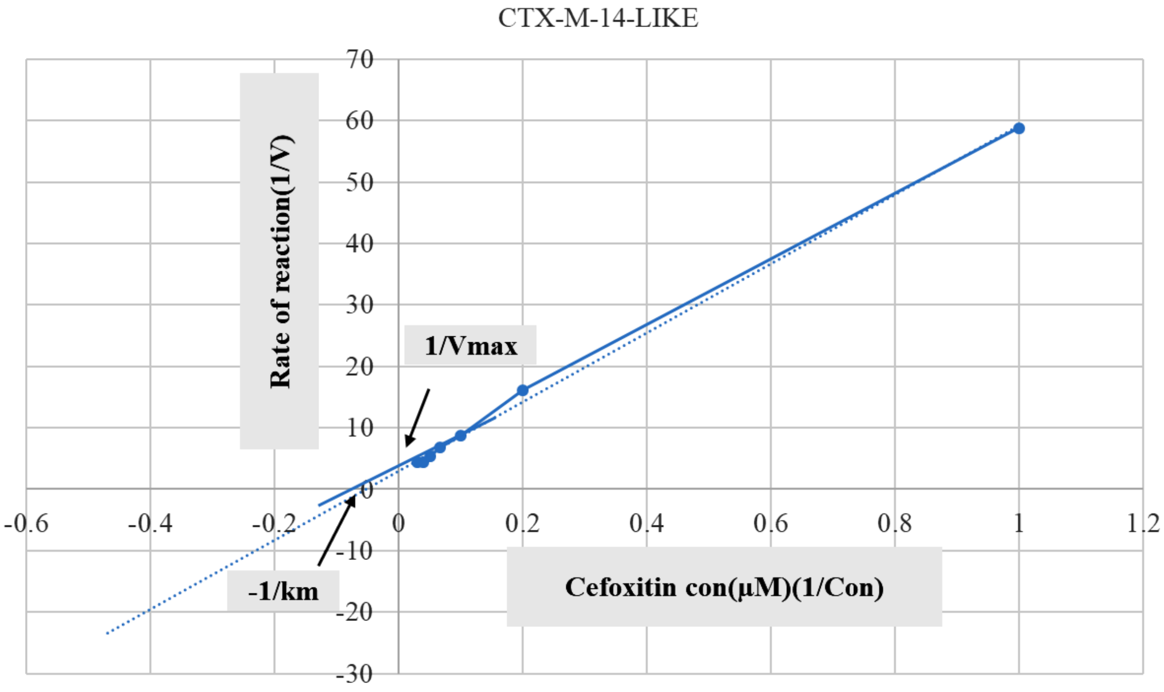
Table 6.12 OD of cefoxitin+CTX-M-14-LIKE mixture

T(min)/con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.013	0.019	0.085	0.177	0.233	0.363	0.411	0.498
1	0.013	0.004	0.072	0.152	0.212	0.345	0.372	0.459
2	0.013	0	0.057	0.138	0.191	0.319	0.332	0.421
3	0.013	0	0.042	0.118	0.173	0.293	0.292	0.383
4	0.013	0	0.025	0.097	0.145	0.265	0.254	0.341
5	0.013	0	0.007	0.069	0.087	0.234	0.216	0.304
6	0.013	0	0	0.042	0.049	0.197	0.177	0.265
7	0.013	0	0	0.018	0.005	0.148	0.139	0.232
8	0.013	0	0	0	0	0.085	0.101	0.192
9	0.013	0	0	0	0	0.014	0.066	0.151
10	0.013	0	0	0	0	0	0.028	0.11
11	0.013	0	0	0	0	0	0	0.075
12	0.013	0	0	0	0	0	0	0.038

Table 6.13 Velocity Values (decline of OD/min) of Cefoxitin+CTX-M-14-LIKE

				Δ/min				
T(min)/Con	**0 µM**	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**20 µM**	**25 µM**	**30 µM**
0	0	0	0	0	0	0	0	0
1	0	0.015	0.013	0.025	0.021	0.018	0.039	0.039
2	0	0.019	0.028	0.039	0.042	0.044	0.079	0.077
3	0	0.019	0.043	0.059	0.06	0.07	0.119	0.115
4	0	0.019	0.06	0.079	0.088	0.098	0.157	0.157
5	0	0.019	0.078	0.108	0.146	0.129	0.195	0.194
6	0	0.019	0.085	0.135	0.184	0.166	0.234	0.233
7	0	0.019	0.085	0.159	0.228	0.215	0.272	0.266
8	0	0.019	0.085	0.177	0.233	0.278	0.31	0.306
9	0	0.019	0.085	0.177	0.233	0.349	0.345	0.347
10	0	0.019	0.085	0.177	0.233	0.363	0.383	0.388
11	0	0.019	0.085	0.177	0.233	0.363	0.411	0.423
12	0	0.019	0.085	0.177	0.233	0.363	0.411	0.46
Average	***0***	***0.017***	***0.062***	***0.114***	***0.148***	***0.188***	***0.227***	***0.229***

Figure 6.12 Km determination of Cefoxitin towards CTX-M-14-LIKE.



Determination of hydrolytic activity of CTX-M-14LIKE enzyme towards Cefoxitin antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 235 nm over 12 min. The concentration of Cefoxitin that corresponded to ½ Vmax (1/0.229≈4.36), solid line, indicates a Km value (1/0.07 ≈ 13 µM).

6.2.3.3 Km of CTXM-14, CTX-M-14-LIKE and CTX-M-15 towards Ceftazidime

Km of Ceftazidime (which had low MIC of the three CTX-Ms) were determined by monitoring Absorbance changes (at λ274 nm) (section 6.2.3.1). (Tables 6.14, 6.15, 6.16, 6.17, 6.18 and 6.19). Figures 6.13, 6.14 and 6.15 show Km values of CTX-M-15, CTX-M-14, and CTX-M14-LIKE respectively.

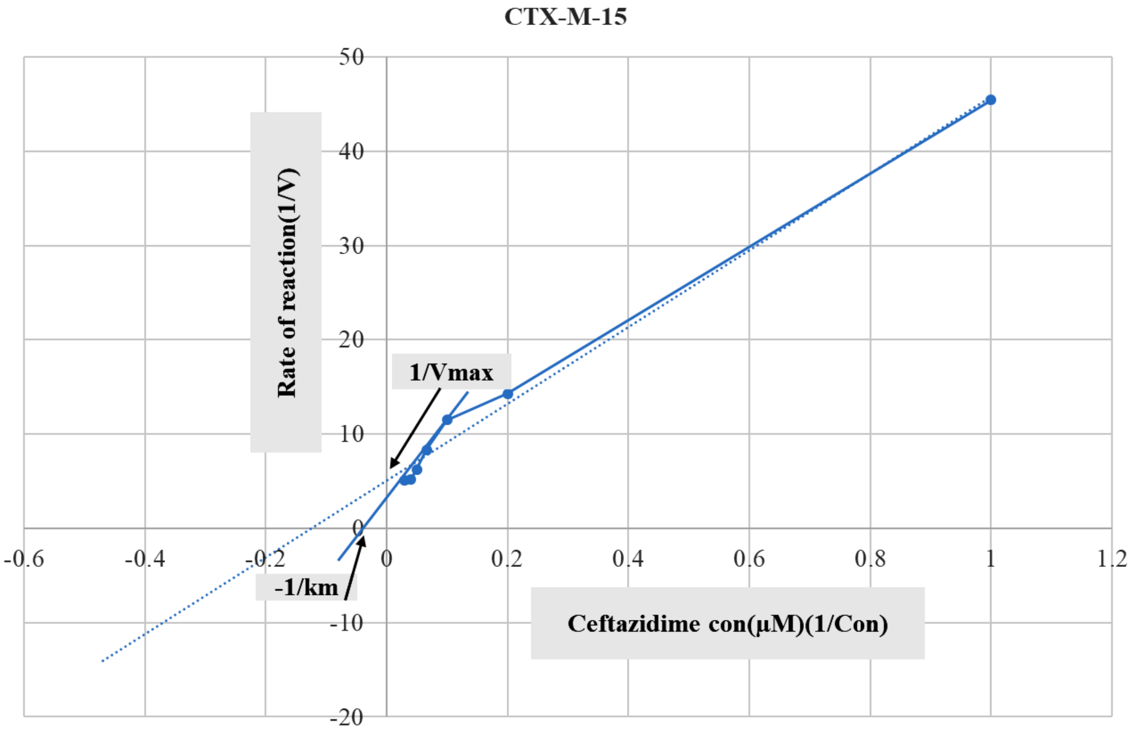
Table 6.14 OD of Ceftazidime+CTX-M-15

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.011	0.026	0.106	0.12	0.188	0.218	0.308	0.349
1	0.011	0.009	0.095	0.117	0.169	0.197	0.268	0.313
2	0.011	0.002	0.083	0.107	0.149	0.179	0.23	0.278
3	0.011	0	0.069	0.099	0.133	0.157	0.197	0.243
4	0.011	0	0.054	0.073	0.108	0.134	0.175	0.208
5	0.011	0	0.039	0.053	0.057	0.106	0.132	0.172
6	0.011	0	0.022	0.029	0.019	0.043	0.098	0.139
7	0.011	0	0	0.009	0	0.013	0.063	0.109
8	0.011	0	0	0	0	0	0.028	0.074
9	0.011	0	0	0	0	0	0	0.036
10	0.011	0	0	0	0	0	0	0
11	0.011	0	0	0	0	0	0	0
12	0.011	0	0	0	0	0	0	0

Table 6.15 Velocity values (decline of OD/min) + CTX-M-15

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0	0	0	0	0	0	0	0
1	0	0.017	0.011	0.003	0.021	0.021	0.04	0.036
2	0	0.018	0.023	0.013	0.039	0.039	0.078	0.071
3	0	0.026	0.037	0.021	0.055	0.061	0.111	0.106
4	0	0.026	0.052	0.047	0.08	0.084	0.133	0.141
5	0	0.026	0.067	0.067	0.131	0.112	0.176	0.177
6	0	0.026	0.084	0.091	0.169	0.175	0.21	0.21
7	0	0.026	0.106	0.111	0.188	0.205	0.245	0.24
8	0	0.026	0.106	0.12	0.188	0.218	0.28	0.275
9	0	0.026	0.106	0.12	0.188	0.218	0.308	0.313
10	0	0.026	0.106	0.12	0.188	0.218	0.308	0.349
11	0	0.026	0.106	0.12	0.188	0.218	0.308	0.349
12	0	0.026	0.106	0.12	0.188	0.218	0.308	0.349
Average	***0***	***0.022***	***0.07***	***0.087***	***0.12***	***0.16***	***0.192***	***0.2***

Figure 6.13 Km determination of Ceftazidime towards CTX-M-15.



Determination of hydrolytic activity of CTX-M-15 enzyme towards the Ceftazidime antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 274 nm over 12 mins. The concentration of Ceftazidime that corresponded to ½ Vmax (1/0.2≈ 5), solid line, indicates a Km value (1/0.036 ≈ 27.5 µM).

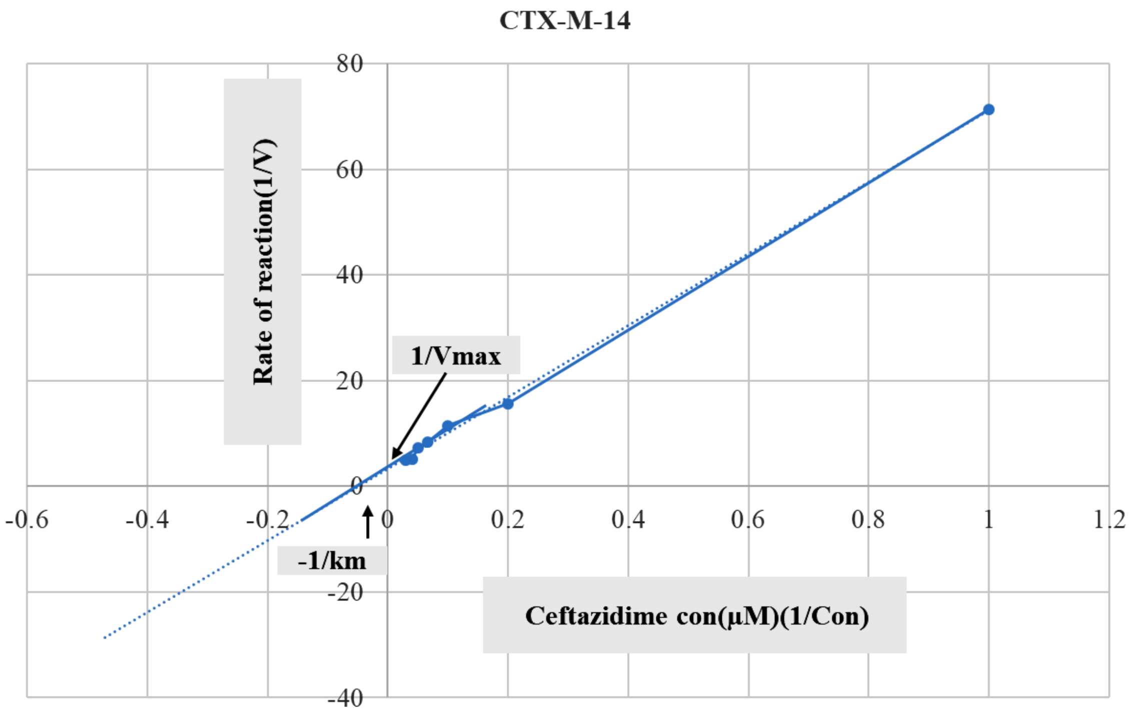
Table 6.16 OD of Ceftazidime+CTX-M-14

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.009	0.015	0.095	0.129	0.179	0.205	0.295	0.339
1	0.009	0.08	0.084	0.105	0.159	0.186	0.267	0.301
2	0.009	0.002	0.072	0.095	0.14	0.168	0.22	0.24
3	0.009	0	0.059	0.079	0.122	0.146	0.185	0.232
4	0.009	0	0.043	0.063	0.098	0.122	0.154	0.194
5	0.009	0	0.029	0.04	0.046	0.095	0.12	0.161
6	0.009	0	0.012	0.022	0.09	0.062	0.088	0.128
7	0.009	0	0	0.003	0	0.015	0.053	0.097
8	0.009	0	0	0	0	0	0.018	0.062
9	0.009	0	0	0	0	0	0	0.025
10	0.009	0	0	0	0	0	0	0.002
11	0.009	0	0	0	0	0	0	0
12	0.009	0	0	0	0	0	0	0

Table 6.17 Velocity values (decline of OD/min) +CTX-M-14

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0	0	0	0	0	0	0	0
1	0	0.007	0.011	0.024	0.02	0.019	0.028	0.038
2	0	0.013	0.023	0.034	0.039	0.037	0.075	0.099
3	0	0.015	0.037	0.05	0.057	0.059	0.11	0.107
4	0	0.015	0.052	0.066	0.081	0.083	0.141	0.145
5	0	0.015	0.067	0.089	0.133	0.111	0.175	0.178
6	0	0.015	0.084	0.107	0.167	0.144	0.207	0.211
7	0	0.015	0.095	0.126	0.179	0.191	0.242	0.242
8	0	0.015	0.095	0.129	0.179	0.205	0.277	0.277
9	0	0.015	0.095	0.129	0.179	0.205	0.295	0.314
10	0	0.015	0.095	0.129	0.179	0.205	0.295	0.337
11	0	0.015	0.095	0.129	0.179	0.205	0.295	0.339
12	0	0.015	0.095	0.129	0.179	0.205	0.295	0.339
Average	***0***	***0.013***	***0.064***	***0.087***	***0.12***	***0.138***	***0.197***	***0.205***

Figure 6.14 Km determination of Ceftazidime towards CTX-M-14.



Determination of hydrolytic activity of CTX-M-14 enzyme towards the Ceftazidime antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 274 nm over 12 mins. The concentration of Ceftazidime that corresponded to ½ Vmax (1/0.205 ≈ 4.87), solid line, indicates a Km value (1/0.036 ≈ 27.5µM).

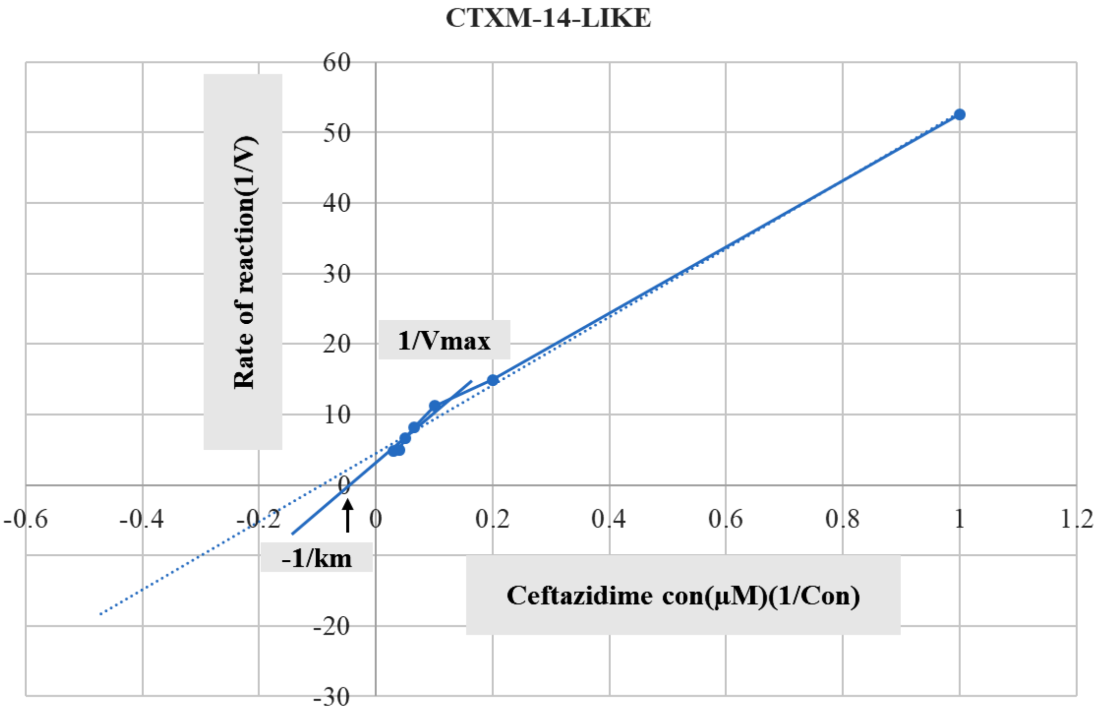
Table 6.18 OD of Ceftazidime+CTX-M-14-LIKE

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0.013	0.021	0.101	0.134	0.183	0.212	0.3	0.346
1	0.013	0.004	0.09	0.112	0.165	0.192	0.264	0.304
2	0.013	0	0.08	0.101	0.146	0.174	0.227	0.27
3	0.013	0	0.061	0.085	0.128	0.152	0.192	0.237
4	0.013	0	0.049	0.069	0.105	0.129	0.16	0.198
5	0.013	0	0.034	0.047	0.051	0.101	0.126	0.167
6	0.013	0	0.017	0.024	0.017	0.069	0.094	0.134
7	0.013	0	0	0.006	0	0.022	0.059	0.105
8	0.013	0	0	0	0	0	0.024	0.069
9	0.013	0	0	0	0	0	0	0.031
10	0.013	0	0	0	0	0	0	0.007
11	0.013	0	0	0	0	0	0	0
12	0.013	0	0	0	0	0	0	0

Table 6.19 Velocity values (decline of OD/min) +CTX-M-14-LIKE

T(min)/Con	0 µM	1 µM	5 µM	10 µM	15 µM	20 µM	25 µM	30 µM
0	0	0	0	0	0	0	0	0
1	0	0.017	0.011	0.022	0.018	0.02	0.036	0.036
2	0	0.021	0.023	0.033	0.037	0.038	0.074	0.09
3	0	0.021	0.037	0.048	0.055	0.06	0.187	0.105
4	0	0.021	0.052	0.064	0.079	0.083	0.14	0.142
5	0	0.021	0.067	0.086	0.132	0.111	0.175	0.175
6	0	0.021	0.084	0.109	0.167	0.144	0.208	0.208
7	0	0.021	0.101	0.128	0.183	0.191	0.3	0.242
8	0	0.021	0.101	0.134	0.183	0.212	0.3	0.274
9	0	0.021	0.101	0.134	0.183	0.212	0.3	0.313
10	0	0.021	0.101	0.134	0.183	0.212	0.3	0.336
11	0	0.021	0.101	0.134	0.183	0.212	0.3	0.346
12	0	0.021	0.101	0.134	0.183	0.212	0.3	0.346
Average	***0***	***0.019***	***0.067***	***0.089***	***0.122***	***0.151***	***0.201***	***0.207***

Figure 6.15 Km determination of Ceftazidime towards CTX-M-14-LIKE.



Determination of hydrolytic activity of CTX-M-14-LIKE towards Ceftazidime antibiotic. The velocity of the reaction is measured by monitoring of the change in absorbance of protein-antibiotic mixture by spectrophotometer at λ = 274 nm over 12 mins. The concentration of Ceftazidime that corresponded to ½ Vmax (1/0.207≈4.83) indicates, solid line, a Km value (1/0.036 ≈ 27.5 µM).

Comparison of km values (Table 6.20) shows that CTX-M-14-LIKE, with the three substitutions (A55T, A273P, R227C), shows lower Km value (higher hydrolytic activity) than CTX-M-14 for Nitrofurantoin which had a high MIC with CTX-M-14-LIKE by E-test.

Table 6.20 Km values of five purified CTX-M enzymes

blaCTX-M	**Nitrofurantoin**		**Cefoxitin**		**Ceftazidime**	
	Vmax	***Km(µM)***	***Vmax***	***Km(µM)***	***Vmax***	***Km(µM)***
CTX-M-15	0.217	**9**	0.214	15.1	0.2	27.5
CTX-M-14	0.214	10	0.198	20	0.205	28.5
CTX-M14-LIKE	0.46	**5**	0.229	15	0.207	28.5

Km values of three CTX-M enzymes;CTX-M-15,CTX-M-14,CTX-M-14-LKE with three selected antibiotics; Nitrofurantoin, Cefoxitin and Ceftazidime which showed high, medium and low MIC respectively with CTX-M-14-LIKE in comparison to CTX-M-14.As it can be observed CTX-M-14-LIKE with the three changes (A55T,A273P,R227C) shows lower Km value (higher affinity) than CTX-M-14 for Nitrofurantoin.

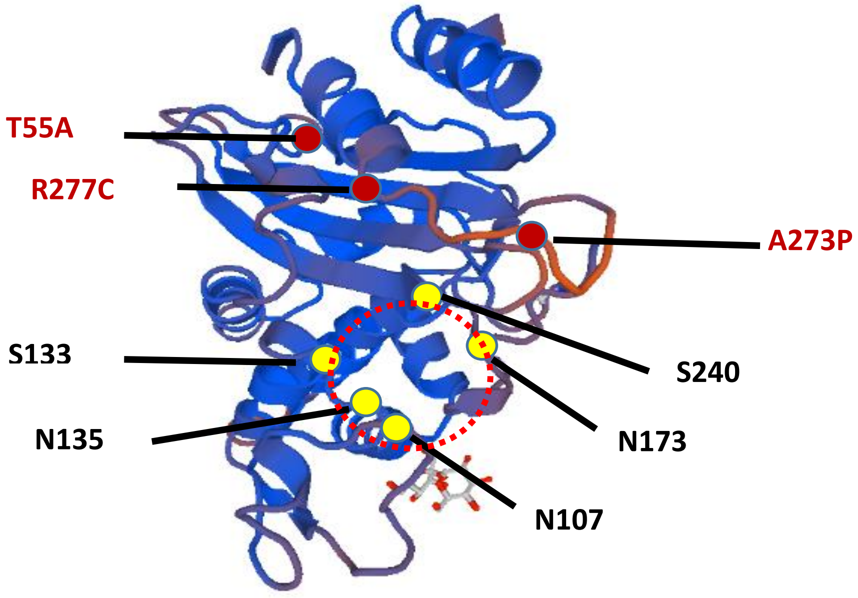
6.3. Discussion

This part of the study aims to determine whether the mutations in CTX-M-14 (T55A, A273P, and R277C) can affect the antibiotic specificity of CTX-M-14 β-lactamase. This was achieved by comparing the minimal inhibitory concentrations (MICs) and the enzyme kinetics in *E. coli* strains expressing either CTX-M-14 or CTX-M-14-LIKE against selected commonly used antibiotics used in the treatment of UTI patients(Nitrofurantoin, Cefotazidime, Cefotaxime, Cefoxitin and Imipenem). The results of the E-tests and in vitro assays show that the three changes in the novel CTX-M (CTX-M-14-LIKE) significantly affect the susceptibility of the strains carrying the recombinant CTX-M-14-LIKE genes makes them more resistant to the Nitrofurantoin antibiotic. In addition, there is a slight effect on Cefoxitin susceptibility in the same strains (i.e. Show bit more resistance to Cefoxitin than those expressing CTXM-14).

As it is shown in Table 6.20, insignificant difference were found in the Km values between CTX-M-14 and CTX-M-14-LIKE with Nitrofurantoin and Cefoxitin. CTX-M-14-LIKE was two folds faster and two fold more affine than CTX-M-14 with Nitrofurantoin and approximately more than one fold faster with Cefoxitin. However, the interesting observation is that the affinities are not significantly different between CTX-M-14 and CTX-M-14-like, although the latter cells are more resistant to Nitrofurantoin (MIC is 64-times higher with CTX-M-14-like than with CTXM-14). This strongly indicates that the three mutations, which are far away from the active site, do not affect the affinity but the turnover for these two antibiotics (Nitrofurantoin and Cefoxitin).

The cells become more resistant because the three mutations seem to promote the conformational changes for Nitrofurantoin and Cefoxitin increasing the rate at which CTX-M-14-like is able to hydrolyse the two antibiotics. This is consistent with the structure model of CTX-M-14 that shows the three amino acid substitutions situated in two loop regions in the beta-strand domain which sits on top of the active site (Figure 6.16). It is therefore not predicted that the three mutated amino acids make direct contact with the β-lactam substrate. Interestingly, two of the substitutions (A273P and R277C) affect the large loop next to the active site. Taken together these findings imply an indirect effect of the substitutions on the conformational changes or the structure of the enzyme.

Figure 6.16 3D structure of the CTX-M-14-LIKE protein.



The 3D structure of CTX-M-14 protein using the SWISS-MODEL tool. The positions of the three changes (T55A, A273P, and R277C) are highlighted. The dotted line highlights the active site with the five amino acids (N107, S133, N135, N173, and S240) that make direct contact with the β-lactam antibiotic substrate. Note that two of the mutated amino acids are predicted to be located in a large loop above the substrate binding site (A273P and R277C).

The catalytic cycle of β-lactamase enzymes is controlled by many structural elements, including as the Ω-loop. The Ω-loop is located at the bottom of the active site entrance and contains highly conserved residues that are important in the catalytic activity of the enzyme. The mutation of these residues results in the loss of enzymatic activity (Pimenta et al., 2014). The effects of structural elements and residues that are located close or in the active site has been demonstrated by many studies. However, few studies have looked into the effect of substitutions in residues distal from the active site on CTX-Ms catalytic activity.

It was reported that the evolution of genetic elements encoding CTX-M enzymes can result from random mutations and recombinant different resistance genes particularly between

CTX-Ms classified under groups 1 and group 9, from which CTX-M-15 and CTX-M-14 originate, respectively (Canton et al., 2012). CTX-M-64 is considered an example of this as it contains the middle part of CTX-M-14, with the N- and C-terminals of CTX-M-15, making it more active than CTX-M-14 (Tian et al., 2014).This example show how can the residues distal to the active site are involved in the catalytic cycle of CTX-M enzymes.

The study conducted by He D et al. (2016) has investigated the molecular basis of the differential mechanisms exhibited by CTX-M-14 and of its hybrid (CTX-M-64), which has a higher catalytic activity when compared to CTX-14, in order to check the contribution of residues located distal from active site, in the N and C terminals, to the enzymatic activity. The study concluded that the greater stability of CTX-M-64, which was analysed by thermal stability assay, was behind the enhanced extended-spectrum activity of CTX-M-64.

In this study the location of the three mutations in CTX-M-14-LIKE, which are far away from the active site, emphasise this conclusion as they do not affect the affinity significantly, but do affect the turnover of the two antibiotics (Nitrofurantoin and Cefoxitin). The greater integrity and stability of CTX-M-14-LIKE, which requires further investigation using thermal stability assays, could be associated with the revealed mutation in CTXM-14-LIKE and may drive the high catalytic activity of this enzyme.

The importance of this observation is that Nitrofurantoin has been considered as a standard treatment for UTIs since 2010 (Gardiner et al., 2019). Since then, Nitrofurantoin has shown high activity against uropathogen, including *E. coli* and *Enterococci*, including most extended spectrum β-lactamase producing bacteria addition to all causative bacterial of nosocomial lower UTIs (McKinnnel & Miller, 2011). Generally, many multidrug resistant organisms are still susceptible to Nitrofurantoin (Sanchez et al., 2016). Hence, the spread of CTX-M-14-LIKE β-lactamase in the local communities is expected to worsen the antibiotic resistance problem among UTI patients as the 3 mutations may affect protein stability and/or turnover rates.

Chapter 7: GENERAL DISCUSSION AND CONCLUSIONS

One of the most significant public health concerns in the 21st century is antibiotic resistance. Many bacteria species have developed a range of physiological mechanisms that confer resistance to many of the most commonly used antibiotics (Munita et al., 2016) Resistance to antibiotics results from developing one of three general mechanisms: (1) revoking the target to which antibiotic use to attach to the bacterial cell; (2) decreasing the permeability barrier of the bacterial cell envelope, which reduces the accumulation of antibiotics in the cell; and (3) producing enzymes that inactivate or export the antibiotic. The family of β-lactamases which degrade β-lactam ring antibiotics belongs to the third group (Babic et al., 2006). It is worth noting that within the family of more than 2800 β-lactamases a rapid evolution continues as individual enzymes undergo random mutation thus generating novel substrate specificities or protein features posing the greatest health concerns (Bush and Karen, 2018).

This research focused on a family of β-lactamase enzymes considered to be the most rapidly evolving type of proteins, the CTX-M-type extended spectrum β-lactamases (CTX-M type ESBL). Specifically, this research investigated the extent to which CTX-M-type ESBLs contribute to the resistance phenomenon detected in isolates from urinary tract infection (UTI) patients in North Wales. It was assessed whether these CTX-M producing bacteria carry the same or different CTX-M genes and the extent to which different CTX-M enzymes vary with regard to the antibiotic resistance spectrum.

The aim of the study was achieved in four main steps: (1) the analysis of UTI patient data from one of the main referral hospitals in North Wales in order to identify the risk factors associated with isolations of ESBL-producing *E. coli* in urine samples from hospitalised patients and outpatients; (2) by determining the contribution of CTX-M-type ESBL, and its dominant type, to antibiotic resistance in 300 ESBL-producing isolates obtained from UTI patients in three referral hospitals in North Wales; Ysbyty Gwynedd, Glan Clwyd Hospital, and Wrexham Maelor Hospital by testing the isolates with multiplex PCR using primers for phylogenic groups of CTX-M family followed by sequencing; (3) by cloning of dominant CTX-Ms in expression plasmids that produce C-terminally affinity tagged protein to facilitate its purification to obtain abundant amount of recombinant CTX-M protein to measure its catalytic activity against commonly used antibiotics for UTI patients; and (4) by determining minimal inhibitory concentrations (MIC) and catalytic activities of the dominant CTX-Ms against antibiotic used to treat UTIs compared to antibiotics used for routine ESBL screening.

7.1. The significantly higher incidence of UTIs in North Wales

Data from Ysbyty Gwynedd (YG) hospital in Bangor indicates a significantly higher number of patients referred for urinalysis due to symptoms of urinary tract infections over the three years of the study in comparison to other regions around the world with approximately the same population size and health care level. The suggested explanation for this high UTI incidence in North Wales is the high percentage of old people in the population of North Wales Which, according to Welsh Government services and information, is expected to rise from 18% (as determined in 2008) to almost 26 % of the Welsh population by 2033. This age-group is more likely to experience UTIs than younger individuals. Another explanation for the increased UTI prevalence in North Wales is the high percentage of rural communities with approximately 80% of Welsh land being managed as farmland, and the close proximity of these communityto antibiotic treated livestock. However, it should be remembered that AB usage is much reduced on the beef and sheep farms typical of North Wales (UK Veterinary Antibiotic Resistance and Sales Surveillance Report, 2019), making this less likely to be the main explanatory factor for our results.

7.2. Uropathogenic *E. coli* (UPEC) as causative organism of nosocomial UTIs

Conclusions of many studies (e.g., Kollef et al., 2016) indicate that uropathogenic *E. coli* represents less than 50% of pathogens causing nosocomial (hospital) acquired UTIs, while community acquired UTIs are caused predominantly by *E. coli*. The outcomes of this study indicates an increased spread of uropathogenic *E. coli* in hospitals as it represents 79% of the detected pathogens of hospital acquired UTIs. The possible reason behind this rise of uropathogenic *E. coli* in hospitals is the divergence of some *E. coli* strains from a commensal to pathogenic form inside the body through the acquisition of specific virulence factors via horizontal DNA transfer of plasmids, transposons, and bacteriophage. The transfer of genetic material also moves antibiotic resistance genes between bacteria thus increasing both the pathogenicity and resistance of *E. coli* (Gyles et al., 2014).

Unlike other uropathogenic bacteria such as *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis* and *Enterococcus* (Hof, 2017), *E. coli* is characterised by the presence of mobile genetic elements, pathogenicity islands that carry specialised virulence factors which provide *E.coli* with a high degree of genetic diversity (Wiles, 2008). The virulence factors of uropathogenic *E. coli* is associated with the development of UTIs making infections more complicated to treat. These factors can be divided into two groups: (1) surface virulence factors, which are characterised by various types of adhesive fimbriae that enable *E. coli* to attach within the urinary tract and (2) secreted virulence factors, which include the toxins produced by colonizing *E. coli* e.g. α-haemolysin which causes the inflammatory response that leads to the symptoms of UTIs (Bien, 2012).

7.3. Remarkable rise of antibiotic resistance conferred by ESBL-producing isolates

The precise definition of extended spectrum β-lactamase has not been agreed, but the commonly used definition is that ESBLs are a group of β-lactamases that are characterised by their broad hydrolytic activity against first, second, and third generations of cephalosporins, in addition to aztreonam, and can be inhibited only by β-lactamase inhibitors (Rahman et al., 2018). This makes ESBL-producing isolates a major problem for clinical therapeutics as many clinically used antibiotics contain a β-lactam ring as the functional element (Shakya et al., 2018).

The data analysed in this study can be added to other studies that show an alarming increase in the threat posed by antibiotic resistance conferred by ESBLs due to the two-fold increase in the number of ESBLs detected over the three years of this study, 2011-2013, (Chapter 3; Table 3.5). One of the possible reasons behind the rapid growth of ESBL-based resistance is the inaccurate phenotypic methods used for ESBL screening in clinical laboratories, e.g. the double-disc synergy test (Bajpai et al., 2017) which is unable to distinguish between approximately 200 types of ESBLs identified to date (Ghafourian et al., 2015). This means that routine screening methods are unable to determine the strain and gene responsible for the production of each ESBL enzyme, which are not all inhibited by the same antibiotic. In addition, ESBL genes are often found on low copy number IncF plasmids with multiple other resistance genes, such as the aminoglycoside/fluoroquinolones resistance conferring gene (Robberts et al., 2009). Together, these factors could lead to treatment failure in many cases due to the use of inappropriate antibiotic resulting in outbreaks of ESBL-based antibiotic resistance. Developing molecular-based techniques with the additional ability to detect low level genetic resistance for detection of ESBL in clinical diagnostic laboratories could decrease the false diagnosis of ESBLs and limit the rapid growing of ESBL-based resistance.

7.4 Dominant CTX-M-type ESBLs in North Wales

Screening of isolates diagnosed in the clinical laboratories of the main three referral hospital in North Wales as ESBL producers for CTX-M genes, using multiplex PCR followed by sequencing, revealed that the most detectable CTX-M among these isolates is CTX-M-15. This isolate represented more than half of all sequenced samples (Chapter 4, Tables: 4.2,4.4 and 4.6). This is a very large increase in CTX-M-15 prevalence in North Wales when compared to that found in previous studies e.g. Mushtaq et al., (2003) who only detected CTX-M-15 in the South and North of England, in addition to some regions of Northern Ireland. North Wales, was part of Mushtaqs et al' study.

Unlike other CTX-M variants, the *CTX-M-15* gene on the conjugative plasmid *IncF*, which facilitates its horizontal transfer by the conjugation process, provides an explanation for the high global prevalence of CTX-M-15 (Orhue et al., 2017).

Some studies (e.g. Laurent Poirel et al., 2013) have investigated the spread of ESBL-producing isolates among companion animals and found through the molecular screening of these isolates high rates of CTX-M-15 producing *Klebsiella pneumonia*. Acquiring resistance confirmed by CTX-M-15 from animal reservoirs is very likely in North Wales hospitals with its large farming community that lives in close proximity to food producing animals. Where resistance in animals are found the resistance plasmid may be transferred horizontally to human. Acquiring resistance genes from animal reservoirs has become an increasingly common practice over recent years and it is used for highly effective antibiotics like colistin. A discussion of this can be found in Wang R et al.'s study conducted in China, which detected the emergence and spreading of colistin resistance gene mcr-1 in pig isolates (Wang R et al., 2018)

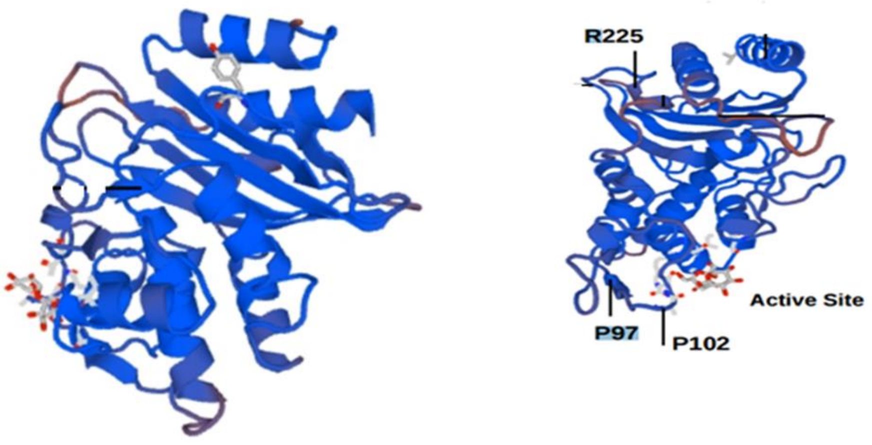
CTX-M-15 is characterised by its high activity against Ceftazidime (Po et al., 2017). The antibiotic profiles of the isolates carrying CTX-M-15 in one the three referral hospitals of the study (Ysbyty Gwynedd hospital at Bangor) shows resistance of these isolates also to other antibiotics like amoxicillin and ciprofloxacin that are known to inhibit the activity of CTX-M-15 (Chapter 3;Table 3.2). The existence of a novel mutation in the *CTX-M-15* gene which was selected for by the misuse of antibiotics for treatment of patients due to inaccurate phenotypic detection, could be the reason behind the development of resistance to these antibiotics.

7.5 The novel CTX-M-14 type ESBL revealed in North Wales

In addition to the spread of CTX-M-15 in the screened samples of this study, sequencing has revealed also a new β-lactamase gene that is closely related to *CTX-M-14* at the protein level, with the substitution of only three amino acids (T55A, A273P, and R227C) in the *CTX-M-14* gene. This novel β-lactamase was detected in approximately 11% of sequenced samples from one of the three hospitals of the study (Ysbyty Gwynedd, Bangor). The presence of this novel CTX-M at only one of three hospital sites included in this study supports the idea that the novel β-lactamase has been transferred horizontally on a plasmid vector from the farming community. Because the close similarity of the novel CTX-M to CTX-14, the novel gene was named *CTX-M-14-LIKE*.

CTX-M14 is the second most frequently reported globally after CTX-M-15. These two variations are classified under two different CTX-M groups: CTX-M group 1 and CTX-M-group 9, respectively (D'andrea et al., 2013). Figure 7.1 shows the 3D structure of the two CTXMs.

Figure 7.1 3D structure of CTX-M-14 and CTX-M-15



CTX-M-14 CTX-M-15

3D structure of CTX-M-14 and CTX-M-15 .The active site is indicated. Proline 97 and Proline 102 and arginine 225 are Key differences between CTXM-15 and CTXM-14 in otherwise highly conserved regions. The images were Generated using Swiss Model (**1ylp.1.A 99.62% identity**).

The second part of this study focused on the comparison between the two CTX-Ms (CTX-M-14 and CTX-M-14-like) in terms of antibiotic resistance and how the substitution between the two enzymes impact on their hydrolytic activity against antibiotics commonly used to treat patients with UTIs.

7.6 Production and purification of recombinant CTX-M proteins

In order to study the enzymatic activity of the dominant CTX-Ms (CTX-M-15 and CTX-M-14-LIKE) and to compare the catalytic activity of the novel β-lactamase (CTX-M-14-LIKE) with the activity of CTX-M-14, recombinant proteins of these CTX-Ms were required. These were obtained by the cloning of the full length CTX-M genes in the *E. coli* expression plasmid *pASK-IBA2C* which allows for the purification of C-terminally strep-tagged recombinant proteins after their induction from the periplasmic space. Among two strategies of protein secretion by pASK-IBA2C; cytosolic and periplasmic expression, the later strategy was used to produce proteins of the three CTX-Ms (CTX-M-15, CTX-M-14, and CTX-M-14-like) because it has several advantages. Among these are, the ability to obtain highly enriched protein extract due to the ease of extraction of proteins from the periplasmic space by osmotic shock that removes the outer membrane and to separate cytoplasmic host cell protease from the recombinant protein product. In addition, the periplasmic expression of the genes reduces aggregation or degradation of unfolded polypeptides as the protein that forms protein multimers is not exported (Van Den Berg et al., 2015).

The export of the recombinant CTX-M proteins from cytoplasm into the periplasmic space is led by a signal peptide included in the pASK-IBA2C plasmid, the OmpA (Outer Membrane Protein A) leader sequence which is fused in the N-terminus of the protein. The export of the CTX-M protein into the periplasmic space enhances their solubility and function as recombinant protein produced in the cytoplasm of *E. coli* predominantly forms insoluble and inactive inclusion bodies (Rosano et al., 2014).

The efficiency of the OmpA leader sequence in exporting the CTX-M protein to the periplasmic space has been checked by western blot analysis of induced cell pellet (i.e., Cytoplasmic produced recombinant protein) in ordered to verify the full exporting of recombinant CTX-M proteins to periplasmic space (Chapter 5 ,Figures;5.9,5.10 and 5.11). This analysis by western blot has shown bands with sizes corresponding to the expected size of the CTX-M protein of 33KD, which indicates that some of protein hasn't been exported despite the high yielded of recombinant CTX-M extracted from the periplasmic space. In addition, a band at approximately 60KD was detected in cell pellet analysed by western blot, this band could be dimeric 60KD which is insoluble and therefore in the pellet.

The produced recombinant CTX-M was C-terminally tagged with the short peptide Strep-Tag (Ala-Trp-Arg-His-Pro-Gln-Phe-Gly-Gly) to purify the protein from the periplasmic extract by its affinity to strep-tactin that is included in prepacked columns and allows for gravity-flow purification of the strep-tagged CTX-M proteins. The purity of the proteins was checked using Coomassie stain to detect additional protein in the preparations that is not Strep-tagged (Chapter 5; Figure 5.14).

7.7 Phosphorylation status analysis of the CTX-M proteins

Protein phosphorylation is responsible for a variety of protein properties, including protein stabilization and degradation, and some biochemical activities (Lin J et al., 2010). Phosphorylation of beta-lactamase produced in *E.coli* has been reported in many studies (e.g. phosphorylated TEM-b-lactamase was determined to be phosphorylated by threonine residue, which effects its secretion to periplasmic space) (Udo H et al. 1995). Analysing of CTX-M proteins sequences bioinformatically using the NetPhos tools revealed that CTX-M-14 and CTX-M-15 both possess some potential phosphorylation sites at serine, threonine, and tyrosine residues (Chapter 5; Figure 5.13). This provided the motivation to analyse the phosphorylation status of the purified CTX-M proteins using two techniques, Phos-tag gel and two dimensional (2D) gel analysis, in order to determine how de-phosphorylation could affect the enzymatic activity of CTX-M proteins against commonly used antibiotics.

Bands detected in the Phos-tag gels (as the only phosphorylated protein can be visualised in this gel as the modified protein moves slower) indicate phosphorylation of the three CTX-M proteins (CTX-M-15, CTX-M-14 and CTX-M-14-LIKE) (Chapter 5; Figure 5.15). This is supported by the detection of at least two isoforms with the expected size of CTX-M proteins (33KD) using western blot analysis of the distributed proteins after isoelectric focusing (2D gel analysis) (Chapter 5; Figure 5.14). It is noteworthy that the 2D technique detects all posttranslational modifications that affect the overall charge of the protein of which phosphorylation is only one form. But combined with the finding that the fraction of the recombinant protein runs more slowly in the presence of the phos-tag that is specific for phosphorylation. It is feasible to conclude that a sub fraction of the recombinant CTX-M protein is phosphorylated. It is however possible that the phosphorylation status of these proteins is a consequence of the overexpression in *E. coli*, which could be further investigated by testing the same proteins from the parental strains under endogenous conditions.

7.8 Comparative analysis of catalytic activity between CTX-M-14 and CTX-M-14-LIKE

Analysis of the catalytic activity of recombinant CTX-M-14 and CTX-M-14-LIKE was conducted in order to determine any marked difference in the minimal inhibitory concentration (MIC) and the kinetics of hydrolysis between these two enzymes to determine whether the three substitutions (T55A, A273P and R277C) change the substrate specificity of CTX-M-14 β-lactamase.

Comparison of the MIC values of strains expressing either CTX-M-14 or CTX-M-14-LIKE against selected antibiotics commonly used in the treatment of UTI patients shows that the three changes in the novel CTX-M (CTX-M-14-LIKE) render cells more resistant to Nitrofurantoin (MIC; ≥512 µg/mL with CTX-M-14-LIKE while ≥8 µg/mL with CTX-M-14) (Chapter 6;Table 6.1). An only two-fold increase in the affinity (Km) of CTX-M-14-LIKE compared to CTX-M-14 was found for Nitrofurantoin and one fold was found for Cefoxitin (chapter 6; Table 6.20). Although the affinity (Km) is independent on the amount of active protein in the in vitro test the maximal velocity (Vmax) is however dependent on the percentage of active protein in the purified preparation, it is difficult to conclude that a factor of two is a significant increase. The reason is that the graphical Laneweaver-Burk method has not such a high resolution.

Given that the affinities are not significantly different between CTX-M-14 and CTX-M-14-like for Nitrofurantoin; instead, MIC is 64-times higher with CTX-M-14-like than with CTXM-14, the three mutations that reside in the beta-strand domain opposite to the active site (Chapter 6; Figure 6.16) affect the conformational changes required for the effective hydrolysis of Nitrofurantoin thus increasing the turnover rate for this antibiotic. Another possibility is that the mutations increase the protein stability or amount in the endogenous coliform as shown for other mutations in β-lactamases (Jacquier et al., 2013).

Modelling the three amino acid substitutions onto the protein structure of CTX-M-14-like revealed that all three substitutions map to the domain opposite the active site. Interestingly, two of the substitutions A273P and R277C affect the large loop next to the active site which may have an effect on the catalytic cycle of the mutated enzyme Taken together these findings imply an indirect effect of the substitutions on the conformational changes or the structure of the enzyme.

The effects of amino acid substitutions on CTX-M β-lactamase hydrolytic activity has been demonstrated by many studies, with the focus of these studies mostly on amino acids located in active site (e.g. Patel et al., 2015). In contrast, few studies have looked into the effect of substitutions in residues distal from the active site on CTX-Ms catalytic activity. He D et al., (2016) detected some differences in residues between CTX-M-14 and one of its variants CTX-M-64 that are located mainly at both the C and N termini (e.g., V29A, L48A, V286A) . These substitutions are reported to increase the hydrolytic activity of CTX-M-14 variant CTX-M-64 toward some B-lactams due to its greater stability which was analysed by thermal stability assay.

The result presented here support the conclusions of the studies regarding the impact of distal residues on CTX-Ms hydrolytic cycle, but expand these findings to other antibiotic agent, most notably Nitrofurantoin. The enhanced activity of CTX-M-14-LIKE associated with the three revealed mutations (T55A, A273P and R277C) could be resulted from the greater integrity and stability of CTX-M-14-LIKE, which requires further investigation using thermal stability assays.

7.9 Conclusion

This study aimed to create an epidemiological snapshot of the antibiotic resistance conferred by CTX-M-type ESBL producing bacteria in UTI patients in North Wales and to determine to what extent do the different CTX-M enzymes vary with regard to the antibiotic resistance spectrum. The data obtained from the three study hospitals; Ysbyty Gwynedd, Glan Clwyd Hospital, and Wrexham Maelor Hospital indicate a significantly high incidence of UTIs in North Wales which may correlate with high percentage of rural communities in the region that live in close proximity to the antibiotic treated livestock.

Multiplex PCR amplification of β-lactamase genes of the *bla*CTX-M groups 1, 2, 9, and 8/25, followed by DNA sequencing of ESBL-producing isolates from three referral hospital in North Wales revealed that CTX-M-15 is the dominant variant of CTX-M found in UTI patients in the study region. In addition, this study revealed a new β-lactamase gene that is closely related to CTX-M-14 at the protein levels and was unique to Ysbyty Gwynedd at Bangor.

Antimicrobial susceptibility testing in addition to determinations on Km constants of CTX-M-14 and CTX-M-14-LIKE with antibiotics commonly used to treat UTI patients indicates enhanced activity of CTX-M-14-LIKE against Nitrofurantoin (significantly) and Cefoxitin (slightly). This is due to the effects of three substitutions A55T, A273P and R277C that reside distal to the active site. This strongly supports the conclusion that, in addition to active-site mutations, the hydrolytic cycle of CTX-M enzymes can be affected by distal residues which are most likely to be associated with the substrate turn-over rates and/or stability of the enzyme.

The impact of the study and the implications for public health policy

Since the most common clinical phenotypic detection methods currently in use are unable to provide detailed information about ESBL gene types, the main impact of this study is to motivate clinical laboratories to invest in the development of quick and accurate molecular techniques. These methods might include PCR-based assays for detecting the genes responsible for the resistance and an efficient molecular-based identification method for the detection of resistant species. This approach could also enable non-expressing ESBL genes to be detected, which would improve the ability of clinicians to diagnose and treat antibiotic resistance UTIs, in addition to creating an epidemiological snapshot of the antibiotic resistance pattern in UTI patients in North Wales and potentially globally.

7.10 Future work

One of the limitations of this study is the difficulty in obtaining clinical samples and data from the study hospitals, which limits the number of samples used in the study. Further, test of Nitrofurantoin resistant isolates will be a priority of future work in order to check whether the novel CTX-M protein exists in the general population of Wales and to investigate the origin of this gene, which to date has only been detected in one of the three study hospitals (Ysbyty Gwynedd). In addition, more information is required on the background of the patients whose samples were provided. This could be difficult to obtain due to issues of data protection and patient anonymity, but at a minimum it would be useful to distinguish "rural" from "urban" samples as this would help to test hypotheses regarding why there was a disproportionately high prevalence of patients with AB-resistant UTIs. Determination of CTX-M-14-LIKE hydrolytic activity against more β-lactam antibiotics should also be a priority for future work.

The phosphorylation analysis of the recombinant CTX-M was promising and further investigation of the phosphorylation status by testing the same proteins from the parental strains under physiological conditions (i.e. not under recombinant conditions) should be a main focus of subsequent studies. This could be achieved by a proteomics approach sequencing endogenous material from Nitrofurantoin resistant isolates. If post-translational modifications also exist, phosphor-specific antibodies could be generated to continue the study under physiological conditions. Further investigation should be conducted to determine how the three revealed residues that are located distal to the active site of CTX-M-14-LIKE result in higher catalytic activity. This work should use thermal stability assays to determine whether these three distal amino acid substitutions can affect the integrity and stability of the protein.

References

Abraham, E.P. & Chain, E. 1988. An enzyme from bacteria able to destroy penicillin. 1940. *Review of Infectious Diseases*. 10 (4). pp. 677.

Ackerman, S. & Gatti, D.L. 2013. Biapenem inactivation by B2 metallo β-lactamases: energy landscape of the hydrolysis reaction. *PloS One*. 8 (1). e55136. doi: 10.1371/journal.pone.0055136.

Agriculture in the United Kingdom 2018 Available at: https://www.gov.uk/government/statistics/agriculture-in-the-united-kingdom-2018

Al Yousef, S.A., Younis, S., Farrag, E., Moussa, H., Bayoumi, F. & Ali, A. 2016. Clinical and laboratory profile of urinary tract infections associated with extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. *Annals of Clinical & Laboratory Science*. 46 (4). pp. 393-400.

Ali, T., Ali, I., Khan, N.A., Han, B. & Gao, J. 2018. The growing genetic and functional diversity of extended spectrum beta-lactamases. *BioMed Research International*. 2018. 9519718. doi: 10.1155/2018/9519718.

Antunes, N.T., Lamoureaux, T.L., Toth, M., Stewart, N.K., Frase, H. & Vakulenko, S.B. 2014. Class D β-lactamases: Are they all carbapenemases? *Antimicrobial Agents and Chemotherapy*. 58 (4). pp. 2119-2125. doi: 10.1128/AAC.02522-13.

Aslam, B., Wang, W., Arshad, M., Khurshid, M., Muzammil, S. & Rasool, M.H. 2018. Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*. 11. pp. 1645-1658. doi: 10.2147/IDR.S173867.

Babic, M., Hujer, A. M. & Bonomo, R. A. 2006. What's new in antibiotic resistance? Focus on β-lactamases. *Drug Resistance Update*. 9. pp. 142-156.

Bajpai, T., Pandey, M., Varma, M. & Bhatambare, G. 2017. Prevalence of TEM, SHV, and CTX-M beta-lactamase genes in the urinary isolates of a tertiary care hospital. *Avicenna Journal of Medicine*. 7 (1). pp. 12-16. doi: 10.4103/2231-0770.197508.

Bakkali, M. 2013. Could DNA uptake be a side effect of bacterial adhesion and twitching motility? *Archives of Microbiology*. 195 (4). pp. 279-289. doi: 10.1007/s00203-013-0870-1.

Bañuelos-Vazquez, L.A., Tejerizo, G.T. & Brom, S. 2017. Regulation of conjugative transfer of plasmids and integrative conjugative elements. *Plasmid*. 91. pp. 82-89. doi: 10.1016/j.plasmid.2017.04.002.

Baumgarten, T., Ytterberg, A.J., Zubarev, R.A. & de Gier, J.W. 2018. Optimizing recombinant protein production in the *Escherichia coli* periplasm alleviates stress. *Applied Environmental Microbiology*. 84 (12). pp. e00270-18. doi: 10.1128/AEM.00270-18.

Beahm, N.P., Nicolle, L.E., Bursey, A., Smyth, D.J. & Tsuyuki, R.T. 2017. The assessment and management of urinary tract infections in adults: Guidelines for pharmacists. *Canadian Pharmacists Journal / Revue des Pharmaciens du Canada*. 150 (5). pp. 298-305. doi: 10.1177/1715163517723036.

Belousoff, M.J., Eyal, Z., Radjainia, M., Ahmed, T., Bamert, R.S., Matzov, D., Bashan, A., Zimmerman, E., Mishra, S., Cameron, D. & Elmlund, H. 2017. Structural basis for linezolid binding site rearrangement in the *Staphylococcus aureus* ribosome. *MBio*. 8 (3). pp. e00395-17. doi: 10.1128/mBio.00395-17.

Bernardini, S., Tiezzi, A., Laghezza Masci, V. & Ovidi, E. 2018. Natural products for human health: an historical overview of the drug discovery approaches. *Natural Product Research.* 32 (16). pp. 1926-1950. doi: 10.1080/14786419.2017.1356838.

Bevan, E.R., Jones, A.M. & Hawkey, P.M. 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *Journal of Antimicrobrial Chemotherapy*. 72 (8). pp. 2145-2155. doi: 10.1093/jac/dkx146.

Bien, J., Sokolova, O. & Bozko, P. 2012. Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *International Journal of Nephrology*. 2012. 681473. doi: 10.1155/2012/681473.

Binda, E., Marinelli, F. & Marcone, G. 2014. Old and new glycopeptide antibiotics: action and resistance. *Antibiotics*. 3 (4). pp. 572-594. doi: 10.3390/antibiotics3040572.

Blaak, H., Hamidjaja, R.A., van Hoek, A.H., de Heer L., de Roda Husman, A.M. & Schets, F.M. 2014. Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. *Applied Environmental Microbiology*. 80 (1). 239. doi: 10.1128/AEM.02616-13.

Blokesch, M. 2016. Natural competence for transformation. *Current Biology*. 26 (21). pp. R1126-R1130. doi: 10.1016/j.cub.2016.08.058.

Bonardi, S., Cabassi, C.S., Longhi, S., Pia, F., Corradi, M., Gillioli, S. & Scaltriti, E. 2018. Detection of extended- spectrum beta-lactamase producing from mesenteric lymph nodes of wild boars (*Sus scrofa*). *Italian Journal of Food Safety*. 7 (4). 7707. doi: 10.4081/ijfs.2018.7707.

Bonnet, R. 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrobial Agents and Chemotherapy*. 48 (1). pp. 1–14. doi:10.1128/aac.48.1.1-14.2004

Bonomo, R.A. 2017. β-lactamases: A focus on current challenges. *Cold Spring Harbor Perspectives in Medicine*. 7 (1). a025239. doi: 10.1101/cshperspect.a025239.

Bradford, P.A. 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews*. 14 (4),. pp.933–951. doi:10.1128/CMR.14.4.933-951.2001

Brandt, C., Makarewicz, O., Fischer, T., Stein, C., Pfeifer, Y., Werner, G. & Pletz, M.W. 2014. The bigger picture: the history of antibiotics and antimicrobial resistance displayed by scientometric data. *International Journal of Antimicrobial Agents*. 44 (5). pp. 424-430. doi: 10.1016/j.ijantimicag.2014.08.001.

Bratzler, D.W., Dellinger, E.P., Olsen, K.M., Perl, T.M., Auwaerter, P.G., Bolon, M.K., Fish, D.N., Napolitano, L.M., Sawyer, R.G., Slain, D. & Steinberg, J.P. 2013. Clinical practice guidelines for antimicrobial prophylaxis in surgery. *American Journal of Health-System Pharmacy*. 70 (3). pp. 195-283. doi: 10.2146/ajhp120568.

Brem, J., Cain, R., Cahill, S., McDonough, M.A., Clifton, I.J., Jiménez-Castellanos, J.C., Avison, M.B., Spencer, J., Fishwick, C.W. and Schofield, C.J. 2016. Structural basis of metallo-β-lactamase, serine-β-lactamase and penicillin-binding protein inhibition by cyclic boronates. *Nature Communications*. 7 (1). pp. 12406. doi: 10.1038/ncomms12406.

Brownie J, Shawcross S, Theaker J, Whitcombe D, Ferrie R, Newton C, Little S. 1997. The elimination of primer-dimer accumulation in PCR. Nucleic Acids Res;25:3235–3241.

Bush, K. & Bradford, P.A. 2016. β-lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbor Perspectives in Medicine*. 6 (8). A025247. doi: 10.1101/cshperspect.a025247.

Bush, K. & Jacoby, G.A. 2010. Updated functional classification of β-lactamases. Antimicrobial *Agents and Chemotherapy*. 54 (3). pp. 969-976. doi: 10.1128/AAC.01009-09.

Bush, K. 2018. Past and present perspectives on β-lactamases. Antimicrobial Agents and *Chemotherapy*. 62 (10). pp. e01076-18. doi: 10.1128/AAC.01076-18.

Cantón, R., González-Alba, J.M. & Galán, J.C. 2012. CTX-M enzymes: origin and diffusion. *Frontiers in Microbiology*. 3. p.110.

Casjens, S. 2003. Prophages and bacterial genomics: what have we learned so far? *Molecular Microbiology*. 49 (2). pp. 277-300. doi: 10.1046/j.1365-2958.2003.03580.x.

Chandra, S. 2013. Extended-spectrum beta-lactamase infections. *Current Emergency and Hospital Medicine Reports*. 1 (3). pp. 145-148. doi: 10.1007/s40138-013-0021-7.

Chaudhry, S.B., Veve, M.P. & Wagner, J.L. Cephalosporins: a focus on side chains and β-lactam cross-reactivity. *Pharmacy*. 7 (3). pp. 103. doi: 10.3390/pharmacy7030103.

Chaves, J., Ladona, M.G., Segura, C., Coira, A., Reig, R. & Ampurdanes, C. 2001. SHV-1 β-lactamase is mainly a chromosomally encoded species-specific enzyme *in Klebsiella pneumoniae*. *Antimicrobial Agents & Chemotherapy*. 45 (10). pp. 2856-2861. doi: 10.1128/AAC.45.10.2856-2861.2001

Cho, Y.H. & Kim, H.W. 2010. UP-2.53: Correlation between post-void residual urine volume and urinary tract infection in asymptomatic men visited for prostate examination. *Urology*. 76 (3). pp. S99. doi: 10.1016/j.urology.2010.07.287.

Climate Change, Environment and Rural Affairs Committee. 2017. The future of land management in Wales. Available at: https://www.assembly.wales/laid%20documents/cr-ld10995/cr-ld10995-e.pdf

Codjoe, F.S. & Donkor, E.S. 2017. Carbapenem resistance: a review. *Medical Sciences*. 6 (1). pp. 1. doi: 10.3390/medsci6010001.

Coertze, R.D. & Bezuidenhout, C.C. 2018. The prevalence and diversity of AmpC β-lactamase genes in plasmids from aquatic systems. *Water Science and Technology*. 2017 (2). pp. 603-611. doi: 10.2166/wst.2018.188.

Croucher, N.J., Mostowy, R., Wymant, C., Turner, P., Bentley, S.D. & Fraser, C. 2016. Horizontal DNA transfer mechanisms of bacteria as weapons of intragenomic conflict. *PLoS Biology*. 14 (3). pp. e1002394. doi: 10.1371/journal.pbio.1002394.

Dallenne C, Da Costa A, Decré D, Favier C, Arlet G.2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae . J Antimicrob Chemother.,65, pp.490-495.

De Sousa, J.M., Alsaadi, A., Haaber, J., Ingmer, H. & Rocha, E.P. 2018. Modelling phage-bacteria interactions driving predation and horizontal gene transfer. *BioRxiv*. pp. 291328. doi: 10.1101/291328.

Delmas, J., Chen, Y., Prati, F., Robin, F., Shoichet, B.K. & Bonnet, R. 2008. Structure and dynamics of CTX-M enzymes reveal insights into substrate accommodation by extended-spectrum β-lactamases. *Journal of Molecular Biology*. 375 (1). pp. 192-201. doi: 10.1016/j.jmb.2007.10.026.

Drawz, S.M. & Bonomo, R.A. 2010. Three decades of β-lactamase inhibitors. *Clinical Microbiology Reviews*. 23 (1). pp. 160–201. doi:10.1128/CMR.00037-09

Drieux, L., Brossier, F., Sougakoff, W. & Jarlier, V. 2008. Phenotypic detection of extended-spectrum β-lactamase production in *Enterobacteriaceae*: review and bench guide. *Clinical Microbiology Infection*. 14. pp. 90–103.

Durojaiye, C.O. & Healy, B. 2015. Urinary tract infections: Diagnosis and management. *Prescriber*. 26 (11). pp. 21-29. doi: 10.1002/psb.1362.

D'andrea MM, Arena F, Pallecchi L, Rossolini GM. CTX-M-type β-lactamases: A successful story of antibiotic resistance. International Journal of Medical Microbiology. 2013;303(6-7):305-317. doi: 10.1016.

Edoo, Z., Arthur, M. & Hugonnet, J.E. 2017. Reversible inactivation of a peptidoglycan transpeptidase by a β-lactam antibiotic mediated by β-lactam-ring recyclization in the enzyme active site. *Scientific Reports*. 7 (1). pp. 9136. doi: 10.1038/s41598-017-09341-8.

El-Gamal, M.I., Brahim, I., Hisham, N., Aladdin, R., Mohammed, H. & Bahaaeldin, A. 2017. Recent updates of carbapenem antibiotics. *European Journal of Medicinal Chemistry*. 131. pp. 185-195. doi: 10.1016/j.ejmech.2017.03.022.

Elnifro, E. M., Ashshi, A. M., Cooper, R. J., & Klapper, P. E. 2000. Multiplex PCR: optimization and application in diagnostic virology. Clinical microbiology reviews, 13(4), 559–570. https://doi.org/10.1128/cmr.13.4.559-570.2000.

Epand, R.M., Walker, C., Epand, R.F. & Magarvey, N.A. 2016. Molecular mechanisms of membrane targeting antibiotics. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 1858 (5). pp. 980-987. doi: 10.1016/j.bbamem.2015.10.018.

EUCAST (European Committee on Antimicrobial Susceptibility Testing). 2018. Breakpoint tables for interpretation of MICs and zone diameters. Available at: http://www.eucast.org (accessed 1 June 2018).

Evans, B.A. & Amyes, S.G. 2014. OXA β-lactamases. *Clinical Microbiology Reviews*. 27 (2). pp. 241–263. doi: 10.1128/CMR.00117-13

Faheem, M., Rehman, M.T., Danishuddin, M. & Khan, A.U. 2013. Correction: Biochemical characterization of CTX-M-15 from *Enterobacter cloacae* and designing a novel non-β-lactam-β-lactamase inhibitor. *PLoS One*. 8 (2). e56926. doi: 10.1371/annotation/049bf1aa-d866-471f-95c1-5939d4461f8c.

Feng H, Liu X, Wang S, Fleming J, Wang D, Liu W. 2017. The mechanism of NDM-1-catalyzed carbapenem hydrolysis is distinct from that of penicillin or cephalosporin hydrolysis. Nat Commun. 8(1):2242. doi: 10.1038/s41467-017-02339-w.

Flores, A.L., Walker, J.N., Caparon, M. & Hultgren, S.J. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 13 (5). pp. 269-284. doi: 10.1038/nrmicro3432.

Foxman, B. 2014. Urinary tract infection syndromes occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious Disease Clinics of North America*. 28. pp. 1–13. doi: 10.1016/j.idc.2013.09.003.

Gąciarz, A., Khatri, N.K., Velez-Suberbie, M.L., Saaranen, M.J., Uchida, Y., Keshavarz-Moore, E. & Ruddock, L.W. 2017. Efficient soluble expression of disulfide bonded proteins in the cytoplasm of *Escherichia coli* in fed-batch fermentations on chemically defined minimal media. *Microbial cell Factories.* 16 (1). pp. 108. doi: 10.1186/s12934-017-0721-x.

Gardiner, B.J., Stewardson, A.J., Abbott, I.J. & Peleg, A.Y. 2019. Nitrofurantoin and fosfomycin for resistant urinary tract infections: Old drugs for emerging problems. *Australian Prescriber.* 42 (1). pp. 14. doi: 10.18773/austprescr.2019.002.

Gozi KS, Froes JR, Deus ALPT, et al. Dissemination of multidrug-resistant commensal Escherichia coli in feedlot lambs in Southeastern Brazil. Front Microbiol. 2019;10:1394.

Goh, H.C., Sobota, R.M., Ghadessy, F.J. & Nirantar, S. 2017. Going native: complete removal of protein purification affinity tags by simple modification of existing tags and proteases. *Protein Expression and Purification*. 129. pp. 18-24. doi: //doi.org/10.1016/j.pep.2016.09.001.

Gilchrist T.1987.Heterocyclic Chemistry. Harlow: Longman Scientific. ISBN 978-0-582-01421-3.

Goodman, C.D., Pasaje, C.F.A., Kennedy, K., Mcfadden, G.I. & Ralph, S.A. 2016. Targeting protein translation in organelles of the Apicomplexa. *Trends in parasitology*. 32 (12). pp. 953-965. doi: 10.1016/j.pt.2016.09.011.

Gonzalez-Estrada A, Radojicic C. Penicillin allergy: A practical guide for clinicians. Cleve Clin J Med. 2015;82(5):295-300. doi: 10.3949/ccjm.82a.14111.

Grigorenko, V., Uporov, I., Rubtsova, M., Andreeva, I., Shcherbinin, D., Veselovsk, A., Serova, O., Ulyashova, M. Ishtubaev, I. & Egorov, A. 2018. Mutual influence of secondary and key drug-resistance mutations on catalytic properties and thermal stability of TEM-type β-lactamases. *FEBS Open Bio.* 8 (1). pp. 117-129. doi: 10.1002/2211-5463.12352.

Grohmann, E., Muth, G. & Espinosa, M. 2003 Conjugative plasmid transfer in Gram-positive bacteria. *Microbiology and Molecular Biology Reviews*. 67 (2). pp. 277-301. doi: 10.1128/MMBR.67.2.277-301.2003.

Gupta, G., Tak, V. & Mathur, P. 2014. Detection of AmpC β lactamases in Gram-negative bacteria. *Journal of Laboratory Physicians*. 6 (1). pp. 1-6. doi: 10.4103/0974-2727.129082.

Gwon, B., Yoon, E.J., Kim, D., Lee, H., Shin, J.H., Shin, J.H., Shin, K.S., Kim, Y.A., Uh, Y., Kim, H.S. & Kim, Y.R. 2019. Differences in Antimicrobial Resistance Phenotypes by the Group of CTX-M Extended-Spectrum β-Lactamase. *Annals of Clinical Microbiolology*. 22 (1). pp. 1-8.

Gyles, C & Boerlin, P. 2014. Horizontally transferred genetic elements and their role in pathogenesis of bacterial disease. *Veterinary Pathology*. 51 (2). pp. 328-340. doi: 10.1177/0300985813511131.

Hangas, A., Aasumets, K., Kekäläinen, N.J., Paloheinä, M., Pohjoismäki, J.L., Gerhold, J.M. & Goffart, S. 2018. Ciprofloxacin impairs mitochondrial DNA replication initiation through inhibition of topoisomerase 2. *Nucleic Acids Research*. 46 (18). pp. 9625-9636. doi: 10.1093/nar/gky793.

Hannan, T.J., Totsika, M., Mansfield, K.J., Moore, K.H., Schembri, M.A. & Hultgren, S.J. 2012. Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic *Escherichia coli* bladder infection. *FEMS Microbiology Review*. 36 (3). pp. 616-648. doi: 10.1111/j.1574-6976.2012.00339.x.

Harada, L.K., Silva, E.C., Campos, W.F., Del Fiol, F.S., Vila, M., Dąbrowska, K., Krylov, V.N. & Balcão, V.M. 2018. Biotechnological applications of bacteriophages: state of the art. *Microbiological Research*. 212. pp. 38-58. doi: 10.1016/j.micres.2018.04.007.

Harms, A., Maisonneuve, E. & Gerdes, K. 2016. Mechanisms of bacterial persistence during stress and antibiotic exposure. *Science*. 354 (6318). pp. aaf4268. doi: 10.1126/science.aaf4268.

Hawkey, P.M. & Livermore, D.M. 2012. Carbapenem antibiotics for serious infections. *British Medical Journal*. 344 (7863). pp. doi: e3236. 10.1136/bmj.e3236.

He, D., Chiou, J., Zeng, Z., Liu, L., Chen, X., Zeng, L., Chan, E.W.C., Liu, J.H. & Chen, S. 2015. Residues distal to the active site contribute to enhanced catalytic activity of variant and hybrid β-lactamases derived from CTX-M-14 and CTX-M-15. *Antimicrobial Agents and Chemotherapy*. 59 (10). pp. 5976-5983.

He D, Chiou J, Zeng Z, Chan EW, Liu J, Chen S. 2016. Comparative characterization of CTX-M-64 and CTX-M-14 provides insights into the structure and catalytic activity of the CTX-M class of enzymes. Antimicrob Agents Chemother. 60(10):6084. doi: 10.1128/AAC.00917-16

Hof, H. 2017. Candiduria! What now? Therapy of urinary tract infections with Candida. *Urologe*. 56. pp. 172–179. doi: doi.org/10.1007/s00120-016-0219-x

Holmes, A.H., Moore, L.S., Sundsfjord, A.,, Steinbakk, M., Regmi, S., Karkey, A., Guerin, P.J. & Piddock, L.J. 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*. 387 (10014). pp. 176-187. doi: 10.1016/S0140-6736(15)00473-0.

Hong, E., Deghmane, A. & Taha, M. 2018. Acquisition of beta-lactamase by *Neisseria meningitidis* through possible horizontal gene transfer. *Antimicrobial Agents and Chemotherapy*. 62 (9). pp. e00831-18. doi: 10.1128/AAC.00831-18.

Hong, W., Zeng, J. & Xie, J.P. 2014. Antibiotic drugs targeting bacterial RNAs. *Acta Pharmaceutica Sinica B*. 4 (4). pp. 258-265. doi: 10.1016/j.apsb.2014.06.012.

House, J., Barnes, V.L., Ersoy, S.C., Heithoff, D.M., Mahan, M.J., Marth. M.J., Smith, J. & Tripp, G. 2017. Correcting a fundamental flaw in the paradigm for antimicrobial susceptibility testing. *EBioMedicine*. 20. pp. 173-181. doi: 10.1016/j.ebiom.2017.05.026.

Irrgang, A., Falgenhauer, L., Fischer, J., Ghosh, H., Guiral, E., Guerra, B., Schmoger, S., Imirzalioglu, C., Chakraborty, T., Hammerl, J.A. & Käsbohrer, A. 2017. CTX-M-15-producing *E. coli* isolates from food products in Germany are mainly associated with an IncF-type plasmid and belong to two predominant clonal lineages. *Frontiers in Microbiology*. 8, pp. 2318. doi: 10.3389/fmicb.2017.02318.

Jacquier H, Birgy A, Le Nagard H, et al. 2013.Capturing the mutational landscape of the beta-lactamase TEM-1. Proc Natl Acad Sci U S A.110(32):13067. doi: 10.1073/pnas.1215206110.

Jelić, D. & Antolović, R. 2016. From erythromycin to azithromycin and new potential ribosome-binding antimicrobials. *Antibiotics*. 5 (3). pp. 29. doi: 10.3390/antibiotics5030029.

Jeon, J., Lee, J., Lee, J., Park, K., Karim, A., Lee, C.R., Jeong, B. & Lee, S. 2015. Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. *International Journal of Molecular Sciences*. 16 (5). pp. 9654-9692. doi: 10.3390/ijms16059654.

Jhang, J.F. & Kuo, H.C. 2017. Recent advances in recurrent urinary tract infection from pathogenesis and biomarkers to prevention. *Tzu-Chi Medical Journal*. 29 (3). pp. 131-137. doi: 10.4103/tcmj.tcmj_53_17.

Johnston, C., Martin, B., Fichant, G., Polard, P. & Claverys, JP. 2014. Bacterial transformation: distribution, shared mechanisms and divergent control. *Nature Reviews Microbiology*. 12(3). pp. 181. doi: 10.1038/nrmicro3199.

Katzung, Bertram; et al. (2015). Basic & Clinical Pharmacology. New York: McGraw Hill Education. pp. 776–778. ISBN 978-0-07-182505-4.

Khan, Z.A., Siddiqui, M.F. & Park, S. 2019. Current and Emerging Methods of Antibiotic Susceptibility Testing. *Diagnostics*. 9 (2). pp. 49. doi:10.3390/diagnostics9020049

Kim, J. 2017. Clinical significance of extended-spectrum beta-lactamase producing bacteria in childhood first febrile urinary tract infection and its differences between age groups. *Pediatric Nephrology*. 32 (9). pp. 1770-1771.

Kim, M.H. & Lee, J.M. 2014. Diagnosis and management of immediate hypersensitivity reactions to cephalosporins. *Allergy, Asthma & Immunology Research*. 6 (6). pp. 485-495. doi: 10.4168/aair.2014.6.6.485.

Knudsen, P.K., Gammelsrud, K.W., Alfsnes, K.W., Steinbakk, M., Abrahamsen, T.G., Müller, F. & Bohlin, J. 2018. Transfer of a bla CTX-M-1-carrying plasmid between different *Escherichia coli* strains within the human gut explored by whole genome sequencing analyses. *Scientific Reports*. 8 (1). 280. doi: 10.1038/s41598-017-18659-2.

Kocaoglu, O. & Carlson, E.E. 2015. Profiling of β-lactam selectivity for penicillin-binding proteins in *Escherichia coli* strain DC2. *Antimicrobial Agents and Chemotherapy*. 59 (5). pp. 2785-2790. doi: 10.1128/AAC.04552-14.

Koksal, E., Tulek, N., Sonmezer, M.C., Temocin, F., Bulut, C., Hatipoglu, C., Erdinc, F.S. & Ertem, G. 2019. Investigation of risk factors for community-acquired urinary tract infections caused by extended-spectrum beta-lactamase *Escherichia coli* and *Klebsiella* species. *Investigative and Clinical Urology*. 60 (1). pp. 46-53. doi: 10.4111/icu.2019.60.1.46.

Kosobokova, E., Skrypnik, K. & Kosorukov, V. 2016. Overview of fusion tags for recombinant proteins. *Biochemistry Moscow*. 81 (3). pp. 187-200. doi: 10.1134/S0006297916030019.

Kuralayanapalya, S.P., Patil, S.S., Hamsapriya, S., Shinduja, R., Roy, P. & Amachawadi, R.G. 2019. Prevalence of extended-spectrum beta-lactamase producing bacteria from animal origin: A systematic review and meta-analysis report from India. *PloS One*. 14 (9). e0221771. doi: 10.1371/journal.pone.0221771.

Kucheria R, Dasgupta P, Sacks SH, Khan MS, Sheerin NS. 2005. Urinary tract infections: new insights into a common problem. Postgrad. Med. J. 81:83–86. doi:10.1136/pgmj.2004.023036.

Lagier, J.C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M. and Raoult, D. 2015. Current and past strategies for bacterial culture in clinical microbiology. *Clinical Microbiology Reviews*. 28 (1). pp. 208-236. doi: 10.1128/CMR.00110-14

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H. & Greko, C. 2013. Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases*. 13 (12). pp. 1057-1098. doi: 10.1016/S1473-3099(13)70318-9.

Lee, D., Das, S., Dawson, N.L., Dobrijevic, D., Ward, J. & Orengo, C. 2016. Novel computational protocols for functionally classifying and characterising serine beta-lactamases. *PloS Computational Biology*. 12 (6). pp. e1004926. doi: 10.1371/journal.pcbi.1004926.

Leigue, L., Montiani-Ferreira, F. & Moore, B.A. 2016. Antimicrobial susceptibility and minimal inhibitory concentration of isolated from septic ocular surface disease in different animal species. *Open Veterinary Journal*. 6 (3). pp. 215-222. doi: 10.4314/ovj.v6i3.9.

Lewis, J.A., Moore, P.C.L., Arnold, D.L. & Lawrance, L.M. 2015. Chromosomal ampC mutations in cefpodoxime-resistant, ESBL-negative uropathogenic *Escherichia coli. British Journal of Biomedical Science.* 2015;72(1):7-11. doi: 10.1080/09674845.2015.11666789.

Liakopoulos, A., Mevius, D. & Ceccarelli, D. 2016. A review of SHV extended-spectrum β-lactamases: neglected yet ubiquitous. *Frontiers In Microbiology.* 7 (1374). doi: 10.3389/fmicb.2016.01374.

Lin, J., Nishino, K., Roberts, M.C., Tolmasky, M., Aminov, R.I. & Zhang, L. 2015. Mechanisms of antibiotic resistance. *Frontiers in Microbiology.* 6. pp. 34. doi: 10.3389/fmicb.2015.00034.

Lin J, Xie Z, Zhu H, Qian J.2010.Understanding protein phosphorylation on a systems level. Briefings in functional genomics.9 (1):32-42. doi: 10.1093/bfgp/elp045.

Lisa, M.N., Palacios, A.R., Aitha, M., González, M.M., Moreno, D.M., Crowder, M.W., Bonomo, R.A., Spencer, J., Tierney, D.L., Llarrull, L.I. & Vila, A.J. 2017. A general reaction mechanism for carbapenem hydrolysis by mononuclear and binuclear metallo-β-lactamases. *Nature Communications*. 8 (1). pp. 538. doi: 10.1038/s41467-017-00601-9.

Llor, C. & Bjerrum, L. 2014. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *Therapeutic Advances in Drug Safety*. 5 (6). pp. 229-241. doi: 10.1177/2042098614554919.

Lobanovska, M. & Pilla, G. 2017. Focus: Drug Development: Penicillin's Discovery and Antibiotic Resistance: Lessons for the Future? *The Yale Journal of Biology and Medicine*. 90 (1). pp. 135-145.

Long, H., Miller, S.F., Strauss, C., Zhao, C., Cheng, L., Ye, Z., Griffin, K., Te, R., Lee, H., Chen, C.C. & Lynch, M. 2016. Antibiotic treatment enhances the genome-wide mutation rate of target cells. *Proceedings of the National Academy of Sciences*. 113 (18). pp. E2498-E2505. doi: 10.1073/pnas.1601208113.

Lowman W. 2018. Minimum inhibitory concentration-guided antimicrobial therapy - the Achilles heel in the antimicrobial stewardship agenda. *South African Medical Journal*. 108 (9). pp. 710-712. doi: 10.7196/SAMJ.2018.v108i9.13285.0

Madec, J.Y., Haenni, M., Nordmann, P. & Poirel, L. 2017. Extended-spectrum β-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? *Clinical Microbiology and Infection*. 23 (11). pp. 826-833. doi: 10.1016/j.cmi.2017.01.013.

Magliano, E., Grazioli, V., Deflorio, L, Leuci, A.I., Mattina, R., Romano, O. & Cocuzza, C.E. 2012. Gender and age-dependent etiology of community-acquired urinary tract infections. *The Scientific World Journal*. 2012. doi: 10.1100/2012/349597.

Manageiro, V., Ferreira, E., Cougnoux, A., Albuquerque ,L., Cançia, M. & Bonnet, R. 2012. Characterization of the inhibitor-resistant SHV β-lactamase SHV-107 in a clinical Klebsiella pneumoniae strain coproducing GES-7 enzyme. *Antimicrobial Agents & Chemotherapy*. 56 (2). pp.1042-1046. doi: 10.1128/AAC.01444-10.

Masi, M., Réfregiers, M., Pos, K.M. & Pagès, J.M. 2017. Mechanisms of envelope permeability and antibiotic influx and efflux in Gram-negative bacteria. *Nature Microbiology*. 2 (3). pp. 17001. doi: 10.1038/nmicrobiol.2017.1.

Matthew, M., Hedges, R.W. & Smith, J.T. 1979. Types of β-lactamase determined by plasmids in gram-negative bacteria. *Journal of Bacteriology*. 138. pp. 657–662.

Matzov, D., Eyal, Z., Benhamou, R.I., Shalev-Benami, M., Halfon, Y., Krupkin, M., Zimmerman, E., Rozenberg, H., Bashan, A., Fridman, M. & Yonath, A. 2017. Structural insights of lincosamides targeting the ribosome of *Staphylococcus aureus*. *Nucleic Acids Research*. 45 (17). pp. 10284-10292. doi: 10.1093/nar/gkx658.

McKinnnel, J.A. & Miller, L.G. 2011. Nitrofurantoin: Preferred empiric therapy for community-acquired lower urinary tract infections. *Mayo Clinic Proceedings*. 86 (12). 1244. doi: 10.4065/mcp.2011.0411.McOsker, C.C. & Fitzpatrick, P.M. 1994. Nitrofurantoin:

Meletis, G. 2016. Carbapenem resistance: overview of the problem and future perspectives. *Therapeutic Advances in Infectious Disease*. 3 (1). pp. 15-21. doi: 10.1177/2049936115621709.

Micali S., Isgro G., Bianchi G., Miceli N., Calapai G. & Navarra M. 2014. Cranberry and recurrent cystitis: more than marketing? *Critical Reviews in Food Science and Nutrition.* 54(8). pp. 1063–1075. doi: doi.org/10.1080/10408398.2011.625574

Miller EL. 2002. The penicillins: a review and update. *Journal of Midwifery and Women's Health*. 47 (6). pp. 426-434. doi: 10.1016/S1526-9523(02)00330-6.

Mishra, B., Srivastava, R., Agarwal, J., Srivastava, S. & Pandey, A. 2016. Behavioral and psychosocial risk factors associated with first and recurrent cystitis in Indian women: A case-control study. *Indian Journal of Community Medicine*. 41 (1). pp. 27. doi: 10.4103/0970-0218.170962.

Moreira, M.A.S., Souza, E.C.D. & Moraes, C.A.D. 2004. Multidrug efflux systems in Gram-negative bacteria. *Brazilian Journal of Microbiology*. 35 (1-2). pp. 19-28. doi: 10.1590/S1517-83822004000100003.

Müller A, Wenzel M, Strahl H, Grein, F., Saaki, T.N., Kohl, B., Siersma, T., Bandow, J.E., Sahl, H.G., Schneider, T. & Hamoen, L.W. 2016. Daptomycin inhibits cell envelope synthesis by interfering with fluid membrane microdomains. *Proceedings of the National Academy of Sciences*. 113 (45). pp. E7077-E7086. doi: 10.1073/pnas.1611173113.

Nathan, C. & Cars, O. 2014. Antibiotic resistance — problems, progress, and prospects. *New England Journal of Medicine*. 371 (19). pp. 1761-1763. doi: 10.1056/NEJMp1408040.

Navon-Venezia, S., Chmelnitsky, I., Leavitt, A. & Carmeli, Y. 2007. Dissemination of the CTX-M-25 family β-lactamases among *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* and identification of the novel enzyme CTX-M-4 in Proteus mirabilis in Israel. *Journal of Antimicrobial Chemotherapy*. 62. pp. 289–295.

Nicola, G., Tomberg, J., Pratt, R.F., Nicholas, R.A. & Davies, C. 2010. Crystal structures of covalent complexes of β-lactam antibiotics with *Escherichia coli* penicillin-binding protein 5: toward an understanding of antibiotic specificity. *Biochemistry*. 49 (37). pp. 8094-8104. doi: 10.1021/bi100879m.

Nielsen, S., Gisev, N., Bruno, R., Hall, W., Cohen, M., Larance, B., Campbell, G., Shanahan, M., Blyth, F., Lintzeris, N. & Pearson, S. 2017. Defined daily doses (DDD) do not accurately reflect opioid doses used in contemporary chronic pain treatment. *Pharmacoepidemiology and Drug Safety*. 26 (5). pp. 587-591. doi: 10.1002/pds.4168.

Nikolaidis, I., Favini-Stabile, S. & Dessen, A. 2014. Resistance to antibiotics targeted to the bacterial cell wall. *Protein Science*. 23 (3). pp. 243-259. doi: 10.1002/pro.2414.

Noeske, J., Huang, J., Olivier, N.B., Giacobbe, R.A., Zambrowski, M. & Cate, J.H. 2014. Synergy of streptogramin antibiotics occurs independently of their effects on translation. *Antimicrobial Agents and Chemotheropy*. 58 (9). pp. 5269-5279. doi: 10.1128/AAC.03389-14.

Noormandi, A. & Dabaghzadeh, F. 2015. Effects of green tea on *Escherichia coli* as a uropathogen. *Journal of Traditional and Complementary Medicine*. 5 (1). pp. 15-20. doi: 10.1016/j.jtcme.2014.10.005.

O'Driscoll, N.H., Cushnie, T.T., Matthews, K.H. & Lamb, A.J. 2018. Colistin causes profound morphological alteration but minimal cytoplasmic membrane perforation in populations of

Escherichia coli and *Pseudomonas aeruginosa*. *Archives of Microbiology*. 200 (5). pp. 793-802. doi: 10.1007/s00203-018-1485-3.

Öztürk, H., Ozkirimli, E. & Özgür, A. 2015. Classification of Beta-lactamases and penicillin binding proteins using ligand-centric network models. *PLoS One*. 10 (2). ep. e0117874. doi: 10.1371/journal.pone.0117874.

Pagès, J.M., Peslier, S., Keating, T.A., Lavigne, J.P. & Nichols, W.W. 2016. Role of the outer membrane and porins in susceptibility of β-lactamase-producing *Enterobacteriaceae* to Ceftazidime-avibactam. *Antimicrobial Agents and Chemotherapy*. 60 (3). pp. 1349-1359. doi: 10.1128/AAC.01585-15.

Palzkill, T. 2013. Metallo-β-lactamase structure and function. *Annals of the New York Academy of Sciences*. 1277 (1). pp. 91-104. doi: 10.1111/j.1749-6632.2012.06796.x.

Palzkill, T. 2018. Structural and mechanistic basis for extended-spectrum drug-resistance mutations in altering the specificity of TEM, CTX-M, and KPC β-lactamases. *Frontiers In Molecular Biosciences*. 5. pp. 16. doi: 10.3389/fmolb.2018.00016.

Papp-Wallace, K., Endimiani, A., Taracila, M.A. & Bonomo, R.A. 2011. Carbapenems: past, present, and future. *Antimicrobial Agents and Chemotherapy*. 55 (11). pp. 4943-4960. doi:

Paterson, D.L. & Bonomo, R.A. 2005. Extended-spectrum β-lactamases: a clinical update. *Clinical Microbiology Reviews*. 18 (4). pp. 657–686. doi:10.1128/CMR.18.4.657-686.2005

Paterson, G.K., Harrison, E.M. & Holmes, M.A. 2014. The emergence of mecC methicillin-resistant *Staphylococcus aureus*. *Trends in Microbiology*. 22 (1). pp. 42-47. doi: 10.1016/j.tim.2013.11.003.

Po, K.H.L., Chan, E.W.C. & Chen, S. 2017. Functional characterization of CTX-M-14 and CTX-M-15 β-lactamases by in vitro DNA shuffling. *Antimicrobial Agents and Chemotherapy*. 61 (12), pp.e00891-17.

Polsfuss, S., Bloemberg, G.V., Giger, J., Meyer, V., Böttger, E.C. & Hombach, M. 2011. Practical approach for reliable detection of AmpC beta-lactamase-producing *Enterobacteriaceae*. *Journal of Clinical Microbiology*. 49 (8). pp. 2798-2803. doi: 10.1128/JCM.00404-11.

Potz, N.A., Hope, R., Warner, M., Johnson, A.P. & Livermore, D.M. 2006. Prevalence and mechanisms of cephalosporin resistance in *Enterobacteriaceae* in London and South-East England. *Journal of Antimicrobial Chemotherapy.* 58 (2). pp. 320–326.

Poulou, A., Grivakou, E., Vrioni, G., Vassiliki, K., Pittaras, T., Pournaras, S. & Tsakris, A. 2014. Modified CLSI extended-spectrum β-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among *Enterobacteriaceae* producing various β-lactamases. *Journal of Clinical Microbiology*. 52 (5). pp. 1483-1489. doi: 10.1128/JCM.03361-13.

Protic, D., Pejovic, A., Djukanovic, N., Toskovic, B., Zdravkovic, M. & Todorovic, Z. 2016. Analysis of the third- and fourth-generation cephalosporin use for the treatment of infections caused by Gram-negative bacteria in hospital settings. *International journal of clinical practice*. 70 (12). pp. 1033-1040. doi: 10.1111/ijcp.12911.

Patel MP, Fryszczyn B, Palzkill T. 2015. Characterization of the global stabilizing substitution A77V and its role in the evolution of CTX-M beta-lactamases. Antimicrob Agents Chemother.59 (11):6741-6748. doi: 10.1128/AAC.00618-15.

Rawat, D. & Nair, D. 2010. Extended-spectrum β-lactamases in Gram negative bacteria. *Journal of Global Infectious Diseases*. 2 (3). pp. 263-274. doi: 10.4103/0974-777X.68531.

Reller, L.B., Weinstein, M., Jorgensen, J.H. & Ferraro M.J. 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical Infectious Diseases*. 49 (11). pp. 1749-1755. doi: 10.1086/647952.

Rennie ,R.P., Turnbull, L., Brosnikoff, C. & Cloke. J. 2012. First comprehensive evaluation of the M.I.C. evaluator device compared to E test and CLSI broth microdilution for MIC testing of aerobic gram-positive and gram-negative bacterial species. *Journal of Clinical Microbiology*. 50 (4). pp. 1147-1152. doi: 10.1128/JCM.05397-11.

Rioseras, B., Yagüe, P., López-García, M.T., Gonzalez-Quiñonez, N., Binda, E., Marinelli, F. and Manteca, A. 2016. Characterization of SCO4439, a D-alanyl-D-alanine carboxypeptidase involved in spore cell wall maturation, resistance, and germination in *Streptomyces coelicolor*. Scientific Reports. 6 (1). pp. 21659. doi: 10.1038/srep21659.

Rodríguez-Rubio, L., Martínez, B., Donovan, D.M., Rodríguez, A. & García, P. 2013. Bacteriophage virion-associated peptidoglycan hydrolases: potential new enzybiotics. *Critical Review of Microbiology*. 39 (4). pp. 427-434. doi: 10.3109/1040841X.2012.723675.

Rowe, T.A. & Juthani-Mehta, M. 2013. Urinary tract infection in older adults. *Aging Health*. 9 (5). pp. 519-528. doi: 10.2217/ahe.13.38. doi:10.2217/ahe.13.38

Rupp, M.E. & Fey, P.D. 2003. Extended spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*: Considerations for diagnosis, prevention and drug treatment. *Drugs*. 63(4). pp. 353-365. doi: doi.org/10.2165/00003495-200363040-00002

Ruppé, É., Woerther, P. & Barbier, F. 2015. Mechanisms of antimicrobial resistance in Gram-negative bacilli. *Annals of Intensive Care*. 5(1). pp. 21. doi:10.1186/s13613-015-0061-0

Sabtu, N., Enoch, D.A. & Brown, N.M. 2015. Antibiotic resistance: what, why, where, when and how? *British Medical Bulletin*. 116 (1). pp. 105-113. doi: 10.1093/bmb/ldv041.

Sader, H.S. & Pignatari, A.C.C. 1994. E test: a novel technique for antimicrobial susceptibility testing. *Sao Paulo Medical Journal*. 112 (4). pp. 635-638. doi: 10.1590/S1516-31801994000400003.

Saha, S. 2016. Adverse weather conditions and fatal motor vehicle crashes in the united states, 1994-2012. *Environmental Health: A Global Access Science Source*.15. pp. 1-10.

Sanchez, G.V., Babiker, A., Master, R.N., Luu, T., Mathur, A. & Bordon, J. 2016. Antibiotic resistance among urinary isolates from female outpatients in the united states in 2003 and 2012. *Antimicrobial Agents and Chemotherapy*. 60 (5). pp. 2680-2683. doi: 10.1128/AAC.02897-15.

Sauvage, E. & Terrak, M. 2016. Glycosyltransferases and transpeptidases/penicillin-binding proteins: valuable targets for new antibacterials. *Antibiotics*. 5 (1). pp. 12. doi: 10.3390/antibiotics5010012.

Sauvage, E., Kerff, F., Terrak, M., Ayala, J.A. & Charlier, P. 2008. The penicillin-binding proteins: Structure and role in peptidoglycan biosynthesis. *FEMS Microbiology Reviews*. 32 (2). pp. 234-258. doi: 10.1111/j.1574-6976.2008.00105.x.

Schmiemann, G., Kniehl, E., Gebhardt, K., Matejczyk, M.M. and Hummers-Pradier, E. 2010. The diagnosis of urinary tract infection: a systematic review. *Deutsches Ärzteblatt International*. 107 (21). pp. 361–367. doi:10.3238/arztebl.2010.0361.

Schumacher, A., Vranken, T., Malhotra, A., Arts, J. & Habibovic, P. 2018. In vitro antimicrobial susceptibility testing methods: agar dilution to 3D tissue-engineered models. *European Journal of Clinical Microbiology & Infectious Diseases*. 37 (2). pp. 187-208. doi: 10.1007/s10096-017-3089-2.

Scriver, S.R., Walmsley, S.L, Kau, C.L., Hoban, D.J., Brunton, J., McGeer, A., Moore, T.C. & Witwicki, E. 1994. Determination of antimicrobial susceptibilities of Canadian isolates of Haemophilus influenzae and characterization of their beta-lactamases. *Antimicrobial Agents and Chemotherapy*. 38 (7). pp. 1678-1680. doi: 10.1128/AAC.38.7.1678.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M. & Kamal, M.A. 2015. Antibiotic resistance and extended spectrum beta-lactamases: types, epidemiology and treatment. *Saudi Journal of Biological Sciences*. 22 (1). pp. 90-101. doi: 10.1016/j.sjbs.2014.08.002.

Shakya, P., Shrestha, D., Maharjan, E., Sharma, V.K. & Paudyal, R. 2017. ESBL Production Among and spp. Causing Urinary Tract Infection: A Hospital Based Study. *The Open Microbiology Journal*. 11 (1). pp. 23-30.

Silhavy, T.J., Kahne, D. & Walker, S. 2010. The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*. 2 (5). pp. a000414. doi: 10.1101/cshperspect.a000414.

Sillankorva, S., Pereira, M.O. & Henriques, M. 2019. Antibiotic alternatives and combinational therapies for bacterial infections. *Frontiers in Microbiology*. 9. pp. 3359. doi: 10.3389/fmicb.2018.03359.

Silveira, M.C., Azevedo da Silva, R., Faria da Mota, F., Catanho, M., Jardim, R., R Guimarães, A.C. & de Miranda, A.B. 2018. Systematic identification and classification of β-lactamases based on sequence similarity criteria: β-lactamase annotation. *Evolutionary Bioinformatics*.14. pp. 1176934318797351. doi: 10.1177/1176934318797351.

Singha, T.K., Gulati, P., Mohanty, A., Khasa, Y.P., Kapoor, R.K. & Kumar, S. 2017. Efficient genetic approaches for improvement of plasmid based expression of recombinant protein in *Escherichia coli*: a review. *Process Biochemistry*. 55, 17-31. doi: //doi.org/10.1016/j.procbio.2017.01.026.

Song, W., Bae, I.K., Lee, Y.N., Lee, C.H., Lee, S.H. & Jeong, S.H. 2007. Detection of extended-spectrum β-lactamases by using boronic acid as an AmpC β-lactamase inhibitor in clinical isolates of *Klebsiella spp*. and *Escherichia coli*. *Journal of Clinical Microbiology*. 45. pp. 1180–1184.

Soucy, S.M., Huang, J. & Johann, P.G. 2015. Horizontal gene transfer: building the web of life. *Nature Reviews Genetics*. 16 (8). pp. 472. doi: 10.1038/nrg3962.

Sultan, I., Rahman, S., Jan, A.T., Siddiqui, M.T., Mondal, A.H. & Haq, Q.M.R. 2018. Antibiotics, resistome and resistance mechanisms: a bacterial perspective. *Frontiers in microbiology*. 9. pp. 2066. doi: 10.3389/fmicb.2018.02066.

Sun, Z., Hu, L., Sankaran, B., Prasad, B.V. & Palzkill, T. 2018. Differential active site requirements for NDM-1 β-lactamase hydrolysis of carbapenem versus penicillin and cephalosporin antibiotics. *Nature Communications*. 9 (1). pp. 4524. doi: 10.1038/s41467-018-06839-1.

Tahlan, K. & Jensen, S.E. Origins of the β-lactam rings in natural products. *The Journal of Antibiotics*. 66 (7). pp. 401. doi: 10.1038/ja.2013.24.

Tamma, P.D. & Simner, P.J. 2018. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *Journal of Clinical Microbiology*. 56 (11). pp. e01140-18. doi: 10.1128/JCM.01140-18.

Tangcharoensathien, V., Chanvatik, S. & Sommanustweechai, A. 2018. Complex determinants of inappropriate use of antibiotics. *Bulletin of the World Health Organization*. 96 (2). pp. 141-144. doi: 10.2471/BLT.17.199687.

Tereshchenkov, A.G., Dobosz-Bartoszek, M., Osterman, I.A., Marks, J., Sergeeva, V.A., Kasatsky, P., Komarova, E.S., Stavrianidi, A.N., Rodin, I.A., Konevega, A.L. & Sergiev, P.V. 2018. Binding and action of amino acid analogs of chloramphenicol upon the bacterial ribosome. *Journal of Molecular Biology*. 430 (6). pp. 842-852. doi: 10.1016/j.jmb.2018.01.016.

Thai, Q. & Pleiss, J. 2010. SHV lactamase engineering database: A reconciliation tool for SHV β-lactamases in public databases. *BMC Genomics*. 11 (563). doi: 10.1186/1471-2164-11-563.

Thom, C. 1945. Mycology presents penicillin. *Mycologia*. 37 (4). pp. 460-475. doi: 10.2307/3754632.

Tian, G.B., Huang, Y.M., Fang, Z.L., Qing, Y., Zhang, X.F. & Huang, X. 2014. CTX-M-137, a hybrid of CTX-M-14-like and CTX-M-15-like β-lactamases identified in an *Escherichia coli* clinical isolate. *Journal of Antimicrobial Chemotherapy.* 69 (8). pp.2081-2085.

Truzzi, J.C., Almeida, F.M., Nunes, E.C. & Said, M.V. 2008. Residual urinary volume and urinary tract infection – When are they linked? *Journal of Urology*. 180 (1). pp. 182–185. doi: doi.org/10.1016/j.juro.2008.03.044.

Udo H, Munoz-Dorado J, Inouye M, Inouye S.1995. Myxococcus xanthus, a gram-negative bacterium, contains a transmembrane protein serine/threonine kinase that blocks the secretion of beta-lactamase by phosphorylation. Genes Dev.9(8):972-983. doi: 10.1101/gad.9.8.972.

UK-VARSS. 2019. UK Veterinary Antibiotic Resistance and Sales Surveillance Report (UK-VARSS 2018). New Haw, Addlestone: Veterinary Medicines Directorate.

US Centers for Disease Control and Prevention. 2016. CDC: 1 in 3 antibiotic prescriptions unnecessary. Available at: https://www.cdc.gov/media/releases/2016/p0503-unnecessary-prescriptions.html.

Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A. Robinson, T.P., Teillant, A. & Laxminarayan, R. 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*. 112 (18). pp. 5649-5654. doi: 10.1073/pnas.1503141112.

van den Berg, B., Bhamidimarri, S.P., Prajapati, J.D., Kleinekathöfer, U. & Winterhalter, M. 2015. Outer-membrane translocation of bulky small molecules by passive diffusion. *Proceedings of the National Academy of Sciences*. 112 (23). pp. E2991-E2999. doi: 10.1073/pnas.1424835112.

van Duin, D. & Doi, Y. 2017. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence*. 8 (4). pp. 460-469. doi: 10.1080/21505594.2016.1222343.

Vandavasi, V.G., Langan, P.S., Weiss, K.L., Parks, J.M., Cooper, J.B., Ginell, S.L. & Coates, L. 2017. Active-site protonation states in an acyl-enzyme intermediate of a class A β-lactamase with a monobactam substrate. *Antimicrobial Agents & Chemotherapy*. 61 (1). pp. e01636-16. doi: 10.1128/AAC.01636-16.

Ventola C.L. 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*. 40 (4). pp. 277–283.

Welsh, M.A., Taguchi, A., Schaefer, K., Van Tyne, D., Lebreton, F., Gilmore, M.S., Kahne, D. & Walker, S. 2017. Identification of a functionally unique family of penicillin-binding proteins. *Journal of the American Chemical Society*. 139 (49). pp. 17727-17730. doi: 10.1021/jacs.7b10170.

White, C., Ismail, H.D. & Saigo, H. 2017. CNN-BLPred: a convolutional neural network based predictor for beta-lactamases (BL) and their classes. *BMC Bioinformatics*. 18 (16). pp. 577. doi: 10.1186/s12859-017-1972-6.

Wiles, T.J., Kulesus, R.R. & Mulvey, M.A. 2008. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Experimental & Molecular Pathology*. 85 (1). 11-19. doi: 10.1016/j.yexmp.2008.03.007.

World Health Organisation. 2009. Defined daily dose: definition and general considerations. Available at: http://www.whocc.no/ddd/definition_and_general_considera/ (accessed 11/8/2015.

World Health Organization. 2018. WHO report on surveillance of antibiotic consumption: 2016-2018 early implementation. Geneva.

Wang R, van Dorp L, Shaw L, et al.2018. The global distribution and spread of the mobilized colistin resistance gene mcr-1. *Nat Commun*..9(1):1179. doi: 10.1038/s41467-018-03205-z.

Welsh Government. Statistics and research department.2019.Available at: https://statswales.gov.wales/Catalogue/Population-and-Migration/Population/Estimates/Local-Authority/populationestimates-by-localauthority-year

Yadav, A.K., Espaillat, A. & Cava, F. 2018. Bacterial strategies to preserve cell wall integrity against environmental threats. *Frontiers in microbiology*. 9. pp. 2064. doi: 10.3389/fmicb.2018.02064.

Yang, D.C., Blair, K.M. & Salama, N.R. 2016. Staying in shape: the impact of cell shape on bacterial survival in diverse environments. *Microbiology and Molecular Biology Reviews: MMBR*. 80 (1). pp.187-203. doi: 10.1128/MMBR.00031-15.

Yeliseev, A., Zoubak, L. & Schmidt, T.G. 2017. Application of Strep-Tactin XT for affinity purification of twin-strep-tagged CB2, a G protein-coupled cannabinoid receptor. *Protein Expression and Purification*.131. pp. 109-118. doi: //doi.org/10.1016/j.pep.2016.11.006.

Yount, N.Y. & Yeaman, M.R. 2013. Peptide antimicrobials: cell wall as a bacterial target. *Annals of the New York Academy of Sciences*. 1277 (1). pp. 127-138. doi: 10.1111/nyas.12005.

Zapun, A., Contreras-Martel, C. & Vernet, T. 2008. Penicillin-binding proteins and β-lactam resistance. *FEMS Microbiology Reviews*. 32 (2). pp. 361-385. doi: 10.1111/j.1574-6976.2007.00095.x.

Zhao, W. & Hu, Z. 2013. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical Reviews in Microbiology*. 39 (1). pp. 79-101. doi: 10.3109/1040841X.2012.691460.

Zhuo, C., Li, X., Zong, Z. & Zhong, N. 2013. Epidemic plasmid carrying blaCTX-M-15 in *Klebsiella penumoniae* in China. *PloS One*. 8 (1). e52222. doi: 10.1371/journal.pone.0052222.

Zygmunt, D.J., Stratton, C.W. & Kernodle, D.S. 1992. Characterization of four beta-lactamases produced by *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 36 (2). pp. 440-445. doi: 10.1128/AAC.36.2.440.

Zhang P., Song Y., Thompson I.P., Ma B., Xu J., Huang W.E. 2015. Single-Cell Biotechnology for Uncultured Microorganisms. In: McGenity T., Timmis K., Nogales B. (eds) Hydrocarbon and Lipid Microbiology Protocols. Springer Protocols Handbooks. Springer, Berlin, Heidelberg.

Appendices

Appendix I

Wrexham hospital samples DNA sequences;

3:

CTGCAGTATAGCGACAATACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTCCCGAT

AAAGTGACGGCGTTTGCTCGCTCGTTGGGTGATGAGACCTTCCGTCTGGACAGAACCGAG

CCCACGCTCAATACCGCCATTCCAGGCGACCCGCGTGATACCACCACGCCGCTCGCGATG

GCGCAGACCCTGAAAAATCTGACGCTGGGTAAAGCGCTGGCGGAAACTCAGCGGGCACAG

TTGGTGACGTGGCTTAAGGGCAATACTACCGGTAGCGCGAGCATTCGGGCGGGTCTGCCG

AAATCATGGGTAGTGGGCGATAAAACCGGCAGCGGAGATTATGGCACCACCAACGACAT

BLAST output: Klebsiella pneumoniae strain KP58 extended-spectrum beta-lactamase **CTX-M-59** (blaCTX-M) gene, blaCTX-M-59 allele, partial cds

Sequence ID: MH661248.1Length: 817Number of Matches: 1

5:

ACGCAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGGTTGTGGGGGGATAAAACCGGCAGCGGTGACTATG

BLAST output: Klebsiella pneumoniae isolate KSH203 plasmid pKSH203-**CTX-M-3**, complete sequence

Sequence ID: CP034325.1Length: 156910Number of Matches: 1

7

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTAAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAA

BLAST output: CTX-M-15 (blaCTX-M-15) gene, partial cds Sequence ID: gb|KR338941.1|Length: 867Number of Matches: 1

9:

CGCAAAAACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTG

AGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGA
CGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGA
ATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGG
GAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCG
ATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGG
GTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCA
CCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCG
GCAGCGGTGGCTATGGCACCACAACCGAATATCGAAAATTTC

BLAST output: Klebsiella pneumoniae strain E161KPMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds

Sequence ID: KY640551.1Length: 632Number of Matches: 1

11:

TTAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAG
CGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCT
GAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCT
TGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGC
GCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCC
CGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGA
GCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCG
CAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTG
ACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGG
TTGTGGGGGATAAA

BLAST output : Proteus mirabilis PM341/03 pPM341/03 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-66**, complete CDS

Sequence ID: NG_049017.1Length: 1076Number of Matches: 1

15:

TTAGGAAGTGTGCCGCTGTATATCATCGATACCGCA
GGCCTGCGCGATGCTAATGACGAAGTGGAACGTATCGGCATCGAGCGCGCCTGGCAGGAG
ATTGCCCAGGCCGATCGGGTGCTGTTTATGGTCGATGGCACCACCAACGATATCG

BLAST output : Klebsiella pneumoniae UHKPC07, complete genome Sequence ID: gb| CP011985.1|Length: 5322270Number of Matches: 1

17:

CGCAAGATTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCG
AATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGC

AAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGC
CGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGCTGAGATCAAAAA
ATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCT
GAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACG
TTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGA
CCGTACCGAGCCGACGTTAAACACCGCTATTCCGGGCGATCCGCGTGATACCACTTCACCTCGG
GCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCG
CAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCT
GCTTCCTGGGTTGTGGGGGATAAAA

BLAST output : *Shigella sp.* **SH223 blaCTX-M-108 (CTX-M-108) g**ene, partial cds
Sequence ID: gb| JF274245.1|Length: 864Number of Matches: 1

19:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGGATAAAACCGGCAGCGGTGGCTATGGCACAGC

BLAST output : Klebsiella pneumoniae subsp. pneumoniae strain KPTR13-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MK113957.1Length: 1027Number of Matches: 1

21:

GCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAC
AGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAG
TAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAA
TCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCA
CGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAA
CGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGC
CCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGC
CATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAA
TCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAA
AGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGG
GGATAAAACCGGCAGCGGTGGCTATGGCACCACCACCGATATC

BLAST output : Escherichia coli strain E78ECMO **CTX-M-15** beta-lactamase (blaCTX-M-15) gene, partial cds

Sequence ID: KY640536.1Length: 641Number of Matches: 1

23:

ACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCC
GTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCA
CTTCACCT

BLAST output : *Escherichia coli* strain IPK192 beta-lactamase **CTX-M-32** (blaCTX-M) gene, blaCTX-M-32 allele, complete cds

Sequence ID: MH900527.1Length: 876Number of Matches: 1

25:

AACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCA
GTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATC
AGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAA
TGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTAC

BLAST output : *Escherichia coli* strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1Length: 876Number of Matches: 1

27:

TACGCAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGT

BLAST output : Klebsiella pneumoniae strain E12KPMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds Sequence ID: KY640528.1Length: 640Number of Matches: 1

29

GATGCCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGG
GCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAA

BLAST output : Escherichia coli strain IPK174 beta-lactamase **CTX-M-55** (blaCTX-M) gene, blaCTX-M-55 allele, complete cds

Sequence ID: MH900523.1Length: 876Number of Matches: 1

31:

TCGAGCCGGAACGTGTCATGCGGGCGTCAGGCTG
CCGTAATGGCGATTTGCGCCCGGACCAGGCCGCACGGGGAAACTCTGCGGCCTTTTTCGT
TCTTACTGCGGGTAAGGCACCCAGTCGCCGCCGTTCAGGCGAACGTACGGTTTATCCTGG
TATTGAATAACTACTGCATTTGAGTTCTCGGAGACCGGTGCTGTTTGTGGTAACCCACTG
GTGAGTTTTTTCCAGTCAACATTGTCTTCGGTGAAAATCTTGCCATCAAGAACGCGAACC
ACCAGATCGGCAGGCTTGA

BLAST output:*Escherichia coli* strain MNCRE44, complete genome

Sequence ID: gb| CP010876.1|Length: 5010884Number of Matches: 3

33:

ATGGTGACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGG
GCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAA
GCAGCGGAGGGCGGCTGGGCGTCGCGCTCATCGATACCGCAGATAATACGCAGGTGCTTTATCG
CGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCCGCGGCGGTGCTTAAG
CAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTA
ACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATGACGCTGGCAGAGCTGAGCGCGG
CCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGG
AGGGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTA
CGCTGAATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCACAGA
CGTTGCGTCAGCTTACGCTGGGTCATGCGCTGGGC

BLAST output: Salmonella enterica strain Sal1426 class A extended-spectrum beta-lactamase **CTX-M-9** (blaCTX-M) gene, blaCTX-M-9 allele, partial cds

Sequence ID: MF797877.1Length: 867Number of Matches: 1

35:

ACGCAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTA
ACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCA
CCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGT
TAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAA
AGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCG
ATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGT
TCG

BLAST output :*Klebsiella pneumoniae* strain KPTR1-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-115 allele, complete cds Sequence ID: MK113960.1Length: 1008Number of Matches: 1

36:

GCTGCTGGGAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAATGCTGGCGGCG
CTGGAGATAAGC

BLAST output : Escherichia coli strain IPK37 beta-lactamase **CTX-M-27** (blaCTX-M) gene, blaCTX-M-27 allele, complete cds

Sequence ID: MH900525.1Length: 876Number of Matches: 1

37:

GCTGGGCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCT
GGAGAAAAGCAGCGGAGGGCGGCTGGGCGTCGCGCTCATCGATACCGCAGATAATACGCA
GGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGG

BLAST output : Escherichia coli strain YSP8-1 plasmid pYSP8-1-**CTX-M-14**, complete sequence Sequence ID: CP037912.1Length: 149304Number of Matches: 1

41:

ACCCCTGCTATTTAGCAGCGCAACGCTGCATGCGCAGGCGAACAGCGTGCAACAGCAGCTGGA
AGCCCTGGAGAAAAGTTCGGGAGGTCGGCTTGGCGTTGCGCTGATTAACACCGCCGATAATTCG
CAGATTCTCTACCGTGCCGATGAACGTTTTGCGATGTGCAGTACCAGTAAGGTGATGGCGGCCG
CGGCGGTGCTTAAACAGAGCGAGAGCGATAAGCACCTGCTAAATCAGCGCGTTGAAATCAAGA
AGAGCGACCTGGTTAACTACAATCCCATTGCGGAGAAACACGTTAACGGCACGATGACGCTGG
CTGAGCTTGGCGCAGCGGCGCTGCAGTATAGCGACAATACTGCCATGAATAAGCTGATTGCCCA
TCTGGGTGGCCCGATAAAGTGACGGCGTTTGCTCGCTCGTTGGGTGATGAGACCTTCCGTCTGG
ACAGAACCGAGCCCACGCTCAATACCGCCATTCCAGGCGACCCGCGTGATACCACCACGCCGCT
CGCGATGGCGCAGACCCTGAAAAATCTGACGCTGGGTAAAGCGCTGGCGGAAACTCAGCGGGC
ACAGTTGGTGACGTGGCTTAAGGGCAATACTACCGGTAGCGCGAGCATTCGGGCGGGTCTGCCG
AAATCATGGGTAGTGGGCGATAAAACCGGCAGCGGAGATTATGGCACCACCAACGATATCGCG
GTTATCTGGC

BLAST output : Klebsiella pneumoniae strain KP72 extended-spectrum beta-lactamase **CTX-M-2** (blaCTX-M) gene, blaCTX-M-2 allele, partial cds

Sequence ID: MH661245.1Length: 826Number of Matches: 1

43:

AACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGC
ACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTG
TTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAA
AAGCACGTCAATGGGA

BLAST output : *Escherichia coli* strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MH900522.1Length: 876Number of Matches: 1

45:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACCGA

BLAST output : Klebsiella pneumoniae strain KPTR1-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (blaCTX-M) gene, **blaCTX-M-115** allele, complete cds Sequence ID: MK113960.1Length: 1008Number of Matches: 1

47:

ATGGTGACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTG
CTGGGCAGCGCGCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGCGGCGCT
GAGAAAAGCAGCGGAGGGCGACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCC
GCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATC
AAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATG
ACGCTGGCAGAGCTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAA
TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT
GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG
AGAGACACCACCACGCCGCGGGCGATGGCACAGACGTTGCGTCAGCTTACGCTGGGTCA
GCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGCAATACGACCGGC
GCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGCAGGTGATAA

BLAST output :*Enterobacter cloacae* strain CERIC152-401 class A extended spectrum beta-lactamase **CTX-M-9** (CTX-M-9) gene, partial cds Sequence ID: KT459753.1Length: 867Number of Matches: 2

49:

TGAGGGCCGCGGCCGCGGTGCTGAAAAAAAGTGAAAGCAAACCGAATCTGTTAAATCAGC
GAGTTGAGATCAAAAAATGTG

BLAST output :*Escherichia coli* strain CR4 plasmid class A extended-spectrum beta-lactamase **CTX-M-15 (blaCTX-M) gene**, blaCTX-M-15 allele, complete cds

Sequence ID: MK405591.1Length: 876Number of Matches: 1

51:

CGCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACA
CAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCA
GTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAA
ATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGC
ACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATA
ACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCG
CCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCG
CCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGA
ATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGA
AAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGG
GGGATAAAACCGCCA

BLAST output : Klebsiella pneumoniae partial **CTX-M-15** gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 327

Sequence ID: LT628520.1Length: 626Number of Matches: 1

54:

ATGGTGACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTG
TGGGCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTG
GAGAAAAGCAGCGGAGGGCGGCTGGGCGTCCGCTCATCGATACCGCAGATAATACGCAG
GTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCC
GCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATC
AAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAAT
AGAGACACCACCACGCCGCGGGCGATGGCACAGACGTTGCGTCAGCTTACGCTGGGTCAT
GCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGCAATACGACCGGC

BLAST output :*Escherichia coli* strain IPK37 beta-lactamase **CTX-M-27** (blaCTX-M) gene, blaCTX-M-27 allele, complete cds

Sequence ID: MH900525.1Length: 876Number of Matches: 2

57:

AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGG
TGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAAT
CTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGG
CTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTG
CTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGT
TCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGG
GCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGA
GCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCT
ATGGCACCAAAAA

BLAST output :*Escherichia coli* strain BA22372 **plasmid pCTX-M-15**_22372, complete sequence Sequence ID: CP040398.1Length: 98470Number of Matches: 1

59:

AAAGTGAAAGGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACTTGG
TTAACTATAATCCGATTGCGGAAAAGCACGTCGATGGGACGATGTCACTGGCTGAGCTTA
GCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTTCTCACGTTG
GCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGGACGAAACGTTCCGTCTCG
ACCGTACACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGA
AACGTTCCTCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGTAATCTG
ACGCTGGGTAAAGCATTGGGTGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGC
AATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAT
AAAACCGGCAGCGGTGACTATGGCACCACCAACA

BLAST output : Escherichia coli strain A19 plasmid beta-lactamase **CTX-M-1** (blaCTX-M) gene, blaCTX-M-CTX-M-1 allele, partial cds Sequence ID: MH037035.1Length: 810Number of Matches: 3

61:

ACGCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC

AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGATAAAACCGGCAGCGGTGGCTATGGACA

BLAST output :*Escherichia coli* strain E78ECMO **CTX-M-15** beta-lactamase (blaCTX-M-15) gene, partial cds Sequence ID: KY640536.1Length: 641Number of Matches: 1

63

TTGCCATCAAGAACGCGAACCACCAGATCGGCAGGCTTGA

BLAST output :*Escherichia coli* strain 4928STDY7071340 genome assembly, chromosome: 1 Sequence ID: LR607331.1Length: 5120867Number of Matches: 1

65 :

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCCGCTGTATGCGCA
AACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGT
GGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATG
TGCAGCACCAGTAAAGTGATGGCCGTGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAAT
CTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAA
AGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACGTACAGCGATAA
CGTGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGAC
GAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGTGATACCACTTCA
CCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAA
CGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGA
CTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATA
TCGCGGTGATCTGGCCAAAAGATCGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCA
ACCTAAGGCAGAAAGCCGTCGCGATGTATTAGCGTCGGCGGCTAAAA

BLAST output :Escherichia coli strain CH-15-5 extended spectrum beta-lactamase **CTX-M-55** (blaCTX-M-55) gene, complete cds; and hypothetical protein gene, partial cds

Sequence ID: MF958462.1Length: 980Number of Matches: 1

67:

TAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGC
GGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTA
TCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTG
AAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTT
GTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCG
CGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCC
GGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAG
CCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGC
AAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGA
CATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGT
TGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCG

BLAST output : Escherichia coli strain F170 **extended-spectrum beta-lactamase CTX-M-15 gene (CTX-M 1 group)** , partial cds Sequence ID: gb|KP325147.1|Length: 843Number of Matches: 1

69:

ATGGTTAAAAAATCACTGAGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAG
GAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAAGCGGCA
GTGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAATACTTTATCG
TGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAG
AAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTA
ACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGC
CGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCT
AGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGA
CGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAAC
TCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATG
GATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCT

BLAST output : *Escherichia coli* strain 1125509 **class A beta-lactamase (blaCTX-M-163) gene**, complete cds Sequence ID: gb|KP681698.1|Length: 876Number of Matches: 1

71:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC

ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGGATAAAACCGGCAGCGGTGGCTATGGCACAGC

BLAST output : Klebsiella pneumoniae subsp. pneumoniae strain KPTR13-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MK113957.1Length: 1027Number of Matches: 1

73:

CTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCC
GAAAAACACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTAC
AGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACG
GCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTG
AATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACG
TTGCGTCAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACG
TGGCTCAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGG
ACTGTGGG

BLAST output : Escherichia coli beta-lactamase CTX-M-16 gene, complete cds AY029068.1

75:

TTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAG
CGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCT
GAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCT
TGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGC
GCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCC

CGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTCCGAG
CCGACGTTAAACACCGCCATTT

BLAST output: *Escherichia coli* strain F147 **extended-spectrum beta-lactamase CTX-M-15 gene**, partial cds . Sequence ID: gb|KP325146.1|Length: 822Number of Matches: 1

77:

ACGCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTA
AATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAAC

BLAST output: Escherichia coli strain E78ECMO **CTX-M-15** beta-lactamase (blaCTX-M-15) gene, partial cds,Sequence ID: KY640536.1Length: 641Number of Matches

80:

ATGGTGACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGG
GCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAA
GCAGCGGAGGGCGGCTGGGCGTCGGCTCATCGATACCGCAGATAATACGCAGTGCTTTATCGCG
GTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCCGCGGCGGTGCTTAGCAG
AGGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCTGCCGATCTGGTTAACTAC
AATCCGATTGCCGAAAAACACGTCAACGGCACAATGACGCTGGCAGAGCTGAGCGCGGCCGCG
TTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGC
GTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGC
TGAATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCACAGACGT
TGCGTCAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCT
CAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGCAGG
TGATAAGGCGACTACGGCACCACCAATGATATTGCG

BLAST output: Salmonella enterica strain Sal1426 class A extended-spectrum beta-lactamase **CTX-M-9** (blaCTX-M) gene, blaCTX-M-9 allele, partial cds

Sequence ID: MF797877.1Length: 867Number of Matches: 1

83:

ATGATGAGAAAAAGCGTAAGGCGGGCGATGTTAATGACGACAGCCTGTGTTTCGCTGCTG
TTGGCCAGTGTGCCGCTGTGTGCCCAGGCGAACGATGTTCAACAAAAGCTCGCGGCGCTG
GAGAAAAGCAGCGGGGGACGACTGGGTGTGCGTTGATTACACCGCCGATAACACGCAG
ACGCTCTACCGCGCCGACGAGCGTTTTGCCATGTGCAGCACCAGTAAAGTGATGGCGGTA
GCGGCGGTGCTTAAGCAAAGTGAAACGCAAAGGGCTTGTTGAGTCAGCGGGTTGAAATT
AAGCCCTCAGACTTGGTTAACTACAACCCCATTGCGGAAAAACACGTCAATGGCACGATG
GACACGTTCCGGCTCGATCGTACCGAGCCGACGCTCAACACCGCGATCCCCGGCGACCCG

BLAST output :Proteus mirabilis 1111641 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-160,** complete CDS .Sequence ID: NG_048945.1Length: 876Number of Matches: 2

84:

AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGG
TGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAAT
CTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGG
CTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTG
CTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGT
TCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGG
GCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGA
GCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAAAAAACCGGCAGCGGTGGCT
ATGGAACACA

BLAST output :Klebsiella pneumoniae partial CTX-M-15 gene for Class A Extended Spectrum beta-lactamase **CTX-M-15,** isolate 122.Sequence ID: LT628516.1Length: 622Number of Matches: 1

86:

GCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGC
AGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTG
ATGGCCGCGGCCGCGGTGCTGAAGAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTG
AGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGAT
GTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTG
ATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGT
TCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCAC
TTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGC
CAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCT

GGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGACGAT

BLAST output :Escherichia coli strain E74ECMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds.Sequence ID: KY640534.1Length: 640Number of Matches: 1

88:

ACGCAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAA

BLAST output : Escherichia coli 275 unnamed blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-225**, complete CDS.Sequence ID: NG_064720.1Length: 1048Number of Matches: 1

90:

AACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCACCGCGTTCGCCCGACAGCTGGGGACGAAACGTTCCGTCTCGACCGTACACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCTCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGTAATCTGACGCTGGGTAAAGCATTGGGTGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACA

BLAST output : Escherichia coli strain RN 1 plasmid cefotaximase class A extended-spectrum beta-lactamase **CTX-M-1 (blaCTX-M) gene**, blaCTX-M-1 allele, partial cds

Sequence ID: MG255315.1Length: 487Number of Matches: 3

91:

AACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTAC

BLAST output : Escherichia coli strain VRES-hospital6495320 genome assembly, plasmid: 1 .Sequence ID: LR607054.1Length: 111743Number of Matches: 1

93

ATGGTTAAAA AATCACTGCG CCAGTTCACG CTGATGGCGA CGGCAACCGT CACGCTGTTG
TTAGGAAGTG TGCCGCTGTA TGCGCAAACG GCGGACGTAC AGCAAAAACT TGCCGAATTA
GAGCGGCAGT CGGGAGGCAG ACTGGGTGTG GCATTGATTA ACACAGCAGA TAATTCGCA
ATACTTTATC GTGCTGATGA GCGCTTTGCG ATGTGCAGCA CCAGTAAAGT GATGGCCGCG
GCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCA
AAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACT
GGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCCGAT
TGCTC ACGTTGGCGG CCCGGCTAGCGTCACCGCGT TCGCCCGACA GCTGGGAGAGAAACGT
TCC GTCTCGACCGTACCGAGCCG ACGTTAAACA CCGCCATTCC GGGCGATCCG
CGTGATACCA CTTCACCTCG GGCAATGGCG CAAACTCTGC GGAATCTGAC
GCTGGGTAAGCATTGGGCG ACAGCCAACG GGCGCAGCTG GTGACATGGA TGAAAGGCAA

BLAST output : Escherichia coli blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-172,** complete CDS

Sequence ID: NG_048957.1Length: 876Number of Matches: 1

95:

AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGG
TGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAAT
CTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGG
CTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTG
CTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGT
TCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGG
GCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCG
AGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGAAAAAACCGGCAGCGGTGGCT
ATGGAACACA

BLAST output : Klebsiella pneumoniae partial **CTX-M-15 gene** for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 122.Sequence ID: LT628516.1Length: 622Number of Matches: 1

97:

ATGGTGACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTG

CTGGGCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTG
GAGAAAAGCAGCGGAGGGCGTCACCGCGTCGCGCTCATCGATACCGCAGATAATACGCAG
GTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCC
GCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATC
AAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATG
ACGCTGGCAGAGCTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAA
TTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGAT
GAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCG
AGAGACACCA

BLAST output : Salmonella enterica strain Sal1426 class A extended-spectrum beta-lactamase **CTX-M-9** (blaCTX-M) gene, blaCTX-M-9 allele, partial cds

.Sequence ID: MF797877.1Length: 867Number of Matches: 1

98:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAAC
ACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACC
AGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTT
AAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAA
GCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGAT
AACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACC
GCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGG
AATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGGATAAAACCGGCAGCGGTGGCTATGGCACAGC

Klebsiella pneumoniae subsp. pneumoniae strain KPTR13-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MK113957.1Length: 1027Number of Matches: 1

100:

ACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGGGCAGCGCGCCGCTTTATGCG
CAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAAGCAGCGGAGGGCGGCTGGG
CGTCGCGCTCATCGATACCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCA
ATGTGCAGTACCAGTAAAGTTATGGCGGCCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAG

CAGCTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCG
AAAAACACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCG
ACAATACCGCCATGAACAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCC
CGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTC
CCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGC
TGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACG

Escherichia coli strain IPK37 beta-lactamase **CTX-M-27** (blaCTX-M) gene, blaCTX-M-27 allele, complete cds.Sequence ID: MH900525.1Length: 876Number of Matches: 1

Appendix II

Glan Clwyd Hospital samples DNA sequences

101:

TGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGA
TAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGT
GATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCG
AGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAA
TGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGC
GATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACA
GCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCC
GGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGAC
GCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAA
TACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAA
AACCGGCAGCGGTGGCTATGGCACCACCAACGATATCG

Blast result : Escherichia coli strain F170 **extended-spectrum beta-lactamase CTX-M-15 gene (CTX-M 1 group)** , partial cds. Sequence ID: gb|KP325147.1|
Length: 843Number of Matches: 1

103:

TGTACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCC
GCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAA
TCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTG
AGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGT
TGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGAC
CGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGG
CAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGC
AGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTG
CTTCCTGGGTTGTGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCCCCA

Blast result : Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cdsequence ID: MH900522.1 Length: 876Number of Matches: 1

106

CGACAATACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTCCCGATAAAGTGACAGC
GTTTGCTCGCTCGTTGGGTGATGAGACCTTCCGTCTGGACAGAACCGAGCCCACGCTCAATACC
GCCATTCCAGGCGACCCGCGTGATACCACCACGCCGCTCGCGATGGCGCAGACCCTGAAAAATC
TGACGCTGGGTAAAGCGATGGCGGAAACTCAGCGGGCACAGTTGGTGACGTGGCTTAAGGGCA
ATACTACCGGTAGCGCGAGCATTCGGGCGGGTCTGCCGAAATCATGGGTAGTGGGCGATAAAA
CCGGCAGCGGAGATTATGGCACCACCAACGA

BLAST output: Klebsiella pneumoniae strain KP10 extended-spectrum beta-lactamase **CTX-M-59** (blaCTX-M) gene, blaCTX-M-59 allele, partial cds

Sequence ID: MH661247.1 Length: 843Number of Matches: 1

108:

ATCAGCGCGTTGAAATCAAGAAGAGCGACCTGGTTAACTACAATCCCATTGCGGAGAAACACG
TTAACGGCACGATGACGCTGGCTGAGCTTGGCGCAGCGGCGCTGCAGTATAGCGACAATACTGC
CATGAATAAGCTGATTGCCCATCTGGGTGGTCCCGATAAAGTGACGGCGTTTGCTCGCTCGTT
GGGTGATGAGACCTTCCGTCTGGACAGAACCGAGCCCACGCTCAATACCGCCATTCCAGGCGAC
CCGCGTGATACCACCACGCCGCTCGCGATGGCGCAGACCCTGAAAAATCTGACGCTGGGTAAA

GCGCTGGCGGAAACTCAGCGGGCACAGTTGGTGACATGGCTTAAGGGCAATATACCGGTAGCG
CGAGCATTTGGGCGGGTCTGCCGAAATCATGGGTAGTGGGCGATAAAACCGGCAGCGGAGATT
ATGGCACCACCAACGATATCGCGGTTATCTGGCCGGAAAACCACGC
ACCGCTGGTTCTGGTGACCTACTTTACCCA

BLAST output: Escherichia coli strain 42.120E1 CTX-M family extended-spectrum beta-lactamase **(blaCTX-M)** gene, partial cds

Sequence ID: MK896928.1 Length: 618Number of Matches: 1

109:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG
TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACC
AACCGA

BLAST output: Klebsiella pneumoniae strain KPTR1-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (blaCTX-M) gene, **blaCTX-M-115** allele, complete cds.Sequence ID: MK113960.1 Length: 1008Number of Matches: 1

111:

CGGTGCCGATGACCCCGTTACAGGAGTTCTGGCACTATTTTAAACGCAACAAAGGCGCGG
TTGTCGGACTGGTTTACGTCGTCATCGTGCCGTTAACGACTGCC

BLAST output: Escherichia coli O1:H42 strain CLSC36 chromosome, complete genome.Sequence ID: CP041300.1 Length: 5083072Number of Matches: 1

113:

TGTACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCC
GCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAA
TCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTG
AGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGT
TGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGAC
CGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGG

CAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAGACC **BLAST output:**Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MH900522.1Length: 876Number of Matches: 1
115: CGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAA **BLAST output:** Escherichia coli strain CR4 plasmid class A extended-spectrum beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MK405591.1 Length: 876Number of Matches: 1
118: ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGG **BLAST output:** Escherichia coli strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MH900522.1 Length: 876Number of Matches: 1
120:

GCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGC
AGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTG
ATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTT
GAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGACGA
TGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCT
GATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACG
TTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCA
CTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAG
CCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGC
TGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCACC
GATATC

BLAST output: Escherichia coli strain E78ECMO **CTX-M-15** beta-lactamase (blaCTX-M-15) gene, partial cds

Sequence ID: KY640536.1 Length: 641Number of Matches: 1

122:

CACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACC
GAATCTGTTAAATCAGCGAGTTGAGATCACAAAATCTGACCTTGTTAACTATAATC
CGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGC
CGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGC
GGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTC
TCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATAC
CACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCA
TTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCG
GTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAGAAC
CGGCA

BLAST output: Escherichia coli ESBL20160070 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-203**, complete CDS

Sequence ID: NG_055269.1 Length: 876Number of Matches: 1

124:

TGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTC
GGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCT

GATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAA
AGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACT
ATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGC
GCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGC
GTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGT
TAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCT
GCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGAT
GAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGG
GATAAA

BLAST output : Proteus mirabilis PM341/03 pPM341/03 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-66**, complete CDS

Sequence ID: NG_049017.1 Length: 1076Number of Matches: 1

128:

AATCAATCGTGCCGTTAACGAGATTAGCGCACCGGTGCCGATGACCCCGTTACAGGAGTT
CTGGCACTATTCTAAACGCAACAAAGGCGCGGTTGTCGGGCTGGTTTACGTCGTCATCGT
GCCGTTAACAAAAC

BLAST output : Escherichia coli strain 4928STDY7071340 genome assembly, chromosome: 1.Sequence ID: LR607331.1 Length: 5120867Number of Matches: 1

129:

ACTGAACCTACGCTGAATACCGCCATTCCCGGCGACACGAGAGACACCACCACGCCGCGGGCG
ATGGCACAGACGTTGCGTCAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAG
TTGGTGACGTGGCTCAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGT
CGTGGACTGCAGGTGATAAGACCGGCAGCGGCGACTACGGCACCACCAATGATATTGCGGTGA
TCTGGCCGCAGGGTCGTGCGCAGCTGGTTCTGGTGACCTATTTTACCCAGCCGCAACAGAACGC
AGAGAGCCGCCGCGATGTGCTGGCTTCAGCGGCGAGAATCA

BLAST output : Salmonella enterica strain Sal1426 class A extended-spectrum beta-lactamase CTX-M-9 (blaCTX-M) gene, **blaCTX-M-9** allele, partial cdsSequence ID: MF797877.1 Length: 867Number of Matches: 1

132:

ACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCG
GTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCT
GACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGC
TTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGG
CGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGT
ACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAA
TGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGC

TGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTC
CTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCGCCC

BLAST output : Klebsiella pneumoniae **KL8-ctx-m-15 gene** cluster, complete sequence

Sequence ID: MH523447.1 Length: 20281Number of Matches:

235:

GTGAAAGGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACTTGGTTAACTAT
AATCCGATTGCGGAAAAGCACGTCGATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCG
CTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTTCTCACGTTGGCGGCCCGGCTAGCG
TCACCGCGTTCGCCCGACAGCTGGGGACGAAACGTTCCGTCTCGACCGTACACGTTGGCGGCCC
GGCTAGCGTCACCGCGTTCGCCTGACAGCTGGGAGACGAAACGTTCCTCCGCGTGATACCACTT
CACCTCGGGCAATGGCGCAAACTCTGCGTAATCTGACGCTGGGTAAAGCATTGGGTGACAGCCA
ACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGG
ACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACA

BLAST output : Escherichia coli strain IPK192 beta-lactamase CTX-M-32 (blaCTX-M) gene, **blaCTX-M-32** allele, complete cds

Sequence ID: MH900527.1 Length: 876Number of Matches: 3

136:

TTCGCATCTACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCG
CGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCA
AAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACT
GGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCT
CACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTC
TCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACC
TCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACG
GGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCG

Escherichia coli strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1 Length: 876Number of Matches: 1

138:

TACGCAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG

TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGT

BLAST output : Klebsiella pneumoniae strain E12KPMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds.Sequence ID: KY640528.1 Length: 640Number of Matches: 1

141:

CAAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACCGATATCGAGA

BLAST output : Escherichia coli strain BA22372 plasmid **pCTX-M-15**_22372, complete sequence.Sequence ID: CP040398.1 Length: 98470Number of Matches: 1

142:

AGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCACCGCGTTCGCCCGACAGCTGGGGACGAAACGTTCCGTCTCGACCGTACACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCTCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGTAATCTGACGCTGGGTAAAGCATTGGGTGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACA

BLAST output : Escherichia coli strain A19 plasmid beta-lactamase **CTX-M-1** (blaCTX-M) gene, blaCTX-M-CTX-M-1 allele, partial cds.Sequence ID: MH037035.1 Length: 810Number of Matches: 3

143:

GTCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGC
GCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGG
TGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCG
ATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCG
AATCTGTTAAATCAGCGAGCTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGG
AAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCG
ATAACGTAGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGC
CCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATT
CCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGC
TGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCA
CCGGTGCAGCGAGCATTCAGGCTGGACTCCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAG
CGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGATCGTGCGCCGCTGATT
CTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGTCGCGATGTATTAGCGTCGG
CGGCTAAAATCGTCA

BLAST output : Shigella sp. SH223 blaCTX-M-108 (CTX-M-108) gene, partial cds

Sequence ID: JF274245.1 Length: 864Number of Matches: 1

145:

ACGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG
TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCAAA

BLAST output : Klebsiella pneumoniae subsp. pneumoniae strain KPTR12-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MK113958.1 Length: 1027Number of Matches: 1

147:

TCGCATCTACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCG
CGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCA
AAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACT
GGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCT
CACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTC
TCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACC
TCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACG
GGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACT
GCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCGCCC

BLAST output : Escherichia coli strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, **blaCTX-M-15** allele, complete cds.Sequence ID: MH900522.1
Length: 876Number of Matches: 1

150:

AGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCG
AGTTGAGATCACAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGG
ACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATA
AGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGA
AACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGAT
ACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCG
ACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTC
AGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAGACCG

BLAST output : Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MH900522.1
Length: 876Number of Matches: 1

151:

CGCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGC
GGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAA
AAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTG
GCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTC
ACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCT
CGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCT
CGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGG
GCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTG
CCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCGCCAA

BLAST output : Escherichia coli strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MH900522.1

Length: 876Number of Matches: 1

157:

GTGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGTGGGTGATAAGACCGGCAGCGGCGACTACGGCACCACCAATGATATTGCGGTGATCTGGCCGCAGGGTCGTGCGCCGCTGGTTCTGGTGACCTATTTTACCCAGCCGCAACAGAACGCAGAGCGCCGCCGCGATGTGCTGGCTTCAGCGCCGGA

BLAST output : Escherichia coli strain Hb-9 plasmid pHb-9 insertion sequence ISEcp1, partial sequence; beta-lactamase **CTX-M-65** (blaCTX-M-65) and truncated transposase of IS903D (tnpA) genes, complete cds; insertion sequence IS26, complete sequence; fosfomycin resistance protein (fosA3), hypothetical protein, CadC-like protein, and putative transcriptional regulator,TetR family genes, complete cds; and insertion sequence IS26, partial sequence

Sequence ID: KX495605.1 Length: 5927Number of Matches: 1

158:

ATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGGGCAGCGCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAAGCAGCGGAGGGCGGCTGGGCGTCGCGCTCATCGATACCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCCGAAAAACACGTCAACGGCACAATGACGCTGGCAGAGCTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTC

BLAST output : Escherichia coli pMSP520 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-51**, complete CDS.Sequence ID: NG_049002.1

Length: 876Number of Matches: 1

159:

TCGAGCCGGAACGTGTCATGCGGGCGTCAGGCTGCCGTAATGGCGATTTGCGCCCGGACCAGGCCGCACGGGGAAACTCTGCGGCCTTTTTCGTTCTTACTGCGGGTAAGGCACCCAGTCGCCGCCGT

TCAGGCGAACGTACGGTTTATCCTGGTATTGAATAACTACTGCATTTGAGTTCTCGGAGACCGGT
GCTGTTTGTGGTAACCCACTGGTGAGTTTTTCCAGTCAACATTGTCTTCGGTGAAAATCTTGCC
ATCAAGAACGCGAACCACCAGATCGGCAGGCTTGA

BLAST output: *Escherichia coli* strain MNCRE44, complete genome**.**Sequence ID: gb| CP010876.1| Length: 5010884Number of Matches: 3

161:

TAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGC
GGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTA
TCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTG
AAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTT
GTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCG
CGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCC
GGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAG
CCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGC
AAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGA
CATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGT
TGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCG

BLAST output: Escherichia coli strain F170 **extended-spectrum beta-lactamase CTX-M-15 gene (CTX-M 1 group)** , partial cds.Sequence ID: gb|KP325147.1| Length: 843Number of Matches: 1

164:

CGCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGC
GGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAA
AAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTG
GCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTC
ACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCT
CGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCT
CGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGG
GCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTG
CCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCGCCAA

BLAST output: Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1 Length: 876Number of Matches: 1

166:

TACGCAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGT

BLAST output: Klebsiella pneumoniae strain E12KPMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds

Sequence ID: KY640528.1 Length: 640Number of Matches: 1

168:

GCGAGAGCGATAAGCACCTGCTAAATCAGCGCGTTGAAATCAAGAAGAGCGACCTGGTTAACTACAATCCCATTGCGGAGAAACACGTTAACGGCACGATGACGCTGGCTGAGCTTGGCGCAGCGGCGCTGCAGTATAGCGACAATACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTCCCGATAAAGTGACGGCGTTTGCTCGCTCGTTGGGTGATGAGACCTTCCGTCTGGACAGAACCGAGCCCACGCTCAATACCGCCATTCCAGGCGACCCGCGTGATACCACCACGCCGCTCGCGATGGCGCAGACCCTGAAAAATCTGACGCTGGGTAAAGCGCTGGCGGAAACTCAGCGGGCACAGTTGGTGACGTGGCTTAAGGGCAATACTACCGGTAGCGCGAGCATTCGGGCGGGTCTGCCGAAATCATGGGTAGTGGGCGATAAAACCGGCAGCGGAGATTATGGCACCACCAACGATATCGCGGTTATCTGGCCGGAAAACCACGCACCGCTGGTTCTGGTGACCTACTTTACCCAACCGGAGCAGAAGGCGGAAAGCCGTCGGGATATTCTGGCTGCGGCGGG

BLAST output: Klebsiella pneumoniae strain KP10 extended-spectrum beta-lactamase CTX-M-59 (blaCTX-M) gene, blaCTX-M-59 allele, partial cds. Sequence ID: MH661247.1 Length: 843Number of Matches: 1

172:

CAAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGTGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACCGATATCGAGA

BLAST output: Escherichia coli strain BA22372 plasmid **pCTX-M-15**_22372, complete sequence.Sequence ID: CP040398.1 Length: 98470Number of Matches: 1

174:

AAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAACCGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAATGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGG

BLAST output: Escherichia coli blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-172,** complete CDS.Sequence ID: NG_048957.1Length: 876Number of Matches: 1

175:

AGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCACAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCAC

BLAST output: Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MH900522.1Length: 876Number of Matches: 1

177:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG

TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCA

BLAST output: Klebsiella pneumoniae subsp. pneumoniae strain KPTR7239-17 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MK113956.1
Length: 1026Number of Matches:

179:

CTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGGTTGTGGGGGGATAAAACCGGCAGCGGTGACTATG

BLAST output: Klebsiella pneumoniae isolate KSH203 plasmid pKSH203-**CTX-M-3**, complete sequence.Sequence ID: CP034325.1Length: 156910Number of Matches: 1

181:

CGCAAAAACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACAACCGAATATCGAAAATTTC

BLAST output: Klebsiella pneumoniae strain E161KPMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds.Sequence ID: KY640551.1Length: 632Number of Matches: 1

184:

TCACTGAGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAAGCGGCAGTGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGG

BLAST output : Klebsiella oxytoca 1125476 blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-162**, complete CDS.Sequence ID: NG_048947.1
Length: 876Number of Matches: 1

185:

TAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCG

BLAST output : Escherichia coli strain F170 **extended-spectrum beta-lactamase CTX-M-15 gene (CTX-M 1 group)** , partial cds.Sequence ID: gb| KP325147.1|
Length: 843Number of Matches: 1

187:

CAAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCACCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTGTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCA

BLAST output : Klebsiella pneumoniae KL8-ctx-m-15 gene cluster, complete sequence

Sequence ID: MH523447.1 Length: 20281Number of Matches: 1

190:

GCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCCAA

BLAST output : Klebsiella pneumoniae partial CTX-M-15 gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 243.Sequence ID: LT628518.1 Length: 626Number of Matches: 1

193:

CGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGCGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCTGTGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACG

CTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCA
GCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGATCGTGCGCCGCTGAT
TCTGGTCACTTACTTCACCCAGCCTCAACC

BLAST output : Escherichia coli strain IPK174 beta-lactamase CTX-M-55 (blaCTX-M) gene, blaCTX-M-55 allele, complete cds

Sequence ID: MH900523.1 Length: 876Number of Matches: 1

195:

CTCAATGTTAACGGTGATGGCGACGCTACCCCTGCTATTTAGCAGCGCAACGCTGCATGCGCAG
GCGAACAGCGTGCAACAGCAGCTGGAAGCCCTGGAGAAAAGTTCGGGAGGTCGGCTTGGCGTT
GCGCTGATTAACACCGCCGATAATTCGCAGATTCTCTACCGTGCCGATGAACGTTTTGCGATGTG
CAGTACCAGTAAGGTGATGGCGGCCGCGGCGGTGCTTAAACAGAGCGAGAGCGATAAGCACCT
GCTAAATCAGCGCGTTGAAATCAAGAAGAGCGACCTGGTTAACTACAATCCCATTGCGGAGAA
ACACGTTAACGGCACGATGACGCTGGCTGAGCTTGGCGCAGCGGCGCTTCAGTATAGCGACAAT
ACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTC

BLAST output : Klebsiella pneumoniae strain KP75 extended-spectrum beta-lactamase **CTX-M-2** (blaCTX-M) gene, blaCTX-M-2 allele, partial cds

Sequence ID: MH661246.1 Length: 829Number of Matches: 1

198:

GTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGGGCAGCGCGCCGCTTT
ATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAAGCAGCGGAGGGCGG
CTGGGCGTCGCGCTCATCGATACCGCAGATAATACGCAGGTGCTTTATCACGGTGATGAACGCT
TTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCCGCGGCGGTGCTTAAGCAGAGTGAAACGCA
AAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATT
GCCGAAAAACACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTAC
AGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCT
TTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCG
CCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGC
TTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGCA
ATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGTGGGTGATAAGA
CCGGCAGCGGCGGCTACGGCACCACCAATGATATTGCGGTGATCTGGCCGCAGGGTCG

BLAST output ::Escherichia coli strain IPK37 beta-lactamase CTX-M-27 (blaCTX-M) gene, blaCTX-M-27 allele, complete cds

Sequence ID: MH900525.1 Length: 876Number of Matches: 1

200:

CGCAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAC
AGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAA
GTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGA
GTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGA
CGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCA

BLAST output : Klebsiella pneumoniae strain KPCSQ15a extended spectrum beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, partial cds

Sequence ID: MH891569.1 Length: 731Number of Matches: 1

Appendix III

YG Hospital samples DNA sequences.

201:

CGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAAC
CGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGC
GGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAG
CGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTC
GCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCC
ATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGA
CGCTGGAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTG
CTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCG

BLAST output : Escherichia coli strain IPK182 beta-lactamase **CTX-M-15 (blaCTX-M)** gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1Length: 876Number of Matches: 1

202

AGCATTCGCCGCTCAATGTTAACGGTGATGGCGACGCTACCCCTGCTATTTAGCAGCGCA
ACGCTGCATGCGCAGGCGAACAGCGTGCAACAGCAGCTGGAAGCCCTGGAGAAAAGTTCG
GGAGGTCGGCTTGGCGTTGCGCTGATTAACACCGCCGATAATTCGCAGATTCTCTACCGT
GCCGATGACAATGTTTTGCGATGTGCAGTACCAGTAAGGTGATGGCGGCCGCGGCGGTGCTT
AAACAGAGCGAGAGCGATAAGCACCTGCTAAATCAGCGCGTTGAAATCAAGAAGAGCGACCTG
GTTAACTACAATCCCATTGCGGAGAAACACGTTAACGGCACGATGACGCTGGCTGAGCTTGGCG
CAGCGGCGCTGCAGTATAGCGACAATACTGCCATGAATAAGCTGATTGCCCATCTGGGTGGTC

BLAST output : Salmonella enterica subsp. enterica serovar Manhattan plasmid **blaCTX-M-2** gene for beta-lactamase CTX-M-2, complete cds, strain: 3377

Sequence ID: LC229068.1Length: 876Number of Matches: 1

205:

TTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAGAAACTTGCCGAATTAGAG
CGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTT
ATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCT
GAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCT
TGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGC
GCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCC
CGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGA
GCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCG
CAAAC
TCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATG
GATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCACCAACGATATCG

BLAST output : Escherichia coli strain EP174a beta-lactamase (blaCTX-M15) gene, partial cds

Sequence ID: MF346615.1Length: 871Number of Matches: 1

207:

AGTTCACGCTGATGGCGACGGCAACCGTCAGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAA
ACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTG
GCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGT

GCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATC
TGTTAAATCAGCGAGCTGAGATCAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAG
CACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAAC
GTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGAC
AGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGG
CGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGT
AAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGT
GCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAA

BLAST output : Shigella sp. SH223 blaCTX-M-108 (CTX-M-108) gene, partial cds

Sequence ID: JF274245.1Length: 864Number of Matches: 1

209:

AACGTGTCATGCGGGCGTCAGGCTGCCGTAATGGCAATTTGCGCCCGGACCAGGCCGCAGGGG
GAAACTCTGCGGCCTTTTTCGTTCTTACTGCGGGTAAGGCACCCAGTCGCCGCCGTTCAGGCGA
ACGTACGGTTTATCCTGGTATTGAATAACTACTGCATTTGAGTTCTCGGAGACCGGTGCTGTTTG
TGGTAACCCACTGGTGAGTTTTTTCCAGTCAACATTGTCTTCGGTGAAAATCTTGCCATCAAGAA
CGCGAACCACCAGATCGG

BLAST output : Escherichia coli strain 3385 chromosome, complete genome

Sequence ID: CP029420.1Length: 4910422Number of Matches: 3

211:

AGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCA
AACGGCGGACGTACAGCAGAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGT
GGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATG
TGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAAT
CTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAA
AGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATA
ACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCG
ACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCG
GGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGG
GTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCG
GTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGT

BLAST output : Escherichia coli strain EP174a beta-lactamase (blaCTX-M15) gene, partial cds Sequence ID: MF346615.1Length: 871Number of Matches: 1

215:

AGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAACCGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGC

Escherichia coli blaCTX-M gene for class A extended-spectrum beta-lactamase **CTX-M-172**, complete CDS Sequence ID: NG_048957.1Length: 876Number of Matches: 1

217:

ACGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGACAGGGT

BLAST output : Escherichia coli strain BA22372 plasmid pCTX-M-15_22372, complete sequence

Sequence ID: CP040398.1Length: 98470Number of Matches: 1

219:

GGATGGCTCGAGTTTTTCAGCAAGATTTAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCATCTTTCTAGAAGATCTCCTACAATATTCTCAGC

TGCCATGGAAAATCGATGTTCTTCTTTTATTCTCTCAAGATTTTCAGGCTGTATATTAAAACTTATATTAAGAACTATGCTAACCACCTCATCAGGAACCGTTGTAGGTGGCGTGGGTTTTCTTGGCAATCGACTCTCATGAAAACTACGAGCTAAATATTCAATATGTTCCTCTTGACCAACTTTATTCTGCATTTTTTTTGAACGAGGTTTAGAGCAAGCTTCAGGAAACTGAGACAGGAATTTTATTAAAAATTTAAATTTTGAAGAAAGTTCAGGGTTAATAGCATCCATTTTTTGCTTTGCAAGTTCCTCAGCATTCTTAACAAAAGACGTCTCTTTTGACATGTTTAAAGTTTAAACCTCCTGTGTGAAATTATTATCCGCTCATAATTCCACACATTATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCAATTGCTTTCCAGTCGGGAAACCTGTCGTGCCA **BLAST output :**Escherichia coli strain CR4 plasmid class A extended-spectrum beta-lactamase CTX-M-**15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds Sequence ID: MK405591.1Length: 876Number of Matches: 1
223: GCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCCAA **BLAST output :**Klebsiella pneumoniae partial CTX-M-15 gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 243 Sequence ID: LT628518.1Length: 626Number of Matches: 1
226: ATGGCATAATGGTCTCAGCGCTCAGCCCTTCGGCGATGATTCTCGCCGCTGAAGCCAGCACATCGCGGCGGCTCTCTGCGTTCTGTTGCGGCTGGGTAAAATAGGTCACCAGAACCAGCGGCGCACGACCCTGCGGCCAGATCACCGCAATATCATTGGTGGTGCCGTAGTCGCCGCTGCCGGTCTTATCACCTACAGTCCACGACGTCGGTAAGCCGGCCCGAATGCTGGCTGCGCCGGTCGTATTGCCTTTGAGCCACGTCACCAACTGCGCCCGCTGGGTTTCGCCCAGCGCATAACCCAGCGTAAGCTGACGCAACGTCTGTGCCATCGCCCGCGGCGTGGTGGTGTCTCTCGGGTCGCCGGGAATGGCGGTATTCAGCGTAGGTTCAGTGCGATCCAGACGAAACGTCTCATCGCCGATCGCGCGGGCAAAAGCCGTCACGCCTCCCGGGCCACCGAGCTGGGCAATCAATTTGTTCATGGCGGTATTGTCGCTGTACTGCAACGCGGCCGCGCTCAGCTCTGCCAGCGTCATTGTGCCGTTGACGTGTTTTTCGGCAATCGGATTGTAGTTAACCAGATCGGCAGGCTTGATCTCGACAGGCTGATTAAGCAGCTGCTTTTGCGTTTCACTCTGCT

TAAGCACCGCCGCGGCCGCCATAACTTTACTGGTACTGCACATTGGAAAGCGTTCATCACCGCG
ATAAAGCACCTGCGTATTATCTGCGGTATCGATGAGCGCGACGCCCAGCCGCCCTCCGCTGCTT
TTCTCCAGCGCCGCCAGCTTTTGCTGCACCGCACTCGTCTGCGCATAAAGCGGCGCGCTGCACA
GCAGCAGCGGAATGCACGCCGCCGCCGCGAACATCATCCGTTGCACTCTCTTTGTCACCATGGC
CTGAGACCATTATGCCAT

BLAST output : Escherichia coli strain SCEC020022 plasmid pCTXM14_020022, complete sequence.Sequence ID: CP032888.1Length: 109553Number of Matches: 1

228:

AACACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACA
ATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCG
CGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATCCGCCATTCCCG
GCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGG
GTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCCAAAGGCAATACGACCGG
CGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTG

BLAST output : Escherichia coli strain IPK37 beta-lactamase CTX-M-27 (blaCTX-M) gene, blaCTX-M-27 allele, complete cds.Sequence ID: MH900525.1Length: 876Number of Matches: 1

229:

GCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGC
CGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGA
GATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAA
TAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGG
AGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGA
TCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGG
TAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCAC
CGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGG
CAGCGGTGGCTATGGCACCCAA

BLAST output : Klebsiella pneumoniae partial CTX-M-15 gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 243
Sequence ID: LT628518.1Length: 626Number of Matches: 1

231:

CGACTTAGAGCGTGCACGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAAT
CGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGC
GGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAA
AAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTG
GCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTC
ACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCT
CGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCT
CGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGG
CGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGC
CTGCTTCCTGGGTTGGGGGGGAAAAAACC

BLAST output : Klebsiella pneumoniae subsp. pneumoniae strain KPTR2-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MK113959.1Length: 1027Number of Matches: 1

238:

CGCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAG
CAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGT
GATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGT
TGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACG
ATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGC
TGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAAC
GTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACC
ACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACA
GCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGG
CTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGCCA

BLAST output : Klebsiella pneumoniae partial **CTX-M-15** gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 327 .Sequence ID: LT628520.1Length: 626Number of Matches: 1

241:

AGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAG
TCGGGAGGAAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATGTGC
TGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGTGGCCGCGGTGCTGAAGAA
AGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACTTGGTTAACT
ATAATCCGATTGCGGAAAAGCACGTCGATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGC
GCTACAGTACAGCGATAACGGCGCGATGAATAAGCTGATTTCTCACGTTGGCGGCCCGGTAGCG

TCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTT
AAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTG
CGTAATCTGACGCTGGGTAAAGCATTGGGTGACAGCCAACGGGCGCAGCTGGTGACATGGATG
AAAGGCA

BLAST output : Escherichia coli strain IPK192 beta-lactamase CTX-M-32 (blaCTX-M) gene, blaCTX-M-32 allele, complete cds.Sequence ID: MH900527.1Length: 876Number of Matches: 1

242:

TAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCA
GCGAGTTGAGATCACAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAAT
GGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATG
AATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAG
ACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCG
TGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTG
GGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGC
ATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCA
CCCCACCCA

BLAST output : Klebsiella pneumoniae partial **CTX-M-15** gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 243

Sequence ID: LT628518.1Length: 626Number of Matches: 1

244:

TATGGGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGA
ACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATT
GCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTAC
AGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGT
TCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGC
CATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTA
CGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATA
CCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGG
CAGCGGTGGCTATGGCACCACCA

BLAST output : Escherichia coli class A extended-spectrum beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MK234851.1

Length: 877Number of Matches: 1

246:

GGTCTCAGCGCTCAGCCCTTCGGCGATGATTCTCGCCGCTGAAGCCAGCACATCGCGGCGGCTCTCTGCGTTCTGTTGCGGCTGGGTAAAATAGGTCACCAGAACCAGCGGCGCACGACCCTGCGGCCAGATCACCGCAATATCATTGGTGGTGCCGTAGTCGCCGCTGCCGGTCTTATCACCTACAGTCCACGACGTCGGTAAGCCGGCCCGAATGCTGGCTGCGCCGGTCGTATTGCCTTTGAGCCACGTCACCAACTGCGCCCGCTGGGTTTCGCCCAGCGCATAACCCAGCGTAAGCTGACGCAACGTCTGTGCCATCGCCCGCGGCGTGGTGGTGTCTCTCGGGTCGCCGGGAATGGCGGTATTCAGCGTAGGTTCAGTGCGATCCAGACGAAACGTCTCATCGCCGATCGCGCGGGCAAAAGCCGTCACGCCTCCCGGGCCACCGAGCTGGGCAATCAATTTGTTCATGGCGGTATTGTCGCTGTACTGCAACGCGGCCGCGCTCAGCTCTGCCAGCGTCATTGTGCCGTTGACGTGTTTTTCGGCAATCGGATTGTAGTTAACCAGATCGGCAGGCTTGATCTCGACAGGCTGATTAAGCAGCTGCTTTTGCGTTTCACTCTGCTTAAGCACCGCCGCGGCCGCCATAACTTTACTGGTACTGCACATTGGAAAGCGTTCATCACCGCGATAAAGCACCTGCGTATTATCTGCGGTATCGATGAGCGCGACGCCCAGCCGCCCTCCGCTGCTTTTCTCCAGCGCCGCCAGCTTTTGCTGCACCGCACTCGTCTGCGCATAAAGCGGCGCGCTGCACAGCAGCAGCGGAATGCACGCCGCCGCCGCGAACATCATCCGTTGCACTCTCTTTGTCACCATGGCCTGAGACCATTATGCCAT

BLAST output : Escherichia coli strain SCEC020022 plasmid pCTXM14_020022, complete sequence.Sequence ID: CP032888.1Length: 109553Number of Matches: 1

248:

AACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGA

Blast result : Escherichia coli strain IPK182 beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1Length: 876Number of Matches: 1

250:

GGATGGCTCGAGTTTTTCAGCAAGATTTAGGAAATGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATG

GGACGATGTCACTGGCTGAGCTTAGCGCGGCATCTTTCTAGAAGATCTCCTACAATATTCTCAGCTGCCATGGAAAATCGATGTTCTTCTTTTATTCTCTCAAGATTTTCAGGCTGTATATTAAAACTTATATTAAGAACTATGCTAACCACCTCATCAGGAACCGTTGTAGGTGGCGTGGGTTTTCTTGGCAATCGACTCTCATGAAAACTACGAGCTAAATATTCAATATGTTCCTCTTGACCAACTTTATTCTGCATTTTTTTTGAACGAGGTTTAGAGCAAGCTTCAGGAAACTGAGACAGGAATTTTATTAAAAATTTAAATTTTGAAGAAAGTTCAGGGTTAATAGCATCCATTTTTTGCTTTGCAAGTTCCTCAGCATTCTTAACAAAAGACGTCTCTTTTGACATGTTTAAAGTTTAAACCTCCTGTGTGAAATTATTATCCGCTCATAATTCCACACATTATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCAATTGCTTTCCAGTCGGGAAACCTGTCGTGCCA

Blast result : Escherichia coli strain CR4 plasmid class A extended-spectrum beta-lactamase CTX-M-**15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MK405591.1Length: 876Number of Matches: 1

252:

TGACTCAGAGCATTCGCCGCTCAATGTTAACGGTGATGGCGACGCTACCCCTGCTATTTAGCAGCGCAACGCTGCATGCGCAGGCGAACAGCGTGCAACAGCAGCTGGAAGCCCTGGAGAAAAGTTCGGGAGGTCGGCTTGGCGTTGCGCTGATTAACACCGCCGATAATTCGCACCGATTCTCTACCGTGCCGATGACCGTTTTGCGATGTGCAGTACCAGTAAGGTGATGGCGGCCGCGGCGGTGCTTAAACAGAGCGAGAGCGATAAGCACCTGCTAAATCAGCGCGTTGAAATCAAGAAGAGCGACCTGGTTAACTACAATCCCATTGCTGAGAAACACGTTAACGGCACTATGACGCTGGCTGAGCTTGGCGCAGCGGCGCTGCAGTATAGCGACAA

Blast result : Escherichia coli strain IPK118 beta-lactamase CTX-M-2 (blaCTX-M) gene, blaCTX-M-2 allele, partial cds.Sequence ID: MH900526.1Length: 793Number of Matches: 1

254:

AGATGCCGTGATTAGCGCTCCGGTGCCGATGACCCCGTTACAGGAGTTCTGGCACTATTTTAAACGCAACACAGGCGCGGTTGTCGGGCTGGTTTACGTCGTCATCGTGCCGTTTACAAGGCACCACCACCAACGAC

Blast result : Escherichia coli strain 4928STDY7071340 genome assembly, chromosome: 1.Sequence ID: LR607331.1Length: 5120867Number of Matches: 1

257:

TCAGCGCTCAGCCCTTCGGCGATGATTCTCGCCGCTGAAGCCAGCACATCGCGGCGGCTCTCTGCGTTCTGTTGCGGCTGGGTAAAATAGGTCACCAGAACCAGCGGCGCACGACCCTGCGGCCAGATCACCGCAATATCATTGGTGGTGCCGTAGTCGCCGCTGCCGGTCTTATCACCTACAGTCCACGAC

GTCGGTAAGCCGGCCCGAATGCTCGCTGCGCCGGTCGTATTGCCTTTGAGCCACGTCACCAACT
GCGCCCGCTGGGTTTCGCCCAGCGCATAACCCAGCGTAAGCTGACGCAACGTCTGTGCCATCGC
CCGCGGCGTGGTGGTGTCTCTCGGGTCGCCGGGAATGGCGGTATTCAGCGTAGGTTCAGTGCGA
TCCAGACGAAACGTCTCATCGCCGATCGCGCGGGCAAAAGCCGTCACGCCTCCCGGGCCACCG
AGCTGGGCAATCAATTTGTTCATGGCGGTATTGTCGCTGTACTGCAACGCGGCCGCGCTCAGCT
CTGCCAGCGTCATTGTGCCGTTGACGTGTTTTTCGGCAATCGGATTGTAGTTAACCAGATCGGCA
GGCTTGATCTCGACAGGCTGATTAAGCAGCTGCTTTTGCGTTTCACTCTGCTTAAGCACCGCCGC
GGCCGCCATAACTTTACTGGTACTGCACATTGGAAAGCGTTCATCACCGCGATAAAGCACCTGC
GTATTATCTGCGGTATCGATGAGCGCGACGCCCAGCCGCCCTCCGCTGCTTTTCTCCAGCGCCGC
CAGCTTTTGCTGCACCGCACTCGTCTGCGCATAAAGCGGCGCGCTGCACAGCAGCAGCGGAATG
CACGCCGCCGCCGCGAACATCATCCGTTGCACTCTCTTTGTCACCATGGCCTGAGACCATTATGC
CAT

BLAST output : Escherichia coli strain SCEC020022 plasmid pCTXM14_020022, complete sequence.Sequence ID: CP032888.1Length: 109553Number of Matches: 1

259:

AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCG
TGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAG
AAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTA
ACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGC
CGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCT
AGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGA
CGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAAC
TCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATG
GATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTG
GGGGAAAAAACCGGCAGCGGTGGCTATGGAACACA

Blast result : Klebsiella pneumoniae partial CTX-M-15 gene for Class A Extended Spectrum beta-lactamase CTX-M-15, isolate 122Sequence ID: LT628516.1Length: 622Number of Matches: 1

262:

GCGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAC
AGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAA
GTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGA
GTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGA
CGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA

CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAAACGGGCAGCGGTGGCTATGGCACCAC
AAC

Blast result : Klebsiella pneumoniae strain KPTR1-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-115 allele, complete cds Sequence ID: MK113960.1Length: 1008Number of Matches: 1

264:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG
TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGGATAAAACCGGCAGCGGTGGC

Blast result : Escherichia coli strain CR4 plasmid class A extended-spectrum beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds
Sequence ID: MK405591.1Length: 876Number of Matches: 1

266:

CGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGC
GGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAA
AAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTG
GCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGCCGATGAATAAGCTGATTGCTC
ACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCT
CGACCGTACCGAGCTCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACC
TCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACG
GGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACT
GCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACC

Blast result : Escherichia coli strain MIAE02105 extended-spectrum class A beta lactamase CTX-M-15 (bla CTX-M15) gene, partial cds.Sequence ID: MF977517.1Length: 867Number of Matches: 1

268:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG
TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGC

Blast result : Escherichia coli strain CR4 plasmid class A extended-spectrum beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MK405591.1 Length: 876Number of Matches: 1

271 :

GCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGC
AGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTG
ATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTT
GAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGA
TGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCT
GATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACG
TTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCA
CTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAG
CCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGC
TGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAAC
GATATCGACGAT

Blast result : Escherichia coli strain E74ECMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds.Sequence ID: KY640534.1Length: 640Number of Matches: 1

272 (lower band):

TAGTTAGGCTCTTCAACCCAGTCGCCCTCTGTTGCCCACCAGTGATTAAATTCGTCGTGATTTGCTGAAACTGCCATACCCTATCGCCTGTCGTTTTTTATTAACGACAATGACTATAGATGTTTAGCTGAGGAAAATCTTAATAATACGTGTTGTATTGACGAGTATCTTATGCCGGGAACAAGTCATCTCG

Blast result : Escherichia coli strain 4928STDY7071340 genome assembly, chromosome: 1

Sequence ID: LR607331.1Length: 5120867Number of Matches: 1

273:

TTCGCATATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCGAC

Blast result : Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds

Sequence ID: MH900522.1Length: 876Number of Matches: 1

275:

AGGGCGGCTGGGCGTCGCGCTCATCGATACCGCAGATAATACGCAGGTGCTTTATCGCGGTGATGAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGTCGCGGCGGTGCTTAAGCAGAGTGAAACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCCTGCCGATCTGGTTAACTACAATCCGATTGCAGAAAAACACGTCAACGGCACAATGACGCTGGCAGAACTGAGCGCGGCCGCGTTGCAGTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGACGGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCTCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGG

Salmonella enterica subsp. enterica serovar California strain GZ680 extended-spectrum beta-lactamase **CTX-M-90** (blaCTX-M-90) gene, partial cds

Sequence ID: MF418175.1Length: 823Number of Matches: 1

277:

GTTAAAAAATCACTGAGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAGGAA
GTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAAGCGGCAGTG
TCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAATACTTTATCGTGC
TGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAA
AAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAAC
TATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCG
CGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAG
CGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACG
TTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTC
TGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGA
TGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACT

BLAST output : Escherichia coli 1125509 blaCTX-M gene for class A extended-spectrum beta-lactamase CTX-M-163, complete CDS.Sequence ID: NG_048948.1Length: 876Number of Matches: 1

279:

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAG
GAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGC
AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCG
TGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAG
AAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTA
ACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGC
CGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCT
AGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGA
CGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAAC
TCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCAACGGGCGCAGCTGGTGACATGG
ATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGG

Blast result : Escherichia coli strain HG16 **extended spectrum beta-lactamase CTX-M-15 (blaCTX-M-15)** gene, partial cds.Sequence ID: gb|KR338941.1|Length: 867Number of Matches: 1

281:

GCGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAC
AGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAA
GTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGA
GTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGA
CGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAAACGGGCAGCGGTGGCTATGGCACCAC
AAC

Blast result : Klebsiella pneumoniae strain KPTR1-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase CTX-M-15 (blaCTX-M) gene, blaCTX-M-115 allele, complete cds.Sequence ID: MK113960.1Length: 1008Number of Matches: 1

283:

ATGGCATAATGGTCTCAGCGCTCAGCCCTTCGGCGATGATTCTCGCCGCTGAAGCCAGCACATC
GCGGCGGCTCTCTGCGTTCTGTTGCGGCTGGGTAAAATAGGTCACCAGAACCAGCGGCGCACGA
CCCTGCGGCCAGATCACCGCAATATCATTGGTGGTGCCGTAGTCGCCGCTGCCGGTCTTATCAC
CTACAGTCCACGACGTCGGTAAGCCGGCCCGAATGCTGGCTGCGCCGGTCGTATTGCCTTTGAG
CCACGTCACCAACTGCGCCCGCTGGGTTTCGCCCAGCGCATAAGCCAGCGTAAGCTGACGCAAC
GTCTGTGCCATCGCCCGCGGCGTGGTGGTGTCTCTCGGGTCGCCGGGAATGGCGGTATTCAGCG
TAGGTTCAGTGCGATCCAGACGAAACGTCTCATCGCCGATCGCGCGGGCAAAAGCCGTCACGCC
TCCCGGGCCACCGAGCTGGGCAATCAATTTGTTCATGGCGGTATTGTCGCTGTACTGCAACGCG
GCCGCGCTCAGCTCTGCCAGCGTCATTGTGCCGTTGACGTGTTTTTCGGCAATCGGATTGTAGTT
AACCAGATCGGCAGGCTTGATCTCGACAGGCTGATTAAGCAGCTGCTTTTGCGTTTCACTCTGCT
TAAGCACCGCCGCGGCCGCCATAACTTTACTGGTACTGCACATTGGAAAGCGTTCATCACCGCG
ATAAAGCACCTGCGTATTATCTGCGGTATCGATGAGCGCGACGCCCAGCCGCCCTCCGCTGCTT
TTCTCCAGCGCCGCCAGCTTTTGCTGCACCGCACTCGTCTGCGCATAAAGCGGCGCGCTGCACA
GCAGCAGCGGAATGCACGCCGCCGCCGCGAACATCATCCGTTGCACTCTCTTTGTCACCATGGC
CTGAGACCATTATGCCAT

BLAST output : Escherichia coli strain SCEC020022 plasmid pCTXM14_020022, complete sequence.Sequence ID: CP032888.1Length: 109553Number of Matches: 1

286:

CGCAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACAA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG

TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA
CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCA

Blast result : Klebsiella pneumoniae strain KPCSQ15a extended spectrum beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, partial cds

Sequence ID: MH891569.1Length: 731Number of Matches: 1

288:

GCAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGC
AGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTG
ATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTT
GAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGA
TGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCT
GATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACG
TTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCA
CTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAG
CCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGC
TGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGCACCACCAAC
GATATCGACGAT

Blast result : Escherichia coli strain E74ECMO beta-lactamase **CTX-M-15** (blaCTX-M-15) gene, partial cds.Sequence ID: KY640534.1Length: 640Number of Matches: 1

290:

CGCAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACA
GCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAG
TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAG
TTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGAC
GATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAA
GCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAA
ACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATA

CCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGA
CAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCA
GGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGGATAAAACCGGCAGCGGTGGCTATGGCACAGC

Blast result : Klebsiella pneumoniae subsp. pneumoniae strain KPTR13-18 insertion sequence ISEcp1, partial sequence; and beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MK113957.1Length: 1027Number of Matches: 1

292:

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGCTGTTGTTAG
GAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAACTTGCCGAATTAGAGCGGC
AGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCG
TGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAG
AAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTA
ACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGC
CGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCT
AGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGA
CGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAAC
TCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCAACGGGCGCAGCTGGTGACATGG
ATGAAAGGCAATACCACCGG

Blast result : Escherichia coli strain HG16 **extended spectrum beta-lactamase CTX-M-15 (blaCTX-M-15)** gene, partial cds.Sequence ID: gb|KR338941.1|Length: 867Number of Matches: 1

293:

ACAAAGAGAGTGCAACGGATGATGTTCGCGGCGGCGGCGTGCATTCCGCTGCTGCTGGGCAGC
GCGCCGCTTTATGCGCAGACGAGTGCGGTGCAGCAAAAGCTGGCGGCGCTGGAGAAAAGCAGC
GGAGGGCGGCTGGGCGTCGGCTCATCGATACCGCAGATAATACGCAGTGCTTTATCGCGGTGAT
GAACGCTTTCCAATGTGCAGTACCAGTAAAGTTATGGCGGCCGCGGCGGTGCTTAGCAGAGGAA
ACGCAAAAGCAGCTGCTTAATCAGCCTGTCGAGATCAAGCTGCCGATCTGGTTAACTACAATCC
GATTGCCGAAAAACACGTCAACGGCACAATGACGCTGGCAGAGCTGAGCGCGGCCGCGTTGCA
GTACAGCGACAATACCGCCATGAACAAATTGATTGCCCAGCTCGGTGGCCCGGGAGGCGTGAC
GGCTTTTGCCCGCGCGATCGGCGATGAGACGTTTCGTCTGGATCGCACTGAACCTACGCTGAAT
ACCGCCATTCCCGGCGACCCGAGAGACACCACCACGCCGCGGGCGATGGCACAGACGTTGCGT
CAGCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAA
GGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGCAGGTGAT
AAGGCGACTACGGCACCACCAATGATATTGCG

BLAST output: Enterobacter hormaechei strain WCHEH020038 plasmid pCTXM9_020038, complete sequence >Sequence ID: CP031724.1Length: 296580Number of Matches: 1

294:

TCAGCGCTCAGCCCTTCGGCGATGATTCTCGCCGCTGAAGCCAGCACATCGCGGCGGCTCTCTGCGTTCTGTTGCGGCTGGGTAAAATAGGTCACCAGAACCAGCGGCGCACGACCCTGCGGCCAGATCACCGCAATATCATTGGTGGTGCCGTAGTCGCCGCTGCCGGTCTTATCACCTACAGTCCACGACGTCGGTAAGCCGGCCCGAATGCTCGCTGCGCCGGTCGTATTGCCTTTGAGCCACGTCACCAACTGCGCCCGCTGGGTTTCGCCCAGCGCATAACCCAGCGTAAGCTGACGCAACGTCTGTGCCATCGCCCGCGGCGTGGTGGTGTCTCTCGGGTCGCCGGGAATGGCGGTATTCAGCGTAGGTTCAGTGCGATCCAGACGAAACGTCTCATCGCCGATCGCGCGGGCAAAAGCCGTCACGCCTCCCGGGCCACCGAGCTGGGCAATCAATTTGTTCATGGCGGTATTGTCGCTGTACTGCAACGCGGCCGCGCTCAGCTCTGCCAGCGTCATTGTGCCGTTGACGTGTTTTTCGGCAATCGGATTGTAGTTAACCAGATCGGCAGGCTTGATCTCGACAGGCTGATTAAGCAGCTGCTTTTGCGTTTCACTCTGCTTAAGCACCGCCGCGGCCGCCATAACTTTACTGGTACTGCACATTGGAAAGCGTTCATCACCGCGATAAAGCACCTGCGTATTATCTGCGGTATCGATGAGCGCGACGCCCAGCCGCCCTCCGCTGCTTTTCTCCAGCGCCGCCAGCTTTTGCTGCACCGCACTCGTCTGCGCATAAAGCGGCGCGCTGCACAGCAGCAGCGGAATGCACGCCGCCGCCGCGAACATCATCCGTTGCACTCTCTTTGTCACCATGGCCTGAGACCATTATGCC

BLAST output : Escherichia coli strain SCEC020022 plasmid pCTXM14_020022, complete sequence.Sequence ID: CP032888.1Length: 109553Number of Matches: 1

296:

CAAACTTGCCGATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGGCTATGGACA

BLAST output: Escherichia coli strain E78ECMO **CTX-M-15** beta-lactamase (blaCTX-M-15) gene, partial cds.Sequence ID: KY640536.1 Length: 641Number of Matches: 1

298:

ACGTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGAAGACTGGGTGTGGCATTGATTAACACAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTGATGGCCGTGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAAAAAATCTGACTTGGTTAACTATAATCCGATTGCGGAAAAGCACGTCGATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTTCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGATGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGTAATCTGACGCTGGGTAAAGCATTGGGTGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGGTGACTATGGCACCACCAACGATATCGCGGTGATCTGGCCAAAAGATCGTGCTCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGCAGAAAGCCGTCGC

BLAST output: Escherichia coli strain A19 plasmid beta-lactamase **CTX-M-1** (blaCTX-M) gene, blaCTX-M-CTX-M-1 allele, partial cds

Sequence ID: MH037035.1Length: 810Number of Matches: 1

300:

TGATGGCCGCGGCCGCGGTGCTGAAGAAAAGATGAAAGCGAACCGAATCTGTTAAATCAGCGAGTTGAGATCACAAAATCTGACCTTGTTAACTATAATCCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCGGCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTGGCGGCCCGGCTAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACGAAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGATACCACTTCACCTCGGGCAATGGCGCAAACTCTGCGGAATCTGACGCTGGGTAAAGCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACCACCGGTGCAGCGAGCATTCAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATAAAACCGGCAGCGG

BLAST output: Escherichia coli strain IPK182 beta-lactamase **CTX-M-15** (blaCTX-M) gene, blaCTX-M-15 allele, complete cds.Sequence ID: MH900522.1Length: 876Number of Matches: 1