# Molecular characterization and antimicrobial resistance profiling of *Salmonella* species isolated from final effluent discharged from the Fort Hare Dairy Farm in Raymond Mhlaba Local Municipality.

**A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY)**


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**Declaration**

"I, the undersigned, declare that this dissertation submitted to the University of Fort Hare for the degree of Master of Science in Microbiology in the Faculty of Science and Agriculture, Department of Biochemistry and Microbiology is my own original work with exception of the citations and has not been previously submitted at any other University for the award of any degree."

Signature/date __ __14/10/2021_____

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Dr N Nontongana (Supervisor)

**Dedication**

This work is dedicated to my daughter Linomtha Luminathi Nogcantsi. Daddy loves you.

**Acknowledgements**

I would like to thank my supervisor, Dr N Nontongana for guiding me through this research and always being there to assist and help me stay focused and motivated, it was great pleasure to work with her.

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***"But if we hope for what we do not see, we wait for it with patience'' Romans 8:25***

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## Abbreviations

**°C - Degree Celsius**

**CDC - Centre for Disease Control and Prevention**

**CFU - Colony Forming Units**

**CLSI - Clinical Laboratory Standards Institute**

**DNA - Deoxyribonucleic acid**

**DWAF - Department of Water Affairs and Forestry**

**DFFE- Department of forestry, fisheries and the environment**

**FDA - Food and Drug Administration**

**I - intermediate**

**MARI - Multiple antibiotic resistance index/indices**

**MDR - Multidrug resistance**

**MH - Mueller-Hinton**



**MIC - Minimum Inhibitory Concentration**

**PCR - Polymerase Chain Reaction**

**R – Resistance**

**RNA- Ribonucleic acid**

**S – Susceptibility**

**TCBS – Thiosulphate Citrate Bile Salt**

**UV – Ultraviolet**

***S. E. enterica - Salmonella Enterica enterica***

***S. E. salamae -Salmonella Enterica salamae***

***S. E. arizonae - Salmonella Enterica arizonae***

***S. E. diarizonae - Salmonella Enterica diarizonae***

***S. E. houtenae - Salmonella  Enterica houtenae***

***S. E. indica - Salmonella  Enterica ndica***

**WHO - World Health Organization**

**WWTP - Wastewater Treatment Plant**

**µl – Microliter**

**TSAP - Typhoid African Fever Surveillance Program**

**UNEP- United Nations Environment Programme**

**IETC - International Environmental Technology Centre**

**iNTS  - invasive non-typhoidal *Salmonella***

# CHAPTER 1


IN LUMINE TUO
VIDE BIMUS LUMEN
University of Fort Hare
Together in Excellence

General Abstract

The exposure of livestock to antimicrobials for treatment, prophylaxis, or development advancement can select for antimicrobial resistant organisms that can be transmitted to humans. *Salmonella* as a significant zoonotic microorganism can go about as a likely supply of antimicrobial resistant determinants. *Salmonella* is a zoonotic pathogen that causes food and waterborne infections. It affects wild and domestic animals, and humans, by causing a number of infections including Salmonellosis. *Salmonella* species infect humans through the consumption of contaminated meat, like beef, chicken, pork etc. This study aimed to determine the molecular characterization and antimicrobial resistance profile of *Salmonella* species isolated from effluent discharged from the Fort Hare Dairy Farm in Raymond Mhlaba Local Municipality. Polymerase chain reaction (PCR) was used for the molecular confirmation of the presumptive *Salmonella* isolates targeting both *ompC* gene and *typh* gene. Standard disc diffusion method was used for the antimicrobial susceptibility testing (AST) as recommended by the Clinical and Laboratory Standards Institute. The confirmed *Salmonella* isolates were tested against 12 test antimicrobial agents and were screened for antimicrobial resistance genes (ARGs) including *bla*$_{TEM}$ and *amp* for beta-lactams, and *tetC* for tetracycline.



The research showed that the effluent discharge from this farm is contaminated with *Salmonella*. Presumptive *Salmonella* densities were ranging between 1,7 $\times 10^2$ to 6,1$\times 10^2$ CFU/100ml, out of 83 presumptive isolates recovered, 61 were molecularly confirmed *Salmonella typhimurium*. The most prevalent *Salmonella* species found in this study was *Salmonella typhimurium*, which was more abundant in the final effluent discharges than in the water samples. This may be due to the contamination from farm animal faeces.

The susceptibility against 12 different antibiotics by the recovered *Salmonella typhimurium* were examined, and *Salmonella typhimurium* isolates was notably resistant to azithromycin, ampicillin, amoxiclav, but less resistance were seen on doripenem , meropenem and ciprofloxacin but none of the isolates were resistant to norfloxacin.

*Antibiotic* results obtained from this research suggest that Quinolones (Norfloxicin, Ciprofloxacin and Nalidixic acid), and Carbapenems (Meropenem and Doripenem.) were the most effective antibiotics against *Salmonella*. Forty-eight percent of isolates were found to be resistant to more than 3 antibiotics from different families thus considering them to be

multidrug resistant. Resistant determinants *ampC*, $bla_{TEM}$ and *tetC* were detected on resistant isolates. Misuse and overuse of antibiotics on animal producing farms put human lives at risk as it promotes the emergency of multidrug resistant bacteria.

Findings of this study revealed that animal producing farm pose a threat to the community as they harbour and promote the emergence of multidrug resistant *Salmonella typhimurium*.

**Keyword**: *Salmonella typhimurium*; multidrug resistant; antimicrobial resistance genes

## 1.1. General Introduction

Microorganisms are known to play an invaluable role in maintaining the natural balance of any ecosystem (Kirby, 2007). The different roles they play include; waste degradation of pollutants and plant matter and then releasing the essential nutrients back into the environment. In a previous study, Arenskotter *et al.,* (2004) suggested that microbial species can also be involved in the food, pharmaceutical and chemical industry. In addition, they can be used for the treatment of waste and also applied in bioremediation. It is of no doubt that microorganisms are a source of great economic importance (Bull *et al*., 2000). However, there are negative impacts microbes have on human and animal health that cannot be overlooked. Based on the research conducted by the World Health Organization (WHO, 2016), practically 10 in 100 people get infected after consuming contaminated food, leading to 420,000 deaths annually. According to a South African health report, gastroenteritis is responsible for 15% of death in children under the age of five (Department of Agriculture Forestry and Fisheries, 2012).

Even though there is no considerable growth in *Salmonella* bacteria in the environment, it has the potential to survive for long periods of time (Chen and Jiang, 2014). Also, the infection cycles may persist independently of any ongoing invasion of *Salmonella* pathogens from farm animals, infections in wild fauna, such as rats, are frequently subsequent to illnesses in farm animals as described by Henzler and Opitz, (1992). In a study by Chigor *et al*., (2010) on *Salmonella* survival in the environment, which states that controlling *Salmonella* must begin with a considerable reduction in the number of organisms released into the environment. When animals and humans contaminate water and soil through faeces, this contributes to the epidemiological cycles and the contamination of farm produce, which can further lead to spread through food (WHO, 2016).



The faecal contamination of water is known to be a significant human health risk and microbiological analysis using coliform bacteria has been reported (Kayombo and Mayo, 2018). Water can be contaminated by animal faeces due to the vulnerability of surface water to constant pollution and this may have adverse public health effects resulting in infections such as gastroenteritis, enteric fever and ear infection or even more harsh illnesses like hepatitis and meningitis (WHO, 2016). Nevertheless, another evolving public health threat is the prevalence of antibiotic resistant strains (ARS) of bacteria in water sources. Such microorganisms have the ability of genetically and phenotypically transferring resistance genes

to previously susceptible strains, particularly those of public health importance, in different environments (Chigor *et al*., 2010).

According to the World Health Organization, (2016); dairy farm effluents generally contains manure used for soil fertility (fertilizer), urine from both animals and humans, pathogenic microorganisms, waste from the feed, unused and used milk spillages and disinfectant residues, and occasionally, minor amounts of veterinary chemicals. It is very important to characterise an effluent of a particular enterprise which will be helpful in the operation and management of that enterprise. Dairy farm operators are urged to collect data with the aim of characterizing effluent (Agrawal *et al*., 2007). A zoonotic pathogenic bacterium such as *Salmonella* species can be transferred from animal production into food and water supply systems which can lead to infections in humans (World Health Organization, 2016).

*Salmonella* can be introduced to dairy farms through many pathways, these include contaminated water, bought cattle, wild animals such as birds, and also human traffic (Nielsen *et al*., 2007). The presence of *Salmonella* on a farm samples have been well established, with previous study by Fossler *et al*., (2005) involving 110 dairy farms in four states reported that, more than 89.9 percent of the dairy farms had at least one *Salmonella* positive culture found during the sequence of five sampling visits over a period of a year. A study done in 2002 by the National Animal Health Monitoring System (NAHMS) during their sampling visit to five herds respectively in 21 states, it was discovered that about 30 percent of herds produced at least one *Salmonella* positive faecal culture (Blau *et al*., 2005). Considering increase in prevalence, it would seem logical that faecal shedders contained by herds characterize a potential point of intervention to mitigate public health risk in humans and animals. *Salmonella enterica* serovar is commonly found in dairy farm effluents, reports have confirmed cases of *Salmonella enterica* serovar specifically *Salmonella typhimurium* in countries like US which exceeded 40,000 in 2005, with an estimated 540 deaths and 1.2 hospitalizations annually (CDC, 2018). Animal farms are typically associated with the presence of diverse *Salmonella* species (Switt *et al*., 2017), hence it is considered as an etiological agent of clinical significance in most humans (Painter, 2013).



The Eastern Cape Province is considered to be the poorest province in South Africa (Katiyatiya *et al*., 2014). The economy of the Eastern Cape is dependent on agriculture and it is known for its livestock production as well (Zungu, 2017). Large amounts of meat and raw milk are

provided by farms in this province to urban and rural areas with an affordable prices. This milk is normally consumed unpasteurised by most people. (Silaigwana *et al*., 2012).

Additionally; rural areas which constitute most of the Eastern Cape depend heavily on surface water sources for domestic use, irrigation and recreational water purposes (Painter *et al*., 2013). The spread of these microorganisms is propelled by the use of polluted surface water, as a result of final effluents that are discharged and does not meet the water quality standards (UNEP, 2002). Regular monitoring of the microbial quality of the final effluents is therefore necessary, so as to avoid risk of contracting waterborne diseases.

## 1.2. Problem statement

*Salmonella* is one of the most common foodborne pathogen constituting a significant public health hazard around the world, with about 93.8 million cases of foodborne disease and 155,000 deaths per year (Dungan *et al.,* 2012). Mohammed *et al*., (2020) reported that more than 2500 *Salmonella* serotypes have been identified to date, with *Salmonella* enterica accounting for more than half of infections caused by *Salmonella* species. Human illnesses caused by *Salmonella enteritidis* have drastically increased worldwide, and by the 1980's *Salmonella enteritidis* had replaced *Salmonella typhimurium* as the primary cause of salmonellosis globally (*WHO,* 2015). In developed countries, Non-Typhoidal *Salmonella* (NTS) usually causes self-limiting gastroenteritis with fewer numbers of deaths in humans compared to developing countries (okoh *et al*., 2010).



*Salmonella* infections are a leading source of morbidity and mortality worldwide; nevertheless, there are significant knowledge gaps in Sub-Saharan Africa about the distribution and incidence of disease caused by *Salmonella typhimurium* and invasive non-typhoidal *Salmonella* (iNTS) (Stanaway *et al*., 2019). Before the African Typhoid Fever Surveillance Program (TSAP*),* which collected data on the Typhoid fever incidences, data from Africa was available from four vaccination trials and one population-based investigation in Kenya, according to a systematic analysis of the incidence and antibiotic resistance patterns of invasive Salmonella infections in Sub-Saharan Africa (Pui *et al.,* 2011). In The Gambia, Malawi, Mozambique, and Kenya, different descriptions of bacteremia in febrile patients led to different estimates of invasive *Salmonella* infections according to reports in 2013 and 2017. In 2013, an outbreak occurred in Kwazulu Natal where people consumed a dead goat, which was infected

by *Salmonella*. However, there were cases of food-borne illnesses reported by private medical practitioners in the same region, which this suggests possible foodborne cases in these villages.

These few, unstandardized, published data are not sufficient for understanding the burden of the disease in sub-Saharan Africa. The TSAP's findings point to the need for preventative measures such as immunizations, better sanitation and hygiene, malaria control, antiretroviral medication programs, and better nutrition. The findings also show that the presence and possible rise of drug resistance can make it difficult to adopt effective antimicrobials. Salmonella strains found in the area (Andino and Hanning, 2015).

Although Igbinosa *et al*., (2009) and Nongogo and Okoh (2014) have reported on the prevalence of various types of *Salmonella* pathogens in the final effluent of dairy farm in the Eastern Cape Province, to the best of my knowledge, no report exists on the presence of pathogenic *Salmonella* species, and evaluating the antimicrobial resistant profiles of the isolated *Salmonella*. Therefore, there is a need to evaluate a molecular characterization and the antimicrobial resistance of *Salmonella* species in farm effluent collected from Fort Hare Dairy farm. To the best capacity of my knowledge, studies on antimicrobial-resistant *Salmonella* species is still an issue to the environment at large due to the spread of antimicrobial resistance. Therefore, the present study would add to the growing chain of knowledge concerning the spatial characterization and antimicrobial-resistant profiling of *Salmonella* species in Raymond Mhlaba Municipality.



## 1.3. Hypothesis

This study is based on a null hypothesis that the effluents discharged from the Fort Hare Dairy Farm in Raymond Mhlaba Local Municipality are devoid of antibiotic resistant *Salmonella* species.

## 1.4. Aim

This study aims to determine the antimicrobial resistance profile and to characterize *Salmonella* species isolated from effluent discharged from the Fort Hare Dairy Farm in Raymond Mhlaba Local Municipality.

1.5. Specific Objectives

- To determine the prevalence of *Salmonella* species from the Fort Hare dairy farm effluent discharge.
- To confirm the identities of the recovered presumptive *Salmonella* isolates by polymerase chain reaction.
- To delineate the confirmed *Salmonella* isolates into their serogroups (A, B, C1, C2 and D) using serogroup specific primers.
- To screen for the presence of relevant virulence genes of the confirmed *Salmonella* isolates.
- To determine the antimicrobial susceptibility patterns of the confirmed *Salmonella* isolates.
- To screen for the presence of putative antimicrobial resistance determinants from the antimicrobial resistant isolates.

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# CHAPTER TWO


## Literature review

Clinical-relevance of pathogenic *Salmonella*: an emerging environmental and public health concern

## 2.1. Salmonellosis

Salmonellosis is an uncommon disease, but it can result in epizootics of high mortality and morbidity. It is primarily a septicemic disease with a brief clinical course, but diarrhea and enteric fever may be observed in some animals and humans (Nasstrom *et al*., 2018). In animals, the disease is normally caused by *Salmonella enterica* serotypes *typhimurium* or *enteritidis,* but other serotypes have been reported (Thompson *et al.,* 2017). The most prevalent serotypes associated with non-typhoidal salmonellosis in people are *Salmonella typhimurium* and *Salmonella enteritidis* (Velge *et al*., 2005). Although presently uncommon, *Salmonella* infections were present in the early 1900s. Many authors reported explosive outbreaks of the disease that resulted in the deaths of large numbers of animals and humans (Lukacs and Malinczak, 2020). In most cases there was rapid death from septicemia, but diarrhea was occasionally seen as well reported by Finke *et al*., (2020). *Salmonella enteritidis* was isolated from this outbreak, which was characterized by abortion and subsequent death. In so many years' salmonellosis has been reported infrequently, but it is not entirely a disease of the distant past (Ferrari *et al*., 2019).

Initially, Karl Eberth visualized *Salmonella* in the Peyer's patches and sleens of typhoid patients in 1880 (Galeev, 2020). Georg Theodor Gaffky was able to effectively grow the pathogen in a pure culture after four years (Ren, 2020). In 1885, Theobald Smith a medical research scientist discovered a pathogenic bacteria called *Salmonella Enterica* while he was working as a research laboratory assistance (FDA, 2020). This means that before 1885 *Salmonella* was not known, that's when Joseph Leon Lignieres based on his research and findings suggested that the pathogen discovered by Salmon's group must be called *Salmonella* in order to honor them (Heymann *et al*., 2018). So from the beginning each *Salmonella* species was termed based on clinical consideration (Porwollik, 2014), for example mouse typhoid fever *Salmonella typhimurium* and *Salmonella cholera-suis.*



## 2.2. Classification and nomenclature of *Salmonella* serovar

The epidemiological characterisation and classification of *Salmonella* serovar described by World Health Organisation scheme WHO, (2018) has been applied for surveillance of *Salmonella* serotype in water, food and food products. *Salmonella* have been defined to contain different types of species by Crosa *et al.,* (1973), using DNA–DNA hybridization. *Salmonella*

are predominantly motile enterobacteria with length between 2-5, diameter from 0.7 and 1.5 (Fàbrega *et al*., 2013). *Salmonella* species are described as a Gram-negative facultative anaerobic bacterium capable of generating adenosine triphosphate (ATP) with oxygen when available or when the oxygen is not available using fermentation or electrons, non-spore forming, rod-shaped which belong to the family Enterobacteriaceae and they are clinical pathogens for animals and humans and can be commonly found in the intestinal tract (Barlow and Hall, 2002). They acquire their energy from reduction and oxidation reaction using organic source as described by (WHO, 2018).

The classification of *Salmonella* has been revised and has the potential to confuse sometimes (Bongiovanni *et al.,* 2019). Reeves *et al.,* (1989) has described *Salmonella* genus to consist of two species being *Salmonella bongori* restricted for cold- blooded animals, predominantly reptiles and *Salmonella enterica* which is widely found in warm-blooded animals globally. *Salmonela enterica* was further divided into six subspecies including 2600 serotypes, *S. e. enterica*, *S. e. salamae*, *S. e. arizonae*, *S. e. diarizonae*, *S. e. houtenae*, and *S. e. indica*. Serotype and the *S. bongori* was considered as its own species because it has no subspecies *(*Brenner *et al., 2000).* They can infect different species of animals and humans (WHO, 2017). *Salmonella*e have been clinically categorized as two basic way typhoidal serotype (invasive) and nontyphoidal serotype (non-invasive) based on host favorite and disease presentation in humans (Okoro *et al., 2012). Salmonella* species thrives inside guts of humans, cold blooded animals and birds by Crosa *et al.,* (1973). It is transferred into the environment through faeces of the infected hosts and can pull through in soil, water and food for extended long of time (Angulo *et al*., 2000). Consumption of contaminated food or contact with infected animals is one of the ways by which Salmonella infections are acquired in developed countries (Charles *et al*., 2008).



2.3. Antigenic types of *Salmonella*

According to Kauffimann White scheme serotyping has for more than 80 years been a primary classification of *Salmonella* species and within the genus around 2600 serotypes have been identified based on their serological reactions to flagellar (H), somatic lipopolysaccharide (O), capsule (Vi) antigens (Grimont and Weill, 2007). Some of these species are specifically for animals while others are strictly for humans but majority of them are phathogenic for both humans and animals and they can be zoonotic (European Food Safety Authority, 2017).

*Salmonella*e are also described as flagellated, facultative anaerobic bacilli possessing three major different antigens with identifying applications as being surface, somatic and flagellar (Grimont and Patrick, 2013*)*. The organism has the ability to change from one phase to another, that H antigen can occur in both of the two forms, called the first phase and second phase.

These organisms have the habit to change from the one phase to another and the O antigens materialize on the surface of the outer membrane and are shown by a specific sugar sequence on the surface of the cell (Limo *et al.,* 2018). The Vi antigen is a superficial O antigen that is present in some serovars, the most important of is *Salmonella typhimurium*, which has cell envelope that contains a complex lipopolysaccharide (LPS) structure that is liberated on cell lysis and to some extent, during culture, unlike other Gram-negative bacilli (Von Seidlein *et al.,*2011).

Based on the research done by Ames and his colleagues in 2015 the *Salmonella* genus evolved into four meeting phases and each of these phases is depended on the available knowledge and methodology stressing, the first one being the clinical evidence followed by antigen specificity then biochemical properties and DNA relatedness. This species has been identified as being one of the major pathogens of global public health concern containing intracellular factors that cause rapid illnesses (Jantsch *et al.,* 2011). *Salmonella* species have been gaining attention due to its outbreak and deaths that are reported each and every year (Fonteneau *et al*., 2017)



## 2.4. *Salmonella* species

*Salmonella* species when grown on Xylose-lysine-deoxycholate (XLD) agar, colonies are red to pink with black centres, this is prevalent in many *Salmonella* species and indicates that the species does not ferment lactose. (de Jong *et al*., 2012). The use of Xylose-lysine-deoxycholate (XLD) agar is designed for detection of these types of bacteria (Faucher *et al*., 2006), this selective agar was developed by Welton Taylor with approximate pH of 7.4 in 1965.

Salmonellae are a well-known speciation model that has paved the way for experimental and genomic studies of recombination and hybridization (Sheppard *et al*., 2018). *Bongori, enterica, salamae, arizonae, diarizonae, houtenae*, and *indica* are the subdivision of genus *Salmonella.* Based on human disease outcomes, *Salmonella* enterica serovars can be separated into two groups: typhoidal Salmonella and non-typhoidal Salmonella (NTS) (Bahroun, 2017). *Salmonella* enterica serovar *Typhi (S.Typhi)* and *Salmonella Paratyphi* are typhoidal

*Salmonella* strains that cause the life-threatening systemic infectious diseases typhoid fever and paratyphoid fever in humans, respectively(Yang *et al*., 2018; Awol *et al*., 2021). *Salmonella typhimurium* and *Salmonella enteritidis* are the most prevalent NTSs responsible for self-limiting gastroenteritis in healthy persons around the world, while *Salmonella javiana* is one of the most common NTSs connected to plant-derived foods. Aside from these cell and tissue adaptations within the same host, typhoidal *Salmonella* has evolved to only infect humans, whereas NTS can infect both humans and animals. *Salmonella typhi* encodes a few distinct virulence components, notably typhoid toxin, in response to the severe illness consequences and rigorous host adaptation (Stepien, 2020). Typhoid toxin, like *Salmonella typhi*, appears to have evolved to adapt in humans (Fowler and Galán 2018).

This species uses a peritrichous flagella arrangement for locomotion, but it does not produce endospores (Winzer *et al*., 2007). Lysine decarboxylase positive, lactose negative, gelatin-hydrolysis negative, citrate positive and the production of hydrogen sulfide these are Some of the metabolic features that can help identify the bacteria (WHO, 2017). *Salmonella typhimurium* can be found on certain food items, drinking water, and also in an infected host's faecal matter (Tuin and Annemarie, 2006). Water samples are assumed to contain pathogens that are isolated and analysed for the prevalence of the genus (Silaigwana *et al*., 2012). Rappaport-Vassiliadis soy broth is the most often used broth for detecting *Salmonella* species. (Allard *et al*., 2016).



## 2.5. Prevalence of *Salmonella* in dairy farm effluents

According to literature, eggs and their products are the key sources of *salmonella* infection with high prevalence of foodborne outbreaks, followed by poultry and pork foodborne outbreaks and the last one is the cheese foodborne outbreaks in a population of 100,000 (EFSA and ECDC, 2017). What is known now is that *Salmonella* is progressively present in dairy cattle around the world (Chlebicz and Slizewska, 2018). This is owing to their ability to survive in a dairy environment for long periods of time, according to the researchers. Salmonella, for example, can live for a year in dry feces, which is the most common source of contamination in cattle (Kwapich *et al.*, 2017). You and his colleagues conducted a laboratory incubation study and found that the organism could survive up to 26 weeks in manure and 47 weeks in manure-amended soils (You *et al*., 2006). *Salmonella* infection is a desease that might

necessitate epidemiologic surveillance due to its clinical implications. This is true despite the fact that they are able to transmit the bacterium without causing any symptoms.

EFSA in 2017 reported *Salmonella* serovars in food, *Salmonella typhimurium*, *Salmonella enteritidis* and variants of *Salmonella typhimurium* (Balcão *et al*., 2014). All of these serovars have been reported to be the most common among human cases around the world, implying that their incidence appears to fluctuate by nation and in significance over time. (EFSA and ECDC, 2017). While *enteritidis* serovars appear to be implicated in half of the infected dairy farms and pork meat, the main serovars are not always the same in different food items. (Lailler *et al*., 2012). The dairy food chain is one of the most contaminated sectors after the egg and meat sectors, it is of greatest importance to try and prevent its contamination, hence several strategies for *Salmonella* biocontrol have been considered (Besnard *et al*., 2018). Despite the huge potential of phages, some studies report phage therapy against *Salmonella* in the dairy sector, yet *Salmonella* phages are quite diverse, abundant and can easily recover from the dairy farm environment (Duenas *et al*., 2017). Therefore, highlighting dairy farms an appropriate reservoir for isolating new *Salmonella* species can minimise the spread of antibiotic resistance of *Salmonella* species (Wongsuntornpoj *et al*., 2014).



## 2.6. Pathogenesis of *Salmonella* species

There are a number of factors that determine *Salmonella* infections in humans, these include the serotype involved, infection dose, the environment where the food is being contaminated at, also the strain and the status of the host in terms of the health (Grassl and Finlay, 2008). According to the World Health Organisation (WHO), individuals with immunosuppressed systems, children less than 5 years and elderly people are more likely to acquire *Salmonella* infection compared to healthy individuals. Le *et al., (*2018) substantiates WHO's statement by clarifying that, almost all *Salmonella* strains are pathogenic and has the ability to invade and replicate inside the host survive inside the host cells and subsequently result in potentially fatal disease.

*Salmonella* commonly shows notable characteristics when it invades non-phagocytic human host cells (Hansen *et al*., 2002). However, in order for it to penetrate the host cell it actually induces its own phagocytosis, the significant genetics underlying this ingenious strategy is found in *Salmonella* pathogenicity islands , gene clusters located at the large chromosomal

DNA region and encoding for the structures involved in the invasion process (Grassl and Finlay, 2008). Bacteria commonly enter the epithelial cells lining the intestinal wall of the digestive tract. Heuchel *et al., (2000)* discovered and reported an increase on *Salmonella* infections, this increase led to a high death rate in developed and developing countries around word wild. High death rates were specifically reported in the poorest nations of the developing countries.

May *et al*., 2017 believe that epidemics caused by *Salmonella* species might have significantly affected the history. Multi-channel proteins that allow *Salmonella* to insert their effectors across the intestinal epithelial cell membrane into the cytoplasm are encoded for by *Salmonella* pathogenicity islands, this also include type III secretion systems (Heuchel *et al*., 2000). The bacterial effector's purpose is to activate the signal transduction pathway and initiate reconstruction of the host cell's actin cytoskeleton, which causes the epithelial cell membrane to ruffle outward and engulf the bacteria. The process of phagocytosis resembles the morphology of the membrane ruffle (Takaya *et al*., 2003).

Diseases caused by *Salmonella* serovars are particular in developing regions like South Africa and Southern America (Food Safety and Inspection, 2016). The emergence of antibiotic resistance *Salmonella* strains posture a significant hindrance to the development of reliable therapies, which makes it hard to control the spread of both typhoidal and non-typhoidal (Ahmadi *et al.,* 2016).



Based on the studies done by Śliżewska and, (2020). amongst the disease that spread between humans and animal caused by curtain germs investigated in the European Union, *Salmonella* when compared to other microorganisms still ranks second after *Campylobacter species.* Over the last ten years, reports indicated between 87,000 and 135,000 of human *Salmonella* infections yearly episodes in Europe (European Food Safety Authority, 2017).

Pathogenic *Salmonella*e engulfed in food invade by passing over the gastric acid walls/barriers and enter the mucosa of the large and small intestine and produce toxins (Zuo and Rothenberg, 2007). Inflammation is caused by the invasion of epithelial cells which then stimulates the release of pro inflammatory cytokines (GBD, 2015). Symptoms such as diarrhea, ulceration, destruction of the mucosa, abdominal cramps are mainly causd by a severe inflammatory response. This can further cause the bacteria to circulate from the intestines to cause systemic disease (CDC*,* 2017*)*.

A statistically significant decrease of about 35 percent in confirmed human *Salmonella* infection cases was observed round about a period of 7 years from 2008 to 2016 (Duenas *et al*., 2017). Furthermore, during the last reported 5 years from 2012 to 2016 the number of *Salmonella* kept on increasing in humans meaning some organisms did acquire resistant genes from the antibiotics that were used to treat *Salmonella* infection. For the period of the year (2016), approximately 9,061 human cases have been associated with 1,067 *Salmonella* infection foodborne outbreaks, which caused for about 40 percent of people to be hospitalized, and the death of 0.250 percent of all the foodborne cases (EFSA and ECDC, 2017).

## 2.7. Clinical manifestations of *Salmonella* Species

Gastroenteritis, enteric fever and septicemia are some of the syndromes caused by *Salmonella* infections (Manassaram *et al*., 2010). Different syndrome is caused by particular serovars *Salmonella typhimurium*, and *Salmonella paratyphi-A* cause enteric fever; *Salmonella choleraesuis* results in septicemia; gastroenteritis is caused by *Salmonella typhimurium* and *Salmonella enteritidis,* however in some cases, any serotype can produce any type of syndromes (Śliżewska and, 2020). Typically, more dangerous infections occur in infants, those over 45, and people suffering from life-threatening illnesses (WHO, 2017). Enteric fevers and gastroenteritis are the two main kinds of gastrointestinal diseases that the term *Salmonella* infection is generally used for. These two diseases are caused primarily by *Salmonella enteritidis* and *Salmonella typhimurium*



## 2.8. Enteric fever

Typhoid fever is a systemic infection caused by bacteria called *Salmonella* enterica serotype *typhimurium* (Newton, 2014). It is a pathogenic organism that penetrate the body through the gastrointestinal tract and gains access to the bloodstream via the lymphatic system and is characterized by severe systemic illness such as fever and abdominal pain (WHO, 2018).Now, for some *Salmonella* serotype particularly *Salmonella* enterica serotypes Paratyphi A, B and sometimes C which grows in the intestines and blood, can cause very similar syndrome but often less severe, since they are host-restricted pathogens whose reservoir is humans. They are different from other *Salmonella* serovars that causes local intestinal inflammation and diarrhea (Crump and Mintz, 2010).

Poverty-stricken areas that have poor access to sanitation are more prone to enteric fever, studies have revealed the above-mentioned. (Global Burden of Disease, 2016). South-central Asia, Southeast Asia, and Southern Africa are areas with high number of *Salmonella* infections with more than 100 cases per 100,000 individuals per year (Mogasale *et al*., 2014). Furthermore, *Salmonella typhimurium* has a remarkable mechanism for persistence in its host, a highly adapted human-specific pathogen that evolved about 50 000 years ago (Wain *et al*., 2015). The enteric fever is estimated to have caused approximately 21.6 million illnesses and 216 600 deaths globally in the year 2000, with paratyphoid fever reported to have caused an estimated 5.4 million illnesses *(*Watson *et al.,* 2015*).* There are now a variety of clinical symptoms associated with these outbreaks, ranging from illnesses needing surgery to long-term discomfort. The monitoring of veterinary diseases and quality control of industrial product are therefore public health concern. Microbial populations in which infection vary depending on transport conditions and manufacturing. Consequently, to prevent such risks it is then important take an important role in the food chain monitoring processes.

## 2.9. Gastroenteritis and Epidemiology of *Salmonella* species

*Salmonella* species are arguably the leading cause of gastroenteritis infections, while other salmonellosis result in gastroenteritis that is temporally which normally does not even require treatment (Scallan *et al*., 2005). Children less than 5 years are more too prone to gastroenteritis that cause morbi-mortalities, because their less-developed immune systems have a limited ability to fight infections, (Kosek *et al*., 2003).

Salmonellosis can be an intermediate stage of infection, this means that the infected person does not experience any intestinal symptoms and the bacteria cannot be removed from faecal specimen, this was reported by Galan and Curtiss in 1989. The development of antibiotics resistant organisms has made a huge positive and negative impact on these species. Long held idea that United States President William Henry Harrison died of pneumonia in the 9th century, but current findings revealed that he died because of typhoid. (Mchugh *et al*., 2017). Due to the unsanitary conditions in Washington, DC in the mid-nineteenth century, this disease may have been a significant factor in the death of 12th century United States President Zachary Taylor. (McHugh and Mackowiak, 2014)

In 2019, The US Center of Disease Control and Prevention investigated the gravity of the infection and whether it remains contained in the intestine or circulates to the bloodstream which might be contingent on the resistance of the patient and the virulence of the *Salmonella* isolate. According to Setia *et al.,* (2010) findings, advise that histological specimens are problematic to obtain, and as a result many diagnoses are missed. Medical literature have reported hundreds of cases since the first description of this entity by Zuo *et al.,* (2007). The largest reported clinical series until today has been that of Talley *et al*., (1990), were they reported a series of 40 cases.

Historically, before the use of antibiotic, the cases of deaths and illnesses caused by typhoid fever were 10 to 20%. In our days with prompt treatment, it is even lesser than 1% (Khan *et al*., 2015). The development of a chronic infection in the gall bladder is can be seen in about 3 to 5% of individuals who are infected. Identification and treatment of these chronic infections can be complicated due to the fact that *Salmonella* species are human-restricted and these chronic carriers become the central reservoir, which can continue for decades for further spread of the disease, (Gonzalez-Escobedo *et al*., 2011). Recently, the study of *Salmonella* species associated with enormous outbreaks and a carrier at the genome level provides new insights into the pathogenesis of the pathogen (Yap *et al*., 2012)

The number of cases has decreased in industrialized nations this is due to improved water sanitation and food handling (Yap *et al*., 2014). Nevertheless, parts of Asia and Africa which are developing nations have been found to have the peak rates of typhoid fever. The high rates are a result of the lack of access to proper sanitation systems, proper health-care facilities and clean water. Unfortunately, for these areas, access to basic public-health services is uncertain for the foreseeable future. The monitoring of veterinary diseases and quality control of industrial product are public health concern. Manufacturing and transport conditions are some of the factors that microbial populations which causes infections depend on for them to vary, this was stated by Poupée, (2016). Therefore, to avoid such risks it is then important take drastic measures in ensuring quality monitoring in the food chain processes.

## 2.10. Antibiotic resistance

### 2.10.1. Spread of resistance

There are a number of ways by which resistance can be spread from one organism to another. A barrier against *Salmonella* pathogen is created by a thick mucus layer (Okoro *et al*., 2012).

Also, a barrier to the bacterial cell wall is created by antibacterial-like antimicrobial peptides of the gastrointestinal tract that are released by cells (Johnson *et al*., 2010). *Salmonella* pathogens are able to break these physical barriers, when this happens they start to invade enterocytes (Addiman *et al*., 2013). This rigger the second line of defence to act. Macrophages and dendritic cells phagocytosing, killing, and signalling other immune cells to the infected site, the pathogen favours to be engulfed in order to continue its infection (Fong *et al.,*2017), Neutrophil influxes, mucosal inflammation are the end results of the internal survival of the pathogenic bacteria. However, *Salmonella* species uses the inflammation that it caused to weaken the human gut microbiota and dictate the infections there (Zhao *et al*., 2003).

Antibiotics have been used in a variety of industries, during the last few years, there has been a lot of pressure on Salmonella pathogens to select for antibiotic resistance in human and veterinary medicine and agriculture, as well as as growth promoters for food animals. (WHO, 2018). The use of selected antimicrobial in animal feed to enhance growth of food for animals has resulted in the emergence of *Salmonella* with antimicrobial resistance (Hyeon *et al*., 2011). Since the creatures are the main reservoir for *Salmonella*, in order to identify people at risk of infection outside of endemic areas, it would be helpful to have a list of places previously travelled whereby there is poor sanitation or there have been typhoid cases, although a specific source or contact is identified in a minority of cases (Marks *et al*., 2017).



Each year in the United State approximately 200 to 300 cases of *Salmonella* species which have become resistant to some organisms were reported (Lynch *et al*., 2006). However, about 80 percent of these cases are reported to occur among people that are travelling to countries where multidrug resistant is endemic, most especially countries in Africa but domestic acquisition still occurs (Imanishi *et al*., 2015). Between 2006 and 2011, the worldwide GeoSentinel Surveillance Network documented 400 cases of enteric fever and gastroenteritis among travelers from resource-rich nations, with 67 percent of cases acquired in south-central Asia (Pullen *et al.,* 2018). A zoonotic pathogenic bacterium such as *Salmonella* species can be transferred from animal production into food and water supply systems which can result to infections (World Health Organization, 2016). Although the sequence of *Salmonella* infection transmission is complex and difficult to detect, improved sanitation and immunization have dramatically reduced the number of serious outbreaks in domestic animals and humans historically (Anderson *et al*., 2009).

## 2.11. Mechanisms of antibiotic resistance

Several studies have shown that the phenotypic multidrug resistance *Salmonella* serotypes have the capacity to produce different types of hybrid plasmids (Gordon *et al*., 2008). Antimicrobial resistance properties of the serotypes against known antibiotics such as streptomycin, chloramphenicol's, ampicillin and tetracyclines are conferred by resistance genes found in gene cassettes on these hybrid plasmids (Guerra *et al*., 2002). Mutation at the quinolone resistance-determining regions of the *gyrA* gene, results in the emergence of *Salmonella* serotypes with reduced ciprofloxacin susceptibility (Chiu *et al*., 2002). Mutated genes that code for extended-spectrum β-lactamases have resulted in the development of serotypes that have developed resistance to broad spectrum cephalosporin (Carattoli *et al*., 2002).

The most well-known resistant mechanism of *Salmonella* is the construction of β-lactam. Cephalosporins are semisynthetic antimicrobials initially derived from cephalosporin C a naturally occurring antimicrobial produced by the fungus *Cephalosporium acremonium* (Oppezzo *et al*., 1991). Cephalosporins act by targeting on different penicillin-restricting proteins that are essential for the synthesis of peptidoglycan, the important part of the bacterial cell wall. This mechanism is similar to that of penicillin and ampicillin. (White *et al*., 2015). The prevalence of a β-lactam ring makes the antimicrobial action of these anti-infection agents possible. The β-lactam ring which carries amino acids with no antimicrobial movement are hydrolysed by β-lactamases which allows them to be resistance (Allen and Poppe, 2002).

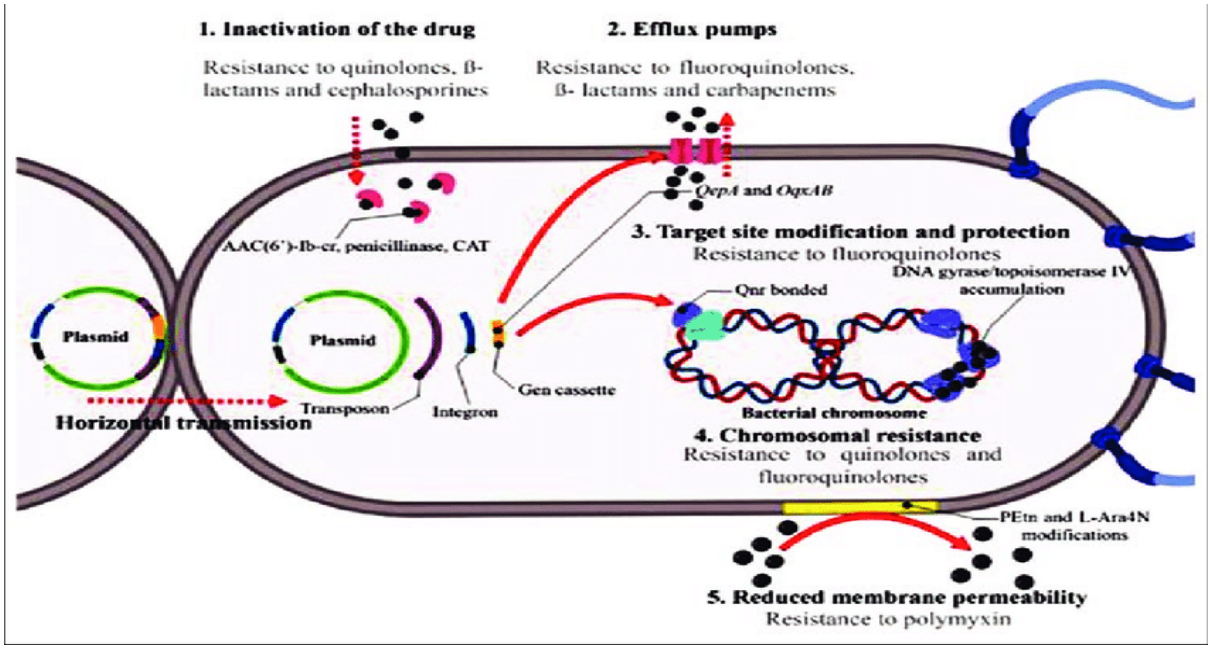


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Figure 2.1: This shows mechanism of antimicrobial resistance in *Salmonella* species. (Jacoby *et al*., 2014)



2.12. Clinical relevance

*Salmonella* gastroenteritis incubation period depends on the dosage of bacteria (Charalabopoulos *et al*., 2004). Symptoms that can be seen include nausea, fever, diarrhea and abdominal pain, these symptoms usually manifest in 7 to 48 hours after the ingestion of contaminated (Zhang *et al*., 2010). Myalgia and headaches are more prevalent, although diarrhea is the most common symptom. Fever (37°C to 39°C) and chills are also frequent, with stomach cramps affecting at least two-thirds of patients. The length of fever and diarrhea varies, although it generally lasts between two and seven days. *Salmonella typhimurium* caused enteric fever which is a severe systemic form of Salmonellosis, although this type of disease can be caused by any species of *Salmonella* (Berkley *et al.,* 2012). After a 10- to 14-day of incubation period, the symptoms appear. Gastroenteritis, which generally cures before the advent of systemic illness, can precede enteric fevers. (Lynch *et al*., 1999). Constipation, headache, anorexia and fever are some of the symptoms enteric fever. If antibiotics are not immediately administered, enteric fever may be fatal. (Lynch *et al*., 2006).

There are two types of *Salmonella* strains, which are typhoid and non-typhoid *Salmonella*, these are based on the clinical patterns in human *Salmonella* infection, (Berkley *et al.,* 2012).

Enteric fever, gastroenteritis, bacteraemia and chronic carrier status are the four clinical manifestations of human infections. (Sheorey and Darby, 2008). *Salmonella*e have been known to be difficult to remove fromthe environment. However, reducing the number of Salmonellae harboured in livestock and poultry would drastically decrease human exposure since these are the major reservoir for human infection.

In other countries nearly all animal feeds are handled to inhibit *Salmonella* before they're dispensed, resulting in a clear discount in Salmonellosis (Mtove *et al*., 2011). Changing animal slaughtering practices to decrease pass-illness of animal carcasses; protecting processed foods from infection; supplying coaching in hygienic practices for all food-dealing with personnel in slaughterhouses, meals processing vegetation, and eating places; cooking and refrigerating meals accurately in meals processing vegetation, restaurants, and houses; and expanding of governmental enteric sickness surveillance packages are one of the crucial measure that may be taken to reduce Salmonellosis (Biggs *et al*., 2014). Immunization and improved sanitation have been proven to reduce the number of serious outbreaks in domestic animals and humans although the sequence of transmission of *Salmonella* infection is difficult. (Berkley *et al.,*2012).



## 2.13. Diagnosis, Treatment and Prevention

The diagnosis of *Salmonella* infection requires bacteriological isolation of the bacteria from a clinical specimen. Identification of the genus *Salmonella* is done in the laboratory by biochemical tests; the serologic type is confirmed by serological testing by International Organization for Standardization (IOS) (2012). Water samples should be plated on several nonselective and selective agar media XLD agar as well as into enrichment Rappaport-Vassiliadis soy broth (LEE *et al*., 2015). Any growth in enrichment broth is subsequently subcultured onto the selected agar plate. The biochemical reactions of suspicious colonies are then determined and a presumptive identification of the organism is made (LEE *et al*., 2015). Nutrient agar is used for the purification of the organism then after PCR is used for the identification of the organism molecularly characterized by the bands, *Salmonella* isolates should then be sent to a reference laboratory for more comprehensive serologic testing and confirmation (Van immerseel *et al*., 2013)

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# CHAPTER 3

## Incidence of *Salmonella* species recovered from selected commercial dairy farm effluents within the Raymond Mhlaba local Municipality, Eastern Cape Province, South Africa.

Abstract

*Salmonella* species was isolated using a selective media and differential media, which is considered as a simple method for isolation. Molecular methods of detecting and evaluating the prevalence of *Salmonella* are of high importance. Samples were collected from seven different sources in Fort Hare dairy farm, these samples were collected from different sources including, two effluents, two wells, two different sprinklers and the dip. The membrane filtration and the enrichment method was used to isolate presumptive *Salmonella* after a selective media was used to identify *Salmonella* species and colonies were picked. Using the genus specific primers ompC and invar, Polymerase Chain Reaction (PCR) was applied to confirm the identification of the recovered *Salmonella* isolates.

A total of 303 presumptive isolates were recovered. effluent 1(28%), effluent 2(32%), well 1(1.3%), well 2(1%), sprinkler 1(0.7%), sprinkler 2(2%), and from washing Boul of cows (Dip)(35%). This study's findings revealed the presence of multidrug resistant *Salmonella typhimurium* in the Fort Hare dairy farm put people of Alice and surrounding areas at risk, which they can contract them through food ingestion, or by contact to animals as *Salmonella* is a zoonotic pathogen. In addition, the rate of *Salmonella* isolates was 24% confirmed as *Salmonella* genus. The PCR technique revealed that 83% of the confirmed genus is *Salmonella typhimurium*. The most prevalent species found in these samples was *Salmonella typhimurium*, which was mostly abundant in final effluents. This information will be valuable in controlling the establishment of better control plans and intervention strategies at the dairy farm level to reduce *Salmonella* contamination of animals before shedding it to the environment.



**Keywords**: Membrane filtration; specific primers; Polymerase Chain Reaction

## 3.0 Introduction

Gastrointestinal infections are known to cause diarrhoea, which is the most common cause for hospitalizations and to a greater extent, death among children and elderly individuals worldwide (Uppal *et al*., 2015). A single bacterial pathogenic infection is less severe than a bacterial infection by more than one pathogenic bacteria (Santosham *et al*., 2013). Diarrhea is known to be one of the most common medical conditions and mild occurrences, which may

not require medical attention sometimes. The treatment progression of such infections may be hindered (Shrivastava *et al*., 2016). Almost every known major group of microorganisms has been linked to the spread of diarrheal illnesses in modern society. (Koenig *et al*., 2011).

The safety of water and quality of food are controlled by the analysing them for the presence of pathogenic microorganisms (Al-Soud *et al.,* 2005). In order to comply with food safety regulations, biological threats need to be identified using new, fast and consistent methods. This will also help in risk management. Water contaminated with pathogenic bacteria has been associated with different ailments in human population. The value and the need for better quality of water cannot be over-emphasized (Tarr *et al.,* 2007). Due to urbanization and population growth, water shortage is becoming more significant, resulting in an increase in demand for water. However, in other developed countries a strategy to reduce water scarcity has been put into considerations, this strategy involves treating and reusing the wastewater or finding antibiotics for those pathogens that are found in water (Igbinosa *et al.,* 2009). The most basic step for the analysis of water quality is the analysis of pathogenic microorganisms present in water.

In the United State *Salmonella* has become the leading cause of death as a foodborne pathogen (Wang *et al*., 2017). *Salmonella enterica* which is a zoonosis bacterial pathogen has induced a noticeable hazard to the health of the public, which caused illness cases close to 1.4 million, with 16 000 hospitalized and deaths ranging between 400 to 600 (Cummings *et al*., 2010). *Salmonella species* has become one of the important pathogens in South Africa, in 2013 it caused an outbreak in KZN whereby a person fell ill from the consumption of dead goat meat, and the second outbreak was reported in Mpumalanga which was associated with food poisoning, in 2014 another *Salmonella* outbreak was reported in TB hospitals, the source of the infection was not confirmed (Muvhali *et al.*, 2017**)**



## 3.1. Materials and methods

### 3.1.1. Description of study site

South Africa has 278 local municipalities with 9 provinces which include the Eastern Cape, this province has two metropolitans and six district municipalities. The district municipalities are further divided into 31 local municipalities. Proper hygiene is very inadequate as this

province is mostly made up of rural areas. This study was conducted within the Raymond Mhlaba Local Municipality, under the Amatole District Municipality. This local municipality comprises of seven towns, namely: Fort Beaufort, Adelaide, Seymour, Hogback, Bedford, Middledrift, and Alice.




Figure 3.1 Eastern Cape Amathole district Municipality showing a region of Raymond Mhlaba Local municipality.

3.1.1.1. Area: 6 358 km²

3.1.1.2. Sampling site: Fort Hare Dairy Farm

The Fort Hare Dairy Trust is a farm in Alice that was established in 2007. It is in partnership with Amadlelo Agriculture and Fort Hare University. It is used for commercial with over 800 cows that are produced annually, it is also part of the Animal Production Industry and has a teaching centre for training students and community members in farm management (Vela, 2016)

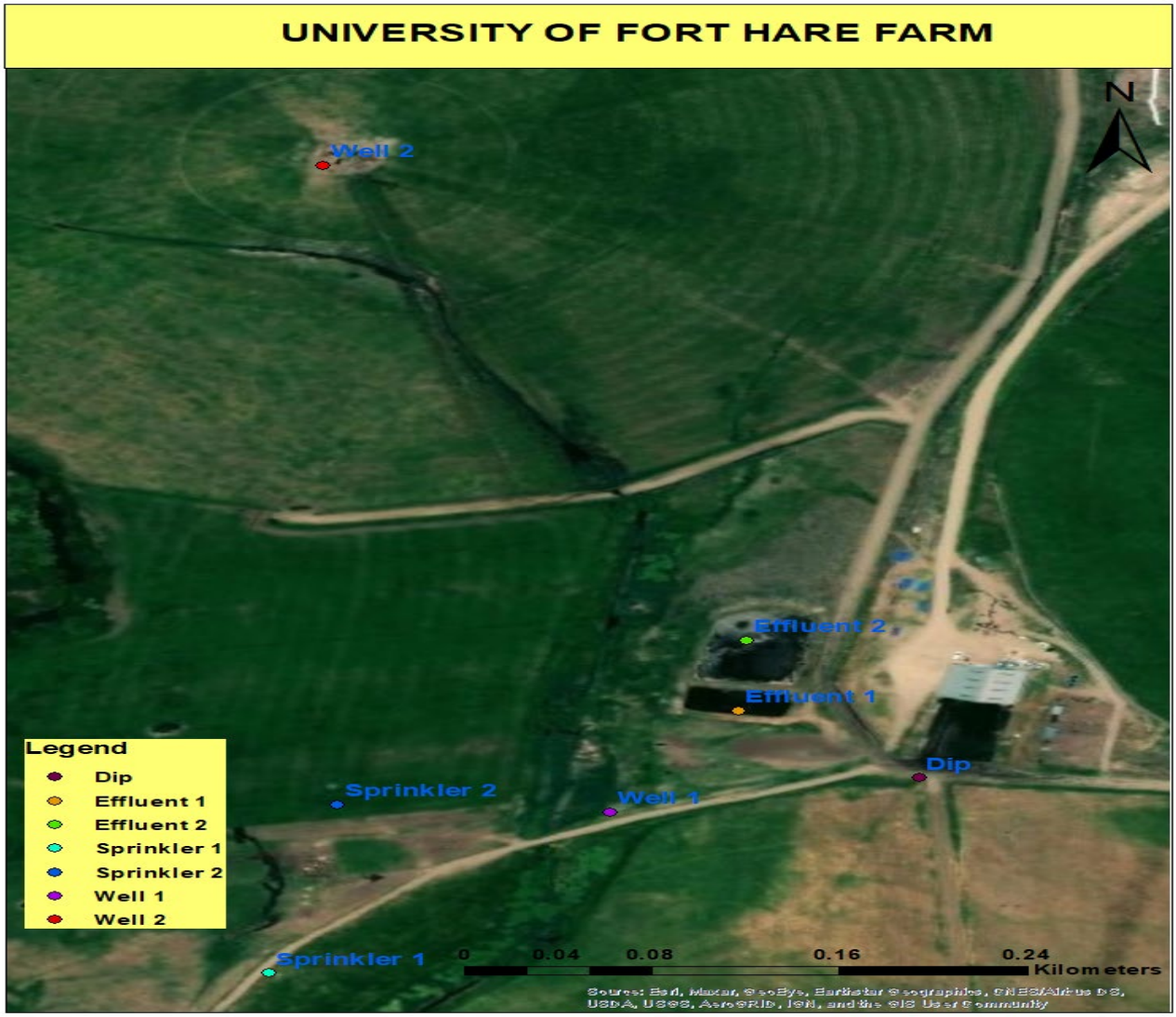


Figure 3.2: The indication of sampling areas in Fort Hare dairy farm

3.1.2. Sample collection and processing

Water samples were collected from Fort Hare Dairy Farm effluent using 1L sterile bottles. Microbiological analysis was conducted at the Applied and Environmental Microbiology Research Group (AEMREG) laboratory for analysis and processing.

3.1.3 Isolation and identification of presumptive *Salmonella* species

Serial dilutions were prepared from the collected effluent samples. Approximately 100ml of the appropriate dilutions were filtered through a 0.45µm filter membrane and were aseptically placed on sterile. Xylose lysine deoxychlate agar (XLD) plates and were incubated 37 ºC for 24 hours. Rappaport vassiladis broth (RVB) was prepared and 9ml was transferred onto test tubes, into that 9ml approximately 1ml of the raw sample was inoculated and incubated at 37 ºC for 24 hours for enrichment of *Salmonella*, 10 µL of the RVB enrichment was then plated onto sterile xylose lysine deoxycholate agar (XLD) and incubated at 37 ºC for 24 hours. Red colonies with black centers characteristic of the desired isolate was picked as presumptive *Salmonella* isolates, and were further purified and subcultured onto fresh sterile XLD Agar plates again for purification. 25% glycerol stocks were prepared from the cultured broths and stored at −80 °C for subsequent analyses.

### 3.1.4. Genomic DNA Extraction

Genomic the boiling method was used to extract DNA, as stated by Gugliandolo, 2011. Presumptive *Salmonella* isolates were grown on fresh sterile nutrient broth then it was incubated at 37ºC for 24 hours. Thereafter 200µl of broth was transferred into eppendorf tube, then the cells were lysed using an Accublock (digital dry bath, labnet) for about 10 minutes at 100 $^{0}$C. The cell debris was then removed by centrifugation at 11,000×g for 2 min using a Mini Spin microcentrifuge (LASEC, RSA). Cell lysates (5 µl) was used as template DNA in polymerase chain reaction (PCR) assays immediately after extraction (Huehn *et al*., 2010).



### 3.1.5. Molecular identification of presumptive *Salmonella* isolates

*Salmonella* species were identified using the polymerase chain reaction. Specific primer sets for the identification of *Salmonella* isolates were used (table 3.1) with optimized cycling conditions to conditions target a specific region within the 16S rRNA (Igbinosa *et al*., 2009). After the cycles were completed, the amplified products were kept at 4°C. 1.5% percent agarose gel electrophoresis was used to separate the amplified products. and thereafter visualized using a UV trans-illuminator (include model and supplier).

3.1.6. Molecular characterization of confirmed *Salmonella* species

The confirmed *Salmonella* isolates were further delineated into different species by PCR using species specific primer sets (Table 3.1). (Oliveira *et al.,* 2003)

Table 3.1: PCR conditions and list of primers that were used for the identification of *Salmonella* species

| Microorganism | Primer(s) | Primer sequence (5'-3') | Amplicon Size (base pair) | Polimerase Chain Reaction conditions | Cycles | Reference (s) |
|---|---|---|---|---|---|---|
| *Salmonella* species | ompC | *F*: ATC GCT GAC TTA TGC AAT CG<br>R: CGG GTT GCG TTA TAG GTC TG | 204 | 94°C, 94°C, 55°C, 72°C, 72°C<br>2', 1', 1', 2',5 | 30 | Oliveira *et al.*, 2003<br><br>Huehn *et al.*, 2010 |
| *Salmonella enteritidis* | SEFA | F- TGT GTT TTA TCT GAT GCA AGA GG<br>R- TGA ACT ACG TTCGTT CTT CTGG | 304 | 95°C, 95°C, 57°C, 72°C, 72°C<br>2', 1', 1', 2', 5, | 30 | (Leo *et al.*, 2013) |
| *Salmonella typhimurium* | TYPH | F-TTG TTC ACT TTT<br>TAC CCC CTG AA | 401 | 95°C, 95°C, 57°C, 72°C, 72°C<br>2', 1', 1', 2', 5, | 30 | (Leo et al., 2015) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | R-CCCTGA<br>CAG CCG<br>TTA GAT ATT | | | | |



3.2. RESULTS

Over the period of nine months sampling a total of 303 phenotypic presumptive *Salmonella* (Gram's stain response and their colonial characteristics) were recovered from Fort Hare dairy farm. 85 from the effluent 1, 97 from effluent 2, 4 from well 1, 3 from well 2, 2 from sprinkler 1, 6 from sprinkler 2, and 106 from washing Boul of cows (Dip). Figure 3.3 shows the number of presumptive *Salmonella* isolates confirmed after preliminary screening using XLD selective media

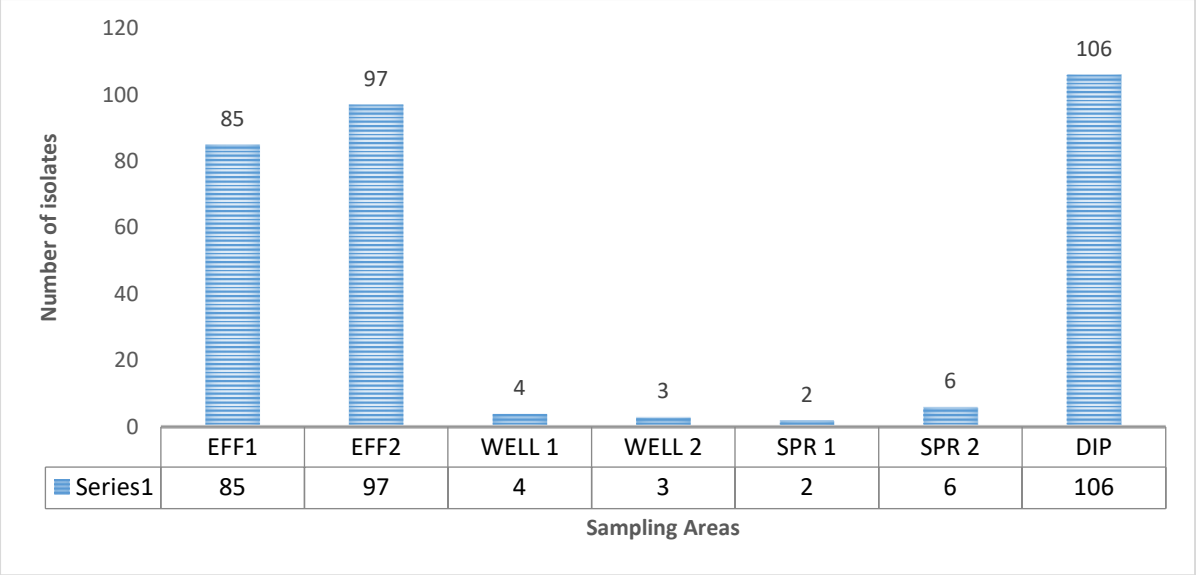


Figure 3.3: Representative of the number of presumptive isolates from different sources



Now, from the 303 presumptive *Salmonella* isolates, 73 were confirmed to be *Salmonella* species using the species-specific primer targeting a specific gene (Fig 3.4 and 3.5). The washing Boul of the caws(Dip) had high number of Salmonella species when confirmed using PCR, followed by Effluent 1 as represented in the Figures below.

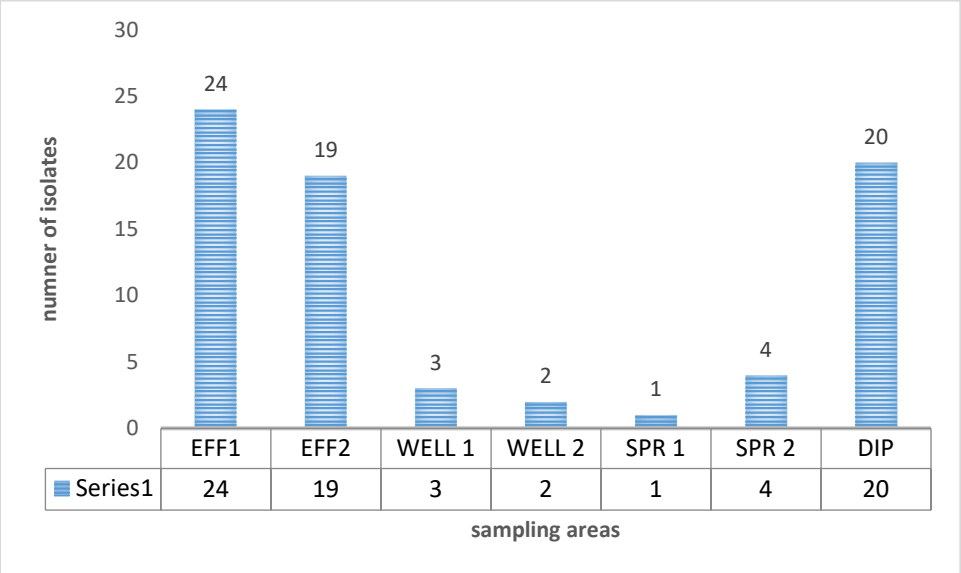


Figure 3.4: Representative of the number of presumptive isolates confirmed using PCR from different sources

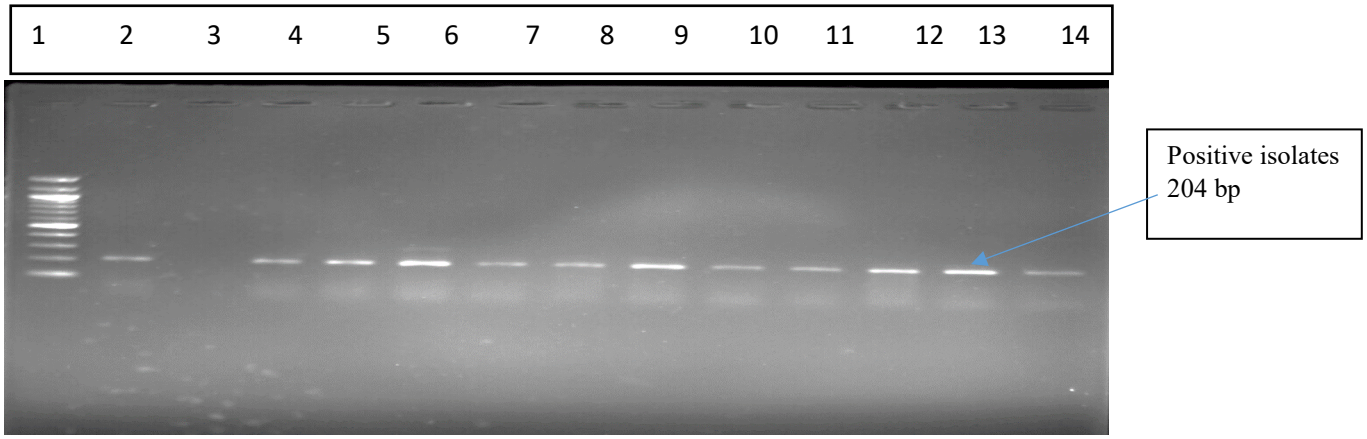


Figure 3.5: Gel picture representing the 204bp PCR amplified *Salmonella* genus 16s rRNA gene. Lane 1: Gene ruler (100 bp); Lane 2: (+ve) control; Lane 3: (-ve) control and Lane 4 -14: (+ve) isolates

3.2.2. Species differentiation

The 73 confirmed isolates to be in the *Salmonella* genus were further delineated into *Salmonella typhimurium* targeting the TYPH gene and *Salmonella enteritidis* targeting the SEFA gene. About 61 (83.6%) were found to be *Salmonella typhimurium* and there was negative for *Salmonella enteritidis*. The remaining 16.4% isolates belonged to other *Salmonella* species that were not evaluated in this study. Figures show gel images of the PCR products used to differentiate the species.

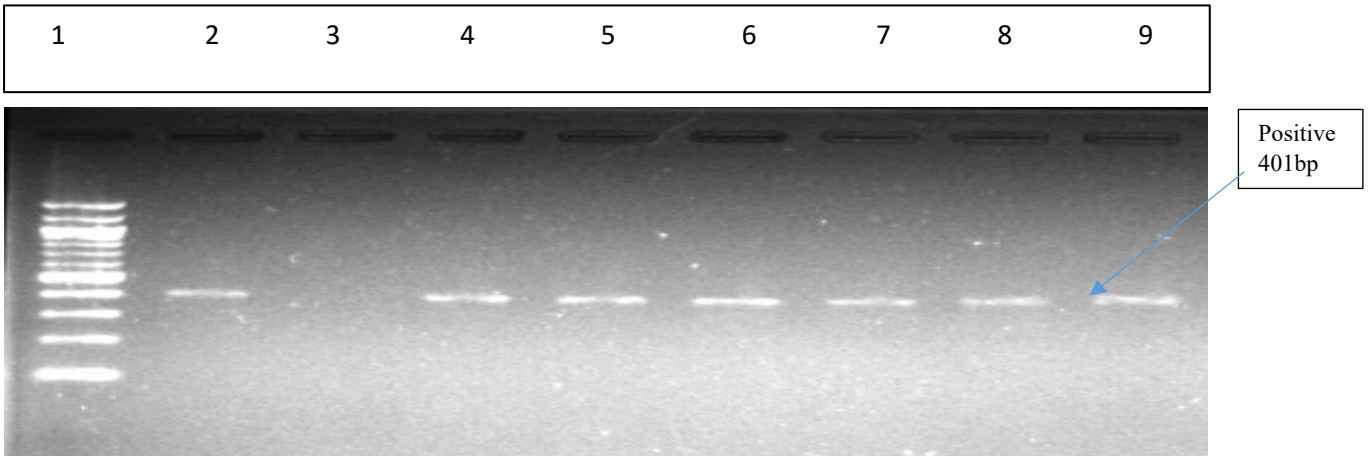


Figure 3.5: Gel picture representing the 401bp PCR amplified Salmonella species 16s rRna gene. Lane 1: Gene ruler (100 bp); Lane 2: (+ve) control; Lane 3: (-ve) control and Lane 4 -9: (+ve) isolates

## 3.3. DISCUSSION

Dairy production is a major concern in South Africa's agricultural sector and contributes to the economic growth and sustainability of the country. All dairy companies use water in their daily steps of the dairy industry, including cleaning, disinfection, heating, cooling and floor cleaning. Dairy product wastewater has a high biological oxygen demand (BOD) and chemical oxygen demand (COD) content, as well as a high content of dissolved or suspended solids, such as fats, oils, and nutrients, such as ammonia or minerals, and phosphoric acid salt, and thus requires special consideration before disposal.

Waterborne feces contamination is a severe environmental health issue that has been linked to outbreaks of a variety of illnesses (Krolik *et al*., 2013). Over a 27-year span, there were 288 reported confirmed water-borne outbreaks of infectious enteric illnesses in Canada, with *Salmonella* being the most common pathogen (Krolik *et al.,* 2013). The most well-known ailment linked to low microbiological water quality is acute gastrointestinal illness, which includes symptoms such as fever and nausea (Macler and Merkle, 2000).

In this study, *Salmonella* species were isolated from different water samples. These include Well 1 (4%), Well 2 (3%), Effluent 1 (33%), Effluent 2 (26%), Sprinkler 1 (1%), Sprinkler 2 (6%) and dip water (27%). PCR confirmed 73 isolates to be positive for the *Salmonella* genus. The confirmed species were further delineated into *Salmonella typhimurium* and *Salmonella enteriditis*, the most prevalent species was *Salmonella typhimurium* with 61 positive isolates present in all the water samples tested.



There were no confirmed isolates for *Salmonella enteriditis*. Out of all the positive isolates, 12 were negative for both *Salmonella typhimurium* and *Salmonella enteriditis*, this means that these isolates belong to other *Salmonella* species that were not tested for in this study. In a study conducted by Sibanda *et al*., (2018), similar observations were reported which stated that *Salmonella typhimurium* is mostly likely to be found in waterbodies that have been in contact with animals, which is the case for the samples collected at Fort Hare Diary farm. This species is, in most cases found in animals and their waste. Now, most of the samples collected from the Diary farm have either been used by cattle for drinking, to shed their waste and to also clean them. According to the results obtained by Msolo (2020), around the same jurisdiction which is Raymond Mhlaba, *Salmonella* has been detected amongst clinical samples. Although this

study focused on water samples, these results show that *Salmonella* is abundant around this area and can be detected in most samples.

## 3.5. CONCLUSION

*Salmonella* species are still among the infections that pose a public health danger. Every year, at least one pathogenic *Salmonella* species has been identified. This is due to a variety of factors including the exploitation of chemotherapeutic agents and antibiotics in agriculture and aquaculture, the effects of global warming on environmental conditions, and *Salmonella* species' improved capacity to pass on acquired resistance genes. These species can be handed down through the ecosystem as a new public health problem as they evolve. It's crucial to know how the disease agent interacts with its environmental reservoir, vector, and other animal hosts in order to understand how these animals generate disease. Our understanding of disease-causing organisms will increase once we have a thorough understanding of their life histories, and we will be able to effectively prevent and control disease outbreaks.

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# CHAPTER 4

## Antibiogram profiles of *Salmonella* species recovered from dairy farm millieu within Raymond Mhlaba local municipality, Eastern Cape Province, South Africa

Abstract
*Salmonella* is a critical pathogen that can be passed between species which can act as a potential supply of antimicrobial resistance determinants. The global problem of antimicrobial resistance has narrowed the range of currently available, inexpensive, and effective antimicrobials. Infections caused by resistant bacteria represent a significant danger to public health and economic stability. Worldwide, almost ten percent of people become ill after drinking contaminated water with *Salmonella*, which has led to continuous deaths annually which is equivalent to the loss of thirty-three million lives. *Salmonella* infection is a common cause of gastroenteritis, typhoid fever, localized infections, and even mortality, making it the most common prevalent foodborne pathogen and a major public health problem. The main aim of this study is to evaluate the antibiogram profiles of *salmonella* species recovered from dairy farm milieu within Raymond Mhlaba local municipality, Eastern Cape Province, South Africa.

The antimicrobial susceptibility patterns of seven members of the Enterobacteriaceae family are presented in Figure: 4.2. High resistance frequencies were observed against Azithromycin (68%), followed by Clavlomic acid (64%), then Minocycline (60%RAmpicillin 30 (54%), Cefoxitin (52%), Chloramphenicol (41%), Nalidixic acid (33%), Cefotaxime (16%), Ciprofloxacin (15%), Doripenem (6%), Meropenem (5%) and Norfloxacin (0%) did not show resistance at all. Results obtained from the confirmed isolates, show that Quinolones and Carbopenem are some of the groups of antibiotics that inhibits the growth of *Salmonella.* According to the findings of this research it can be recommended that the above-mentioned antibiotics can highly be used for the treatment of Salmonellosis.



**Keywords:** Antimicrobial Resistance Determinants; Antibiogram profiles

4.1. Introduction

A broad range of virulent determinants is known to have been acquired by *Salmonella* species, which has necessitated their implication in various diseases in humans which may also help them to tolerate stressful conditions (Brown *et al*., 2019). Some of the virulence factors are capsule polysaccharide, lipopolysaccharide, endotoxin, iron sequestering systems, pili, cytolytic haemolysin, elastase, flagellum, phospholipase, and other exotoxins. Pathogenic strains of *Salmonella* species have been found in final effluents of dairy farm causing an increase in the number of death and disease outbreaks around Raymond Mhlaba local municipality. The use of antimicrobials in farm animals for treatment and infection prevention, Antimicrobial resistance develops as a result of growth stimulation, which can lead to widespread transmission of antimicrobial-resistant bacteria across the food chain, posing a substantial threat to worlwide public health.

During the year 2016, the incidences of *Salmonella* infections were 15.4 per 100, 00 people, this occurred in the United States (CDC, 2017). In 2015, disease outbreaks that are caused by other foodborne bacteria around the world were the lowest compare to *Salmonella* outbreaks. The number of reported *Salmonella* infections outbreak were 23% while other *Salmonella* associated illness were 62 %(Kemal, 2014). Farm environments, notably dairy farms, have been linked to *Salmonella* isolates responsible for the reported outbreaks. (CDC, 2016)). Direct contact with contaminated cattle, the consumption of contaminated water or food are some of the things that contribute to the spread of Salmonellosis from cattle to people (CDC, 2017). As one of the main reservoirs of *Salmonella,* cows are estimated to share almost 73% of *Salmonella* species by faeces annually and the number does not show any decrease as from 2012 (Himathongkham *et al*., 1999). *Salmonella* species have emerged as a serious pathogen in South Africa, necessitating increased molecular surveillance of this pathogen.



## 4.1. Methods and materials

4.1.1. To determine the antimicrobial susceptibility patterns of the confirmed *Salmonella* isolates.

The standard disc diffusion method established by the Clinical and Laboratory Standards Institute was used to determine antimicrobial susceptibility using Mueller-Hinton agar (MH) (Merck, SA) (CLSI, 2017). Normal saline was prepared and 5ml was transferred to test tubes.

Fresh cultures were inoculated onto the test tubes until the turbidity of the suspension was 0.5 McFarland standards. MH agar plates were also prepared and inoculated with the bacterial suspension. This was done by dipping sterile swabs into the suspension thereafter the swabs were spread on the surface of the agar plates, then the antibiotic discs were inserted onto the plates. The plates were then at 35 ± 2°C for 18 to 24 hrs (Li *et al*., 2015). The antibiotics that were used in this study are shown in Table 4 below. An antimicrobial susceptibility test was performed for all the confirmed isolates using a list of antibiotic used for the treatment of *Salmonella* infections. Plates were checked for zones of inhibition after incubation, which were quantified and analyzed using the disc-diffusion method (CLSI, 2017).

Table 4: Antibiotics used and their concentrations are as follows

| Antimicrobial Agent | Disk Content in (μg) | Breakpoints in zone diameter and interpretive categories, to the nearest whole mm | | |
|---|---|---|---|---|
| | | S | I | R |
| Cephalothin | 30.0 | ≥ 18.0 | 15.0–17.0 | ≤ 14.0 |
| Norfloxacin | 30 .0 | ≥ 17.0 | 13.0–16.0 | ≤ 12.0 |
| Nalidixic acid | 30 .0 | ≥ 19.0 | 14.0–18.0 | ≤ 13.0 |
| Cefotaxime | 30 .0 | ≥ 23.0 | 15.0–22.0 | ≤ 14.0 |
| Cefoperazone | 75 .0 | ≥ 21.0 | 16.0–20.0 | ≤ 15.0 |
| Clavulanate-Amoxicillin | 10.0/20.0 | ≥ 20.0 | - | ≤ 19.0 |
| sulbactam -Ampicillin | 10.0/10.0 | ≥ 15.0 | 12.0–14.0 | ≤ 11.0 |
| Cefotetan | 30.0 | ≥ 16.0 | 13.0–15.0 | ≤ 12.0 |
| Cefotaxime | 30.0 | ≥ 23.0 | 13.0–15.0 | ≤ 12.0 |
| Cefazolin | 30.0 | ≥ 15.0 | - | ≤ 14.0 |
| Cefotaxime or Ceftriaxone | 30.0 | ≥ 26.0<br>≥ 23.0 | 23.0–25.0<br>20.0–22.0 | ≤ 22.0<br>≤ 19.0 |
| Cefuroxime (parenteral) | 30.0 | ≥ 18.0 | 15.0–17.0 | ≤ 14.0 |

4.1.2. To screen for the presence of relevant virulence genes of the confirmed *Salmonella* isolates.

The *Salmonella* isolates which shows resistant to antibiotics were further examined for antibiotic resistant determinant using specific primers, depending on the antibiotic to which resistance was shown.

Table 5: Antibiotic resistance determinants are detected using these listed primers.

| Antimicrobial family | Primer | Primer sequence (5' – 3') | Expected band size (bp) | PCR conditions | Reference |
|---|---|---|---|---|---|
| **SULFONAMIDES** | *sul1* | F: TTCGGCATTCTGAATCTCAC<br>R: ATGATCTAACCCTCGGTCTC | 822 | 94 °C, 94 °C, 55 °C, 72 °C, 72 °C.<br>35cycles<br>5', 1', 1', 5',5 | Maynard *et al*. (2004) |
| | *sul2* | F: CGGCATCGTCAACATAACC<br>R: GTGTGCGGATGAAGTCAG | 625 | 94 °C, 94 °C, 50 °C, 72 °C, 72 °C<br>30cycles<br>5', 30", 30", 1', 5' | Falbo *et al*. (1999) |
| **TETRACYCLINES** | *tetA* | F: GCTACATCCTGCTTGCCTTC<br>R: CATAGATCGCCGTGAAGAGG | 201 | 94 °C; 94 °C, 55 °C, 72 °C, 72°C<br>35cycles | Ng *et al.* (2001) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 5', 30", 60", 1', 5' | |
| **Antimicrobial family** | **Primer** | **Primer sequence (5' – 3')** | **Expected band size** | **PCR conditions** | **References** |
| | *tetM* | F: AGT GGA GCG ATT ACA GAA<br>R: CAT ATG TCC TGG CGT GTC TA | 158 | 94 °C; 94 °C, 55°C, 72 °C, 72°C<br>35cycles<br>5', 60", 60", 1', 5' | Strommenger et al. (2003) |
| **AMINOGLYCOSIDES** | *StrA* | F: CTTGGTGATAACGGCAATTC<br>R: CCAATCGCAGATAGAAGGC | 348 | 94 °C; 94 °C, 50°C, 72 °C, 72°C<br>30cycles | Bailey *et al*. (2010) |
| | *tetC* | F: CTTGAGAGCCTTCAACCCAG<br>R: ATGGTCGTCATCTACCTGCC | | 4', 45", 45", 45", 7' | |
| | *strB* | F: GGCACCCATAAGCGTACGCC<br>R: TGCCGAGCACGGCGACTACC | 470 | 94 °C; 94 °C, 50°C, 72 °C, 72°C<br>30cycles<br>4', 45", 45", 45", 7' | Bailey *et al*. (2010) |

| Antimicrobial family | Primer | Primer sequence | Expected band size | PCR conditions | References |
|---|---|---|---|---|---|
| **B-Lactams** | *BlaTEM* | F:ACTTCAACACCTGCTGCTTIC<br>R:TCACCACTTTTATCAGCAACC | 690 | 94 °C; 94 °C, 55°C, 72 °C, 72°C<br>35cycles<br>5', 60", 50", 90", 5' | Bailey *et al*. (2010) |
| | *ampC* | F: TTCTATCAAMACTGGCARCC<br>R:CCYTTTTATGTACCCAYGA | 550 | | |

4.2. Results

Antimicrobial susceptibility teste was used in this study to measure the ability of the antibiotic to inhibit the *Salmonella* species after the confirmation of the organism 12 antibiotics were used namely, doripenem (65%S, 2%I, 6%R), Meropenem (67%S, 1%I, 5%R) , Azithromycin (5%S, 0%I, 68%R), Chloramphenicol (26%S, 6%I, 41%R), Cefotaxime (53%S, 3%I, 16%R), Cefoxitin (17%S, 4%I, 52%R), Clavlomic acid (8%S, 1%I, 64%R), Ampicillin 30(14%S, 5%I, 54%R), Minocycline (9%S, 4%I, 60%R), Ciprofloxacin (16%S, 42%I, 15%R), Nalidixic acid (35%S, 5%I, 33%R) and Norfloxacin (19%S, 0%I, 0%). Based on the results gathered from this study, Macrolides (93.2%), tetracycline (83.2%), Beta-lactam (80.9%) were the major groups of antibiotics that high resistance was observed for all the confirmed isolates this is shown in figure 4.1

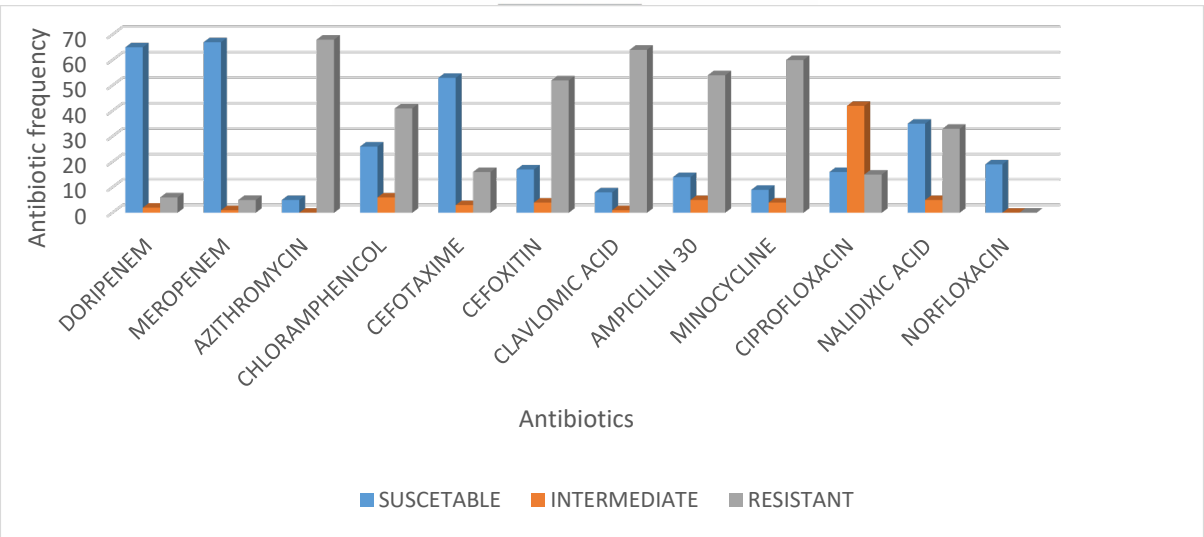



Figure 4.1: Representation of antimicrobial resistance shown by zone of inhibition using CLSI 2017

The Kirby–Bauer disc diffusion technique was used to put all of the identified Salmonella species through AST utilizing 12 panels of antibiotics. Following the CLSI recommendations, the data were interpreted appropriately as determined in the table below

Table 4.3: Classification of antimicrobial resistant genes among *Salmonella* resistant species

| | | n=73 (frequency/percent %) | | |
|---|---|---|---|---|
| CLASS OF ANTIBIOTICS | ANTIBIOTICS | SUSCEPTIBLE | INTERMEDIATE | RESISTANT |
| B-lactam | AMPICILLIN 30 | 14/19.2 | 5/6.8 | 54/74 |
| | CLAVLOMIC ACID 30 | 8/11 | 1/1.4 | 64/87.7 |
| Phenicols | CHLORAMPHENICOL 30 | 26/35.6 | 6/8.2 | 41/56.2 |
| Carbapenem | MEROPENEM 10 | 67/91.8 | 1/1.4 | 5/6.8 |
| | DORIPENEM 10 | 65/89 | 2/2.7 | /8.26 |
| Tetracyclines | MINOCYCLINE 30 | 9/12.3 | 4/5.5 | 60/83.2 |
| Macrolides | AZITHROMYCIN 15 | 5/6.8 | 0/0 | 68/93.2 |
| Cephems | CEFOTAXIME 30 | 54/74 | 3/4.1 | 16/21.9 |

| | | | | |
|---|---|---|---|---|
| | CEFOXITIN<br>30 | 17/23.3 | 4/5.5 | 52/71.2 |
| Fluoroquinolones | NALIDIXIC ACID<br>10 | 35/47.9 | 5/6.8 | 33/45.2 |
| | CIPROFLOXACIN<br>5 | 16/21.9 | 42/57.5 | 15/20.5 |
| | NORFLOXACIN<br>10 | 45/61.6 | 4/5.5 | 24/32.9 |

Phenotypic antimicrobial resistant isolates obtained from the sampling sites were analysed for the genetic variability of related resistance genes namely *blaTEM* gene, which showed high number of resistance compared to other classes as represented in figure 4.2 beow. The amplicon size for the gene *blaTEM* is 690 base pair.

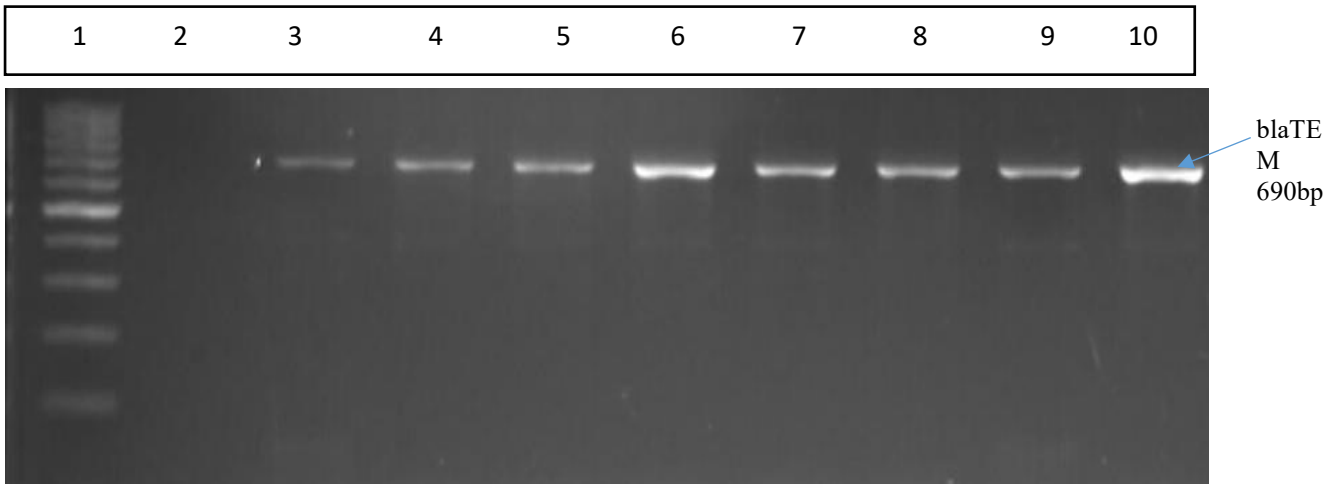



Figure 4.2: Representative gel showing the 690bp PCR amplified *Salmonella typhimurium blaTEM* gene. Lane 1: Gene ruler (100 bp), lane 2: (-ve) control, lane 3: (+ve) control lanes 4–10: (+ve) isolates.



tetracycline (83.2%) were the second highest antibiotics to show resistance represented by the gene *Tet*C with 418 base pair, figure 4.3 as show below

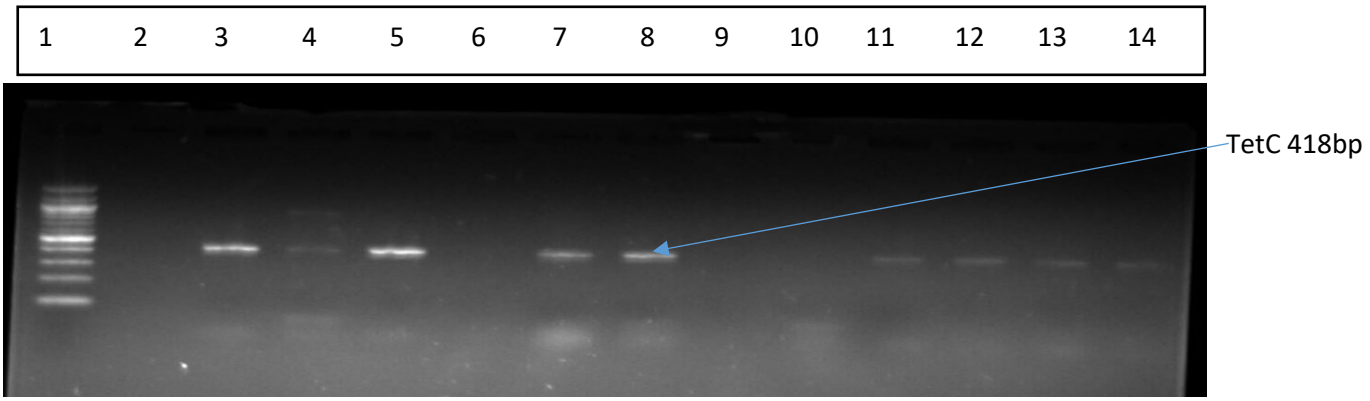
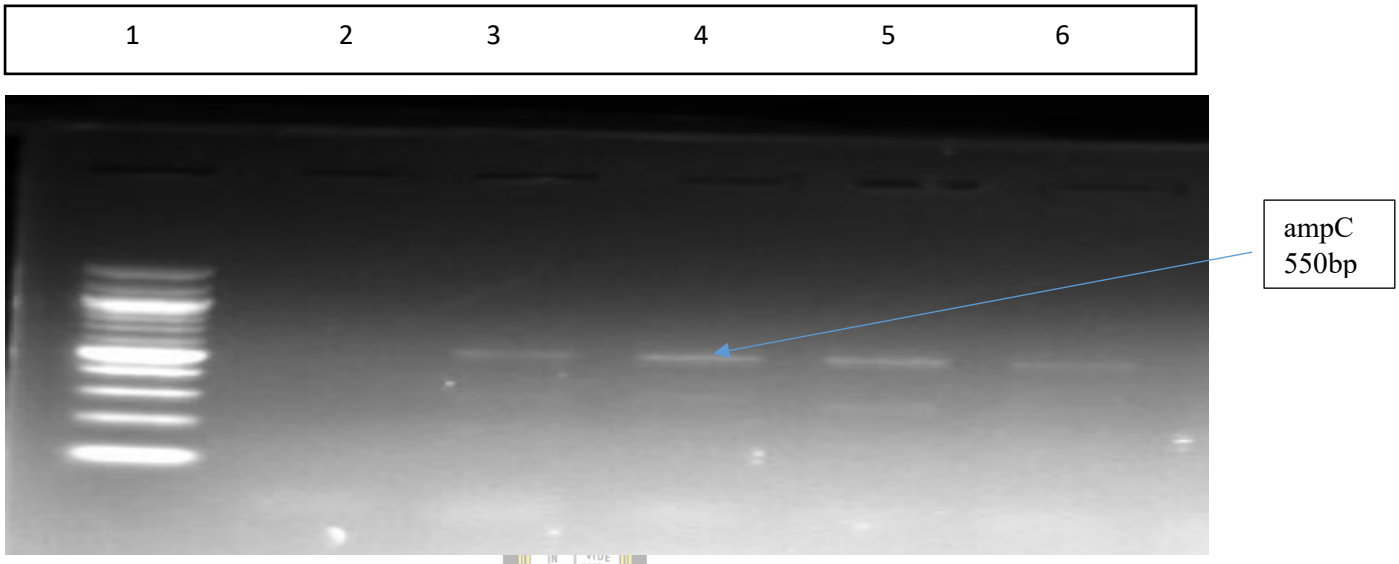



Figure 4.3: Representative gel showing the 418bp PCR amplified *Salmonella typhimurium* TetC gene. Lane 1: Gene ruler (100 bp), lane 2: (-ve) control, lane 3: (+ve) control, lanes 3-4, 5,7-8,11-14-14: (+ve) isolate

Beta lactam (80%) were the second highest antibiotics to show resistance represented by the gene *amp*C with 418 base pair, figure 4.4 as show below




=

Figure 4.4: Representative gel showing the 550bp PCR amplified *Salmonella typhimurium ampC* gene. Lane 1: Gene ruler (100 bp), lane 2: (-ve) control, lane 3: (+ve) control, lanes 4– 6: (+ve) isolates

In figure 4.5 is the duplex Agarose gel electrophoresis of two genes of the same members of the Enterobacteriaceae family *TetC 418 base pair and TetA 201 base pair*

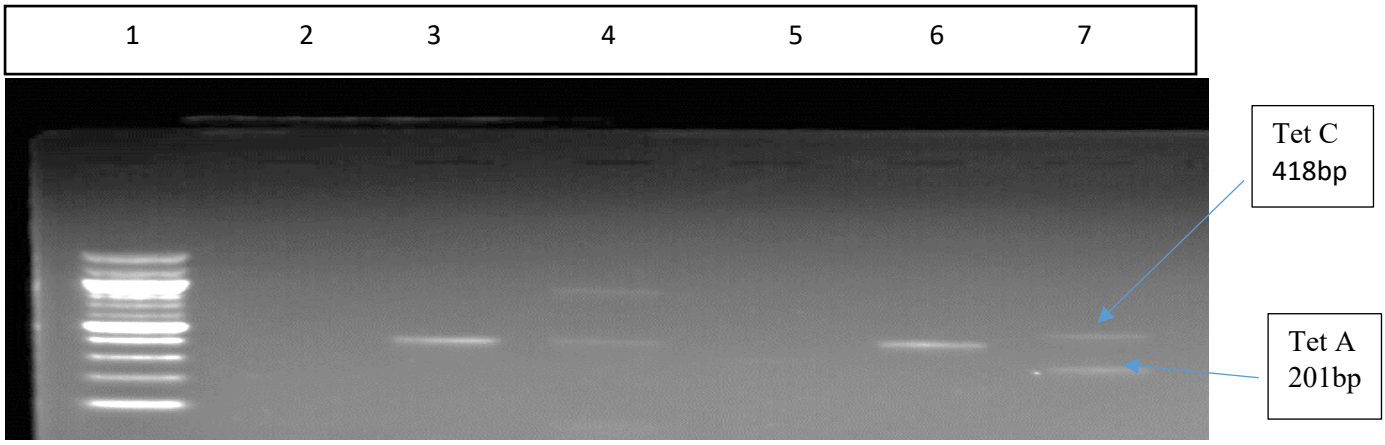



Figure 4.5: Representative gel showing duplex 418bp and 201bp PCR amplified *Salmonella typhimurium Tet C and Tet A* gene. Lane 1: Gene ruler (100 bp), lane 2: (-ver) control, lane 3: (+ve) control, lane 5: (+ve) isolates.



4.3. Discussion

Antimicrobial resistance is a critical issue; as the prevalence of resistance rises, doctors are faced with one of the most difficult issues in the management of infectious diseases. Most bacterial species, without exception, will develop resistance given enough time and antibiotic use. Now, from the results obtained there was no antibiotic that showed 100% susceptibility for all the confirmed isolates. Based on the results gathered from this study, Macrolides (93.2%), tetracycline (83.2%), Beta-lactam (80.9%) were the major groups of antibiotics that high resistance was observed for all the confirmed isolates.

These antibiotics have been one of the major antibiotics used for Salmonellosis treatment, the resistance of these organism to above-mentioned antibiotics can be explained by mechanisms developed by *Salmonella* to fight the functions of these antibiotics making *Salmonella* somewhat resistant to all of them. These mechanisms, as described in literature include the enzymatic degradation of the antibiotic, alteration of the targeted site and changes in membrane permeability to antibiotics. The above-mentioned are some of the mechanism developed by *Salmonella* to fight the function of the antibiotics, making them less active. *Salmonella* species

obtained from the collected water samples show multiple drug resistance as their resistant to more than 1 type of antibiotic, a pattern of multiple drug-resistance is an indication of the over-use of those drugs, fertilisers and other chemicals on the farm. The use of the same antibiotics for the treatment of bacteria results in bacteria acquiring resistance to those antibiotics and the spread of the antibiotic resistance genes within the bacteria.

The high level of resistance within the isolates to Tetracyclines, Macrolides and B-lactams antibiotics could mean that the resistance genes of these antibiotics are widespread in the gene pool of these bacteria within the sample sites. This could result in the spread of multiple-drug resistant bacteria within the farm and to humans. Resistance genes can be transferred by direct contact of the bacterium, with one being the donor and the other a receiver (conjugation), when a bacterium takes a piece of DNA floating in its environment (transformation) and lastly when DNA is transferred from one bacterium to another by a virus. Based on the results obtained resistance was very low to fluoroquinolones displaying high susceptibility. According to Mather *et al*, (2018), this is due to its broad-spectrum activity, nalidixic acid and ciprofloxacin, which are compounds of fluoroquinolone, are used for the treatment Salmonellosis. These are the antibiotics that are currently recommended by clinicians for the treatment of *Salmonella* infection in humans which also include third generation cephems and carbopenems (Kagambèga *et al*., 2018).



Antimicrobial resistance gene can enter the environment through the discharge of untreated water, animals waste or sewage. These genes can spread throughout the environment and result in antimicrobial resistant bacteria. In this study, the occurrence of antimicrobial resistance genes was investigated amongst *Salmonella* isolates from different water samples at a dairy farm. Tetracyclines and B-lactams were one of the antibiotics that had the highest genes present amongst the isolates. The *Amp*C, *bla*TEM were the genes targeted for B-lactam, *bla*TEM was the most prevalent gene (65,3%), followed by *Amp*C (50%). For tetracycline, tetA and tetC were targeted with tetC (50%) being the highest followed by tetA (20%). Tetracyclines and B-lactams are the most commonly used antibiotics, which could easily result in high resistance. In previously studies, the above-mentioned antibiotics have been highly investigated (Kagambèga *et al*., 2018). and results obtained are similar to those of this study which show high prevalence of the Tetracycline and B-lactam genes amongst bacteria. This is could be due to the high presence of these antibiotics in the environment. The only suitable

way to drastically reducing the abundance of these antibiotics is to stop their use (Pan *et al*., 2018). According to the results obtained by Msolo (2020), around the same jurisdiction, which is Raymond Mhlaba, *Salmonella* has been detected amongst clinical samples. Although this study focused on water samples, these results show that *Salmonella* is abundant around this area and can be detected in most samples.

The development of resistance has been observed globally, although susceptibility patterns can differ between different environments caused by different conditions in that particular area. Different rates of resistance are frequently recorded even within a single geographic location.. There are also variations in susceptibility according to the observations analysed from water in different aspects. In most geographic areas, for example, *Salmonella* species in most cases: Clinical Implications for Pediatric.

*Salmonella* strains in this study were unable to grow in Ampicillin (AMP), Minocycline (MH) and Clavulanic acid (AMC). *Salmonella* species were susceptible to some antibiotics most specially fluoroquinolones the ciprofloxacin, nalidixic acid and norfloxacin based on the research done by (Hall *et al*., 2015), which proves that the results obtained from this research were correct.



## 4.4. Conclusion

Based on the findings of this study, it is clear that new antibiotics are needed, as well as joint efforts from higher authorities to provide and distribute new vaccinations as soon as possible to battle the spread of these *Salmonella* bacteria. Extension of contributions from public health sectors is required to reach even the most remote areas. These environmental strains should be identified with caution since they represent a substantial pool of various virulence genes in the environment and play a critical role in pathogenicity and horizontal gene transfer, according to the findings of this study. *Salmonella* species were also shown to have a significant level of resistance to antibiotics. However, isolates from this area were susceptible to Ampicillin (AMP), Minocycline (MH) and Clavulanic acid (AMC) which could be used against the disease caused by *Salmonella.*

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# CHAPTER FIVE

# General discussion and conclusion



## 5.1 General discussion

Dairy farms form a huge part of the environment, they have both a negative and positive impact on the environment and its surroundings (Manyi-Loh *et al*., 2016). A lot of activities can be seen in dairy farms, these include the production of milk, soil and pasture management, farm management and animal health, research for agricultural purposes. All the above-mentioned activities require the use of water, e.g in order for animals to produce sufficient milk they need to drink as much water as possible, also the management of the farm requires the use of water.

This indicates the importance of water in everyday activities, clean and purified water (Imran *et al*., 2019). The major problem occurs when water becomes contaminated by pathogens that can cause infections in both animals and humans. Treatment of water becomes a major priority, as untreated water can pose a huge threat to animals and human dependent on those waterbodies. Contaminated water cannot be seen by the naked eye; microbiological methods are imperative for the analysis of water that is presumed to be contaminated (Garcia *et al*., 2018).

In this study, water samples were collected from different areas of the dairy farm for analysis. *Salmonella* species were isolated and identified in these water samples. *Salmonella* species are pathogenic organisms that are mostly found in water, and are mostly infectious to humans. *Salmonella* species found in these water samples were further delineated into *Salmonella typhimirium* and *Salmonella enteriditis,* the most prevalent species was *Salmonella typhimirium* with 61 out of 73 confirmed isolates being positive for this species. In a study done by Msolo (2020), which was based on *Salmonella* species on clinical samples at Raymond Mhlaba, *Salmonella typhimurium* was detected in almost all the samples. These finding are similar to the findings of this study although focused on water samples, it can be concluded that *Salmonella typhimurium* can be found in samples taken around this jurisdiction whether it is on water or clinical samples.



Also, in a study done by Akinbankole *et al*., (2015) on the over-use of antibiotics has contributed to the resistance of bacteria to multiple-drug. This is seen in this study as samples collected from water samples displayed high resistance to more than one drug. Beta-lactams (80,9%), Tetracyclines (83,2%) and Macrolides (93,2%) are one of the major antibiotics that showed resistance for most of the confirmed isolates. These antibiotics have been previously used for the treatment of infections caused by *Salmonella*, which could explain the resistance of these bacteria to antibiotics. When antibiotics are in excessive use, bacteria develop different mechanisms to fight the purpose of each antibiotic (Otang and Afolayan, 2016). There are certain mechanisms developed by bacteria, these include the enzymatic degradation of the antibiotic, alteration of the targeted site and changes in membrane permeability to antibiotics. Once bacteria acquire any of these mechanisms, it comes impossible for an antibiotic to act on it and perform its purpose of getting rid of bacteria. Also, the high resistance of these bacteria to *Salmonella* is also an indication of the prevalence of their resistance genes in the gene pool of *Salmonella* found in the water samples. Resistant genes can also be transferred from one bacterium to the other by different method. These methods include conjugation, transformation

and transduction, all these methods make horizontal gene transfer possible. Once genes are transferred, resistance is spread within different bacterium, this results in the spread of multiple-drug resistant strains and resistance determinants in the environment and amongst humans.

Antimicrobial resistance genes are known as determinants of antibiotic resistance; these can be detected in isolates that confer resistance for those specific antibiotics. In this study, resistance determinants for tetracyclines and beta-lactams were investigated. For tetracycline, tetA(10%) and *tetC* (50%) were targeted and for beta-lactams *ampC* (50%) and *blaTEM* (63,5%) were targeted. As seen on the results, *tetC* was the most prevalent for tetracyclines while *blaTEM* was the most prevalent for beta-lactam. The presence of these resistance genes on the confirmed *Salmonella* isolates shows the resistance of the bacteria to tetracyclines and beta-lactams, this suggests that clinicians should avoid the use of these antibiotics to treat infections caused by *Salmonella* (Guerrant *et al*., 2015). A study done by Wang *et al.,* (2018), *Salmonella typhimurium* was found to be resistant to multiple-drugs including tetracyclines, this correlates to the findings of this study as *Salmonella typhimurium* was the most prevalent species confirmed from the isolates and tetracyclines showed high antibiotic resistance to the confirmed isolates. This study and that of Igbinosa *et al.,* (2017) revealed that flouroquinoles amongst other drugs show high susceptibility to *Salmonella* species and can be recommended for use by medical practitioners to treat infections.



5.2 Conclusion and recommendations

It is evident that there is dire need to perform direct analysis of specific pathogens that pose a threat to water quality. It is also important that these pathogens are frequently monitored. When these pathogens are monitored, they do not form part of the many outbreak-related pathogens. In order for public health to be maintained, identification of important techniques for the detection of *Salmonella* pathogens is needed. Recommendations have been made, following the detection of pathogenic *Salmonella* species in final effluent discharge, to ensure that environmental standards are followed and that receiving water bodies are not harmed. For the exact purpose, different strategies must be applied to ensure effective monitoring of pathogenic *Salmonella* species in water through efficient management that complies with set guidelines.

Based on the research conducted and findings i recommend that better surveillance systems should be developed, specifically in inland areas of South Africa. Also, that better monitoring systems for water and food outlets be improved and that health education awareness campaigns regarding water management and food safety be conducted in rural areas. Now, the presence

of *Salmonella* species in surface water collected from Fort Hare dairy farm poses a health risk to people around the Raymond Mhlaba local municipality using the domestic water supply for drinking and other domestic purposes. The findings of this study highlight the human health risk associated with drinking contaminated water due to the presence of antibiotic resistant *Salmonella* with pathogenic *Salmonella*-specific resistance genes. As a result, the existence of these antimicrobial-resistant and dangerous *Salmonella* strains in these surface waters necessitates improved risk assessment and prevention methods to protect public health. Because antibiotic resistance is a complex problem involving many interconnected elements, single and isolated interventions have minimal impact, necessitating coordinated efforts.

- The Department of forestry, fisheries and the environment (DFFE) including health departments must be informed on the rise of *Salmonella* species in dairy farm effluents and be proactive in preventing upcoming outbreaks of diseases from *Salmonella* species

- Environmental guidelines and precautionary measures of the water quality from final effluents should be enforced. However, different approaches should be implemented to make sure that monitoring of *Salmonella* species in dairy farms is successful through effective management that complies with set guidelines.

- When prescribing antibiotics, clinicians must also provide patients with sufficient infection treatment and control information and guidelines to eliminate antibiotic abuse.

- Careful regulation and monitoring of the wastewater disposed along with antibiotic should be done in order to control the dissemination of drug resistant bacteria and genes in environment.

- To have an understanding of the health risks, epidemiological studies should be carried out frequently and control strategies for reduction of possible human infections should be implemented.

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