PREVALENCE, SEROTYPES AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATED FROM BEEF AND ANIMAL FEED IN NAMIBIA

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ABSTRACT

The occurrence of *Salmonella* is a global challenge in the public health and food production sectors. Contaminated beef and animal feed with *Salmonella* may lead to infections of humans through food chain. The present study investigated the prevalence, serovar and antimicrobial resistance of strains of *Salmonella* serovars isolated from beef (meat cuts, meat fluid and carcass swabs) and animal feed (meat-and-bone, and blood meal) samples from the abattoirs in Namibia. Beef (9508) and animal feed (827) samples were examined for the presence of *Salmonella*. The data showed that 0.85 % (*n*= 81) of beef were positive for *Salmonella*. In animal feed, 11.73 % (n = 97) were positive for *Salmonella*. The prevalence was higher in carcass swab samples than meat cuts and meat fluid but did not differ significantly ($p > 0.05$) between the products. However, there was a significant difference ($p < 0.05$) on the prevalence of *Salmonella* in blood meal as compared to meat-and-bone meal. Forty-four different types of *Salmonella* serovars were isolated and identified in this study. However, 23 isolated *Salmonella* serovars could not be identified conclusively. Of the *Salmonella* serovars isolated, *S.*Chester was the most common isolated serovar (18.54 %) followed by *S.* Schwarzengrund (7.30 %), *S.* Anatum (5.06 %), *S.* Typhimurium (4.49 %), *S.* Braenderup (4.49 %) and *S.* Reading (4.49 %). From 19 *Salmonella* serovars which exhibited resistance to one or more types of antimicrobials, 15 different *Salmonella* serovars exhibited resistance to at least two antimicrobials. Most *Salmonella* were resistant to sulfisoxazole followed by trimethoprim-sulfamethoxazole and tetracycline

whereas, 80.3% (*n*= 57) were susceptible to all 16 antimicrobials tested. Resistance to sulfisoxazole and the trimethroprim-suflamethoxazole combination were the most common. Of all *Salmonella* isolated, 29.21 % (n = 178) exhibited resistance to the antimicrobials used. The resistant isolates belonged to 19 different *Salmonella* serovars of which 15 different types of serovars showed multidrug resistance (MDR). From the study it was found that the prevalence of *Salmonella* in Namibia is comparable to some studies in other countries. Few or no similar studies are available in the Southern African region for comparison. The susceptibility of *Salmonella* to the antimicrobials tested indicated that antimicrobial resistance is not as common and extensive in Namibia as has been reported in many other countries. It also appears that there is a range of antimicrobials available that are effective in managing *Salmonella* infections in Namibia. However, there is some evidence that resistance is developing and this will need further monitoring to ensure it does not escalate to a problem. This study has helped to understand the prevalence of *Salmonella* serovars in Namibia and how their antimicrobial resistance pattern may influence on the selection of drugs and the treatment of salmonellosis in humans and animals. It is recommended that continuous studies to be done to monitor the link between the *Salmonella* serovars and their resistance pattern between food animals and humans.

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ABBREVIATIONS

AVMA	-	American Veterinary Medical Association
ATR	-	Acid Tolerance Response
CDC	-	The US Center for Disease Control and Prevention
CLSI	-	Clinical and Laboratory Standards Institute
CVL	-	Central Veterinary Laboratory
EFSA	-	European Food Safety Authority
EU	-	The European Union
FDA	-	Food and Drug Administration
HACCP	-	Hazard Analysis Critical Control Point
H_2S	-	Hydrogen Sulfide
ISO	-	International Standards Organization
LPS	-	Lipopolysaccharide
MIC	-	Minimal Inhibitory Concentration
MDR	-	Multidrug Resistance
NARMS	-	The US National Antimicrobial Resistance Monitoring System
NMRC	-	Namibia Medicines Regulatory Council
NCLLS	-	National Committee for Clinical Laboratory Standards
NEMLIST	-	Namibia Essential Medicine List
NTS	-	Non Typhoidal *Salmonella*
RNA	-	Ribonucleic Acid

RSA	-	Republic of South Africa
RVS	-	Rappaport Vassiliadis medium with soya
TSI	-	Triple Sugar Iron
UK	-	United Kingdom
USA	-	United States of America
VP	-	Voges Proskauer
WHO	-	World Health Organization
XLD	-	Xylose Lysine Desoxycholate agar

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DEDICATION

This dissertation is dedicated to my late father Mzee Peter Shilangale who passed on while I was pursuing my degree. To wife, Harriet Brenda for her love, support and continuous encouragement throughout the period of my studies. To our beautiful daughters, Karen Wande and Maureen Aika for bringing love and happiness to our lives. May the Almighty God let this work be the source of inspiration for their future education accomplishment.

DECLARATION

I, Renatus Peter Shilangale, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Renatus Peter Shilangale

CHAPTER 1: INTRODUCTION

1.1 Introduction

Salmonella is a genus of bacteria that are a major cause of foodborne outbreaks in humans throughout the world. Due to genetic and environmental diversity *Salmonella* serotypes are adapted to live in a various range of hosts and habitats using pathogenic and non-pathogenic means of surviving (Callaway, Edrington, Byrd, Anderson & Nisbet, 2008). The prevalence of this pathogen presents major challenges in the food production and public health sectors in their efforts to supply safe foods as consumers' food safety awareness is also on the increase.

The *Salmonella* bacteria are generally transmitted to humans through consumption of mainly contaminated food of animal origin. The contamination is usually caused by the intestinal materials which often contain *Salmonella* bacteria that pollute the surface of the carcasses during the slaughtering process as a result lead to *Salmonella* contamination of meat and meat products (Oosterom, 1991). The contamination by *Salmonella* is associated with popular foodborne disease for humans known as salmonellosis. Animals may also become infected from other *Salmonella* infected animals, directly or via a contaminated environment, including contaminated feed (European Food Safety Authority [EFSA], 2008).

In fact, the global transformation and intensification of agriculture production has led to increasing reliance on manufactured feed products as food for food animals (Crump,

Griffin & Angulo, 2002). Animal feed is usually made up from bone, meat trimmings, blood and other slaughter animal by-products. However, there are evidences of the possible transmission of *Salmonella* from animal feed to animals consuming the feed, and then to food products derived from the animals (EFSA, 2008). These pathogens may be transmitted to humans through the food chain and cause illness (Crump, Griffin & Angulo, 2002).

Even though common *Salmonella* serotypes occurring in humans are rarely found in animal feedstuffs, similar serotypes found in feed have been detected in humans (EFSA, 2008). The movement of people, food animals and food stuffs across national boundaries through importation and exportation increase the risk of salmonellosis and other zoonotic foodborne diseases (Molla, Alemayeh & Salah, 2003). Therefore, in order to reduce the risk of cross border contamination between countries it is important to understand the distribution and mode of introduction into a country.

Currently, the best way for minimizing humans' illness from food animals is a strategic introduction of interventions throughout from the farm to the table. A periodic surveillance of the level of *Salmonella* contamination in the different food animals, food products and environment is also regarded necessary to control the spread of the pathogens and infection to humans (Molla *et al*., 2003). Hazard Analysis Critical Control Point (HACCP), a food safety management tool which is mandatory in many developed countries is currently being used to reduce the occurrences of foodborne

outbreaks. In Namibia, application of HACCP system is not mandatory for food companies producing for local markets but is widely applied by food companies producing for the export markets i.e. the beef and fish industry.

However, the control of infectious diseases is seriously threatened by the steady increase in the number of microorganisms that are resistant to antimicrobial agents (Okeke *et al*., 2005). The emergence and spread of antimicrobial resistance are complex problems driven by various interrelated factors, many of which are linked to the misuse of antimicrobials (World Health Organization [WHO], 2002). On the other hand, concern is growing about the possible transfer of bacterial resistance to antibiotics from animals to humans. This was first illustrated by the ban of avoparcin use as a growth promoter in livestock which took effect in the European Union (EU) on 1 April 1997 (Editorial Committee, 1997).

Studies have since demonstrated that the increase in the number of infections and the use of antimicrobials directly correlate with the increase in prevalence of resistance (WHO, 2002). Although the selection of effective antibiotics is critical for the treatment of invasive infections, treatment has also become more difficult as antimicrobial resistance has increased (Angulo, Johnson, Tauxe & Cohen, 2000). It has since been concluded that the dominant factor in the emergence and spread of antibiotic resistant pathogens is the intensive use of antimicrobial agents (Nweneka, Tapha-Sosseh & Sosa, 2009).

1.2 Statement of the problem

1.2.1 Global food safety concern

The global threat of foodborne diseases especially those associated to *Salmonella* has been on the increase. This trend has caused some markets to introduce some strict conditions on imported foodstuffs of animal origin because of food safety concern and for economic reasons. For example, the Norway National Food Law has a zero tolerance policy on *Salmonella* (Isakbaeva *et al*., 2005). This policy suggests that a documentation of *Salmonella* testing program is required to satisfy this quality requirement for all imported meat before accepted in that country.

Currently, Namibia exports meat and animal feed to other countries such as the EU, Republic of South Africa (RSA) and Norway. The meat industry earns the country about N$ 1.7 billion (Approx. US$ 220 million) per annum and is estimated to contribute about 3.1 % of the national Gross Domestic Product (GDP). However, if these products are contaminated with *Salmonella* they may cause serious food safety concern. This is because of the ability of *Salmonella* to cause foodborne illness in humans. On the other hand, *Salmonella* contamination may negatively affect the acceptability of products in importing countries which in turn may affect the local meat industry. The EU has the Rapid Alert System for Food and Feed (RASFF), an effective tool to exchange information, which helps the Member States to act more rapidly in response to a health threat caused by food or feed in a coordinated manner. This is because movement of food animals and food stuffs across the borders through the import and export are the

factors that are believed to increase the risk of salmonellosis and other zoonotic foodborne diseases (Molla *et al*., 2003). According to Taddele, Rathore and Dhama (2012), livestock and livestock products alone may contribute up to 96 % of the *Salmonella* infections in humans. Although there are few countries that report data on the disease, salmonellosis constitutes a major public health burden causing approximately 15 000 hospitalizations and 580 deaths annually in the USA alone (WHO, 2005).

Lack of studies and knowledge on the prevalence of *Salmonella* in Namibia has made it difficult to estimate the potential health threat they may have locally and the impact they might have internationally due to trade. This is because the prevalence of *Salmonella* in Namibia is yet to be investigated or documented. Therefore, this research provides information on the prevalence of *Salmonella* serovars which may be used to estimate the potential threat in food production chain and public health in Namibia.

1.2.2 Antimicrobial resistance of *Salmonella*

Salmonella which are resistant to a range of antimicrobials used in both humans and animals have emerged and are becoming a serious public health problem (WHO, 2005). There are reports of increasing incidences of multidrug resistant (MDR) *Salmonella* in both humans and cattle (Hogue *et al*., 1997). Reports of antimicrobial resistant *Salmonella* isolated from beef have been reported in different African countries such as Botswana (Samaxa, Matsheka, Mpoloka & Gashe, 2012), Ethiopia (Dabassa & Bacha,

2012) and Algeria (Mezali & Hamdi, 2012). According to Taddele *et al.* (2012), in developing countries, the problem of antimicrobial resistance is also characterized by the easy availability of drugs together with inadequate health services which result in increased proportions of drugs that are used as self-medication compared to prescribed drugs.

The development of drug resistant and MDR bacteria complicates the treatment of infections because the available drugs become less effective. There is also a growing global concern on the danger of transferring bacterial resistance to antibiotics from animals to humans (Editorial Committee, 1997). This fear has lead to several countries such as the United Kingdom (UK), United States of America (USA), Denmark, France and Canada to monitor the resistance of antimicrobials in livestock and (Hogue *et al.*, 1997).

Since Namibia is a meat producing country and a tourism destination for many countries there is a big possibility of importing and exporting antimicrobial resistant pathogens therefore significantly contributing to the global problem. Consequently, the size of the problem remains unknown in Namibia because there are no studies which have been conducted on the antimicrobial resistance of *Salmonella* in the country. This study therefore investigated the antimicrobial resistance pattern of *Salmonella* strains isolated from beef and animal feeds in Namibia and contributes to the global knowledge of *Salmonella* antimicrobial resistance.

1.2.3 Selection of antimicrobials

According to WHO (2002), the discoveries of antimicrobial substances had reduced the global threat posed by infectious diseases. Nevertheless, these gains have been seriously jeopardized by the emergence and spread of microbes that are resistant to drugs (WHO, 2002). In order to maintain the advantages of antimicrobial substances there is need to have more studies on the effectiveness of antimicrobials through diagnostic laboratories and improve the surveillance on the emergence of resistance in pathogens. Furthermore, better regulation on the use of antibiotics and more education in the appropriate use of the drugs for public, medical practitioners and veterinarians is required (Hart & Kariuki, 1998).

When infections become resistant to first-line antimicrobials, treatment has to be switched to second then to third line drugs. However, this practice is always much more expensive, sometimes more toxic and the cost of such replacement drugs is usually unaffordable (WHO, 2002). Poverty and inadequate health care systems are also reported to have greatly limited the benefits of drugs in controlling infectious diseases in developing countries (Byarugaba, 2004). The poor health system has led to drugs abuse and self medication in most developing countries (Taddele *et al.*, 2012). Nevertheless, the failures of antimicrobials to overcome infections complicate the treatment and the choice of suitable antimicrobial for treatment of a particular illness.

The loss of susceptibility by organisms to drugs due to development of resistance affects the selectivity of effective drugs to control infectious diseases. This suggests that there is a need for developing newer compounds albeit more knowledge is still required on the effectiveness of available drugs in treating diseases and controlling emerging organisms. According to WHO (2005) fluoroqunolines and the third-generation cephalosporins are the recommended drugs of choice for treatment of *Salmonella* infection for both adults and children respectively while chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole are used as alternative drugs. However, as microorganisms are becoming resistant to antimicrobials the effectiveness of available drugs in the treatment of infectious diseases may only be known through laboratory research and investigations.

One of the areas of research interest is on the susceptibility test for bacteria such as *Salmonella* on different antimicrobials. Susceptibility test provide information on how much the bacteria is susceptible towards different antimicrobial substances in the laboratory conditions and help on the selection of effective antimicrobial for treatment of infections. Therefore, the knowledge and results obtained from the susceptibility tests of *Salmonella* from this study may contribute to proper selection of effective antimicrobials that may be used in the treatment of humans and livestock in Namibia. Similarly, the findings may further help to reduce the costs of health care in Namibia if only effective drugs will be used for treatment of *Salmonella* infections.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study was to determine the prevalence and antimicrobial resistance of *Salmonella* isolated from beef and animal feed in Namibia.

1.3.2 Specific objectives

This study had the following specific objectives:

1. To determine the prevalence of *Salmonella* serovars isolated from beef and animal feed in Namibia.
2. To determine the diversity of *Salmonella* serovars isolated from beef and animal feed in Namibia.
3. To determine the antimicrobial resistance profile of *Salmonella* serovars isolated from beef and animal feed in Namibia.
4. To determine the prevalence of multi-drug resistance (MDR) and non MDR *Salmonella* serovars isolated from beef and animal feed in Namibia.
5. To determine if available antimicrobials may effectively be used for treatment of salmonellosis in Namibia.

1.4 Significance of the study

The presence of *Salmonella* in food, animal feed and their products remain to be a major global food safety concern because of its potential to cause foodborne illnesses in humans. On the other hand, the emergence of *Salmonella* strains that are resistant to available drugs complicates the selection of available drugs for effective treatment of humans and food animals. In order to tackle these problems it is important to know the prevalence, diversity and antimicrobial resistance status of pathogens that exist in a country. The zoonotic nature of *Salmonella* and their connection with food poisoning and their threats due to multidrug resistance suggest that in addition to prevalence it is important that the serotypes and antimicrobial resistance pattern of the isolates is correctly understood. This is because of the public health risk posed by these pathogens when they enter food production chain. This study therefore determines the prevalence, serovars and antimicrobial resistance pattern of *Salmonella* isolated in beef and animal feed produced in Namibia. The study further helps to increase the knowledge of available *Salmonella* serovars and may be used to start database of the *Salmonella* spp. that are prevalent in Namibia which currently does not exist. Furthermore, the information obtained from this study on the antimicrobial resistance profile of *Salmonella* serovars could be used for successful selection of drugs and treatment of both humans and livestock. Proper selection of drugs for effective treatment of diseases may also help to reduce the cost in health care. In addition, the information obtained from this study could help the local meat industry to control the prevalence of *Salmonella* in their establishments. The effective control of *Salmonella* will make it

easier for their products to have access to lucrative markets with stringent food safety requirements such as the EU, USA and Norway.

CHAPTER 2: LITERATURE REVIEW

2.1 Historical aspects of *Salmonella*

The early history of the *Salmonella* is confused because at that time some members of *Salmonella* serovars were either inadequately characterized or confused with members of other bacterial genera. Therefore, the historical aspects on when exactly the bacterium was discovered seem to remain unclear. However, many scholars seem to agree that *Salmonella* was first isolated by the bacteriologist D.E. Salmon (Lin-Hui & Cheng-Hsun, 2007; Wray, 2003; Grimont, Grimont & Bouvet, 2000).

According to Lin-Hui and Cheng-Hsun (2007), Smith (1894) reported that the genus *Salmonella* is named after Dr. D. E. Salmon, who first isolated the bacteria in 1884. Wray (2003) stated that the organism now known as *Salmonella choleraesuis* was first isolated in 1885 from pigs by Salmon and Smith, who considered the organism to be the cause of swine fever (hog cholera). Grimont *et al.* (2000) stated that the organism now known as *S. choleraesuis* was first isolated from pigs by Salmon and Smith in 1886 when they considered the organism to be the cause of swine fever. On the other hand, a different publication suggests that *S. choleraesuis* was discovered as early as 1880 and was first isolated in a culture media in 1884 by a scientist known as Gaffky (Mølbak, Olsen & Wegner, 2006).

Further developments on the historical aspects of *Salmonella* came in 1892 when Loeffler described the causative agent of murine typhoid (then known as *Bacillus typhi* but now *S. enterica* serotype Typhimurium) that caused an epidemic typhoid fever-like disease in mice (Santos *et al*., 2001). Infact, the organism we know today as *Salmonella* was originally called "*Bacillus choleraesuis*," and was later changed to "*Salmonella choleraesuis*" by Lignieres in 1900 (Lin-Hui & Cheng-Hsun, 2007). The name "*choleraesuis*," refers to both a species and serotype which causes confusion, and also, the name does not represent the majority of the serotypes (Brenner, Villar, Angulo, Tauxe & Swaminathan 2000). Initially, *Salmonella* strains isolated from different clinical conditions or hosts were considered to be different species and were given names such as '*Eberthella typhosa*' (*S. typhi*), *S. enteritidis*, '*S. abortusovis*', *S. choleraesuis* or *S. typhimurium* (Grimont *et al.*, 2000).

2.2 Evolution of *Salmonella*

Salmonella is a bacterium belongs to a genus of the family *Enterobacteriaceae* and comprises a large and closely related population of medically important pathogens (Lin-Hui & Cheng-Hsun, 2007). Among the bacteria in the family *Enterobacteriaceae Salmonella*, *E. coli* and *Shigella* strains have been shown to have close identity of gross chromosome organization (Brenner, Fanning, Johnson, Citarella & Falkow, 1969). Based on the close DNA relatedness among *Salmonella* serotypes, it is estimated that a

common ancestor of the genus existed about 25 to 40 million years ago (Bäumler, Tsolis, Ficht & Adams, 1998).

On the other hand, on an estimated rate of 16S rRNA divergence of nearly 1% per 50 million years, it is speculated that *Escherichia coli* and *Salmonella* diverged from the last shared common ancestor between 120 and 160 million years ago (Ochman, Elwyn & Moran, 1999). This estimation was based on the time scale constructed by Ochman and Wilson for bacteria evolution. In a different study, O antigen clusters from two sero group O58 show that *S. enterica* strains had approximately 85 % identity with the *E. coli* O123 O antigen region over their entire length (Clark *et al*., 2009).

It is further suggested that during their separate evolution, *Salmonella* serotypes acquired many genes by phage or plasmid-mediated horizontal transfer (Bäumler, Heffron & Reissbrodt, 1997). The acquired genes now distinguish *Salmonella* species from each other. Point mutations leading to the modification, inactivation or differential regulation of existing genes are believed to have contributed to the diversification of microorganisms on an evolutionary timescale (Ochman, Lawrence & Groisman, 2000).

Unlike other pathogens *Salmonella* has managed to adapt and survive in different hosts and environmental conditions during the evolution process. The question of how bacteria are able to overcome species barriers and adapt to new hosts is central to the understanding of both the origin of infectious diseases and the emergence of new

pathogens (Bäumler, Tsolis, Ficht & Adams, 1998). However, the answer could be due to the result of the inactivation of single genes, as well as the acquisition or loss of single genes or large islands of DNA to host adaptation of the bacteria (Papagrigorakis, Synodinos & Yapijakis, 2007).

A strain such as *S.* Typhi which is now restricted to humans is thought to have had the ability to infect animals as well in ancient times. This theoretical possibility has now turned into a working hypothesis after the isolation of *S.* Typhi in ancient skeleton from the mass grave connected to the Plague (a typhoid fever like) (Papagrigorakis *et al*., 2007). The disease devastated Athens in 430 - 426 B.C. and reported to have killed both humans and animals. However, it is thought that the accumulation of single mutations, insertions or deletions within the genome of modern-time *S.* Typhi appears to have generated various pseudogenes, suggesting its recent evolutionary origin (Papagrigorakis *et al*., 2007).

2.3 *Salmonella* nomenclature

Since their discovery, *Salmonella* nomenclature has been controversial because the original taxonomy of the genus was not based on DNA relatedness (Todar, 2008). Each serotype was then considered a separate species (for example, *S.* Paratyphi A, *S.* Newport and *S.* Enteritidis) (Brenner *et al*., 2000). Other taxonomic proposals had given names according to clinical considerations based on the clinical role of a strain, on

the biochemical characteristics that divide the serotypes into subgenera, and ultimately, on genomic relatedness (Todar, 2008; Brenner *et al*., 2000). In that case, the nomenclature of these bacteria has changed many times and is still unstable.

The confusion in *Salmonella* nomenclature started when the serological analysis was adopted into the Kauffmann-White scheme in 1946 where *Salmonella* species were defined as "a group of related fermentation phage-type" with the result that each *Salmonella* serovar was considered as species. The proposed Kauffmann concept was based on the serological identification of O (somatic) and H (flagellar) antigens (Brenner *et al.,* 2000). The current nomenclature of the genus *Salmonella* has therefore evolved around this concept which is known as one serotype-one species concept. Since the host-specificity suggested by some for the earlier names does not exist (e.g., *S.* Typhimurium, *S.* Cholerae-suis are in fact ubiquitous), names derived from the geographical origin of the first isolated strain of the newly discovered serovars were next chosen, e.g. *S.* London, *S.* Panama and *S.* Stanleyville (Todar, 2008).

Since *Salmonella* nomenclature is complex, scientists use different systems to refer to and communicate about this genus (Brenner *et al*., 2000). The current taxonomy and nomenclature of the genus *Salmonella* has been the subject of debate since the 1980s when Le Minor and Popoff (1987) proposed changes not only in the nomenclature of the species of this genus but also on the formal taxonomic interpretations (Tindall, Grimont, Garrity & Euzêby, 2005). Two other proposals were later submitted by Euzêby *et al*.

and, Ezaki and Yabuuchi to deal with the problem of the serious discrepancies between the nomenclatures of Le Minor and Popoff (Tindall *et al*., 2005).

A study by Crosa, Brenner, Ewing and Falkow (1973) which provided a major development in *Salmonella* taxonomy used DNA-DNA hybridization to differentiate *Salmonella* species. Their findings indicated that all serotypes of *Salmonella* were highly related and might have been considered a single species (Crosa *et al*., 1973). However, on the Le Minor & Popoff proposal the genus *Salmonella* was proposed to be changed to *Salmonella enterica* (Tindall *et* al., 2005; Brenner *et al*., 2000) because no serotype shares this name (Brenner *et al*., 2000). This proposal came after a recommendation by Subcommittee of *Enterobacteriaceae* of the International Committee on Systematic Bacteriology in 1986 on the name change to *Salmonella enterica* (Brenner *et al*., 2000).

In 2005, *Salmonella enterica* finally gained official approval as the type species of the genus *Salmonella* (Lin-Hui & Cheng-Hsun, 2007). The genus *Salmonella* encompasses other two species; *Salmonella bongori* and *Salmonella subterranean*, a new species which was also recognized in 2005 (Lin-Hui & Cheng-Hsun, 2007). The subspecies of *Salmonella enterica* are now divided into six subspecies which are represented by Roman numerals in the scheme: *S. enterica* subsp. *enterica* = I; *S. enterica* subsp. *salamae* = II; *S. enterica* subsp. *arizonae* = IIIa; *S. enterica* subsp. *diarizonae* = IIIb; *S. enterica* subsp. *houtenae* = IV; *S. enterica* subsp. *indica* = VI (Euzéby & Barrett, 2007). *Salmonella enterica* subspecies are differentiated biochemically (Brenner &

McWhorter-Murlin, 1998; Popoff & Le Minor, 1997) and by genomic relatedness (Popoff & Le Minor, 1997; Reeves *et al*., 1989). *Salmonella bongori* is represented by Roman numeral V (Euzêby & Barrett, 2007).

Currently, the nomenclature system used at the US Center for Disease Control and Prevention (CDC) for the genus *Salmonella* is based on recommendations from the WHO Collaborating Centre. The current CDC nomenclature system used is summarized in Table 1. According to this system, the genus *Salmonella* contains two species, *S. enterica*, the type species, and *S. bongori* (CDC, 2008). However, a third species "*Salmonella subterranea*" which was recognized in 2005 is yet to be incorporated in CDC nomenclature system (Lin-Hui & Cheng-Hsun, 2007).

Table 1. *Salmonella* Nomenclature (Lin-Hui & Cheng-Hsun, 2007).

Taxonomic position (writing format) and nomenclature				No. of serotypes in each species or subspecies
Genus (Capitalized, italic)	Species (italic)	Subspecies (italic)	Serotypes (or serovars) (Capitalized, not italic)*	
Salmonella	*enterica*	*enterica* (or subspecies I)	Choleraesuis, Enteritidis, Paratyphi, Typhimurium	1504
		Salamae (or subspecies II)	9, 46: z: z39	502
		Arizonae (or subspecies IIIa)	43: z29:-	95
		diarizonae (or subspecies IIIb)	6, 7:l, v: 1,5,7	333
		houtenae (or subspecies IV)	21: m,t:-	72
		indica (or subspecies VI)	59: z36:-	13
	bongori	Subspecies V	13,22: z39:-	22
	subterranae			

*Some selected serotypes (serovars) are listed as examples.

Although *Salmonella* nomenclature is complex, the uniformity is still necessary for communication between scientists, health officials and the public. CDC uses names for serotypes in subspecies I and uses antigenic formulas for the remaining subspecies described after 1966 including *Salmonella bongori*. The names given usually refer to

associated diseases, geographical location, or usual habitat where the serotype was first isolated.

To avoid confusion between serovars and species, the serovar name is not italicized and starts with a capital letter. When cited for the first time in a report, the genus name is given followed by the word "Serotype" (or the abbreviation "ser.") and then the serotype name, e.g., *Salmonella* serotype or ser. Choleraesuis. Afterward the name may be shortened with the genus name followed directly by the serotype name, e.g. *Salmonella* Choleraesuis or *S.* Choleraesuis. Because the type species name, enterica was not approved before 2005, serotype names are used directly after the genus name without mention of the species.

2.4 Morphology and characteristics of *Salmonella*

The present defined *Salmonella* species are very closely related to one another, with their genomic DNA re-association rates estimated to be as higher as 90% (Liu *et al*., 2002). Morphologically, *Salmonella* are Gram-negative, straight rods shaped bacteria that measure between 0.7 and 1.5 μm in diameter and 2 to 5 μm in length. They are usually motile with peritrichous flagella and are facultative anaerobes. *Salmonella* ferment mostly glucose with the formation of gas and also reduce nitrate to nitrite.

Salmonella grow optimally between 35 °C to 37 °C and catabolizes a variety of carbohydrates into acid and gas. They use citrate as the sole carbon source, and decarboxylate lysine and ornithine to cadaverine and putrescine respectively. They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. Most *Salmonella* species produce H_2S, which can readily be detected by growing them on media containing ferrous sulfate such as triple sugar iron (TSI) where *Salmonella* is able to produce H_2S from thiosulphate. The production of H_2S on TSI media is one of the common biochemical characteristics which are widely used when identifying the bacteria.

Historically *Salmonella* was mostly confirmed if it catabolized glucose and lysine but failed to metabolize lactose, sucrose and urea. However, some *Salmonella* have shown to have no reaction on these substances. Atypical biotypes such as *Salmonella enteric* serova Enteritidis that cannot decarboxylate lysine (Morita *et al*., 2006), *Salmonella enterica* serova Typhi that readily use lactose (Kohbata, Takahashi & Yabuuchi, 1983) and *Salmonella enterica* serova Mbandaka that have the ability to ferment sucrose (Reid, Porter & Ball, 1993) have been isolated.

The usual habitat of different *Salmonella* species and subspecies is the intestines of both cold and warm blooded animals (Brenner *et. al*., 2000). The bacteria can also be found throughout the natural environment. Environmental sources of the organism include water, soil, insects, factory surfaces, kitchen surfaces, animal feces, raw meats, raw

poultry and raw sea foods (Bad Bug Book [BBB], 2009). Even though *Salmonella* cannot multiply outside the host digestive tract, the bacteria can live for number of weeks in water and some years in soil if there are favorable conditions such as temperature, pH and humidity (Todar, 2008).

2.5 Pathogenesis and clinical syndrome of *Salmonella*

Enteric fever is a systemic illness usually caused by human adapted pathogen, *S.* Typhi (typhoid) (Edgeworth, 2001; Tankhiwale, Agrawal & Jalgaonkar, 2003) or *S.* Paratyphi (paratyphoid), and occasionally by other *Salmonella s*erotypes (Edgeworth, 2001). A typhidal *S.* Typhi infects humans only; there is no animal reservoir, and transmission occurs following consumption of food or water contaminated with human faeces (Ohl & Miller, 2001; Parry, Hien, Dougan, White & Farrar, 2002). Clinical manifestations of the disease include fever, abdominal pain, transient diarrhoea or constipation, and occasionally a maculopapular rash (Ohl & Miller, 2001).

As pointed out above, all *Salmonella* infections begin with the ingestion of organisms in contaminated food or water. The infectious dose of *S.* Typhi in a study done on volunteers varies between 1000 and 1 million organisms where the Vi-negative strains of *S.* Typhi are less infectious and less virulent than Vi-positive strains (Parry *et al*., 2002). When the pathogen enters and moves through its host, it encounters changes in chemical and physical properties, such as temperature, pH, osmolarity, and nutrient

availability in each new environment as well as the host innate immune system (Ohl & Miller, 2001). For the infections to occur, *Salmonella* must survive the gastric acid barrier and a low pH which is an important defense mechanism of the host to reach the small intestine (Parry *et al*., 2002). Gastric acidity presents a significant initial barrier to infection by possibly altering the bacterial infection dose (Giannella, Broitman & Zamcheck, 1972).

For survival, the pathogens must sense these changing surroundings and respond with coordinated programs of gene expression that provide an adaptive advantage in each new host environment (Ohl & Miller, 2001). However, a study by Garcia-del Portillo, Foster and Finlay (1999) found that the acid tolerance response (ATR) in *Salmonella* is not required to overcome low pH stress in the stomach. These findings suggest that salmonellae exhibit an adaptive ATR on exposure to low pH, possibly promoting survival in acidic host environments such as the stomach (Ohl & Miller, 2001).

After entering the small bowel, salmonellae traverse the intestinal mucus layer before encountering and adhering to cells of the intestinal epithelium. Infection is initiated when invasive organisms pass through the epithelial surface of the small bowel (Jones, Ghori & Falkow, 1994). This happen when *Salmonella* invades the intestinal mucosa via surface epithelial cells of the small intestine, particularly M cells overlying Peyer's patches (Edgeworth, 2001). The M cells are specialized epithelial cells overlying Peyer's patches. These cells are thought to be the site of the internalization of *S.* Typhi and its

transport to the underlying lymphoid tissue (Parry *et al.*, 2002). Previous findings suggest that a specific interaction between these cells and invasive *Salmonella* initiates infection to the host (Jones, Ghori & Falkow, 1994).

The organisms then migrate within infected macrophages to intestinal lymph nodes and then via the lymphatics to the systemic reticulo endothelial system, particularly of the spleen, liver and bone marrow (Edgeworth, 2001). After penetration, the invading microorganisms translocate to the intestinal lymphoid follicles and the draining mesenteric lymph nodes, and some pass on to the reticuloendothelial cells of the liver and spleen. Liver, spleen, bone marrow, gallbladder, and Peyer's patches of the terminal ileum are the most common sites of secondary infection. Organisms excreted in the bile may either reinvade the intestinal wall or are excreted in the feces (Parry *et al*., 2002).

Shortly, after bacteria adherence to the apical epithelial surface, profound cytoskeletal rearrangements occur in the host cell, disrupting the normal epithelial brush border. The process induces the subsequent formation of membrane ruffles that reach out and enclose adherent bacteria in large vesicles (Ohl & Miller, 2001). This process resembles the membrane ruffling and macropinocytosis induced in many cells by growth factors, and it is morphologically and functionally distinct from receptor-mediated endocytosis, the mechanism by which many other pathogens enter nonphagocytic cells (Ohl & Miller, 2001).

Bacteria are then phagocytosed by resident macrophages but, instead of being killed, they survive and replicate within the endosomal compartments (Edgeworth, 2001). At a critical point that is probably determined by the number of bacteria, their virulence, and the host response, bacteria are released from this sequestered intracellular habitat into the bloodstream (Parry *et al*., 2002). The study by Wain *et al*. (2001) indicates that at this stage the concentrations of *Salmonella* in the bone marrow are considerably higher than in peripheral blood. Migration of infected phagocytes to other organs of the reticuloendothelial system probably facilitates dissemination of bacteria in the host (Ohl & Miller, 2001).

2.6 Salmonellosis

Salmonellosis a disease caused by the bacteria *Salmonella* has emerged to be one of the global widespread foodborne diseases even though the incidences tend to vary between countries (Molla, *et al.,* 2003). *Salmonella* cause enteric fever, a salmonellosis disease which commonly known as typhoid fever is transmitted between human to human. However, a similar but often less severe disease is caused by a non typhoidal *S.* Paratyphi A and, less commonly, by *S.* Paratyphi B and *S.* Paratyphi C (Bhutta, 2006).

It is estimated that 80 % of typhoidal and 95 % of on non typhoidal *Salmonella* (NTS) infections are transmitted through the consumption of contaminated food and food products (Mead *et al.*, 1999). The great majority of these infections are associated with

the consumption of food such as meat, poultry, eggs, milk and seafood contaminated with *Salmonella* (Foley, Lynne & Nayak, 2008; Voetsch *et al*., 2004). On the other hand, fruits and vegetables that have been contaminated with animal manure can also cause infections (Voetsch *et al*., 2004). However, many pathogens transmitted through food are also spread through water or from person to person, thus obscuring the role of foodborne transmission (Mead *et al*., 1999).

In general, *Salmonella* can cause a number of different disease syndromes including gastroenteritis, bacteremia, and typhoid fever, with the most common being gastroenteritis (Foley & Lynne, 2008). Although most *Salmonella* infections cause mild to moderate self-limited illness, serious disease resulting in death does occur (Voetsch *et al*., 2004). There is also a danger that the bacteria may not be detected from the person who may handle food because bacteriological examination of faeces is not completely reliable (WHO/FAO, 1967).

The second group features *Salmonella enterica* Enteriditis and *Salmonella enterica* Typhimurium, the two most important serovars that can be transmitted from animals to humans. Many NTS strains, such as *S.* Enteritidis *and S.* Typhimurium which infect a wide range of animal hosts, including poultry, cattle and pigs usually cause per-acute septicaemia, acute enteritis or chronic enteritis to humans (Ohl & Miller, 2001). *Salmonella* Enteritidis caused the most outbreaks, which peaked in humans in 1992 in

many European countries (WHO, 2005) and in North America (Center for Diseases Control and Prevention [CDC], 2012).

While all *Salmonella* serotypes can potentially cause disease in humans, they are however often classified according to their adaptation to animal hosts (WHO, 2005). A few *Salmonella* serotypes have a limited host-spectrum (affect only one or a few animal species). *Salmonella enterica* Typhi cause infections in primates, *Salmonella enterica* Dublin in cattle and *Salmonella enterica* Choleraesuis in pigs (WHO, 2005).

2.7 Salmonellosis in humans

Salmonella infections in humans vary with the serovar and the infectious dose, the nature of the contaminated food, and the host status (Todar, 2008). Although the infectious dose varies among *Salmonella* strains, a large inoculum of 10^6 bacterial cells is thought to be necessary to overcome stomach acidity and to compete with normal intestinal flora (Klotchko & Wallace, 2009). Studies by Toprak and Erdoğan (2008) of healthy previously unvaccinated men showed that ingestion of 10^7 *S.* Typhi bacilli caused disease in 50% of the volunteers. Further investigations on the same study on the disease outbreak indicated that an inoculum of as few as 200 organisms may lead to the disease (Toprak & Erdoğan, 2008).

While salmonellosis is a self limiting disease and has low mortality rate in humans it can occasionally be life threatening especially in very young children and very old adults (WHO/FAO, 1967). Salmonellosis is also reported to be problematic in a wide variety of immuno-compromised hosts (Woc-Colburn & Bobak, 2009; Morpeth, Ramadhani & Crump, 2009). This includes but not limited to patients with malignancy, human immunodeficiency virus, diabetes, and those receiving corticosteroid therapy or treatment with other immunotherapy agents (Hohmann, 2001).

The mode of transmission usually occurs when the causative organisms pass through the food chain from primary production to households or food-service establishments and institutions (WHO, 2005). Although the source of infection may vary, person to person transmission through poor hygiene and sewage contamination of water supply are the most important source of transmission (Bhutta, 2006). Therefore, hygiene is the best way of minimizing the transmission of this disease in a community. For example, the provision of clean water and good sewage systems has led to a dramatic decrease in the incidence of typhoid in the US and Europe (Parry *et al.*, 2002).

The widespread distribution of *Salmonella* in the natural environment and its prevalence in the global food chain has increased the concern regarding the economic and public health consequences from this pathogen (Kapperud, Lassen & Hasseltvedt, 1998). On the economic impact of the disease, salmonellosis constitutes a major public health burden and represents a significant cost to society in many countries (WHO, 2005). The

global epidemiology based on regional extrapolations suggest that although typhoid fever illnesses have increased from 16 million to 22 million, the number of deaths have decreased from 600 000 to 200 000 of which paratyphoid fever caused about 5 million illnesses a year (Crump, Luby & Mintz, 2004). However, these estimates were based on the global population of 6.1 billion in the year 2000 from that of 4.1 billion 16 years earlier.

2.8 Salmonellosis as a zoonotic disease

Salmonella infection is a zoonotic disease which is a major challenge for both animal production and food safety. It is believed that over 80 percent of *Salmonella* and Campylobacter infections in humans are acquired from food animals (Gorbach, 2001). However, what is particularly intriguing about salmonellae is that their ability to cause disease in animals encompasses a spectrum of host specificity and disease severity (Chan *et al*., 2003).

Studies also suggest that there is an overlap of *Salmonella* serovars which cause disease in human and those found in food producing animals. Many nontyphoidal *Salmonella* strains such as *S.* Typhimurium and *S.* Enteritidis infect a wide range of animal host including poultry, cattle and pigs (Ohl & Miller, 2001) and could also cause severe illnesses in humans. *Salmonella* serovar Typhimurium DT 104 first isolated in cattle in 1984 in the United Kingdom have later been isolated from humans and many other food

animals (Van Duijkeren, Wannet, Houwers & Van Pelt, 2002). Unlike typhoidal *Salmonella* which usually affect humans, nontyphoidal serotypes generally cause self limiting gastrointestinal infections in many animals **(**Chan *et al*., 2003**).**

A study by CDC comparing common serovars from human infections with those most commonly isolated from different food animal sources found that there are a number of instances of overlap (Foley & Lynne, 2008). Of the top 10 most common serovars causing human infections, 8 are also in the top 10 most identified serovars from at least 1 of the major food animal (Foley & Lynne, 2008). Another study in the US found that of the top 20 most common *Salmonella* serovars associated with human *Salmonella* infections, 4 are commonly isolated from swine (Foley, Lynne & Nayak, 2008).

The study in the Netherland on the distribution of *Salmonella* between 1984 and 2001 found the serovar Typhimurium to be frequently isolated from both humans and animals (Van Duijkeren *et al*., 2002). During the same study *Salmonella* serovar Dublin, Enteritidis and Typhimurium were found to be prevalent serotype in cattle and humans. Because of the commonality of serotype overlap between food animals and human infections, there is a likelihood of pathogen spread through the food supply chain (Foley *et al*., 2008). It has since been suggested that food containing products from farm animals including cattle are seemingly to be an important source of human *Salmonella* infections (Van Duijkeren *et al*., 2002).

One of the prevailing questions in *Salmonella* research today concerns the identification of genetic factors that confer upon these highly related serovars their ability to colonize, and in some cases to cause disease in a wide variety of animal hosts (Chan *et al*., 2003). Certain zoonotic diseases such as brucellosis or tuberculosis can be eradicated in animals so that food of animal origin is free of the pathogens. However, the eradication of other animal borne pathogens such as *Salmonella* is currently not possible, even if good animal husbandry is strictly applied. Knowledge of how *Salmonella* is disseminated through the food chain is important in understanding how food animals and/or food processing procedures contribute to product contamination and to subsequent human infection by this pathogen (Zou *et al*., 2010).

2.9 Animal feed as a source of *Salmonella* infections

The major global improvements and the growth in animal production have increased the reliance on a range of manufactured feed as food for food animals (Crump, Griffin & Angulo, 2002). The production of animal waste into animal feed has been practiced for over 40 years as a means of cutting feed costs (Sapkota, Lefferts, McKenzie & Walker, 2007). Feed mills also combine ingredients of plant origin to produce a feed mix suited to animals of a particular species and/or age (Crump *et al*., 2002). Feed ingredients are believed to represent a major risk for *Salmonella* contamination in feed mills (Papadopoulou, Carrique-Mas, Davies & Sayers, 2009).

Since food of animal origin is a good source of food borne pathogens, animal feed derived from animal by-products can also be a good source of *Salmonella* contamination. According to Sapkota *et al*. (2007), available data demonstrates that the quality of animal derived products is directly related to animal feeding practices. This is due to the fact that contamination of animal feed before arrival at and while on the farm contributes to infection and colonization of food producing animals with food borne pathogens (Crump *et al*., 2002).

According to Crump *et al*. (2002), there is significant evidence that animal feed is often contaminated with foodborne bacterial pathogens including salmonellae. However, the contamination of animal feed is believed to happen during the post processing handling including storage and transportation. This is because the process of producing animal feed requires a heat treatment stage which is effective against most bacterial pathogens (Johnston, 2000). Previous findings suggest that contaminated animal feed can under certain conditions allow *Salmonella* to survive for as long as 16 months (Papadopoulou *et al*., 2009).

There are many ways of which *Salmonella* may have access to food animals and humans. Available evidence suggests that there is a possibility of transmission of *Salmonella* from animal feed to animals consuming the feed, and to food products derived from the animals (EFSA, 2008). This could probably be due to the fact that animals may become infected from other *Salmonella* infected animals, directly or via a

contaminated environment of which the original source could have been contaminated by feed. These pathogens may then be transmitted through the food chain and cause illness to humans (Crump *et al*., 2002).

Given the current high consumption of animal based food in the world, animal feed production becomes essentially important on the human health due to its potential to contaminate food through the food supply chain (Sapkota *et al*., 2007). This is the reason why food animal producers have ethical obligations to reduce the risk of foodborne hazards in animals under their care (Davies *et al*., 2004). In the US, the FDA has the power to ensure that animal feed is appropriately labeled, is safe for intended use, and does not pose any potential human health risks when fed to food producing animals (Crump *et al*., 2002). However, several countries in the world are reported to have banned the use of feed derived from animal products in ruminants including the US (Denton, Coon, Pettigrew & Parsons, 2005), the EU (EFSA, 2008) and Namibia due to the potential exposure of humans to transmissible spongiform encephalopathies (TSE) related illnesses.

As animal feed is a known source of *Salmonella* for food producing animals it may therefore act indirectly as a cause of human infections if enters the food supply chain (Hald, Wingstrand, Brondsted & Lo Fo Wong, 2006; Papadopoulou *et al*., 2009). Several incidents which are traced back to 1958 have linked the human *Salmonella* infections to contaminated animal feed (Crump *et al*., 2002; Faranco, 2006). Based on

these previous findings, *Salmonella* have now been used by scientists as a prototype to heighten the relationship of animal feed, food animal production, food processing, public health and global trade as early as the eighties (Faranco, 2006).

Several countries have regulations and guidelines regarding the control of *Salmonella* in animal feed. In the US, the product is considered adulterant when *Salmonella* is present (Faranco, 2006). This is because *Salmonella* contamination in animal feeds is has the potential to cause infection and disease in animals. In Sweden where the control of *Salmonella* started as early as 1961, its presence in feed is a notifiable case by law followed by immediate action to eliminate the pathogen (Österberg, Vågsholm, Boqvist & Lewerin, 2006).

However, there is still a disagreement in the scientific community as to whether feed is a major source of *Salmonella* contamination for animals. This is because some studies the serovars isolated from feed do not always correspond with those from animals (Papadopoulou *et* al., 2009). Nevertheless, a proper control mechanism should be put in place in order to eliminate any potential risk of food-borne pathogens to enter the food chain through animal feed.

2.10 Epidemiology and distribution of *Salmonella*

Typhoidal and nontyphoidal salmonellae are all important cause of food-borne infection in humans. Non-typhoidal *Salmonella* outbreaks are reported to be more common in developed countries. In contrast with the situation in developed countries *S.* Typhi is the most commonly isolated species in many developing countries (Tauxe, 1986). In the US, *S.* Typhimurium and *S.* Enteritidis are most prevalent, with each currently responsible for 16 to 17 % of infection cases (Andrews-Polymenis, Bäumler, McCormick & Fang, 2010). In the European Union, the human cases caused by *S.* Enteritidis decreased markedly in 2008, while an increase in *S.* Typhimurium cases was observed (EC, 2009).

In fact, it appears that the distribution of *Salmonella* differs in different parts of the world depending on the geographical location. It also appears that there are some differences between the regions in the predominant serotypes of *Salmonella* associated with human infections. According to Foley and Lynne (2008), in the US the most common *Salmonella* serovars are Typhimurium, Enteritidis, Newport, Heidelberg and Javiana. In the EU, *S.* Enteritidis is the most predominant serovar where in many parts of Asia, *S.* Choleraesuis is one of the top serovars which cause most humans infections (Foley & Lynne, 2008). *Salmonella enterica* serotypes Typhi, Paratyphi A, Paratyphi B, and Paratyphi C which cause typhoid (enteric) fever are endemic in Africa, Asia and South America (Santos *et al.*, 2001).

In Namibia, although there are no publications on the prevalence of *Salmonella*, the infections caused by these microorganisms are prevalent in the country. According to Chikukwa (2013), the laboratory confirmed culture at the Oshakati state hospital laboratory for the period of 1 July 2008 to 15 June 2009 were 68 cases of typhoid fever of which the majority of the cases (n = 54) occurred between November 2008 to April 2009. However, most of the non typhoidal *Salmonella* infections cases remain unknown in Namibia because most of these cases are treated based on the clinical diagnosis.

Although thyphoidal *Salmonella* outbreaks are reportedly to be more prevalent in the developing world, the two non-thyphoidal *Salmonella* namely; *S.* Typhimurium and *S.* Enteritidis are also reported to cause disease outbreak in developing countries including sub-Sahara countries. A study in Malawi on children and adults during the period of 1998 to 2004 reported an isolation rate of 75 % for *S.* Typhimurium and 21 % for *S.* Enteritidis (Gordon *et al*., 2008; Crump & Mintz, 2010). Other cases of non typhoidal *Salmonella* infections in sub-Sahara Africa have been reported in rural Kenya and Mozambique (Morpeth, Ramadhani & Crump, 2009).

Generally, in sub-Saharan Africa, studies indicate that non-Typhi serotypes of *Salmonella* particularly *S.* Enteritidis and *S.* Typhimurium, greatly outnumber *S.* Typhi and *S.* Paratyphi as the cause of infection (Crump & Mintz, 2010). However, the reports of outbreak virulent drug resistant *S.* Kentucky in Europe, Africa and the Middle East is becoming a global concern in public health because of its occurrence across the three

continents (Le Hello *et al*., 2011). The new *S.* Kentucky is a multidrug resistance strain and has shown to have high level of resistance to Ciprofloxacin, a second generation drug used in the treatment of *Salmonella* infections.

The epidemiological studies in different parts of the world suggest that the incidence rate on *Salmonella* isolation is more on infants (Olsen *et al.*, 2001). A study in the US shows that rates of *salmonella* isolations are low through the adult years, and are also higher among old persons (Olsen *et al*., 2001). In the same study the rate of *Salmonella* isolation seem to be affected by season with higher rates of isolation recorded in summer time. These findings suggest that weather or climate variability is thought to be a factor in *Salmonella* seasonality. Meanwhile, previous findings suggest that *Salmonella* isolation rates and diversity in the environment vary temporally and are strongly influenced by season and temperature (Haley, Cole & Lipp, 2009).

2.11 *Salmonella*: Global public health and economic challenges

Globally, incidences of food-borne infections are thought to be on the increase in many parts of the world over the years. Among other food-borne pathogens, salmonellae are important causes of reportable food-borne infection. According to Foley, Lynne and Nayak (2008), the dynamics of *Salmonella* infections are variable and are mainly affected by changes in human demographics and lifestyles, human behavior, changes in industry and technology, changes in travel and commerce. Other factors that influence to

Salmonella infections are the shift toward global economy, microbial adaptation, breakdown in the public health infrastructure, and the lack of knowledge on food safety and handling practices among consumers (Foley *et al*., 2008).

Based on its importance to human public health, *Salmonella* is one of the eight micro-organisms which are mandatory to monitor in the EU food safety programs (Jong & Ekdahl, 2006). However, a recent report suggests that there have been a significant decreasing trend in the notification rate of the salmonellosis cases among the EU Member States (European Committee [EC], 2009). This could be due to the fact that HACCP is a mandatory in the food production within the EU Member States. Although there is a decrease in the EU, *Salmonella* infections are reportedly to be the second leading cause of bacterial foodborne illness in the US (Foley *et al*., 2008).

Like other food-borne diseases *Salmonella* infections usually occur through food consumption. It is estimated that about 95% of human salmonellosis cases are associated with the consumption of contaminated products (Foley & Lynne, 2008). A recent CDC National Outbreak Reporting System 2004 - 2008 report shows that foods which associates with *Salmonella* infection are poultry (29 %), eggs (18 %), pork (12 %), beef (8 %), vine vegetables, fruits and nuts (13 %) and other foods (20 %) (CDC Vitalsigns, 2011). In the EU, *Salmonella* has been often detected in fresh broiler, turkey and pig meat, on the average levels of 5.1%, 5.6% and 0.7%, respectively (EC, 2009). A report on the increase in per capita consumption of meat consumption since 1910 in the US

suggests that there is increased potential exposure to *Salmonella* through meat commodities (Foley *et al*., 2008).

Besides the importance of this micro-organism to public health, another aspect is the cost generated by human infections due to *Salmonella*. Although the current global *Salmonella* impact on public health is not very clear, the available data estimates that there are 93.8 million cases of *Salmonella* infections and 155,000 deaths each year (Majowicz *et al*., 2010). The CDC estimates the annual number of cases of *Salmonella* infections in 2010 in the US to be at 1.4 million with an estimated cost of US$ 2.7 billion (United States Department of Agriculture [USDA], 2011). In Europe, during 1999, the cost linked to foodborne salmonellosis ranged between € 560 million and 2.8 billion, where *Salmonella* was estimated to be responsible for nearly 166 000 cases (Korsak *et al*., 2006).

In Denmark, the annual estimated cost of foodborne salmonellosis in 2001 was US$ 15.5 million representing approximately 0.009% of the Gross Domestic Product (GDP) (WHO, 2005). This report further suggests that although *Salmonella* control program in Denmark has been in place for several years the annual estimated cost was at US$ 14.1 million. In Canada, there are about 11 million cases of food-borne illness yearly with an estimated annual cost of $ 1089 million (Holley, 2010).

Comparatively, there are few established surveillance systems exists in the developing world, which make it difficult to estimate the global true burden of *Salmonella* infections (Buttha, 2006). In 2004, the global estimate of typhoid fever alone which usually affect developing world was reported to be at 21.7 million cases with about 200,000 deaths annually (Crump, Luby & Mintz, 2004). Other consequences of salmonellosis in humans include the long recovery period, which may result in loss of working capacity of patients. However, ongoing changes in the food supply, the identification of new foodborne diseases, and the availability of new surveillance data make published data obsolete over time (Mead *et al*., 1999).

Estimates available from the Foodnet data suggest that approximately 1.4 million cases of non typhoidal *Salmonella* infections are causing approximately 15,000 hospitalizations and 400 deaths each year in the US (Voetsch *et al*., 2004). In the EU, a total of 5,332 food-borne outbreaks reported in 2008 causing 45,622 human cases, 6,230 hospitalisations and 32 deaths were due to *Salmonella* infections amounting to 35.4% of all reported cases (EC, 2009). In Austria, of 1,255 *Salmonella* outbreaks reported within five years, *Salmonella* Enteritidis accounted for more than 82% of all cases (Hrivniaková *et al*., 2011).

On the other hand, a report published by CDC Vitalsigns (2011) suggests that since the mid of 1990s the recall of ground beef contaminated with a dangerous type of *E. coli* has been cut to almost half. However, during that same period *Salmonella* infections have

never been on a decrease. These results came following the CDC national goal which was set in the beginning of 2000 to reduce NTS cases by 50 % to 6.8 culture confirmed cases per population of 100,000 by 2010. This achievement on *E. coli* reduction came after the reported infection rate reached a plateau of 15 cases per population of 100,000 in the previous 20 years (Andrews-Polymenis *et al*., 2010).

Nevertheless, apart from the introduction of new food safety measures worldwide, *Salmonella* remain a challenge in both developing and developed countries. Recent outbreaks of non-typhoidal *Salmonella* from conventional sources, such as raw meats, and unconventional food sources not previously known to transmit *Salmonella* have required increased active epidemiologic surveillance and created new challenges in tracing and controlling these outbreaks (Andrews-Polymenis *et al*., 2010). The increasing centralization and industrialization of the global food supply have also increased the distribution of *Salmonella* (Hohmann, 2001). Never the less, the disease still remains prevalent in many parts of the developing countries while it is virtually decreased in much of the developed countries mostly due the advances in public health and hygiene (Bhutta, 2006).

2.12 Antimicrobial and Multidrug Resistance (MDR)

2.12.1 Emergence of drug resistance

Chloramphenicol, the first broad-spectrum antibiotic was discovered in 1947 in a Venezuelan soil sample containing the *Streptomyces venezuelae*, which produces the antibiotic substance $C_{11}H_{12}Cl_2N_2O_5$ known as chloramphenicol. From 1948 to the mid 1970s, chloramphenicol was the first-line drug of choice, and in developed countries its use resulted in a reduction in mortality rates from 10 % to less than 2% (Threlfall & Ward, 2001). Ealier research by Woodward, Smadel and Lay (1950) found that despite the obvious benefits of chloramphenicol therapy in typhoid fever, the facts suggest that chloramphenicol is primarily bacteriostatic rather than bactericidal in the human body. This conclusion was based on the findings where a relapse of clinical symptoms on treated patients, traces of *S.* Typhosa in stools and urine from patients after drug therapy was discontinued, and the failure of larger dose of chloramphenicol to eradicate a carrier state for over a two weeks period (Woodward *et al*., 1950).

Chloramphenicol resistant *S.* Typhi was first reported in 1950 (Colquhoun & Weetch, 1950) followed by a few sporadic isolations in the UK from samples sent from Chile and Kuwait, and from infected patient from Aden (Anderson & Smith, 1972). However, there were no accounts of epidemic until 1972 when the first outbreak in Mexico followed by the one in Kerala, India caused a high mortality rate (Rowe, Ward & Threlfall, 1997). Since those extensive outbreaks in Mexico and India, in which endemic strains were resistant to chloramphenicol, the efficacy of this antimicrobial has been in

doubt (Threlfall & Ward, 2001). The failure of chloramphenicol resulted to newer drugs such as trimethoprim-sulfamethoxazole and ampicillin to be the choice drugs for treatment of enteric fever (Mandal, 1991).

However, later outbreaks in developing countries including South Africa in the late 1980s and early 1990s, in which causative strains were resistant to alternative drugs such as ampicillin and trimethoprim in addition to chloramphenicol, the efficacy of these antimicrobial agents has also been impaired (Rowe *et al*., 1997). As it happened to chloramphenicol the use of ampicillin and trimethoprim were similarly impaired due the proliferation of resistant strains (Rowe *et al*., 1997). The emergence of strains such as *S.* Typhi with resistance to trimethoprim and ampicillin has caused many problems in developing countries. This is due to the fact that these antibiotics were being used extensively for the treatment of patients infected with chloramphenicol-resistant strains since the 1980s (Mandal, 1991).

Since the 1990s, *Salmonella* strains exhibiting MDR to antimicrobials that were previously clinically effective have been reported worldwide. Although MDR (to four or more drugs) became common in *S.* Typhimurium in the mid-1960s but there was a dramatic increase in resistance in the 1990s (Threlfal, 2002). These include Pakistan (Bhutta, 1996), Indian subcontinent and the UK where resistant strains were linked to travelers returning from endemic areas (Threlfall & Ward, 2001). The emergence of resistant *Salmonella* strains to antimicrobials which were previously effective has placed

tremendous pressure on public health systems in developing countries. This is because the problem of antimicrobial resistance results to the limitation on the treatment options. A few antimicrobials that are effective against these MDR strains are expensive and not readily available (Kairuki *et al.*, 2004).

However, after the development of resistance to chrolamphenicol, alternative drugs, fluoroquinolones, such as ciprofloxacin, became the drug of choice for the treatment of most infection diseases (Renuka, Sood, Das & Kapil, 2005). Fluoroquinolones is licensed in many countries for use in food animals since the binging of the 1990s (Aarestrup, Wiuff, Mølbak & Threlfall, 2003). However, the recent concern about the poor efficacy of fluoroquinolones as the first-line therapy for enteric fever is legitimate, especially for those in endemic areas (Arya & Agarwal, 2006). The concomitant emergence and increased incidence of quinolone-resistant *Salmonella* have been an issue of intense worldwide debate. The increasing resistance to nalidixic acid and decreasing susceptibility to fluoroquinolones among *Salmonella* from food animals and humans has been used as an argument against the continued usage of fluoroquinolones for food animals (Aarestrup *et al*., 2003).

Currently, the National Committee for Clinical Laboratory Standards (NCCLS) breakpoint for resistance to the fluoroquinolone ciprofloxacin is ≥ 4 g/ml and ≥2 g/ml for its veterinary equivalent, enrofloxacin (Aarestrup *et al*., 2003). These breakpoints are widely used by clinicians, veterinarians, microbiologists and others for drug selection

even though the NCCLS does not provide specific breakpoints for bacteria associated with gastrointestinal infections. Some recently available studies suggest that *Salmoella* strains exhibiting a level of resistance to ciprofloxacin are being isolated worldwide (Renuka *et al*., 2005; EC, 2011).

According to EC (2011) report on antimicrobial susceptibility testing, there are clinical evidences which indicate a poor response of ciprofloxacin in systemic infections caused by *Salmonella* with low-level fluoroquinolone resistance (MIC > 0.064 mg/l). While the available data relate mainly to *S.* Typhi there are also case reports of poor response with other *Salmonella* species (EC, 2011). Another study suggests that a strain of *S*. Typhi with high-level resistance to ciprofloxacin has been isolated in India (Renuka *et al*., 2005).

Although it is regrettable that resistance to ciprofloxacin is now emerging, ciprofloxacin still remains the drug of choice for the treatment of MDR typhoid fever (Rowe *et al*., 1997). Fluoroquinolones or ceftriaxone are recommended drugs for treatment of *Salmonella* septicemia. However, fluroquinolones are not approved for use in children under the age of 16 due to concerns about cartilage damage (Foley & Lynne, 2008).

In contrast to the situation in developed countries where the increase in antimicrobial resistance is associated with their use in animal production, in developing countries the increase has been almost entirely associated with the use in human medicine, both in

hospitals and the community (Threlfall, 2002). The application of antimicrobials for prophylaxis in food animals has been a significant factor in the emergence of microorganisms with resistance to certain antimicrobials (Threlfall, Ward, Frost & Willshaw, 2000). Contrary, the UK published data on veterinary drugs sales demonstrates that changes in the incidence of antimicrobial resistance do not correlate with changes in veterinary use (Threlfall, Day, de Pinna, Charlett & Goodyear, 2006).

2.12.2 Antimicrobial substances and bacterial drug resistance

The use of antimicrobial substances in humans combined with improvements in sanitation and nutrition and the widespread immunization programs have significantly reduced the number of deaths by helping to bring many infectious diseases under control (WHO, 2002). These antimicrobial substances have been beneficial in the treatment of many bacterial diseases including foodborne infections such as salmonellosis. However, overtime the effectiveness of available antimicrobials has been declining due to emergency of bacteria that are resistant to such antimicrobials.

So far, septicemic salmonellosis can be treated with a number of antibiotics including ampicillin, amoxicillin, gentamicin, trimethoprim/sulfamethoxazole, third generation cephalosporins, chloramphenicol and fluoroquinolones (World Organisation for Animal Health [OIE], 2005). The group of fluoroquinolones is most widely regarded as optimal for the treatment of salmonellosis in adults where the earlier drugs such as chloramphenicol, ampicillin and amoxicillin and trimethoprim-sulfamethoxazole are

occasionally used as alternatives (WHO, 2005). Fluoroquinolones are very potent antimicrobials and active against a wide range of pathogenic organisms and well distributed in the body after administration (EMEA, 2006). Third-generation cephalosporins (which need to be given by injection) are widely used in children with serious infections, as quinolones are not generally recommended for this age group (WHO, 2005). However, many bacterial isolates are becoming resistant to one or more antibiotics, and the choice of drugs is now recommended, if possible, be based on susceptibility testing (OIE, 2005).

However, the trend of antimicrobial resistance has changed since the start of the 21st century. The rate of emergence and spread of bacterial pathogens resistant to antimicrobial substances has threatens to return us to an era when common infections were untreatable (Laxminarayan, Malani, Howard & Smith, 2007). The first finding that antibiotic use in livestock could result to antibiotic-resistant bacteria was first documented in 1951 where Starr and Reynolds reported streptomycin resistance in generic intestinal bacteria from turkeys that had been fed antibiotic (Marler, 2010). After sporadic outbreaks of chloramphenicol resistant typhoid between 1970 and 1985, many strains of *S.* Typhi developed plasmid mediated MDR to the three primary antimicrobials used; ampicillin, chloramphenicol, and co-trimoxazole (Rowe *et al*., 1997).

On the other hand, some of the antibiotics are originated from bacterial species such as Pennicillium, Cephalosporium and Streptomyces. This is because some bacterial species have the ability to produce antimicrobial compounds as part of its protection or defencing mechanisms. This ability to produce antimicrobial agents occurs naturally to give the bacteria surviving advantage over other microorganisms in the same environments (Aavitsland, 2008). Moreover, antibiotic-resistant bacteria happen as the natural result of mutation and natural selection within a population of organisms i.e. an infection in a human host-faced with an agent that eliminates most of its members. The resistance to the antimicrobials usually occurs as a result of mutations in the bacterial genome (DNA) where resistance to other antimicrobials often spread by transfer of DNA between bacterial strains (WHO, 2005). Plasmids are readily mobilizable between taxa and represent the most common method of acquiring antibiotic resistance determinants (Ochman *et al*., 2000).

Bacteria are particularly efficient at enhancing the effects of resistance, not only because of their ability to multiply very rapidly but also because they can transfer their resistance genes, which are passed on when the bacteria replicate (WHO, 2002). In some cases MDR in the same bacterial strain is transferred through a plasmid which is a coherent piece of DNA (WHO, 2005). The resistance to antimicrobials can spread in a different ways. The bacteria that survive because of mutations may avoid the effect of the antibiotic and can multiply thereby increasing the numbers of antibiotic-resistant bacteria (Laxminarayan *et al.*, 2007).

When antibiotic use became the norm in both human and animal medicine, selection pressure increases the bacterial advantage of maintaining and developing new resistance genes. The new resistance gene could be shared among bacterial populations and increase the likelihood of disease transmission (Marler, 2010). In the absence of alternative antibiotics or other control mechanisms, these antibiotic-resistant bacteria can spread to other people just like any other bacterial infection (Laxminarayan *et al.*, 2007). This is because the antimicrobial resistance genes allow a microorganism to expand its ecological niche, allowing its proliferation despite the presence of certain noxious compounds (Ochman *et al*., 2000).

2.12.3 Antimicrobial resistance and MDR *Salmonella*

Antimicrobial resistance is still a growing problem worldwide even though different medical and public health organizations have recognized the need to reduce inappropriate use of antibiotics in humans as well as in animal production (Bronzwaer *et al*., 2002). Strains of *Salmonella* with resistance to antimicrobial drugs are now widespread in both developed and developing countries (Threlfall, 2002). One of the concerns of increasing antimicrobial resistance is that when severe infections occur, treatment with antimicrobials is thought to be life-saving but is difficult if the organism is resistant to the antimicrobials used (Cohen & Gangarosa, 1978).

While it has become more difficult as antibiotic resistance strains have increased, the selection of effective antibiotics is critical for the treatment of invasive infections

(Angulo *et* al., 2000). The increase of antibiotic resistance strains narrow down the selectivity of effective antimicrobials as few drugs become available for treatment. According to Tauxe (1986), there are several reports in developing countries where MDR *Salmonella* strains have been found to be resistant to virtually all available antimicrobials. These MDR *Salmonella* are also associated with a high frequency of invasive and fatal infections.

According to Threlfall (2002), available information suggests that there have been increases in the occurrence of resistance in both non-typhoidal and typhoidal *Salmonella* in developing countries. The increase of antimicrobial resistance in developing countries is possibly caused by drug abuse and availability of drugs without prescription. Meanwhile, in developed countries the situation of MDR in non-typhoidal *Salmonella* is linked to the consequences on the use of antimicrobial drugs in food producing animals (Threlfall, 2002). However, other scholars argue that the emergence of MDR strains in developed countries have been introduced by the returning travelers (Rowe *et al.*, 1997).

However, *Salmonella* with multi-resistance pattern usually carry a gene that produces AmpC-type enzymes that cause much of the drug-resistance; thus they are referred to as MDR-AmpC which has been found in *Salmonella* Newport (CDC, 2002). Of particular importance since the begining of 1990s has been a MDR strain of *Salmonella* Typhimurium definitive phage type (DT) 104. This strain displays resistance to up to six

commonly used antimicrobials, with about 15% of the isolates exhibiting decreased susceptibility to ciprofloxacin (Threlfall, 2002).

Salmonella phage typed DT104 is reported to be resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (Lee & Lee, 2007; Mølbak *et al*., 1999). The DT104 which was first isolated in the UK (Cloeckaert & Schwarz, 2001; Poppe *et al*., 1998) and later in several other European countries is known to be a virulent pathogen for humans and animals particularly cattle (Poppe *et al.*, 1998; Besser *et al*., 2000). These multidrug-resistant DT104 isolates have a chromosomal gene cluster that codes for resistance to these substances (Winokur *et al*., 2000). At the same time, an increasing number of *S.* Enteritidis isolates submitted to National Antimicrobial Resistance Monitoring System (NARMS) have been reported to be resistant to nalidixic acid; a drug closely related to ciprofloxacin, or cipro, the most commonly prescribed antibiotic for *Salmonella* infections (Marler, 2010).

According to CDC (2002), since 1996 the NARMS has identified increasing numbers of *Salmonella* isolates resistant to nine of the 17 antimicrobial agents tested. These include important antimicrobials such as amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. These isolates also have decreased susceptibility to ceftriaxone, an antimicrobial used to treat serious infections in children (CDC, 2002). The rising rates of resistance to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol has caused for these substances to

no longer be effectively used for the treatment of severe *Salmonella* infections (Winokur *et al*., 2000).

2.12.4 Antimicrobial usage in humans and food animals

Antimicrobials have been used in food animals in North America and Europe for more than half a century (Gorbach, 2001). In modern agricultural farming antimicrobials are specifically used in food animals for treatment of sick animals, and prophylaxis to prevent illness during times of increased risk of disease (Shaikh, 2011). In the absence of disease, antimicrobial agents are given to food animals for subtherapeutic purposes with the goals of growth promotion and improved feed utilization (improved nutritional benefits of the animal feed) (Gorbach, 2001; Shaikh, 2011).

The use of these antimicrobial substances in food animals is thought to have improved the yields in animal production (Marler, 2010) and as the well being of both humans and animals (Institute of Food Technologies [IFT], 2006). Among the most common are drugs used in different food animals that are either identical to or related to those administered to humans, including penicillins, tetracyclines, cephalosporins (including ceftiofur, a third-generation cephalosporin), fluoroquinolones, avoparcin (a glycopeptide that is related to vancomycin), and virginiamycin (a streptogramin that is related to quinupristin-dalfopristin) (Gorbach, 2001). As livestock and poultry production has increased over the years due to the increased population, the demand for better disease management has also increased. This demand for better disease management may have

resulted to the significant increase on the use of antimicrobial substances in food animal production and subsequently affected the microbial resistance pattern (Marler, 2010).

As it has been pointed out earlier, similar classes of antimicrobial agents that are used in food animals for growth promotion, disease prevention, and therapy are also used in humans. Although antibiotic-resistant bacteria are a natural consequence of antibiotic use, but development and spread of resistant pathogens can be controlled by the way antibiotics are used (Laxminarayan *et al*., 2007). In the US, it is estimated that 50 percent of all antimicrobials produced are administered to animals (Gorbach, 2001).

The US data estimates that 24.6 million pounds (11.2 million kg) of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds (1.3 million kg) consumed by humans (White *et al*., 2001). However, the extensive use of antimicrobials in animals has raged an intense debate over the years on the impact on health in humans of the use of antimicrobial agents in food animals (Gorbach, 2001). One of the negative effects of increasing the use of such antimicrobial agents in food animals is the likelihood of developing a cross-resistance to drugs approved for use in human medicine (Angulo, Baker, Olsen, Anderon & Barrett, 2004).

The available scientific findings are linking the significant increase in the prevalence of antimicrobial resistant bacteria to humans and the use of antimicrobial substances in

animal production. This is because both the prudent and inappropriate use of antibiotics in human medicine, veterinary medicine, and animal husbandry create selective pressure that favors the emergence of antibiotic resistant microbes (IFT, 2006). In developed countries it is now increasingly accepted that for the most part such strains are zoonotic in origin and acquire their resistance in the food-animal host before onward transmit to humans through the food chain (Threlfall, 2002).

For example, research findings suggest that when fluoroquinolones were first licensed for human therapy, no immediate rise in *Salmonella* resistance was observed. Until the mid 1990s only one case of fluoroquinolone resistant *Salmonella* was reported in the US. The second case which was reported in 1997 was linked to the Philippines, where quinolones have been available without prescription (Herikstad *et al*., 1997). In contrast, when fluoroquinolones were subsequently licensed for use in food animals, the rates of fluoroquinolone resistant *Salmonella* in animals and food, and then subsequently in human infections, rapidly increased in several countries (Joint FAO/OIE/WHO Expert Workshop, 2003).

2.12.5 Control of antimicrobials usage

The rising prevalence of antimicrobial resistant bacteria such as *Salmonella* complicates the treatment of *Salmonella* infections in both humans and animals. This could be the reason why antimicrobial therapy is not recommended for routine treatment of salmonellosis although appropriate antimicrobial therapy can be life saving for patients

with persistent disease. Since antimicrobial agents are essential for treating some *Salmonella* infections, isolates from such infections should be monitored for antimicrobial resistance (Herikstad *et al*., 1997). The complications of treatments due to antimicrobial resistance suggest a call for prudent use of antibiotics in both human and animal medicine the measures which have been taken in developed countries with some positive results (Marler, 2010).

Several European countries have demonstrated that restricting the use of antimicrobial agents in food animals can result in a decrease in antimicrobial resistance in humans without compromising animal health or significantly increasing the cost of production (Angulo *et al*., 2004). However, many developed countries have introduced legislations trying to control the overall usage of antimicrobials in food producing animals. Despite these efforts there still significant increases in the occurrence of resistance in non-typhoidal *Salmonella* in developed countries (Threlfall, 2002).

In 2005, the US Food and Drug Administration (FDA) decided to ban the use of enrofloxacin in poultry because of the risk that it promotes drug-resistant bacteria that are harmful to human health (Center for Infectious Disease Research and Policy [CIDRAP], 2005). According to Keep Antibiotic Working [KAW] (2009), the FDA also proposed a ban in July 2008 on the use of cephalosporins animal production after the routine surveillance showed that cephalosporin resistance in *Salmonella* had risen rapidly over the last 10 years. The FDA was concerned that the extra-label use of

cephalosporins in food-producing animals is likely to lead to the emergence of cephalosporin resistant pathogenic strains and affect its effectiveness for treating disease in humans especially children (KAW, 2009).

However, the American Veterinary Medical Association (AVMA) challenged the FDA's proposal citing that the available scientific evidence failed to show that the use of cephalosporins in veterinary medicine impairs human medicine (Webster, 2009). The FDA later withdrew the proposal in order to reconsider all available scientific evidence on the subject. However, according to the CDC data, a year later, an estimated 68,000 people including 5,000 young children were infected in the US with cephalosporin-resistant *Salmonella* because of the removal of the ban on cephalosporin use (KAW, 2009).

2.12.6 MDR *Salmonella* and human infections

The use of antibiotics in humans and animals is a predisposing factor which has been observed in enteric pathogenic bacteria including *Salmonella* (Hogue *et al*., 1997). The rate of increase of antimicrobial resistance in *Salmonella* isolates have been reported from a number of countries worldwide (Winokur *et al.,* 2000). The trend is currently showing that the antimicrobial resistance in salmonellae isolated from both food and clinical sources are on increase in many countries (Cui, Ge, Zheng & Meng, 2005).

According to CDC information in the US the resistance of non-Typhi *Salmonella enterica* to nalidixic acid significantly increased from 0.4% in 1996 to 2.3% in 2003 (Stevenson *et al*., 2007). In France, until early 1990s, no or few cases of antibiotic resistant *Salmonella* were isolated in clinical practice and the antibiotic treatment (not given routinely) of salmonellosis was rarely a therapeutic issue (Brisabois, Cazin, Breuil & Collatz, 1997).

In the US, most of the MDR humans infections threats through food animals are now being linked two *Salmonella* strains; *S.* Typhimurium, definitive phage type 104 (DT104) and *S.* Newport. In 2006, *S.* Typhimurium and Newport were reported to cause most of the *Salmonella* outbreaks in the US with *S* Typhmurium catching up with the number of Enteriditis outbreaks for the first time (CDC, 2008). A study in Denmark looking at antibiotic-resistant *S* Typhimurium has shown that patients with MDR infections were 4.8 times more likely to die than the general population, and patients with quinolone-resistant infections were 10.3 times more likely to die (Helms, Vastrup, Gerner-Smidt & Molbak, 2002). However, the increase of bacterial strains which are resistance to multiple-antimicrobial substances may be of severe health consequences in the public health sectors especially in developing world where the increase in infections and mortality are still difficult to control.

In developing countries poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and

bad governance have been linked with the growing problem of antimicrobial resistance (Byarugaba, 2004). According to Okeke *et al*. (2005), several developing countries in Asia have reported multiple resistant *Salmonella* strains which are resistant to virtually all available antimicrobials and are associated with a high frequency of invasive and fatal infections. However, lower rates of resistance were observed in African countries like Zimbabwe and Kenya (Okeke *et al*., 2005). A report suggests that MDR *S* Typhi (resistant to chloramphenicol, ampicillin, and trimethoprim sulphamethoxazole) and isolates with reduced susceptibility to fluoroquinolones have caused epidemics and became endemic in southern Vietnam during the 1990s (Parry, 2004). In Africa, several researches have been done and reported on the isolation of *Salmonella* serovars but the only problem is that the resistance among *Salmonella* isolates has less systematically being investigated.

2.13 *Salmonella* isolation

Salmonellae can be isolated using numerous low-selective media (MacConkey agar, deoxycholate agar), intermediate-selective media (*Salmonella-Shigella* [SS] agar, Hektoen [HE] agar), and highly selective media (selenite agar with brilliant green) (Klotchko & Wallace, 2009). Salmonellae are oxidase-negative and predominantly lactose-negative. Less than 1% of nontyphoidal *Salmonella* isolates are lactose-positive (pink on MacConkey agar), but most produce hydrogen sulfide, which is detectable on HE or SS agar. As facultative anaerobes, they grow well both in bottles of standard automated systems for blood cultures and on culture media routinely used for urine,

tissue, and respiratory cultures. Individual isolates can then be distinguished with serogrouping, pulsed-field gel electrophoresis, bacteriophage serotyping and molecular typing techniques.

2.14 *Salmonella* serotyping and sub typing

Salmonella serotyping is a subtyping method which has proven to be very useful in differentiating isolates of the different species of *Salmonella*, particularly for public health purposes such as surveillance and outbreak investigations. Epidemiologically, it is increasingly becoming important to be able to distinguish *Salmonella* isolates. This is because definitive typing of *Salmonella* may assist in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated with a particular type (Yan *et al*., 2003).

Epidemiological typing of bacterial strains can be carried out by a variety of techniques, including serotyping, biotyping, bacteriophage and bacteriocin typing. Traditionally, conventional antigen based serotyping technique has been widely used for typing bacteria. However, phage typing and other advanced molecular techniques are currently being used for *Salmonella* serotyping and subtying. Comparing conventional method and advanced molecular techniques, the later are thought to be rapid and inexpensive. However, the choice of which typing method is the most efficient is usually based on a number of factors such as typability, reproducibility and discrimination (Hunter & Gaston, 1988).

2.14.1 Conventional typing

Salmonella serological identification and classification are based on the immune-reactivity of antigens by following the Kauffmann-White scheme. *Salmonella* express polysaccharide (O), flagellar (H) and capsular (Vi) antigens which determine strain pathogenicity (Mortimer, Peters, Gharbia, Logan & Arnold, 2004). According to CDC (2005), O antigen is a carbohydrate comprising the outermost component of lipopolysaccharide (LPS). It consists of a polymer of O subunits; each O subunit typically composed of four to six sugars depending on the O antigen. H antigen is the filamentous portion of the bacterial flagella; H antigen is made up of protein subunits called flagellin (CDC, 2005). The Vi antigen occurs in only three *Salmonella* serovars namely; *S.* Typhi, *S.* Paratyphi C, and *S.* Dublin (Todar, 2008). However, the strains of these three serovars may or may not have the Vi antigen. The variation of these antigens that are present in the cell surface has therefore formed the basis for *Salmonella* serotyping.

O-antigens differ both in the composition of the polysaccharides of the repeating units and the linkage between the individual sugar molecules (Broadbent, Davies & Van der Woude, 2010). The lipid A tail and the core polysaccharide of *Salmonella* LPS have little structural or compositional variation compared with the high degree of variability in the O-antigen. Further variation of *Salmonella* O-antigen composition can occur by modification of these repeating units, specifically by linkage of an acetyl group or glucose moiety (Broadbent *et al*., 2010). Some of these variable O-antigen modifications

which are recognized in the serotyping scheme contribute to the large number of *Salmonella* serovars.

Serotyping uses differences in the polysaccharide portion of lipopolysaccharide layer (O antigen) and the filamentous portion of the flagella (H antigen) present on the surface of *Salmonella* to separate strains into distinct serotypes (Foley & Lynne, 2008). Currently, the Kauffmann-White scheme for serotyping *Salmonella* recognizes 46 somatic (O) antigen groups (Fitzgerald, Sherwood, Gheesling, Brenner & Fields, 2003; Clark *et al*., 2009), which together with detection of the flagellar (H) antigens form the basis for serotype identification (Fitzgerald *et al*., 2003). The O factors determine the serogroup and the H factors define the serotype of a *Salmonella* strain. Unlike most of the other serovars of *Salmonella* that expresses a capsular polysaccharide antigen known as Vi (Wain *et al*., 2005).

Salmonella serotyping methods recognize 63 distinct phase 1 flagellar antigenic factors and 37 phase 2 flagellar antigenic factors although the latter are not always present (Mortimer, Peters, Gharbia, Logan & Arnold, 2004). Through the Kauffmann-White scheme which was first published in 1929, *Salmonella* are divided into more than 2500 serotypes according to their antigenic formulae. According to Grimont and Weill, by the year 2007 the actual numbers of registered *Salmonella* serotypes were 2579 (Clark *et al*., 2009).

Serotyping is usually done in a suspension, which is incubated with antisera (monoclonal or polyclonal) that recognize O and H antigens. Monoclonal and Polyclonal antisera are used routinely in *Salmonella* serotyping. Monoclonal antibodies (MAbs) with their monoepitopic specificity have many advantages over monospecific polyclonal sera (Iankov *et al*., 2002). The agglutination profiles generated are used to determine the particular serotype of the isolate being tested. Serovars of subspecies *enterica* are given a name, whereas the remaining serovars are designated by only their antigenic formula (Euzêby & Barrett, 2007).

In most laboratories, initial serotyping is done using polyvalent O antisera to allow *Salmonella* isolates to be grouped into different O groups designated in capitalized letters e.g. *S.* Typhimurium belongs to Group B and *S.* Enteritidis to Group D. Principally, the method is mainly based on agglutination with specific sera to identify antigenic variants of the flagellar antigen (H factor) and the O-antigen of the LPS, which defines the O factor (Broadbent *et al*., 2010). The strains of *Salmonella* which are serologically classified into serovars (serotypes) are defined and maintained by the WHO Collaborating Centre for Reference and Research at the Pasteur Institute in Paris, France.

Although serological typing has become an invaluable typing method for epidemiological investigations of *Salmonella*, it does have some practical limitations. The limitation of the methods are time consuming, difficult to standardize, and often has

to be done in a reference laboratory (Christensen, Moller, Vogensen & Olsen, 2000). In additional, the availability of antisera is limited considering the ethics and cost, lack of trained personnel and quality control measures necessary to maintain such a supply are also the disadvantages of the method (Mortimer *et al*., 2004). However, the advantages of serotyping methods are that they are stable, reproducible and have high typeability (Mortimer *et al*., 2004).

2.14.2 Phage typing

Phage typing is a means of differentiating bacteria on the basis of the susceptibility of the bacterial host to specific bacteriophages. A typing system for salmonellae is based on the lytic activity of certain bacteriophages on the strains of *S.* Typhi, and was first introduced by Craigie and Yen in 1938 (Kasatiya, Caprioli & Champoux, 1978). The method has since being used for differentiating organisms belonging to the same serotype (Jegathesan, 1983).

Phage typing utilizes the selective ability of bacteriophages to infect certain strains of *Salmonella* thus enabling the user to differentiate unique isolates (Yan *et al*., 2003). Its application depends on the most important property of the phages, that is, their host specificity (Castro *et al*., 1992). Different bacteriophages are able to selectively infect *Salmonella* isolates due to differences in the phage and phage receptor present on the surface of the bacterium (Yan *et al*., 2003). When an appropriate phage receptor is located on the surface of the cell, the phage infects the bacterium and lyses the cell. A

phage type designation is assigned to the specific strain of bacteria based upon the array of typing phages that are able to lyse the cells and form plaques in the bacterial lawns (Snyder & Champness, 1997).

Phage typing has been a useful laboratory tool for investigating *Salmonella* outbreaks such as *S.* Typhi and *S.* Typhimurium (Hickman-Brenner, Stubbs & Farmer, 1991). The drug resistance properties of some phage type can also be used for differentiation of *Salmonella* strains (Hickman-Brenner *et al*., 1991). Many other phage typing systems have been proposed and widely applied to the identification of *Salmonella* serotypes including *S.* Adelaide, *S.* Anatum, *S.* Bareilly, *S.* Blockley, *S.* Bovis-morbificans, *S.* Braenderup, *S.* Dublin, *S.* Enteritidis, *S.* Heidelberg, *S.* Minnesota and *S.* Newport (Castro *et al*., 1992).

Phage typing has proven to be epidemiologically valuable in strain differentiation within a particular *Salmonella* serotype. This is due to the ability of phage to distinguish varieties among apparently identical bacterial serotypes (Gershman, 1976). Epidemiological investigations of any bacterial infection must be able to differentiate among the strains of the same species of organisms (Castro *et al*., 1992).

Because phage typing is a technically demanding technique that requires the maintenance of multiple biologically active phage stocks, it is usually performed only by public health and reference laboratories. This is due to the fact that the wide variety of

phages involved in each phage typing set and its complex methodology make the system based on individual sets inappropriate for use in a routine microbiology laboratory (Castro *et al*., 1992). On the other hand, phage typing systems have the advantage of providing results easily and rapidly (within 24 h) once the systems are set up (Hickman-Brenner *et* al., 1991).

2.14.3 Molecular typing

Traditionally, phenotypic methods such as serotyping have been used for identification of *Salmonella* isolates in outbreak investigations. According to Olsen, Skov, Threlfall and Brown (1994), phenotypic methods have limited utility for epidemiologic analysis of *Salmonella* transmission because of their poor discriminatory ability for closely related isolates. They further called for the use of different typing methods in parallel with molecular techniques when performing phylogenetic or epidemiological studies.

However, Yan *et al.* (2003) suggest that differentiation of *Salmonella* isolates and source identification of foodborne outbreaks can be accomplished by using molecular typing techniques. These typing methods utilize restriction endonuclease digestion, nucleic acid amplification, or nucleotide sequencing techniques. Subsequently, selection of the most appropriate molecular typing method is made considering factors like sample size, turn-around time, and resources available to perform the typing (Yan *et al.*, 2003).

Although it is believed that DNA sequencing of highly variable genes will become the method of choice for molecular epidemiologists in the future, currently the "gold standard" technique used in this discipline is Pulsed-field gel electrophoresis (PFGE). Compared to other genotypic typing methods in previous studies, PFGE alone offer an improved level of discrimination as compared to ribotyping and provide ease interpretation than Restriction Endonuclease Analysis (Kristjansson *et al.,* 1994). According to Zou *et al.* (2010), PFGE is considered to be a possible alternative method in serotype determination or screening isolates for possible serotypes before actual serotyping especially where coventional method is unable to serotype. This is because PFGE method has potentially the ability to classify isolates that are "untypeable" based on conventional serotyping (Zou *et al.*, 2010).

The limitations of PFGE relate primarily to the relatively long processing time, require specialized equipment (Kristjansson *et al.,* 1994), it is labour intensive and serotyping reagents are usually expensive (Zou *et al.,* 2010). Due to its limitations, many studies have compared PFGE to other genetic typing methods in attempts to identify more powerful tools for epidemiological investigations and evolutionary analyses. Comparisons have been made with various typing schemes, including serotyping and phage typing, repetitive-element PCR, multilocus variable-number tandem repeat analysis, amplified fragment length polymorphism, antibiotic susceptibility typing, and DNA sequence typing (Harbottle, White, McDermott, Walker & Zhao, 2006).

CHAPTER 3: METHODOLOGY

This study was conducted on beef samples (meat cuts, meat fluid and carcass swabs) and animal feed samples (bone-and-meat meal and blood meal) from three local meat companies in the southern of the cordon fence in Namibia. A total number of 10335 of which 9508 from beef and 827 from animal feed were analyzed starting from January 2008 to December 2009 and, January 2008 to June 2010 respectively. The analyses were done in order to determine the prevalence, identifying the existing serovars and antimicrobial resistance pattern of *Salmonella* in Namibia. Isolation procedure for *Salmonella* was done at the Central Veterinary Laboratory, Windhoek, Namibia while serotyping and antimicrobial resistance susceptibility tests were done at the Istituto G. Caporale, Teramo, Italy as part of a scientific collaboration between the two laboratories.

3.1 Research design

This study was done in order to find out the prevalence and antimicrobial resistance of *Salmonella* in beef (meat cuts, meat fluid and carcass swabs) and feed (bone-and-meat meal and blood meal) samples. In the abattoir samples for *Salmonella* analysis were selected randomly based on individual abattoir slaughtering schedules. The number of samples depended on the number of heads presented for slaughtering on each production day from each abattoir and quantities of boxes packaged per day.

Since this study was a laboratory based research, the isolation and identification of *Salmonella* contamination was done on all beef and feed samples presented for routine tests. The convenient sampling method was used whereby all samples received were regarded as samples for this study. The minimum of sample size of 138 for each product i.e. beef and feed was estimated based on the sample calculation formula given below (See 3.1.2.1). Isolates of confirmed *Salmonella* serovars were also tested for antimicrobial resistance in order to establish the resistance of the organisms against antimicrobials. The antimicrobial resistance of *Salmonella* serovars was tested against selected antimicrobials which are used in both human and animal production.

3.1.1 Population

The population of this study was defined as samples presented for routine analysis for screening for presence of *Salmonella* in beef and animal feed from the three local abattoirs from the period of January 2008 throughout June 2010. All isolated *Salmonella* strains in the tests were subjected for serotyping and antimicrobial susceptibility test. The minimum sample size was determined based on the formula presented in section 3.1.2.1.

3.1.2 Samples

Samples used in this study were from beef and animal feed samples. The beef samples presented were in three different form; meat cuts, meat fluid and carcass swabs. The

animal feed samples were of two different types; bone-and-meat meal and blood meal. All samples presented for routine testing for the stated period were used in this study.

3.1.2.1 Sampling strategy

The abattoirs used in this study use a sampling strategy based on the sampling plan proposed by EUROSTAT known as a simple random sampling (SRS). The SRS is often regarded as the basic form of probability sampling and is applicable to situations where there is no previous information available on the population structure. Simple random sampling choose directly from the frame population ensures that each population element has an equal probability of selection. Thus, SRS is an equal-probability sampling design. In simple random sampling of *n* elements, every element *k* in the population frame of *N* elements has exactly the same inclusion probability. Therefore, the formula used in this regard was as follows;

$$\pi_k = \pi = n/N$$

3.1.2.2 Determination of sample size

The minimum sample size for this study was calculated as follows; with the confidence level of 95 % and a confidence interval of 5 %, the minimum sample size is calculated using the formula below assuming that the prevalence rate of *Salmonella* is below 10 %.

$$N = \frac{Z^2 * (P) * (1 - P)}{C^2}$$

Where:

Z = 1.96 for Confidence level of 95%

P = percentage picking a choice (Prevalence rate = 10 %)

C = Confidence interval of 5 %

N = Minimum number of sample required

From this formula, a minimum of 138 samples for each type of sample (beef and animal feed) were expected to be used in this study.

3.2 Procedure

3.2.1 *Salmonella* isolation

Salmonella serovars were isolated from routine samples for *Salmonella* analysis. The conventional methods for the isolation of *Salmonella* was performed according to the ISO 6579: 2002 for Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella*. The procedure has four main stages; pre-enrichment, enrichment, plating and confirmation as summarized in **Figure 1** in this chapter.

3.2.1.1 Non-selective pre-enrichment

Meat and animal feed samples

Meat or animal feed sample (25 g) was weighed and transferred into a sterile stomacher bag. Approximately, 225 ml of buffered peptone water (BPW) (Merck, Darmstadt, Germany) was added into the sample. The sample was then homogenized with a stomacher machine for about 2 minutes followed by incubation at 37 °C ± 1 °C for 18 to 24 hours.

Carcass swab samples

Carcass swabs were sampled from unspecified surface area and pooled to the maximum of five individual samples. The minimum sampling surface area was at least 100 cm^2 per individual sample. The pooled samples were incubated in 500 ml of BPW (Merck, Darmstadt, Germany) using a 500 ml media bottles. The samples were then incubated at 37 °C ± 1 °C for 18 to 24 hours.

Meat fluid samples

Approximately 100 ml of meat fluid sampled from 10 vacuum packed meat. About 50 ml of the meat fluid sample was transferred into a 500 ml media bottle. Then 450 ml of BPW (Merck, Darmstadt, Germany) was added to the sample and incubation at 37 °C ± 1 °C for 18 to 24 hours.

3.2.1.2 Selective enrichment

After the incubation time samples from the pre-enrichment broth were mixed and then inoculated into a selective media. Approximately 0.1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium with soya (RVS) broth (Scharlau Chemie S.A. Barcelona, Spain). Another 10 ml of the pre-enrichment broth was transferred into a tube containing 100 ml Selenite cystine broth (Merck, Darmstadt, Germany). Selenite cystine broth was used instead of Mueller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth (Merck, Darmstadt, Germany). Inoculated RVS broth (Scharlau Chemie S.A. Barcelona, Spain) was incubated at 41.5 °C ±1 °C for 18 to 24 hours where inoculated Selenite cystine broth was incubated at 37 °C ±1 °C for 18 to 24 h.

3.2.1.3 Culture and identification

After incubation, a loopful of the enriched cultures of RVS broth and selenite cystine broth was streaked separately onto two selective agar plates: Xylose Lysine Desoxycholate (XLD) (Merck, Wadeville, South Africa) and Brilliant Green Agar (BGA) (Scharlau Chemie S.A. Barcelona, Spain). These plates were incubated in an inverted position at 37 °C ±1 °C for 18 to 24 h. Following incubation, the black and pink colonies with or without black center on XLD agar and the colorless or opaque-white colonies surrounded by pink or red zone on BGA were identified as suspect *Salmonella.* Such colonies were picked out and subcultured on Nutrient agar (Merck, Wadeville, South Africa) and incubated at 37 °C ±1 °C for 18 to 24 hours.

3.2.1.4 Biochemical confirmation

Using a sterile inoculating wire loop, two or more colonies of typical suspicious *Salmonella* were selected from the Nutrient agar. Selected colonies were used to perform biochemical confirmation tests. The following biochemical tests were performed:

Triple sugar iron agar (TSI agar)

The TSI agar slant was inoculated by streaking slant and stabbing the butt with pure culture of typical suspicious *Salmonella* from the Nutrient agar. After inoculation the TSI agar was incubated at 37 °C ±1 for 18 to 24 hours. The inoculated tubes were caped loosely to maintain aerobic conditions while incubating in order to prevent excessive Hydrogen Sulphide (H_2S) production.

For interpretation of the TSI results, typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas formation (bubbles) and (in about 90% of the cases) formation of hydrogen sulfide (blackening of the agar). When lactose-positive *Salmonella* is isolated the TSI agar slant is yellow. The preliminary confirmation of *Salmonella* cultures was not based on the results of the TSI agar test only.

Urea agar test

The urea agar slant surface was inoculated by streaking the agar slope surface and stabbing the butt with pure culture of typical suspicious *Salmonella* from the Nutrient agar. The Urea agar slants were then incubated at 37 °C ±1 °C for 18 to 24 hours

followed by results interpretation. The positive reaction showed, splitting of urea which liberated ammonia, with changes of the color from phenol red to rose pink, and later to deep cerise (moderate red). The reaction is often apparent after 2 to 4 hours. For a negative reaction, the color of the Urea media remained unchanged.

$_{L}$-Lysine decarboxylation medium

Typical suspicious *Salmonella* from the Nutrient agar was inoculated in the $_{L}$-Lysine decarboxylation medium just below the surface of the liquid medium. The medium was then incubated at 37 °C ±1 °C for 18 to 24 hours. Turbidity and a purple color after incubation indicated a positive reaction. A yellow color indicated a negative reaction.

Detection of β-galactosidase

A loop-full of the suspected colony of *Salmonella* from the Nutrient agar was inoculated in a tube containing 0.25 ml of the saline solution. One drop of toluene was added and the tube was shaken to mix the solution. The tube was put in a water bath set at 37 °C ±1 °C for several minutes (approximately 5 minutes). Approximately 0.25 ml of the reagent was added for detection of β-galactosidase and mixed with the solution. The tube was replaced in the water bath and was left for 24 ±3 hours, being examined at intervals. A yellow color indicated a positive reaction. The reaction was often apparent after 20 minutes.

Medium for Voges-Proskauer (VP) reaction

A loop-full of the suspected colony of *Salmonella* from the Nutrient agar was suspended in a sterile tube containing 3 ml of the Voges-Proskauer (VP) medium and incubated at 37 °C ±1 °C for 24 ±3 hours. After incubation 2 drops of creatine solution was added followed by 3 drops of the ethanolic solution of 1-naphthol and then 2 drops of potassium hydroxide solution. The solution was shaken after each addition of the reagent. The formation of pink to bright red color within 15 minute indicated a positive reaction.

During the VP reaction, bacteria fermenting sugars via the butanediol pathway produce acetoin (i.e. acetyl methyl carbinol or 3-hydroxybutanone) as an intermediate which can be further reduced to 2,3-butanediol. In the presence of KOH the intermediate acetoin is oxidized to diacetyl, a reaction which is catalyzed by a-naphthol. Diacetyl reacts with the guanidine group associated with molecules contributed by peptone in the medium, to form a pinkish-red-colored product.

Medium for indole reaction

A tube containing 5 ml of the tryptone/tryptophan medium was inoculated with the suspected colony of *Salmonella* from the Nutrient agar. The medium was then incubated at 37 °C ±1 °C for 24 ±3 hours. After incubation, 1 ml of the Kovacs reagent was added in the medium. The formation of a red ring indicated a positive reaction. A yellow-brown ring indicated a negative reaction.

Table 2. Summary of typical biochemical reactions for *Salmonella*.

Test or Substrate	**Positive**	**Negative**	***Salmonella* reaction**
Glucose (TSI)	Yellow butt	Red butt	+
Lysine decarboxylase	Purple	Yellow	+
H_2S (TSI)	Blackening	No blackening	+
Urease	Purple-red color	No color change	-
Lysine decarboxylase broth	Purple	Yellow	+
Indole test	Red color at surface	Yellow color at surface	-
Voges-Proskauer (VP) test	Pink-to-red color	No color change	-
β-galactosidase	Yellow	No color change	-

3.2.1.4 Serological Confirmation

Elimination of auto-agglutinatable strains

This is a procedure that was done to eliminate auto-agglutinable microbial strains. One drop of saline solution was placed onto a clean glass slide. Part of the colony to be tested was dispersed in the drop in order to obtain a homogeneous and turbid suspension. The slide was then gently rocked for 30 to 60 seconds and the result was observed against a dark background. If the bacteria are clumped, the strain was considered auto-agglutinable and was not submitted to the serological tests as the detection of the antigens is impossible.

Examination for O- and H-antigens

One drop of the anti-O serum (Merck, Marburg, Germany) or anti-H serum (Merck, Marburg, Germany) was placed onto a clean glass slide. Part of the non-auto agglutinating pure colony to be tested was dispersed in the drop in order to obtain a homogeneous and turbid suspension. The slide was then gently rocked for 30 to 60 seconds and the result was observed against a dark background. If agglutination occurred, the reaction was considered positive for the respective antigen.

3.2.1.4 Interpretation of biochemical and serological reactions

All reactions for both biochemical and serological reactions for the presence of *Salmonella* were interpreted according to the ISO 6579: 2002 for Microbiology of food and animal feeding stuffs horizontal method for the detection of *Salmonella*. The summary for the interpretation is presented in **Table 3**.

Table 3. Interpretation of biochemical and serological confirmatory tests.

Biochemical reactions	Auto-agglutination	Serological reactions	Interpretation
Typical	No	O- or H-antigen positive	Strain considered to be *Salmonella*
Typical	No	All reaction negative	
Typical	Yes	Auto-agglutination	May be *Salmonella*
None typical	No / No	O- or H-antigen positive	
None typical	No / Yes	All reaction negative	Not considered to be *Salmonella*

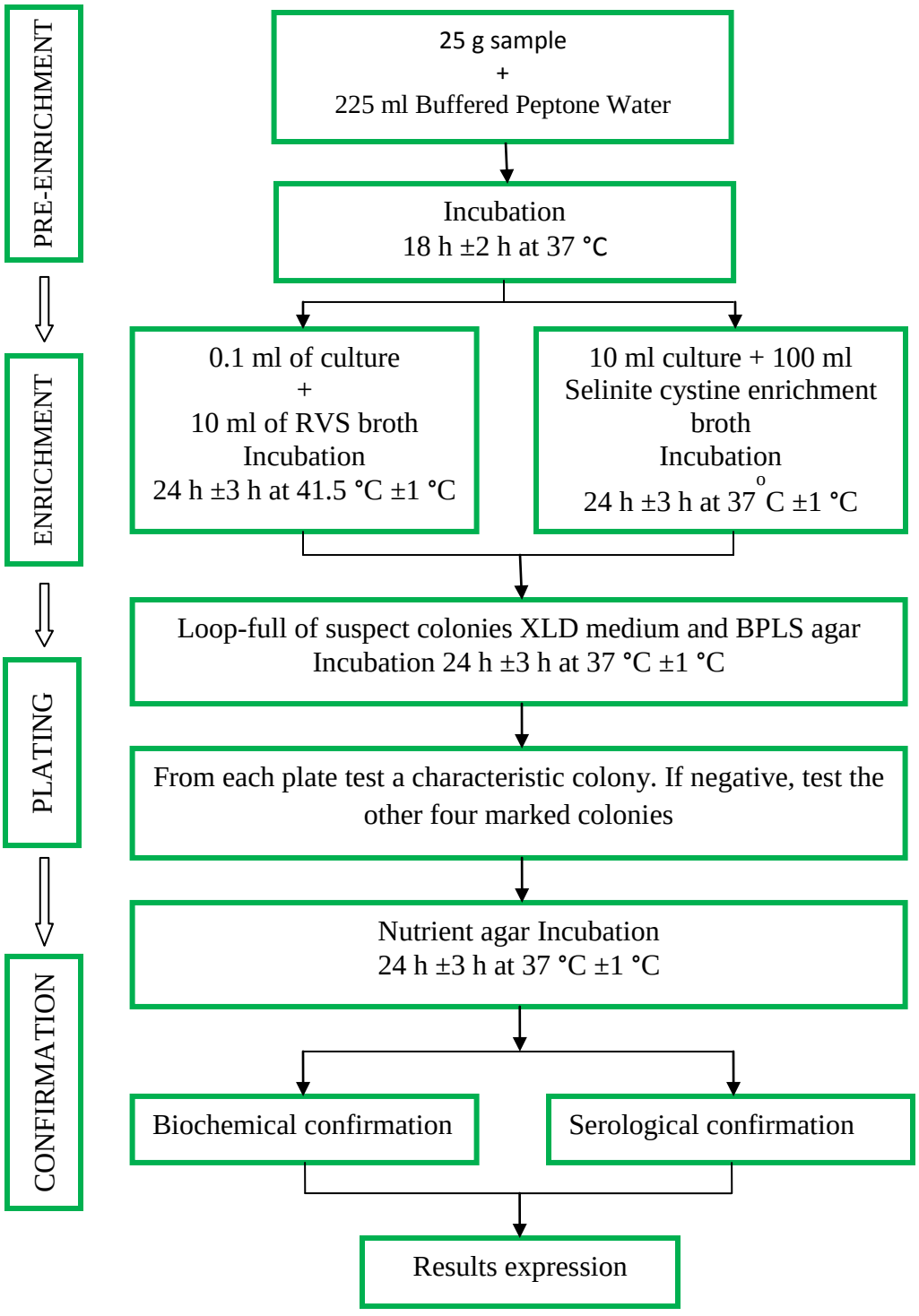

Figure 1. Flow diagram summarizing the procedure for the isolation of *Salmonella* (ISO 6579: 2002).

3.2.2 *Salmonella* serotyping

Salmonella isolated and confirmed according to the ISO 6579:2002; Microbiological of food and animal feeing stuffs – Horizontal method for the detection of *Salmonella* spp. were serotyped. The serotyping was a serological test done according to the Kauffmann-White scheme. *Salmonella* serotyping activities were carried out at the Bacteriology section, Istituto G. Caporale, Teramo, Italy.

3.2.3 Antimicrobial susceptibility testing

All *Salmonella* serovars which were isolated, confirmed and serotyped according to the Kauffmann-White scheme were tested for antimicrobial susceptibility. The antimicrobial susceptibility testing was done using the agar disk diffusion method as described by the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards [NCLLS], 2002), now known as the Clinical and Laboratory Standards Institute (CLSI). The zone of inhibition (sensitive, intermediate and resistant) was interpreted according to NCLLS (2002), Popoff (2001) and Kirby, Bauer, Sherris & Turck (1966). Strains were classified as resistant if they grew at the following antibiotic concentrations: Nalidixic acid (30 μg/ml), Ampicillin (10 μg/ml), Amoxicillin-clavulanic acid (20 / 10 μg/ml), Cefazolin (30 μg/ml) Gentamicin (10 μg/ml), Kanamycin (30 μg/ml), Enrofloxacin (5 μg/ml), Trimethoprim-sulfamethoxazole (1.25 / 23.75 μg/ml), Tetracycline (30 μg/ml), Cefotaxime (30 μg), Sulfisoxazole (250 -

300 µg/ml), Colistin (10 µg/ml), Streptomycin (10 µg/ml), Chloramphenicol (30 µg/ml), Cephalothin (30 µg/ml) and Ciprofloxacin (5 µg/ml).

3.2.3.1 Preparation of Mueller-Hinton agar

The Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) was prepared according to the manufacturer's instructions. Briefly, after autoclaving at 121 ℃ for 15 minutes, the media was cooled to 45 – 50 ℃. Approximately 30 to 50 ml of media was poured into the 12 x 120 mm Petri dishes with the depth of the agar in the Petri dishes maintained to approximately at 4 mm.

3.2.3.2 Preparation of the inoculum

At least 2 -3 well isolated colonies of the same morphological type were selected from the agar plate culture. The top of each colony was touched with a sterile swab, and the growth was transferred into a tube containing 4 ml saline water. The culture was directly adjusted to the McFarland standards (BioMérieux, Marcy-l'Étoile, France) until it achieved the turbidity of between 0.5 - 1 McFarland standards.

3.2.3.3 Inoculation of test plates

Optimally within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab then was rotated several times pressed firmly on the inside wall of the tube above the fluid level. This removed excess inoculum from the swab. The dried surface of a Mueller-Hinton agar

(Oxoid, Basingstoke, Hampshire, England) plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum. The procedure was done under laminar flow to avoid contamination.

3.2.3.4 Application of disks to inoculated agar plates

The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. The discs were pressed down to ensure complete contact with the agar surface using a mult-antibiotic dispenser (Becton Dickinson). The plates were inverted and incubated at 37 °C for 16 to 24 h within 15 minutes after the disks were applied.

3.2.3.5 Reading plates and interpreting results

Each plate was examined after 16 to 24 hours of incubation time. The resulting zone of inhibition was uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition (judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured using a ruler which was held on the back of the inverted Petri plate. The petri plate was held a few inches above a black, nonreflecting background and illuminated with reflected light. Transmitted light from the colony counter was used to examine the zones for light growth wherever indicated, within apparent zones of inhibition.

The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, was ignored. The sizes of the inhibition zones were interpreted by referring to zone diameter interpretive standards from NCCLS 2000, and the equivalent minimal inhibitory concentration (MIC) breakpoints for Enterobacteriaceae, and organisms were reported as susceptible, intermediate or resistant to the antimicrobial substance tested. The summary for the interpretation of antimicrobial resistance is given in the **Table 4**.

Table 4. Summary for interpretation of antimicrobial resistance of *Salmonella*.

Antimicrobial substance	Concentration (μg/ml)	S (mm)	I (mm)	R (mm)
Nalidixic acid (NA)	30	≥ 18	15 - 17	≤ 14
Ampicillin (AM)	10	≥ 17	14 - 16	≤ 13
Amoxicillin-clavulanic acid (AMX+ACL)	20 / 10	≥ 18	14 - 17	≤ 13
Cefazolin (CFZ)	30	≥ 18	15 - 17	≤ 14
Gentamicin (GM)	10	≥ 15	13 - 14	≤ 12
Kanamycin (K)	30	≥ 18	14 - 17	≤ 13
Enrofloxacin (ENR)	5	≥ 23	17 - 22	≤ 16
Trimethoprim-sulfamethoxazole (SXT)	1.25 /23.75	≥ 16	11 - 15	≤ 10
Tetracycline (TE)	30	≥ 19	15 - 18	≤ 14
Cefotaxime (CTX)	30	≥ 14	15 - 17	≤ 18
Sulfisoxazole (SULF)	250 - 300	≥ 16	13 - 15	≤ 12
Colistin (COL)	10	≥ 11	9 - 10	≤ 8
Streptomycin (S)	10	≥ 15	12 - 14	≤ 11
Chloramphenicol (C)	30	≥ 18	13 - 17	≤ 11
Cephalothin (CF)	30	≥ 18	15 - 17	≤ 14
Ciprofloxacin (CFX)	5	≥ 21	16 – 20	≤ 15

S: Susceptible; I: Intermediate; R: Resistant

3.3 Data analysis

The prevalence of *Salmonella* strains was evaluated in terms of percentage occurances, in which the denominator is the total number of *Salmonella* isolates within a group. The

antimicrobial resistance of a group of isolates was calculated as the percentage of isolates among the group that were resistant to a single antimicrobial or a number of antimicrobials (Johnson, Rajic & McMullen, 2005). The differences between observations were analyzed using a Chi-square method with the confidence interval of 95 %. Similar method wad used by Akoachere, Tanih, Ndip and Ndip (2009) to compare the prevalence in the different anatomical sites and biotypes. The differences were considered significant at $p < 0.05$. The Statistical Package for the Social Sciences [SPSS], version 17.0 was used for the data analysis. The findings were presented as descriptive statistics. Tables, graphs and charts were used to display the relationships of various variables and also for comparative analysis of data on the prevalence and antimicrobial resistance pattern of *Salmonella* strains.

CHAPTER 4: RESULTS

This is the first study on the prevalence of *Salmonella* and antimicrobial resistance in Namibia. The study assessed the frequency of contamination and determined the antimicrobial resistance pattern of *Salmonella* serovars in beef and animal feed in Namibia. A total of 9508 and 827 of beef and animal feed samples were analyzed during the period of over two years from of January 2008 throughout to December 2009 and, January 2008 throughout to June 2010 respectively. The presentation of the results in this study is divided into three major sections; Prevalence of *Salmonella* and Antimicrobial Resistance in beef, Prevalence of *Salmonella* and Antimicrobial Resistance in animal feed and, Prevalence and Antimicrobial Resistance of *Salmonella* in beef and animal feed. Some sections of the results of this thesis have been published as: **Shilangale, R.P,** Di Giannatale, E., Chimwamurombe, P.M. & Kaaya, G.P. (2012). Prevalence and antimicrobial resistance pattern of *Salmonella* in animal feed produced in Namibia. *Veterinaria Italiana* 48 (2), 125 – 132.

4.1 Prevalence of *Salmonella* and antimicrobial resistance in beef

4.1.1 Prevalence of *Salmonella* in beef

From a total of 9508 samples of meat samples examined for the presence of *Salmonella*, 0.85 % (*n* = 81) were found to be positive for *Salmonella*.The prevalence of *Salmonella* in beef was analyzed in three different products; meat cuts, meat fluid and carcass

swabs. The prevalence of *Salmonella* per individual product and total prevalence of *Salmonella* in beef is presented in **Table 5**. There was no significant difference ($p > 0.05$) on the prevalence of *Salmonella* between all three types of samples.

Table 5. The prevalence of *Salmonella* per individual product and total prevalence of *Salmonella* in beef

Product	No. *Salmonella* isolates	% prevalence rate per product	% Total prevalence rate (N = 9508)
Meat cuts	17	0.50 (N = 3424)	0.18
Meat fluid	19	0.43 (N = 4396)	0.20
Carcass swabs	45	2.67 (N = 1688)	0.47
Total	**81**	**-**	**0.85**

The prevalence of *Salmonella* serovars in meat cuts, meat fluid and carcass swabs samples are reported in **Table 6, Table 7** and **Table 8** respectively. The prevalence rate of *Salmonella* in meat cuts samples (*n* = 3424) was 0.50 %, whereas in meat fluid samples (*n* = 4396), the prevalence rate was 0.43 %. The prevalence rate of *Salmonella* in carcass swabs samples (*n* = 1688) was 2.67 %. There was no significant difference ($p > 0.05$) of the prevalence of *Salmonella* between the three types of samples. A total of 31 different types of *Salmonella* serovars were identified from one or both sample types, with *S.* Chester being the most frequently isolated with 12 isolates, followed by *S.* Reading and *S.* Bredeney with 6 serovars isolates each and *S.* Typhimurium with 5

isolates. Six strains were found to be positive for *S. enterica* subsp. Salamae, two for *Salmonella* group K, one *Salmonella* Group I, one each for *Salmonella* Group C1 and D2 respectively. These groups could not be identified further because they did not express the phase 2 'H' antigens.

Table 6. Distribution of *Salmonella* serovars isolated in meat and the prevalence rate.

Salmonella serotype	Number of isolates	Prevalence (%) (N = 17)
S. Schwarzengrund	1	5.88
S. Bredeney	1	5.88
S. Saintpaul	1	5.88
S. Parkroyal	1	5.88
S. Chester	5	29.41
S. Winston	1	5.88
S. Bahrenfeld	1	5.88
Salmonella group K*	1	5.88
S. Dublin	1	5.88
S. Lamberhurst	1	5.88
S. Uganda	1	5.88
S. Anatum	1	5.88
S. Sao	1	5.88
Total	**17**	**100.00**

* non-typeable isolates of *Salmonella*

Table 7. Distribution of *Salmonella* serovars isolated in meat fluid and the prevalence rate.

Salmonella serotype	Number of isolates	Prevalence (%) (*N* = 19)
S. Braenderup	2	10.53
S. Ball	1	5.26
S. Chester	1	5.26
S. Fischerkietz	1	5.26
S. Cannstatt	2	10.53
S. Petahtikve	1	5.26
S. Bredeney	3	15.79
S. Anatum	3	15.79
Salmonella enterica subsp. *salamae**	1	5.26
S. Winston	1	5.26
Salmonella group K*	1	5.26
S. Vaertan	1	5.26
S. Cerro	1	5.26
Total	**19**	**100.00**

* non-typeable isolates of *Salmonella*

Table 8. Distribution of *Salmonella* serovars isolated in carcass swabs and the prevalence rate.

Salmonella serotype	Number of isolates	Prevalence (%) (*N* = 45)
S. Typhimurium	5	11.11
S. Saint-paul	2	4.44
S. Chester	6	13.33
S. Minnesota	1	2.22
S. Braenderup	3	6.67
S. Parkroyal	1	2.22
S. Djermaia	1	2.22
S. Petahtikve	1	2.22
S. Fischerkietz	1	2.22
S. Kaapstad	1	2.22
Salmonella group I*	1	2.22
S. Cannstatt	1	2.22
S. Reading	6	13.33
S. Bredeney	2	4.44
S. Chichiri	1	2.22
S. Newport	1	2.22
S. Kintambo	1	2.22
S. Banana	1	2.22
Salmonella enterica subsp. salamae *	5	11.11
S. Anatum	1	2.22
Salmonella Group D2*	1	2.22
Salmonella Group C1*	1	2.22
S. Schwarzengrund	1	2.22
Total	**45**	**100.00**

* non-typeable isolates of *Salmonella*

4.1.2 Prevalence and antimicrobial resistance pattern of *Salmonella* in beef

In total, 20 of 81 isolates from beef samples belonging to 15 different *Salmonella* serovars showed antimicrobial resistance to one or more of the antimicrobials tested (**Table 9, 10 and 11**). Ten (12.35 %) of 81 isolates that belonged to eight of 29 different serovars showed resistance to sulfisoxazole and the trimethoprim-sulfamethoxazole combination. Two isolates of *S.* Chester isolated in two different samples showed a multidrug resistance of up to four different antibiotics. Only one strain of *S.*Chester isolated from carcass swabs was found to be resistant to Colistin, Cephalothin, Amoxicillin-clavulanic acid and tetracycline (**Table 9**). The other strain of *S.* Chester isolated from meat cuts showed to be resistant to trimethoprim-sulfamethoxazole, sulfisoxazole, tetracycline and Chloramphenicol (**Table 10**). Only one strain of *S.* Schwarzengrund was isolated from carcass swabs. However, this strain showed a multidrug resistance to three different antibiotics used, namely; trimethoprim-sulfamethoxazole, sulfisoxazole and tetracycline. All other *Salmonella* serovars showed resistance to one or two antibiotics used. The two *Salmonella* serovars isolated in meat fluids samples showed to be resistant to two antibiotics each with both serovars being resistant to trimethoprim-sulfamethoxazole (**Table 11**).

Table 9. *Salmonella* antimicrobial resistance pattern and the prevalence of resistant strains isolated from carcass swabs.

Salmonella serovar	Antimicrobial Resistance pattern	No. of resistant Strains	Prevalence of resistant strain (%)
S. Typhimurium	SXT^R, $SULF^R$	2 (5)	4.44
S. Saint-paul	$SULF^R$	1 (2)	2.22
S. Chester	COL^R, CF^R, $AMX+ACL^R$, TE^R	1 (6)	2.22
S. Fischerkietz	SXT^R, $SULF^R$	1 (1)	2.22
S. Kaapstad	SXT^R, $SULF^R$	1 (1)	2.22
S. Reading	COL^R	1 (6)	2.22
Salmonella enterica subsp. salamae	$SULF^R$	1 (5)	2.22
S. Chester	$SULF^R$	2 (6)	4.44
Salmonella Group D2	SXT^R, $SULF^R$	1 (1)	2.22
Salmonella Group C1	$SULF^R$	1 (1)	2.22
S. Schwarzengrund	SXT^R, $SULF^R$, TE^R	1 (1)	2.22
		13 (45)	

Values in the brackets shows the total number of isolates for a particular strain

Table 10. *Salmonella* antimicrobial resistance pattern and the prevalence of resistant strains in meat.

Salmonella serovar	Antimicrobial resistance pattern	No. of resistant strains	Prevalence of resistant strain (%)
S. Chester	$SULF^R$	1 (5)	5.88
S. Chester	SXT^R, TE^R, $SULF^R$, C^R	1 (5)	5.88
S. Chester	SXT^R, $SULF^R$	1 (5)	5.88
S. Uganda	SXT^R, $SULF^R$	1 (1)	5.88
S. Anatum	SXT^I, $SULF^R$	1 (1)	5.88
S. Sao	$SULF^R$	1 (1)	5.88
		6 (17)	35.29

Table 11. *Salmonella* antimicrobial resistance pattern and the prevalence of resistant strains in meat fluid.

Salmonella serovar	Antimicrobial resistance pattern	No. of resistant strains	Prevalence of resistant strain (%)
S Petahtikve	SXT^R, $SULF^R$	1 (1)	5.26
S Cerro	SXT^R, $SULF^R$	1 (1)	5.26
		2 (19)	10.53

4.2 Prevalence of *Salmonella* and antimicrobial resistance in animal feed

4.2.1 Prevalence of *Salmonella* in animal feed

From a total of 827 samples of animal feed examined for the presence of *Salmonella*, 11.73 % (*n* = 97) were found to be positive for *Salmonella*. The prevalence of

Salmonella in animal feed were analysed in two different animal feed products; blood meal and meat-and-bone meal. The comparison of prevalence of *Salmonella* for individual product and total prevalence of *Salmonella* is presented in **Table 12**. There was a significant difference ($p < 0.05$) in the prevalence of *Salmonella* between the two types of samples.

Table 12. The prevalence of *Salmonella* per individual product and total prevalence of *Salmonella* animal feed.

Product	No. *Salmonella* isolates	% prevalence rate per product	% Total prevalence rate (N = 827)
Blood meal	19	17.4^{a} (N = 109)	2.30
Bone-and-meat meal	78	10.86^{b} (N = 718)	9.43
Total	97	-	11.73

Values in the same column with different letters differ significantly from each other ($p < 0.05$)

The prevalence of *Salmonella* in blood meal and in meat-and-bone meal samples are reported in **Table 13** and **Table 14** respectively. The prevalence rate of *Salmonella* in meat-and-bone meal samples (*n* = 718) was 10.86 %, whereas in blood meal samples (*n* = 109), the prevalence rate was 17.43 %. A total of 35 different *Salmonella* serovars were identified from one or both sample types, with *S.* Chester being the most frequently isolated, followed by *S.* Schwarzengrund and *S.* Chartres. Six strains were found to be positive for *Salmonella* group C1, one *Salmonella* group D2 and five *S. enterica* subsp.

salamae but could not be identified because they did not express the phase 2 'H' antigens.

Table 13. Distribution of *Salmonella* serovars isolated in blood meal and the prevalence rate.

Salmonella serotype	Number of isolates	Prevalence (%) (N = 19)
S. Chester	2	10.53
S. enterica subsp. salamae	1	5.26
S. Schwarzengrund	6	31.58
S. Group C1	1	5.26
S. Bredeney	1	5.26
S. Sao	1	5.26
S. Charters	1	5.26
S. Anatum	2	10.53
S. Kaapstad	1	5.26
S. Typhimurium	2	10.53
S. Bahrenfeld	1	5.26
Total	**19**	**100.00**

* non-typeable isolates of *Salmonella*

Table 14. Distribution of *Salmonella* serovars isolated in bone-and-meat meal and the prevalence rate.

Salmonella serotype	Number of isolates	Prevalence (%) (*N* = 78)
*S.*Eppendorf	2	2.56
S. Reading	2	2.56
S. Charters	3	3.85
S. Braenderup	3	3.85
S. Chester	19	24.36
S. Anatum	2	2.56
S. Sandiego	1	1.28
S. Onderstepoort	2	2.56
*S.*Beaudesert	1	1.28
*S.*Fischerkietz	2	2.56
S. Lamberhurst	1	1.28
S. enterica subsp. salamae	4	5.13
*S.*Ball	1	1.28
*S.*Sajam	1	1.28
*S.*Brezany	2	2.56
*S.*Stanley	1	1.28
*S.*Southbank	1	1.28
*S.*Djugu	1	1.28
*S.*Petahtikve	2	2.56
*S.*Vaertan	1	1.28
*S.*Mbandaka	1	1.28
*S.*Djermaia	1	1.28
*S.*Infantis	3	3.85
*S.*Parkroyal	1	1.28

*S.*Typhimurium	1	1.28
*S.*Svedvi	2	2.56
*S.*Aflao	1	1.28
S. Saintpaul	1	1.28
S. Newlands	1	1.28
S. Group D2	1	1.28
S. Cerro	1	1.28
S. Uganda	1	1.28
S. Group C1	5	6.41
S. Schwarzengrund	5	6.41
S. Sao	1	1.28
Total	**78**	**100.00**

* non-typeable isolates of *Salmonella*

4.2.2 Prevalence and antimicrobial resistance pattern of *Salmonella* in animal feed

In total, 31 of 97 isolates from feed samples belonging to 15 different *Salmonella* serovars showed antimicrobial resistance to one or more of the antimicrobials tested (**Table 15 and 16**). This figure did not include the *Salmonella* serovars that could not be identified conclusively. Fourteen (45.16 %) of 31 isolates that belonging to eight different *Salmonella* serovars showed resistance to sulfisoxazole and the trimethoprim-sulfamethoxazole combination. Two isolates of *S.* Chester and one of *S.* Schwarzengrund isolated from bone-and-meat meal samples showed a multidrug resistance of up to three different antibiotics (**Table 15**). Both serovars showed multidrug resistance to trimethoprim-sulfamethoxazole, sulfisoxazole and tetracycline.

Table 15. *Salmonella* antimicrobial resistance pattern and the prevalence of resistant strains in meat-and-bone meal.

Salmonella serovar	Antimicrobial resistance pattern	No. of resistant strains	Prevalence of resistant strain (%)
S. Eppendorf	AN^R	2 (2)	100.00
S. Schwarzengrund	SXT^R, $SULF^R$	2 (5)	40.00
S. Reading	SXT^R, $SULF^R$	2 (2)	100.00
S. Chester	SXT^R, $SULF^R$	2 (19)	10.53
S. Sandiego	SXT^R, $SULF^R$	1 (1)	100.00
S. Onderstepoort	SXT^R, $SULF^R$	1 (2)	50.00
*S.*Beaudesert	SXT^R, $SULF^R$	1 (1)	100.00
S. Fischerkietz	SXT^R, $SULF^R$	1 (2)	50.00
S. Newlands	$SULF^R$	1 (1)	100.00
S. Chester	$SULF^R$	3 (19)	15.79
Salmonella enterica subsp. salamae	$SULF^R$	1 (4)	25.00
S. Chester	SXT^R, $SULF^R$, TE^R	2 (19)	10.53
Salmonella enterica subsp. salamae	SXT^R, $SULF^R$	1 (19)	5.26
S. Schwarzengrund	SXT^R, $SULF^R$, TE^R	1 (5)	20.00
		24 (78)	**30.77**

One isolates of *S.* Sao isolated from blood meal samples showed a multidrug resistance of up to three different antibiotics (**Table 16**). This serovar exhibited a multidrug resistance to trimethoprim-sulfamethoxazole, sulfisoxazole and nalidixic acid.

One strain of *S.* Schwarzengrund isolated from bone-and-meat meal showed a multidrug resistance to three different antimicrobials used. The same strain isolated from both bone-and-meat meal and blood meal exhibited a multidrug resistance to two antimicrobials. The antimicrobials which the *S.* Schwarzengrund exhibited MDR were; trimethoprim-sulfamethoxazole, Sulfisoxazole and tertracycline. In general, all other *Salmonella* serovars isolated from both meat-and-bone meal and blood meal exhibited resistance to one or two antibiotics used.

Table 16. *Salmonella* antimicrobial resistance pattern and the prevalence of resistant strains in blood meal.

Salmonella serovar	Antimicrobial resistance pattern	No. of resistant strains	Prevalence of resistant strain (%)
S. Chartres	SXT^R, $SULF^R$	1 (1)	100.00
S. Chester	$SULF^R$	1 (2)	50.00
S. Chester	$SULF^R$	1 (2)	50.00
S. Typhimurium	SXT^R, $SULF^R$	1 (2)	50.00
S. Virchow	NA^R	1 (1)	100.00
S. Schwarzengrund	$SULF^R$, TE^R	1 (6)	16.67
S. Sao	SXT^R, $SULF^R$, NA^R	1 (1)	100.00
		7 (19)	**36.84**

4.3 Prevalence and antimicrobial resistance of *Salmonella* in beef and animal feed

4.3.1 Prevalence of *Salmonella* in beef and animal feed

From a total of 10335 samples of beef and animals feed examined for the presence of *Salmonella*, 1.72 % (*n* = 178) were found to be positive for *Salmonella*. The prevalence of *Salmonella* was compared in two different products; beef and animal feed. The prevalence for the two products and total prevalence of *Salmonella* is presented in **Table 17**. There was no significant difference ($p > 0.05$) of the prevalence of *Salmonella* between the two types of samples.

Table 17. The prevalence of *Salmonella* per individual product and total prevalence of *Salmonella* in beef and animal feed.

Product	No. *Salmonella* isolates	% prevalence rate per product	% Total prevalence rate (N = 10335)
Beef	81	0.85 (N = 9508)	0.78
Animal feed	97	11.73 (N = 827)	0.94
Total	178	-	1.72

The percentages of *Salmonella* prevalence in individual beef samples (Meat, Meat fluid and Carcass swabs) and animal feed samples (Bone-and-meat meal and Blood meal) are presented in **Figure 2** below. There were 44 different types of *Salmonella* serovars that

were isolated and identified in this study (**Table 18**). Among all confirmed *Salmonella* isolates 23 *Salmonella* serovars could not be identified. These *Salmonella* serovars belongs to *Salmonella* group K, *Salmonella enterica* subsp. Salamae, *Salmonella* group I, *Salmonella* Group D2 and *Salmonella* Group C1.

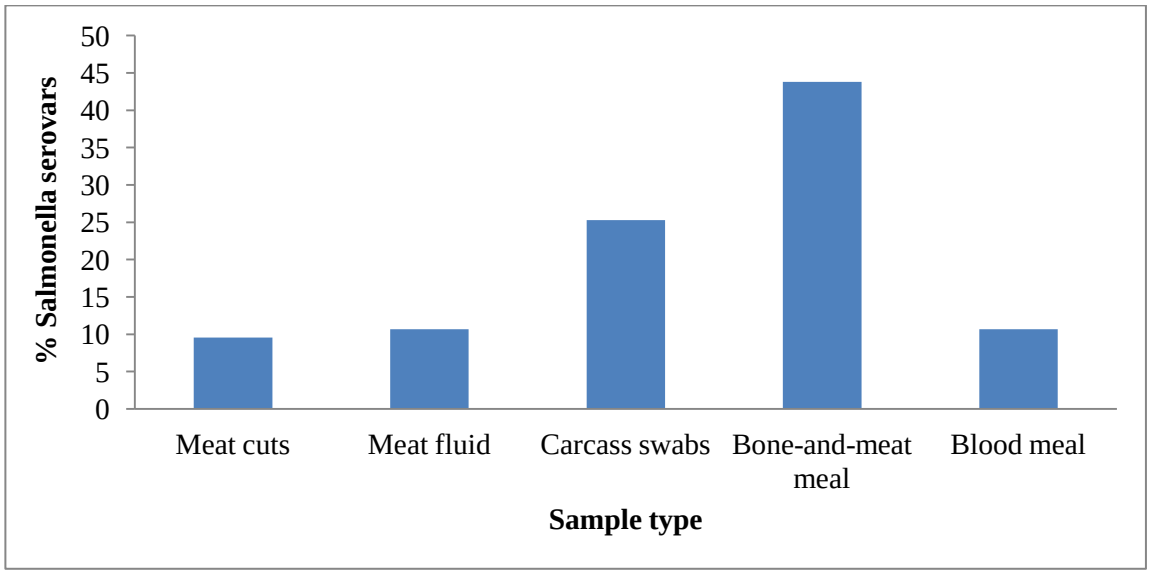

Figure 2. Percentage of *Salmonella* serovars isolated in different samples in beef and animal feed samples.

Table 18. Distribution of *Salmonella* serovars and the prevalence rate of individual *Salmonella* serovar from beef and animal feed samples.

Salmonella serotype	Number of isolates (*N* =178)	% of all serovars isolated
S. Schwarzengrund	13	7.30
S. Bredeney	7	3.93
S. Saint-paul	4	2.25
S. Parkroyal	3	1.69
S. Chester	33	18.54
S. Winston	2	1.12
S. Bahrenfeld	2	1.12
Salmonella group K*	2	1.12
S. Dublin	1	0.56
S. Lamberhurst	2	1.12
S. Uganda	2	1.12
S. Anatum	9	5.06
S. Sao	3	1.69
S. Braenderup	8	4.49
S. Ball	2	1.12
S. Fischerkietz	4	2.25
S. Cannstatt	3	1.69
S. Petahtikve	4	2.25
Salmonella enterica subsp. Salamae*	11	6.18
S. Vaertan	2	1.12
S. Cerro	2	1.12
S. Typhimurium	8	4.49
S. Minnesota	1	0.56

S. Djermaia	2	1.12
S. Kaapstad	2	1.12
Salmonella group I*	1	0.56
S. Reading	8	4.49
S. Chichiri	1	0.56
S. Newport	1	0.56
S. Kintambo	1	0.56
S. Banana	1	0.56
Salmonella Group D2*	2	1.12
Salmonella Group C1*	7	3.93
*S.*Eppendorf	2	1.12
S. Charters	4	2.25
S. Sandiego	1	0.56
S. Onderstepoort	2	1.12
*S.*Beaudesert	1	0.56
*S.*Sajam	1	0.56
*S.*Brezany	2	1.12
*S.*Stanley	1	0.56
*S.*Southbank	1	0.56
*S.*Djugu	1	0.56
*S.*Mbandaka	1	0.56
*S.*Infantis	3	1.69
*S.*Svedvi	2	1.12
*S.*Aflao	1	0.56
S. Newlands	1	0.56
Total	**178**	**100.00**

* non-typeable isolates of *Salmonella*

4.3.2 Antimicrobial resistance in beef and feed

To determine the antimicrobial resistance pattern of *Salmonella* all isolated serovars were subjected into a qualitative antimicrobial susceptibility test. All *Salmonella* isolates that tested positive for antimicrobial resistance showed resistance to one or more of the 16 antimicrobials employed (**Figure 3**). The antimicrobials which *Salmonella* serovars exhibited resistance were trimethoprim-sulfamethoxazole, sulfisoxazole, Colistin, Cephalothin, Amoxicillin-clavulanic acid, tetracycline and/or Chloramphenicol. From 19 *Salmonella* serovars which exhibited resistance to one or more types of antimicrobials, 15 different *Salmonella* serovars exhibited resistance to at least two antimicrobials (**Table 19**). Five different antimicrobial resistance patterns were displayed among the *Salmonella* that exhibited MDR in both beef and feed.

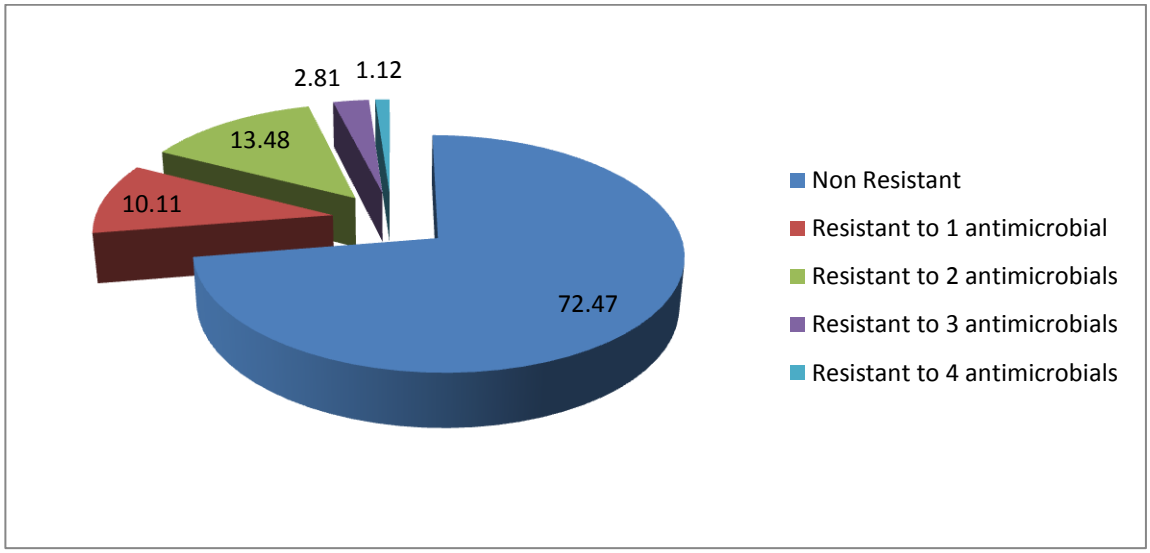

Figure 3. Percentage of *Salmonella* serovars that showed resistance to one or more antimicrobials used.

Table 19. Resistance pattern exhibited by *Salmonella* resistant to two or more types of antimicrobials.

Resistance Type	Resistance pattern	Serovars
Two types of Antimicrobials	SXT, SULF	*S.* Typhimurium, *S.* Fischerkiertz, *S.* Kaapstad, *Salmonella* Group D2, *S.* Chester, *S.* Uganda, *S.* Anatum, *S.* Petahtikve, *S.* Cerro, *S.* Schwarzengrund, *S.* Reading, *S.* Sandiego, *S.* Onderstepoort, *S.* Beaudesert, *Salmonella* enterica subsp. salamae, *S.* Chartres
Three types of Antimicrobials	SXT, SULF, TE	*S.* Schwarzengrund, *S.* Chester
	SXT, SULF, NA	*S.* Sao
Four types of Antimicrobials	COL, CF, AMX+ACL, TE	*S.* Chester
	SXT, TE, SULF, C	*S.* Chester

Results for antimicrobial susceptibility of all *Salmonella* isolates (n = 178) as determined by the qualitative method are presented in **Table 20**. Among all the antimicrobials tested, *Salmonella* serovars showed to be most resistant to Sulfisoxazole followed by Trimethoprim-sulfamethoxazole and Tetracycline. Some serovars also showed resistance to Nalidixic acid, Colistin, Amoxicillin-clavulanic acid, Chloramphenicol and Cephalothin. Off all the isolates 77.53 to 100 % showed to be sensitive against the antimicrobials tested where 0.56 to 3.93 % showed intermediate reaction to all antimicrobials tested. Cefazolin, Gentamicin, Kanamycin, Enrofloxacin and Cefotaxime showed maximum sensitivity and were the only antimicrobials that the isolates did not show any resistance. In addition, all *Salmonella* isolates showed 100 % susceptibility to Cefazolin, Gentamicin, Kanamycin, Enrofloxacin, Cefotaxime, Streptomycin and Ciprofloxacin.

Table 20. Antimicrobial susceptibility testing results of 178 *Salmonella* isolates as determined by a qualitative method.

Antimicrobial substance	Concentration (µg/ml)	% Resistant	% Intermediate	% Susceptible
Nalidixic acid (NA)	30	1.69(3)	1.12 (2)	97.19
Ampicillin (AM)	10	0	0.56 (1)	99.44
Amoxicillin-clavulanic acid (AMX+ACL)	20 / 10	0.56 (1)	0	99.44
Cefazolin (CFZ)	30	0	0	100
Gentamicin (GM)	10	0	0	100
Kanamycin (K)	30	0	0	100
Enrofloxacin (ENR)	5	0	0	100
Trimethoprim-sulfamethoxazole (SXT)	1.25 /23.75	13.48 (24)	0.56 (1)	85.96
Tetracycline (TE)	30	3.37 (6)	0.56 (1)	96.07
Cefotaxime (CTX)	30	0	0	100
Sulfisoxazole (SULF)	250 - 300	21.91 (39)	0.56 (1)	77.53
Colistin (COL)	10	1.12 (2)	0	98.88
Streptomycin (S)	10	0	3.93 (7)	96.07
Chloramphenicol (C)	30	0.56 (1)	0.56 (1)	98.88
Cephalothin (CF)	30	0.56 (1)	0.56 (1)	98.88
Ciprofloxacin (CFX)	5	0	0.56 (1)	99.44

Nineteen different *Salmonella* serovars showed resistance against the antimicrobials tested (**Table 21**). Of all *Salmonella* serovars that showed resistance 29.21 % (n = 178) exhibited resistance to antimicrobials used. The types of *Salmonella* serovars that showed most resistance to antimicrobials tested were S. Chester, *S.* Schwarzengrund, *S.* Fischerkietz, *S.* Typhimurium and *S.* Reading. Three serovars of *Salmonella* enterica subsp. Salamae exhibited resistance but could not be identified.

Table 21. Antimicrobial resistance of isolated *Salmonella* in beef and animal feed.

Salmonella serovar	No. of resistant strains	% Prevalence rate of resistant strain (N = 52)
S. Typhimurium	3	5.77
S. Saint-paul	1	1.92
S. Chester	16	30.77
S. Fischerkietz	3	5.77
S. Kaapstad	1	1.92
S. Reading	3	5.77
Salmonella enterica subsp. Salamae	3	5.77
S. Group D2	1	1.92
S. Group C1	1	1.92
S. Schwarzengrund	5	9.62
S. Uganda	1	1.92
S. Anatum	2	3.85
S. Sao	2	3.85
S. Petahtikve	1	1.92
S. Cerro	1	1.92
S. Eppendorf	2	3.85
S. Sandiego	1	1.92
S. Onderstepoort	1	1.92
S. Beaudesert	1	1.92
S. Newlands	1	1.92
S. Chartres	1	1.92
S. Virchow	1	1.92
Total	**52**	100.00

CHAPTER 5: DISCUSSION

5.1 Prevalence of *Salmonella* and antimicrobial resistance in beef

Previous reports have suggested that meat and meat products may be contaminated with potentially pathogenic *Salmonella* with antimicrobial resistance characteristics (Dabassa & Bacha, 2012; Ejeta *et al*., 2004; Samaxa *et al*., 2012). The study on the prevalence of *Salmonella* and antimicrobial resistance is the first of its kind in Namibia. This study assessed the prevalence of and the antimicrobial resistance pattern of *Salmonella* serovars in beef in Namibia.

5.1.1 Prevalence of *Salmonella* in beef

Salmonella is the major cause of foodborne illness in humans (Patrick *et al*., 2004; Mølbak & Neimann, 2002) commonly known as salmonellosis and can also lead to loss of product shelf life. The epidemiology of salmonellosis is mostly known as a result of outbreak investigations which are commonly associated with beef consumption that are contaminated with *Salmonella* (Bosilevac, Guerini, Kalchayanand & Koohmaraie, 2009). In Namibia, commercial abattoir test for *Salmonella* on beef carcass as part of the quality control criteria in order to meet the stringent conditions for the export markets such as Norway, the EU and RSA. Unlike the EU and RSA, Norway has a zero tolerance policy on *Salmonella* based on the Norwegian National Food Law **(**Isakbaeva *et al*., 2005).

In this study the prevalence of *Salmonella* in beef was investigated. The prevalence of *Salmonella* was found to be 4.97 % for beef samples derived from meat cuts, meat fluid and carcass swabs. These findings are comparable with other studies done in the region and elsewhere outside the region. However, there is little information in the sub Sahara region on the prevalence of *Salmonella* in beef from the slaughter houses which can be used to make a comparison with this study. Most of the studies in Africa are related to clinical cases and from retail markets.

A study done in Ethiopia on the prevalence of *Salmonella* from beef carcasses in abattoirs found the prevalence rate to be 13.3 % (Dabassa & Bacha, 2012). Another study in Ethiopia found the prevalence rate of *Salmonella* contamination of minced beef to be 14.4 % (Ejeta *et al.*, 2004). A study done in Botswana in raw beef sausages found the prevalence rate of *Salmonella* to be as high as 25.3 % (Samaxa *et al.*, 2012). However, the two later studies were done on naturally contaminated raw beef from retail outlets where the level of bacterial contamination is likely to be higher than the levels at the abattoir due to post handling or processing conditions.

As compared to other studies done elsewhere, *Salmonella* isolation was 2.6 % in beef and 4.1 % in veal carcasses during the years 1983 to 1986 in samples collected from federally inspected abattoirs across Canada (Lammerding *et al*., 1988). In a study in one of a commercial abattoir in Ireland the prevalence of *Salmonella* in cattle carcass was reported to be 7.6 % (McEvoy, Doherty, Sheridan, Blair & McDowell, 2003).

A different study in Australia on cattle and carcass during processing found the prevalence of *Salmonella* to be 2 and 3 % of pre-chill and post-chill carcasses respectively (Fegan, Vanderlinde, Higgs & Desmarchelier, 2005).

When the present study was compared with available information elsewhere it was found that the prevalence of *Salmonella* in beef carcasses in Namibia is similar or lower to that of some developed countries. These findings indicate that the good hygiene practice during slaughtering, processing and handling is functioning at the slaughtering facilities. This could probably be due to the fact that beef samples for this study were obtained from commercial abattoirs where the HACCP system is fully implemented and functional as part of the export requirements. HACCP is a safety tool that is used in food production in order to prevent, reduce or eliminate risks to occur through food production.

Although some of the studies used for comparison are a bit old, the fact that there have been some developments recently with regard to food safety and the control of pathogens in the slaughtering process it is possible that the prevalence rate in most developed countries is also on a decline. Available information on the national incidence crude rate (CIR) for salmonellosis in Ireland for 2008 and 2009 suggests that the rate of *Salmonella* infection is decreasing (National Disease Surveillance Centre [NDSC], 2010). This information may probably suggests that the decrease is due to application of improved control measures by employing the HACCP system in order to reduce the

contamination of *Salmonella* from various steps during harvesting, transportation, processing and storage.

Recent studies in Iran found the prevalence of *Salmonella* on beef carcass to be zero percent (Movassagh, Shokoori & Zolfaghar, 2010) while a different study in Algeria found the prevalence rate to be as high as 26.61 % (Mezali & Hamdi, 2012). However, the later study was done from red meat and meat products collected from various retail outlets where the contamination is likely to be higher. However, the possible general reasons for the differences between the present study and others could be due the differences in the sampling methods, slaughtering techniques, handling, hygiene practices and isolation techniques.

There was no significant difference ($p > 0.05$) observed on the prevalence of *Salmonella* when the carcass swabs method was used as compared to meat cuts and meat fluid. However, the rate of *Salmonella* isolation was higher when the carcass swab method was used as compared to other two sampling methods. These findings suggest that the carcass swab sampling method may be the best suitable method as compared to other two methods. Nevertheless, the findings of this study suggest that the rate of *Salmonella* isolation on the beef carcasses may be the same when the three sampling techniques are applied.

The findings of this study on the differences of the sampling methods between swabbing and excision methods were not different from other studies anywhere. According to Gill and Jones (2000), the statistics indicates that the numbers of bacteria recovered on pig or beef carcasses by swabbing and excision methods are similar. However, different study by Palumbo, Klein, Capra, Eblen and Miller (1999) found that swabbing method gave higher microbial count than excision when three sites were sampled as opposed to lower numbers obtained than excision when one site was sampled. These findings were also in agreement with the findings by Pearce and Bolton (2005) where the bacteria were recovered from a greater number of samples using the swabs than excision.

These findings may probably explain why the rate of *Salmonella* isolation in the current study was observed to be higher with a swabbing method as compared to other two methods. The reason for the higher prevalence rate with the swabbing method would be due to the lager sampling area used as compared to excision method. The sampling surface area for the swabbing method is a minimum of 100 cm^2 per site (approximately 400 cm^2) as opposed to 25 g (approximately 5 cm^2 per site) when excision method is used. This idea is also supported with the findings of other scholars. According to Gill and Jones (2000), the analysis of larger sampling areas of carcasses has shown to relatively increase the rate of recovery of bacteria.

The other reason for the efficiency of the swabbing technique could be due to the application of two swabs; wet and dry per site when using this method. Pearce and

Bolton (2005) findings suggest that apart from the role of the surface area the abrasiveness of the material used may influence the bacterial recovery. In the present study cotton gauze swabs which are abrasive materials were used in the swabbing method. However, there is a limitation in the comparison of the present study with others because unlike other studies the present study did not quantify the bacteria recovered.

The low rate of *Salmonella* isolation in meat fluid samples could be due to the result of the packaging method used. Meat fluid samples were obtained from the vacuum packaged meat. The vacuum packaging conditions may have probably inhibited or reduced the microbial load due to reduced oxygen levels in cold storage conditions. According to Buick and Damoglou (1987), vacuum packaging may significantly extends the shelf-life of the product stored at 4 °C for up to 8 days.

In the present study, *S.* Chester was isolated more frequently in meat cuts and carcass swab as compared to other serovars. However, in meat fluid *S.* Typhimurium was isolated more frequently as opposed to *S.* Chester. These isolates were among the 31 different *Salmonella* serovars isolated in the three types of samples. The finding of more *S.* Typhimurium in meat fluid could be due to the ability of this strain to tolerate in vacuum packaging conditions than *S.* Chester. According to a study by Tu and Mustapha (2002), vacuum packaging did not significantly reduce *S.* Typhimurium counts in beef stored at 4 °C for a period of 25 day.

When the results of the three products were analyzed together the study found out that *S.* Chester was the most frequently isolated strain with 12 isolates, followed by *S.* Reading and *S.* Bredeney with 6 serovras isolates each and *S.* Typhimurium with 5 isolates. These results were not comparable with other available findings in the region because of the differences in the type of serovars that are reported to be the most frequently isolated in those countries. A study done in Botswana found *Salomella enterica* subsp. salamae II, *S.* Thompson and *S.* Anatum to be the most frequently isolated *Salmonella* strains in that country. However, this study was done in different raw meat sausages made from beef, mutton, chicken and pork meat (Samaxa *et al*., 2012).

Of these 4 most frequently isolated serovars in Namibia, *S.* Typhimurium is the only serovar that is often being reported to be among the most frequently isolated elsewhere as compared to other serovars. A different study in Sweden done between 1993 and 1997 on the isolation of *Salmonella* from animals and animal feed found *S.* Typhimurium to be the second most frequently isolated serotype in cattle (Boqvist *et al*., 2003). However, 78 of the 115 isolates originated from infected herds where the remaining isolates were collected at autopsies, sanitary slaughter and surveillance at slaughterhouses. In a different study in Ireland on the prevalence of *Salmonella* in cattle carcass *S.* Typhimurium was found to be the third most frequently isolated serotype after *S.* Dublin and *S.* Agona (McEvoy *et al.*, 2003). In the US, *S.* Typhimurium was found to be the second most common isolate after *S.* Montevideo (Schlosser *et al*., 2000). In a

different study in meat and meat products, *S.* Typhimurium was among the top five most frequently isolated in Algeria (Mezali & Hamdi, 2012).

However, the differences of the present study and other studies could be due to the differences in the geographical locations where these studies were carried out. This idea may be supported by the findings of other researchers who have suggested that the distribution of different types *Salmonella* varies based on the geographical location. According to Hendriksen *et al.* (2011), the majority of salmonellosis cases in humans are caused by a limited number of *Salmonella* serovars which may vary over time from one country to another. According to McEvoy *et al.* (2003), the higher prevalence in the incidence of *Salmonella* may be among other factors due to geographical variation. These suggestions imply that different types of *Salmonella* serovars may be expected to be more prevalent in this region and in Namibia in particular as opposed to other regions. Namibia weather pattern is different from many countries and is among of the driest countries in the world which means may have different growth conditions as compared to other countries.

Nevertheless, *S.* Typhimurium was the most frequently isolated strain in meat fluid and was among the top four when the three beef products were analyzed together. This finding suggests that *S.* Typhimurium is an important bacterium of public concern in Namibia as it is in other parts of the world. In the US, 43 % of all *Salmonella* isolates from human sources are only from three types of strains; *S.* Enteritidis, *S.* Newport and

S. Typhimurium which the later contributes to 11 % of all *Salmonella* outbreaks (Morbility and Mortality Weekly Report [MMWR], 2013). However, there is not information that can be used to estimate the potential threat of *S.* Typhimurium in Namibia.

During 2009 - 2010, a total of 1,527 foodborne disease outbreaks were reported, resulting in 29,444 cases of illness, 1,184 hospitalizations, and 23 deaths of which *Salmonella* accounted for 30 % of the foodborne outbreaks in the US (MMWR, 2013). These findings suggest that *S.* Typhimurium could be among the pathogenic strain in relation to salmonellosis in humans as compared to other strains. Although other serovars exist this type of strain could be among the majors cause of illnesses in Namibia like elsewhere in the world.

The most notable *Salmonella* strain in this study was *S.* Chester. Although this strain is not frequently reported among the most frequently isolated strain in foods worldwide, this serovars seem to be the most common stain in Namibia. Similarly, *S.* Chester strain may be the major cause of foodborne illnesses in Namibia as opposed to other countries. This is because there is a possibility of the most frequently isolated strains in foods to be transmitted to humans through the food chain due to cross contamination. In this case the infection can happen through the consumption of contaminated beef or beef products. Although the official records on the per capita meat consumption are not available, Namibians regard themselves as among the high meat consumers at least in

Africa. According to the CDC Surveillance for Foodborne Disease Outbreaks for the year 2009 – 2010, beef contributes up to 11 % of the foodborne outbreaks in the US (MMWR, 2013).

The argument of the link between foodborne infections and food consumption can be supported by the findings of other scholors elsewhere. According to a study in Australia, wide diversity of *Salmonella* serovars, all of which have been isolated from humans, was identified in both cattle and sheep (Vanselow *et al*., 2007). In the US and Canada, *S.* Heidelberg is among the most frequently isolated serovars both in clinical cases of salmonellosis and from retail meats and food animals (Zhao *et al*., 2008) but *S.* Typhimurium and *S.* Enteritidis remain to be the most common serovars in both countries (CDC, 2012; National Enteric Surveillance Program [NESP], 2010). Another study done in food animals, meat products and slaughterhouses personnel in Ethiopia suggests the likely link of human *Salmonella* infections and food of animal origin (Ejeta *et al*., 2004).

Nevertheless, although *S.* Chester is not often linked to most outbreaks but can still be considered as one of the *Salmonella* serovars of public concern worldwide. This is because of its recent links to several salmonellosis outbreaks in North America. In 2010, *S.* Chester caused a multistate outbreak of human infections in 18 States in the US (CDC, 2012). The outbreak resulted to 37 illnesses with 7 people being hospitalized. The outbreak was linked to the consumption of commercially processed cheesy chicken

and rice product. In the same year, another outbreak of *S.* Chester in Canada which involved 26 people was due to the consumption of luncheon meat, sausage and head cheese (Food Illness Outbreak Database [FIOD], 2013).

Yet, *S.* Chester is considered to be a rare serotype of *Salmonella* in Canada with regard to human infections (Taylor *et al*., 2012). Available data from the Canadian National Enteric Surveillance Program show that there has been an increase in cases of *S.* Chester from 0.16 % (10 of 6351) in 2008 (Taylor *et al.,* 2012) to 0.58 % (42 of 7251) reported in 2010 (NESP, 2010, p. 22). However, so far there are no clinical records to determine if *S.* Chester is a major cause of most salmonellosis cases in Namibia apart of being the most frequently isolated strain in beef. However, the findings of this study suggest that the level of prevalence of *S.* Chester in beef is higher in Namibia than the ones found elsewhere in available publications.

5.1.2 Prevalence and antimicrobial resistance pattern of *Salmonella* in beef

The use of antimicrobials and antimicrobial resistance in food animals are areas of food safety concern worldwide. This is because there is an increase in the consumer awareness on the issues related to food safety and public health. On the other hand, the danger of food as a vehicle to transmit antimicrobial resistant pathogens to humans is widely reported (Barza, 2002). Although many foodborne bacteria are capable of causing illness in humans the antimicrobial resistance pathogens may however increase the severity of the disease.

In the present study the prevalence and antimicrobial resistance pattern was investigated. The study found that 24.69 % of isolates displayed resistance to at least one of the antimicrobial agents tested, with 13.58 % (N = 81) exhibiting resistance to two or more antimicrobial agents. The *Salmonella* isolates that exhibited resistances were from 12 different serotypes. However, other three isolates could not be identified. Most of the serotypes that exhibited resistance were *S.* Chester (n = 6) with three each isolated from meat and carcass swabs followed by *S.* Typhimurium with 2 isolates from carcass swabs while the rest of the serotypes had only 1 isolate that exhibited resistance. The resistance was most commonly observed to sulfisoxazole (23.46 %), trimethoprim-sulfamethoxazole (13.58 %), tetracycline (3.7 %), amoxicillin-clavulanic acid (1.34 %), cephalothin (1.34 %) and chloramphenicol (1.34 %). Most of the *Salmonella* isolates that showed resistance to two or more antimicrobials had a common resistance pattern of trimethoprim-sulfamethoxazole and sulfisoxazole.

These findings are not similar to other studies done elsewhere in the region and in other parts of the world. In a study in Botswana all *Salmonella* isolates were resistant to amikacin, cefuroxime, gentamicin, and tombramycin (Samaxa *et al.*, 2012). The four antimicrobials are common aminoglycoside antibiotics. In the same study *Salmonella* isolated from beef showed antimicrobial resistance of 90%, from pork 71%, from mutton 63%, and from chicken 35%. In Ethiopia, the report suggests that *Salmonella* are resistant mostly to ampicillin (100 %), nalidixic acid and streptomycin (87.5 %), tetracycline (50 %) and chloramphenicol (12.5 %) (Dabassa & Bacha, 2012). A study

done in Algeria found a resistance of *Salmonella* against selected 32 antimicrobials to be as much as 90.32 % with 32.26 % to be resistant to two or more antimicrobial agents (Mezali & Hamdi, 2012). Another study in developing countries on animals and food of animal origin in Malaysia, Thailand and Vietnam found the antimicrobial resistance to range between 22 – 49 %, 41 – 92 % and 17 – 68 % respectively (Hao Van, Nguyen, Smooker & Coloe, 2012).

A study in the US found the isolates from food animals to have resistance to antimicrobial agents used of up to 72 % with 24 % exhibiting resistance to eight or more antimicrobial agents used (Lynne, Kaldhone, David, White & Foley, 2009). The same study also found most of the isolates to have resistance to tetracycline (71 %), streptomycin (62 %), kanamycin (52 %) and ampicillin (33 %). In Finland, of the 73 domestic isolates, only 1 isolate from a cattle farm (strain = 2476) recovered in 1997 showed resistance to any of the antimicrobials tested (chloramphenicol and tetracycline). Five of the 37 foreign isolates were resistant to tetracycline and streptomycin was the most common (Lindqvist, Siitonen & Pelkonen, 2002).

When comparing the findings of the present study with the findings within the region, other developing and developed countries, it is clear that the problem of antimicrobials resistance of *Salmonella* in Namibia is not as bad as it in other countries. The percentage of antimicrobial resistance of *Salmonella* isolates in Namibia is lower as compared to other countries in the region, developing and developed countries. The differences

between the findings of this study and others could be due to the differences in the geographical location (Lynne *et al*., 2009), the type of antimicrobial agents which the animals often being exposed to and the extent of which such antimicrobial are used in food animals production.

In addition, this study showed that most of the antimicrobials can be used effectively for treatment of salmonellosis in Namibia. Antimicrobials such as tetracycline, streptomycin and ampicillin which seem to be less effective in countries like the US (Lynne *et al*., 2009) and Ethiopia (Dabassa & Bacha, 2012) could effectively be used to treat salmonellosis in Namibia. The current advantage of Namibia could probably be due to the strict control measures on the use of antimicrobials in both humans and animals. In Namibia antimicrobials are secured at the pharmacy or a veterinary medicine shop through a prescription from a registered a medical practitioner or veterinarian. The Namibia Medicines Regulatory Council (NMRC) is a statutory body that regulates the use of medicines in Namibia.

However, the fact that there are *Salmonella* that are resistant to antimicrobials in Namibia there is a danger that the problem may gradually grow if there are no new mechanisms to control the use of antimicrobials in food animals production and humans. In addition, there is a possibility of the transfer of antibiotic resistance determinants or pathogenic bacteria from food animals to humans which can be a major threat to public

health. However, the connection between the occurrences of the same drug-resistant bacteria between people through the use of antibiotic in food animals is not yet clear.

Several studies have shown same type of *Salmonella* strains with similar resistance pattern to have been isolated in both food animals and humans. According to a study done in the US in 2003, *S.* Newport isolates resistant to at least nine antimicrobials emerged as pathogens in both animals and humans throughout the country (Zhao *et al*., 2003). In a different study in Spain which was done in humans, food and animals found that the most frequently isolated *Salmonella* (*S.* Enteritidis, *S.* Tyhimurium and *S.* Hadar) had similar antimicrobial resistance pattern with the level of resistance to individual drugs among *Salmonella* isolated from animal samples being significant higher than the rest except for cefotaxime (Cruchaga *et al*., 2001).

Furthermore, available data by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) suggests that there is a temporal association between changing levels of contamination of retail chicken drug resistant *Salmonella* and the incidences of human infections with the same type of strain (Dutil *et al*., 2012). According to Molla, Mesfin and Alemayehu (2003), the increasing proportion of antimicrobial-resistant *Salmonella* strains isolated from human salmonellosis cases has been associated with the widespread use of antimicrobial agents in food animal production. However, the risk assessments studies done in the US on the use of animal antibiotics consistently found very low risk to human health or an increase in foodborne

illness if no antibiotics are used to control diseases (Animal Health Institute [AHI], 2013).

5.2 Prevalence of *Salmonella* and antimicrobial resistance in animal feed

The prevalence and antimicrobial resistance of *Salmonella* in feed and feed ingredients is a problem worldwide and has been well documented (Hofacre *et al*., 2001; Jones, 2011). The problem relates to a considerable health risk of salmonellosis in animals, and subsequently in the consumers of the animal products (Crump, Griffin & Angulo, 2002). This is the reason why large resources are put into the fight against *Salmonella* in feed- and fishmeal factories (Vestby, Møretrø, Langsrud, Heir & Nesse, 2009).

5.2.1 Prevalence of *Salmonella* in animal feed

Animal feed (or at least the pre-cursors of animal feed) can be a common source of *Salmonella* infection in humans (Crump, Griffin & Angulo, 2002). This may happen when the food supply becomes contaminated with *Salmonella* and the pathogen subsequently comes into contact with humans. In Namibia, animal feed made from animal protein such as blood meal, meat-and-bone meal, and carcass meal are produced locally, but they are not allowed to be fed to animals produced for food. These types of feeds are processed for pet food and for export to other countries.

However, pets may also be a source of *Salmonella* infection for humans (CDC, 2010). This may happen through a direct contact with animals or indirectly through contaminated environment by the animal excreted materials. Several other countries in the world including the US are reported to have banned the use of feed derived from animal products in ruminants (Denton *et al.*, 2005). The ban is aimed to prevent the potential occurrence of transmissible spongiform encephalopathy (TSE) in humans. Thus, microbial monitoring of animal feeds remains important in food animal production.

In this study the prevalence of *Salmonella* in feed was investigated. A significant difference ($p < 0.05$) on the prevalence of *Salmonella* was observed between blood meal and bone-and-meat meal. The reason for the higher prevalence rate in blood meal as compared to that of bone-and-meat meal is not clear. This is because all products are processed at higher temperatures that could destroy most of microorganisms including *Salmonella*. Nevertheless, the differences on the prevalence rate could probably be due to the post processing conditions of the products such as pH, nutrients and moisture content. However, these factors were not investigated in this present study.

Nevertheless, the results obtained found that the prevalence of *Salmonella* in feed was lower than in previously reported findings from other studies (Newell, McClarin, Murdocka, MacDonalda & Hutchinsona, 1959; Sartorelli, Bertechini, Fassani, Kato & Fialho, 2003). A study conducted by Sartorelli *et al.* (2003), on the nutritional and

microbial evaluation of meat-and-bone meal, found *Salmonella* contamination to be as high as 90% in the samples tested. In an Australian study, the prevalence rate of *Salmonella* in bone-and-meat meal samples was as high as 69.5% (Bensink, 1979). However, a study by Gopo and Banda (1997) found no *Salmonella* contamination in all bone-and-meat meal samples processed in Zimbabwe for export purposes.

The differences between these findings could be due to the species of animals slaughtered, the relative prevalence of *Salmonella* serovars in different animal species, processing methods and possible recontamination during handling, storage and transportation after processing. However, recontamination of animal by-products after processing is usually believed to be the principal factor accounting for the presence of *Salmonella* in the final product (Denton *et al.*, 2005; Franco, 2006). This is because *Salmonella* is heat labile such that it cannot survive in higher temperatures that are used in the production of animal by-product feed meal. The recontamination of bacteria could be due to the fact that bacteria seem to be able to persist in the factory because biofilm formation that protects bacteria against environmental stress, e.g. disinfection and air drying at surfaces (Vestby, Møretrø, Langsrud, Heir & Nesse, 2009). In the biofilm, the cells are embedded in a self produced matrix which may act as a barrier to protect them against chemical and other unfavorable conditions in the surroundings (Vestby *et al.*, 2009).

The prevalence of the isolated *Salmonella* strains in feed samples in Namibia showed *S.* Chester to be isolated more often than other serovars. The six most frequently isolated serovars were *S.* Chester with 21 isolates, followed by *S.* Schwarzengrund with 11, *S.* Chartres and *S.* Anatum with 4 isolates each and then by *S.* Infantis, *S.* Typhimurium and *S.* Braenderup with each having the 3 isolates. *Salmonella* Enteritidis was not isolated at all.

These findings were different from other studies where *S.* Enteritidis was found to be the most commonly isolated *Salmonella* serovar. Bouchrif *et al*. (2008) reported *S.* Enteritidis and *S.* Anatum to be the most common serovars isolated in Africa, whereas *S.* Infantis followed by *S.* Enteritidis and *S.* Typhimurium constituted the largest proportion of isolates in Europe. However, few studies, if any, have been done on this subject in this part of Africa to enable comparison of the present results with previous findings.

Previous findings reported in Kenya, Malawi and Mozambique (Kariuki *et al*., 2002; Gordon *et al*., 2008; Morpeth *et al.*, 2009) showed that *S.* Enteritidis and *S.* Typhimurium were the most common clinical isolates from humans. However, the present study findings showed that *S.* Enteritidis may not be the most common *Salmonella* serovar in this part of the southern African region. A research in Sweden between 1993 and 1997 found *S.* Livingstone to be the most common serotype where

more prevalent serotypes in animal production such as *S.* Typhimurium, *S.* Enteritidis or *S.* Dublin were rarely detected in the feed production (Boqvist *et al*., 2003).

Although the present study did not focus on the biomedical side, the present findings indicate that *Salmonella* in animal feed may be a significant cause of bacterial diseases in Namibia. The association between contaminated animal feed and *Salmonella* infections in humans has been previously reported by different authors (Crump *et al*., 2002; EFSA, 2008; Molla *et al.*, 2003). According to these scholars the association is through the food chain.

While *S.* Chester is not commonly isolated from or linked to outbreaks of salmonellosis in humans in Africa, a recent report of the CDC showed that 44 people were infected with this serovar in 18 states in the US from April to August 2010 (CDC, 2010). These findings show that *S.* Chester could be an important food borne pathogen of public health concern worldwide. Such conclusions cannot be reached in this particular study unless further clinical studies are done on the pathogens isolated from humans in Namibia. On the other hand, there are few or no studies available that have isolated *S.* Chester in animal feed that could be used for comparison in this study. The isolation of *S.* Onderstepoort, which is usually referred as the 'mutton type' of *Salmonella,* was not surprising because the meat-and-bone meal was produced from bovine and ovine meat by-products.

5.2.2 Antimicrobial resistance pattern of *Salmonella* in animal feed

Concerning the antimicrobial resistance pattern of *Salmonella* strains, the susceptibility reported in this study was not similar to the findings reported previously. A study in the US has reported *Salmonella* isolates to have a susceptibility of 40% towards the 13 antimicrobials used (Arthur *et al*., 2008). A different study in Alberta, Canada found that of 3553 antimicrobial susceptibility tests conducted with 17 antimicrobials on each of 209 *Salmonella* strains isolated from food animals and foods, 11.8% of *Salmonella* isolates were resistant (Johnson *et al*., 2005). Like in beef the comparison on the prevalence and antimicrobial resistance was not done from *Salmonella* isolated from similar products. It appears that in the region there are few production plants and studies in animal feed production that can be used to make a comparison. However, comparing the findings of this study with other available studies, it appears that the prevalence of *Salmonella* in animal feed in Namibia does not appear to be similar to that observed in many other countries where fewer effective antimicrobials are available for treatment of *Salmonella* infections.

The findings of the present study regarding patterns of antimicrobial resistance were different from those reported elsewhere. In other studies, most of the *Salmonella* strains were shown to be resistant to tetracycline (Speer, Shoemaker & Salyers, 1992). This study showed that only 11 of the 23 resistant strains were resistant to sulfisoxazole and the trimethoprim-sulfamethoxazole combination, whereas two strains were additionally resistant to tetracycline and one to nalidixic acid. These results were unexpected

especially for tetracycline which has long and extensively been used in Namibia as it has been in many other parts of the world (Speer *et al*., 1992). The findings of this study suggest that there is a need to control the introduction of tetracycline resistant pathogens into Namibia. The country should further ensure the prudent use of tetracycline, in order to avoid development of resistant strains towards this antimicrobial.

Resistance to nalidixic acid was present in three isolates of two different serovars and to sulfisoxazole and trimethoprim-sulfamethoxazole in isolates of twelve serovars. In fact, sulfisoxazole and, sulfisoxazole and trimethoprim-sulfamethoxazole were the most *Salmonella* resistance pattern observed. These findings may probably suggest that sulfisoxazole and trimethoprim-sulfamethoxazole may not be used to effectively treat salmonellosis in Namibia for both humans and animals. This is based on the facts that some of the strains showed up to 100 % resistance to these drugs.

Although these frequencies of *Salmonella* that are resistant to antimicrobials were low, the resistance observed may still be of a public health concern if these foodborne pathogens enter the food chain. This is because the animal feed used in this study was produced from the by-products of meat which suggest the possibility of such pathogens being present in the food chain. Although *S.* Chester was found to be the most frequently isolated strain in feed, most of the isolates of this strain showed to be susceptible to most of the antimicrobials used in this study. These findings suggest that a range of

antimicrobials could be effectively used to treat salmonellosis cases caused by *S.* Chester in Namibia.

5.3 Comparing the prevalence and antimicrobial resistance of *Salmonella* in beef and animal feed

Animal feed is one of the major sources of *Salmonella* infection in both humans and food producing animals. *Salmonella* can pass through the entire food chain from animal feed, primary production, and all the way to households or food-service establishments and institutions. On the other hand, rendered bacteria contaminated animal protein products used in animal feeds production can potentially serve as a source of antibiotic-resistant bacteria (Hofacre *et al*., 2001). The link between animal feeds and both human and animal salmonellosis has been investigated and established with many scholars (Jones, 2011).

5.3.1 Prevalence of *Salmonella*

The general prevalence rate of *Salmonella* in both beef and animal feed was 1.72 % (n = 10 335). Although animal feed had a higher prevalence rate of *Salmonella* than that observed in beef, there was no significant difference ($p > 0.05$) on the prevalence between the two products. The reason for this is not clear but the two products were different and were subjected to different processing, storage and packaging conditions.

However, good hygine practice during processing, handling and the storage conditions at the plants could have been the reason for the similar prevalence rate.

Of the *Salmonella* serovars isolated in this study, *S.* Chester could probably be the most prevalent *Salmonella* serovar in Namibia. This is because *S.* Chester represented 18.54 % of all *Salomella* isolates in both beef and animal feed samples. Other *Salmonella* strains that showed high prevalence rate in Namibia are *S.* Schwarzengrund, *S.* Anatum, *S.* Typhimurium, *S.* Braenderup and *S.* Reading. These serovars were also found to be individually the most frequently isolated *Salmonella* in both beef and animal feed samples.

The findings that *S.* Chester is the most prevalent serovars could also mean that the same serovars is among the top serovars that cause salmonellosis in Namibia. In the US, the outbreak implicating *S.* Chester in 1990 affected at least 245 persons in more than 30 States (Tauxe *et al*., 1997). The fact that no *S.* Entritidis was isolated in all samples does not suggest that the strain is not prevalent in Namibia. This is because *S.* Entritidis may be prevalent in other products which were not investigated in this study. However, these findings suggest that *S.* Entritidis is not among the top *Salmonella* strains that cause infections in both humans and animals. *Salmonella* Entritides has been reported to be among the top most frequently isolated *Salmonella* in many countries.

However, the results of this study suggest that the rate of *Salmonella* prevalence is significantly higher in animals feed samples as compared to beef samples. The reason here is not clear because *Salmonella* is exposed to higher temperatures during the production of animal feed. These production temperatures are lethal and are expected to produce a product that is free from bacterial contamination such as *Salmonella*. The only possible explanation for *Salmonella* contamination in feed is due to post processing handling practices.

Unlike animal feed production, beef did not undergo any process that would have inhibited or prevented *Salmonella* growth. The lower prevalence rate in beef could be due to the good slaughtering practices, proper implementation of the HACCP system and proper maintenance of the cold chain conditions during the process, packaging and storage. On the other hand, the prevalence of *Salmonella* on animal feed could be the result of post processing contamination during handling, packaging and storage.

The findings of this study show that some of the most frequently isolated strains were the same in both beef and animal feed. These findings suggest that *Salmonella* strains can survive different conditions in the production chain and be able to infect the human through the food chain. Nevertheless, a number of researchers have questioned this link between *Salmonella* serotypes in feed and those commonly cause diseases (Jones, 2011). However, the findings of the present study show the possibility of such link to exist if contaminated animal feed is used in food production.

5.3.2 Antimicrobial resistance

Overall, *Salmonella* isolates exhibited resistance to only nine of the 16 (56.25 %) of the antimicrobials used. At least 27.53 % these serovars exhibited resistance to antimicrobials tested of which 10.11 % exhibited resistance to at least one of the antimicrobials where the rest exhibited resistance to at least two or more antimicrobials tested. These findings are higher than those observed in Kenya where only 5 % of *Salmonella* exhibited MDR characteristics although the total prevalence of *Salmonella* antimicrobial resistance in Kenya was higher (31.3 %) as compared to that observed in the present study. However, the study in Kenya was done on *Salmonella* obtained from clinical cases where the rate of resistance is likely to be higher because of the repeatedly exposure of same bacteria to same drugs. Nevertheless, the findings of the current study found the resistance of *Salmonella* isolates to be lower as compared to other findings elsewhere.

According to Dabasa and Bacha (2012), more than 80 % of the *Salmonella* isolates from an abattoir in Ethiopia exhibited resistance to at least one type of antimicrobial tested where at least 50 % of the isolates exhibited resistance to two or more antimicrobials tested. A study done in Ghana on the susceptibility tests of pathogenic bacteria isolated from milk found that 100 % of *Salmonella* isolates were resistant to all antimicrobials used (Mahami, Odonkor, Yaro & Adu-Gyamfi, 2011). The antimicrobials used included; Ampicillin, Tetracycline, Chloramphenicol, Gentamycin and cefotaxime.

The fact that 72.47 % of the resistant strains did not show antimicrobial resistance suggests that the problem of antimicrobials resistance among *Salmonella* is still low in Namibia. However, 10.11 % of *Salmonella* were resistant to one antimicrobial, 13.48 % were resistant to two antimicrobial, 2.82 % were resistant to three antimicrobial and 1.12 % were resistant to four antimicrobial. The later findings suggest that the development of *Salmonella* resistance to antimicrobials may also be increasing in Namibia.

One of the interesting findings on the resistance pattern of *Salmonella* in this study was on the two strains of *S.* Schwarzengrund. The two strains of *S.* Schwarzengrund one isolated from swabs and the other from bone-and-meat meal exhibited the same MDR pattern to Trimethoprim-sulfamethoxazole, Sulfisoxazole and Tetracycline. These findings may suggest that there is an exisisting problem of *S.* Schwarzengrund resistant strains in Namibia towards these three antimicrobials. Multi-drug resistant *S.* Schwarzengrund has been reported to cause illnesses in some countries including Denmark and the US (Aarestrup *et al*., 2007). The international spreading source of *S.* Schwarzengrund strain has been linked to imported foods from Thailand. *Salmonella* Schwarzengrund is among the few *Salmonella* serovars that cause invasive salmonellosis and have limited number of available antimicrobials for treatment.

Comparing previous findings with those of the present study, the strain obtained in Namibia does not have similar resistance pattern. A study done in Denmark, Thailand and the US found high frequencies of Nalidixic acid resistant *S.* Schwarzengrund with

reduced susceptibility to ciprofloxacin (Aarestrup *et al*., 2007). In a different study done in Taiwan, *S.* Schwarzengrund exhibited multidrug-resistance and demonstrated high resistance to ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, nalidixic acid, trimethoprim-sulfamethoxazole and chloramphenicol (Chen *et al*., 2010). On the other hand, a more recent study in the US found *S.* Schwarzengrund exhibiting resistance to fluoroquinolone (Akiyama & Khan, 2012). *Salmonella* Schwarzengrund exhibiting resistance to fluoroquinolone was reported as early as 1996 in the US (Olsen *et al*., 2001). Unlike the present study these studies were done from other foods and clinical samples.

Comparatively, the desirable attribute about the findings of the present study was that most of the *Salmonella* showed resistance only to antimicrobials from the first and second generation. This could probably reflect on the prudent use of antimicrobials in Namibia. The control on the use of antibiotic in humans, animals and feed may positively help to reduce the rate of antibiotic resistance. According to Yavar (2012), the efficient policy towards controlling the antibiotic resistance by effective management and regular prevention programs has successfully managed to control the antimicrobial resistance in Sweden.

The fact that all strain of *Salmonella* did not show resistance to Cefotaxime, a broad spectrum third generation antibiotic, shows that Namibia may have little problem in controlling antimicrobial resistance *Salmonella* infections at the moment. The

emergence of *Salmonella* strains resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole suggest that third generation cephalpsporin such as Cefotaxime be considered as drug of choice for treatment of *Salmonella* infections (Mahami *et al.*, 2011). This antimicrobial is also one of the cephalosporins which is frequently recommended for treatment of *Salmonella* infections in children because of their pharmacodynamic properties and their very low prevalence of resistance. *Salmonella* isolates resistant to Cefotaxime have been isolated in a number of countries and reported in various studies including Ghana in Sub Sahara Africa (Mahami *et al.*, 2011). According to Fey *et al*. (2000), the resistance of *Salmonella* to Ceftriaxone was also reported in other several countries as early as 1991. These countries include Argentina, Turkey, Algeria, Saudi Arabia, Greece, Tunisia and France.

A study done in Petersburg, Russia found 100 % of *S.* Typhimurium exhibiting resistance to Cefotaxime (Gazouli *et al*., 1998). *Salmonella* resistant to Cefotaxime has also been rarely isolated in Hong Kong (Cheung *et al*., 2005). The 100 % of *Salmonella* susceptibility to Cefazolin, Gentamicin, Kanamycin, Enrofloxacin, Cefotaxime, Streptomycin and Ciprofloxacin suggest that the proportion antimicrobials that are effective for treatment of *Salmonell*a is larger than in other countries.

Another interesting finding of this study was the small percentage of *Salmonella* isolates that exhibited resistance to tetracycline as compared to available findings elsewhere. Tetracycline has long and extensively been used in the treatment of various bacterial

diseases Namibia as it has in many parts of the world. A study done in Ethiopia on the antimicrobial resistance of *Salmonella* isolated from abattoir between December 2009 and May 2010, showed resistance to tetracycline to be 50 % (Dabassa & Bacha, 2012). A different study done in Algeria on *Salmonella* isolated from meat and meat products showed the resistance to tetracycline was at 12.90 % (Mezali & Hamdi, 2012).

According to Johnson *et al*. (2005), a study on the resistance of selected *Salmonella* from food animal and food in Alberta found the resistance to be highest on tetracycline (35.4 %) as compared to other 17 antimicrobials tested. In a different study conducted in Vietnam in retail raw foods, 40.2 % of *Salmonella* isolated showed to be resistance to tetracycline. In Australia, the resistance to antimicrobial agents is as low as 0 - 5% of isolates for most of the antimicrobials, with the exception of resistance to tetracycline which the resistance is as high as 16% of isolates (Page, 2009).

However, the results of this study found that 37.50 % of *S.* Typhimurium strains were resistant to only trimethoprim-sulfametoxazol and sulfisoxazole. This outcome suggests that probably there are no *S.* Typhimurium phages typed as DT104 strains in Namibia that is commonly known to exhibit a common MDR pattern. The epidemic strain of DT104 is consistently resistant to at least five antimicrobial drugs, ampicillin, chloramphenicol, streptomycin, sulphonamide, and tetracycline (R-type ACSSuT) (Besser *et al.*, 2000). The *S.* Typhimurium DT104 is reported to be food borne pathogen of public health significance in Europe, North America and the Middle East (Humphrey,

2001). A study done by McEvoy *et al*. (2003) on the prevalence of *Salmonella* in bovine faecal, rumen and carcass samples at a commercial abattoir found all *S*. Typhimurium DT104 isolates to exhibit this type of multi-drug resistant pattern.

Although the *S.* Typhimurium isolated in this study did not have the ACSSuT profile the resistance of the three *S.* Typhimurium strains to trimethoprim-sulfametoxazol and sulfisoxazole in this study could be comparable to other findings on the DT104 strains. Most of the studies have found the DT104 strain to be resistant sulfisoxazole, where in UK the DT104 have shown to have increasing trend of additional resistance to trimethoprim (Hogue *et al*., 1997). These findings suggest the existence of some strains of *S.* Typhimurium that do not have the ACSSuT profile. However, this study could not determine if the *S.* Typhimurium strains isolated were of DT104 type.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In the present study, the prevalence of *Salmonella* in beef and animal feed appear to be lower than those observed in both developed and developing countries. This could be due to effective implementation and maintenance of the HACCP system in the beef and animal feed production plants in abattoirs involved in this study. The HACCP system is mandatory in Namibia for all export beef processing abattoir as per EU requirements. On the other hand, most of the *Salmonella* strains isolated in beef and animal feed were susceptible to most of the antimicrobials tested. This finding suggests that widespread antimicrobial resistance is not a significant problem in Namibia, as it is in other parts of the world. This is because in Namibia both veterinary and humans drugs can only be obtained by prescription, thereby reducing the potential misuse of antimicrobials unless such use is deliberate. However, the evidence of antimicrobial resistance in some strains emphasizes that resistance is developing and that further monitoring will be required to ensure that it does not become a problem in Namibia.

Unlike other parts of the world, *S.* Chester appears to be the most prevalent serovar in food animals in Namibia. This was evident as this strain was most prevalent in both beef and animal feed samples. However, 7 of the 33 strains of this serovar exhibited multi drug-resistant of which only 4 the serovars exhibited resistance to more than two antimicrobials used. These findings suggest that in case of infections from *Salmonella*

serovars a range of antibiotics available in Namibia may still be used to control the disease. Yet there is little information, if any, on either the prevalence of *S.* Chester in beef and animal feed, or the antimicrobial resistance pattern of this serovar in other countries which could be compared with the findings of this study. Although the surveillance of food borne pathogens in Namibia and other developing countries is minimal, antimicrobial resistance is still a major public health concern in Africa.

Although the purpose of this study was to determine the prevalence and resistance of *Salmonella* isolated from beef and animal feed against a wide range of antimicrobials, some of the drugs may have not been used in food producing animals in Namibia. However, the prevalence and the antimicrobial resistance observed among *Salmonella* strains isolated from beef and animal feed may provide evidence on the general antimicrobial resistance trend exhibited by *Salmonella* spp. in Namibia.

Overall, the findings show that the prevalence of *Salmonella* serovars and the antimicrobial resistance pattern observed from this study may vary from one source to another and from region to and region. This was evident for strain like *S.* Enteritides which is the most common strain in many countries but was never isolated in this study. Nevertheless, the findings of the present study may help improve the prudent use of antimicrobials in Namibia and may help direct the proper selection of antimicrobials for the treatment of infections in both humans and animals in Namibia. Similarly, these data

may provide important information for future control of important antimicrobial agents used in humans and animals.

6.2 Recommendations

1. The findings of this study showed that there are 44 of different types of *Salmonella* species that are prevalent in beef and animal feed. These findings suggest that at least 44 different types of *Salmonella* serovars can be a major source of infections in humans in Namibia. In order to establish if the *Salmonella* strains that were isolated from beef and animal feed are the same with the strains that cause infections in humans it is recommended that different studies be done in order to establish the similarities between the strains that cause infections in humans and those are found in beef and animal feeds in Namibia.

2. The present study found out that using these methods of isolation and identification the rate of prevalence of *Salmonella* in Namibia is lower in beef and animal feed as compared to other studies. The fact that there was *Salmonella* in these products suggests that consumers are still exposed to health risks due to *Salmonella* infections. However, lower levels than those reported in other findings suggest that the HACCP system may be the useful tool to minimize the risks. Therefore, it is recommended that the HACCP system should be mandatory in all food production establishments in Namibia as a tool to reduce the health risks to consumers. Unlike

now where HACCP is mandatory to the EU export abattoirs, the system should be extended to all local slaughtering abattoirs, food processing and retail outlets. Food safety education and awareness from farm to fork may help to reduce the prevalence of *Salmonella* and health risks to consumers.

3. The problem of antimicrobial resistance seems to be small in Namibia as compared to other countries although the presence of antimicrobial resistant *Salmonella* showed that the problem is still developing. Therefore, in view of this study finding it is recommended that Namibia should develop and implement new comprehensive measures and policies on the use of antimicrobials. The new measures and policies should focus on ensuring the safe and prudent use of antimicrobials in both humans and animals in order to prevent the growth of antimicrobials resistance in Namibia.

4. On the other hand, the MDR bacteria are becoming a worldwide problem causing fewer antimicrobials be available for treatment making it difficult in deciding the effective antimicrobials to be used for treatment of both humans and animals. The availability of the information on the antimicrobial resistance pattern may assist in decision making during treatment of humans and animals. It is, therefore, recommended that medical practitioners and Veterinarians use available information on the susceptibility tests of different bacteria during treatment. The information of this study may help on the selection of antimicrobials and effective treatment of *Salmonella* infections for both humans and animals in Namibia.

5. The investigation on the prevalence of this study has helped to understand what types of *Salmonella* serovars are the most prevalent in Namibia beef industry. The opening of *Salmonella* data bank will help to preserve the current *Salmonella* serovars in Namibia and the same strains may be used for further future studies. This is because the available data from the present study suggests that *Salmonella* serovars that are most prevalent in other parts of the world are not the most prevalent serovars in Namibia. Similarly, the antimicrobial resistance patterns of *Salmonella* serovars observed in this study were different from those found in other studies elsewhere.

6. Some of serovars (12.92 %) could not be conclusively identified using the serological method according to Kauffman-White scheme. This observation suggests that some other techniques may be useful to complement conventional methods when doing this type of study. In future studies, it is recommended that some molecular techniques be considered when doing the identification of *Salmonella* serovars. This will ensure that all untyped strains are conclusively identified.

6.3 Unique science contribution of the study

1. The study found the most prevalent *Salmonella* serovars in Namibia are different from that reported to be the most frequently isolated elsewhere in the world. This finding probably implies that there are differences in the distribution of *Salmonella*

serovars from one geographical region to another. This could be due to the differences in climatic conditions and other diversity on the geographical conditions that exist from one region to another.

2. This study found that *S.* Chester as the most prevalent *Salmonella* serovar in beef and animal feed. This finding is different from other studies done elsewhere. The findings from other studies have linked the prevalence of *S.* Chester with other products other than beef or animal feed. Although *S.* Chester was found to be the most prevalent strain in Namibia this strain is considered to be a rare strain among the *Salmonella* isolates in other countries and is not linked to beef and animal feed.

3. The rate of prevalence of antimicrobial resistance in *Salmonella* and the MDR problem is low in Namibia as compared to both developed and developing countries. Similarly, *Salmonella* isolated in this study showed resistance to fewer antimicrobials than what have been reported in other countries. This includes tetracycline, a common antimicrobial which has been long widely used in the treatment of different infections. Unlike other countries there a range of antimicrobials that can be selected for treatment of *Salmonella* infections in Namibia. These findings further suggest that the prevalence of antimicrobial resistance may be managed with the prudent use of antimicrobial agents. Unlike in Namibia, in developed countries there is extensive use of antimicrobials in food

animal production where in most developing countries there is poor management on the use of antimicrobials in both humans and animals.

CHAPTER 7: REFERENCES

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Appendix 1

Salmonella serotypes, antigenic formula and antimicrobial resistance pattern for the first batch

SEROTYPES AND ANTIGENIC FORMULAR	AM	CFZ	K	SXT	COL	AN	CTX	CF	AMX + ACL	GM	ENR	TE	SUL	S	C	CFX
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Eppendorf (4; d; 1,5)	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Chartres (4; e,h; l,w)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
*S. S*chwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chartres (4; e,h; l,w)	S	S	S	R	S	S	S	S	S	S	S	S	R	I	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chartres (4; e,h; l,w)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S

S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10; e,h; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Saintpaul (4,5; e,h; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Eppendorf (4; d; 1,5)	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Sandiego (4; e,h; e,n,z15)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Onderstepoort (6,14,25; e,h; 1,5)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Beaudesert (6,14,25; e,h; 1,7)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Fischerkietz (6,14,25; y; e,n,x)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Bredeney (4;l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Minnesota (21; b; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
*S. S*aintpaul (4,5; e,h; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Ball (4; y; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Ball (4; y; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Sajam (4,27; d; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Brezany (4; d; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Stanley (4; d; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Fischerkietz (6,14,25; y; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S
S. Southbank (3,10,15; m,t; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Saintpaul (4,5; e,h; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Djugu (6,7; z10; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Petahtikve (3,19; f,g,t; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Vaertan (13,22; b; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Mbandaka (6,7; z10; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Parkroyal (3,19; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Onderstepoort (6,14,25;e,h;1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Braenderup (6,7; e,h; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 enterica sub. Enterica (6,7; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 enterica sub. Enterica (6,7; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 enterica sub. Enterica (6,7; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	I	S	S	S	R	S	S	R	R	S	S	R	S	S	I	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 enterica sub. Enterica (6,7; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Fischerkietz (6,14,25; y; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Parkroyal (3,19; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Djermania (28; z29; -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Petahtikve (3,19; f,g,t; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Infantis (6,7; r; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Winston (16; m,t; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Braenderup (6,7;e,h;e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Infantis (6,7; r; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Parkroyal (3,19; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Salamae (18; z4,z23;)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Cannstatt (3,19; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Fischerkietz (6,14,25; y; e,n,x)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Kaapstad (4,12; e,h; 1,7)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Petahtikve (3,19; f,g,t; 1,7)	S	S	S	R	S	S	S	S	S	S	S	S	R	I	S	S
S. Anatum (3,10; e,h; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Kaapstad (4,12; e,h; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
*S.*Typhimurium (4,5;i;1,2)	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bahrenfeld (6,14,24; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4; d; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella enterica sub. Enter (18; z4,z23;)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group I (16; -; -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Cannstatt (3,19; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bredeney (4; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bredeney (4; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chichiri (6,14,24; z4,z24; -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bredeney (4; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Dublin (9,12; g,p)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bredeney (4; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10; e,h; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10; e,h; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10; e,h; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Svedvi (3,19; l,v; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Svedvi (3,19; l,v; e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Winston (16; m,t; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Cannstatt (3,19; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Typhimurium (4,5; i; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group K (18;-;-)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4; e,h; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Newport (6,8; e,h; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Kintambo (13,23; m,t; -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Vaertan (13,22; b;e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Bredeney (4; l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	I	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S
S. Reading (4; e,h; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 enterica sub. Enterica (6,7; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Banana (4; m,t)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Uganda (3,10: l,z13: 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chartres (4; e,h; l,w)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Djermania (28; z29; -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Aflao (6,14,25; l,z28; e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Brezany (4; d; 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Infantis (6,7; r; 1,5)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Appendix 2

Salmonella serotypes, antigenic formula and antimicrobial resistance pattern for the second batch

SEROTYPES AND ANTIGENIC FORMULAR	AM	AMC	CZ	GM	K	ENO	SXT	TE	CAZ	CL	SUL	NA	S	C	CF	CIP
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Lamberhurst (3,10: e,h: e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. enterica subsp. salamae (9,12: g,m,t: -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. enterica subsp. salamae (6,7: a: z42)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	S
S. enterica subsp. salamae (6,7: a: z42)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Newlands (3,10: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. enterica subsp. salamae (4: z: z39)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. enterica subsp. Salamae (9,12: g,m,t: -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 (6,7: r: -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Saintpaul (4,5; e,h; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. enterica subsp. *salamae* (13,22: z29: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. enterica subsp. *salamae* (13,22: z29: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Sao (3,19: e,h: e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	I	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	I	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Schwarzengrund (4: d: 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Salmonella Group C1 (6,7: r: -)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. Bredeney (4;l,v; 1,7)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. enterica subsp. *salamae* (6,8: z29: -)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. Schwarzengrund (4: d: 1,7)	S	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	R	I	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I

Salmonella Group D 2 (9,46:-:1,7)	S	S	S	S	S	S	R	S	S	S	R	S	I	S	I	S
S. enterica subsp. salamae (6,7: a: z42)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Sao (3,19: e,h: e,n,z15)	S	S	S	S	S	S	R	S	S	S	R	R	S	S	I	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	R	I	S	S	R	I	S	R	S	S
S. Lamberhurst (3,10: e,h: e,n,z15)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	R	R	S	S	R	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	R	I	S	S	R	S	S	S	S	S
S. Cerro (6,18: z4,z23: -)	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S
S. Cerro (6,18: z4,z23: -)	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S
S. Uganda (3,10: l,z13: 1,5)	S	S	S	S	S	S	R	S	S	S	R	S	S	S	S	S
Salmonella Group C1 (6,7: -: 1,7)	S	S	S	S	S	S	S	S	S	S	R	S	I	S	S	S
S. enterica subsp. salamae (9,12: g,m,t: -)	S	S	S	S	S	S	I	S	S	S	R	S	S	S	S	S
S. Schwarzengrund (4: d: 1,7)	S	S	S	S	S	S	R	R	S	S	R	S	S	S	S	S
S. Schwarzengrund (4: d: 1,7)	S	S	S	S	S	S	R	R	S	S	R	S	S	S	S	S
S. Anatum (3,10: e,h: 1,6)	S	S	S	S	S	S	I	S	S	S	R	S	I	S	S	S
S. Chester (4: e,h: e,n,x)	S	S	S	S	S	S	I	S	S	S	R	S	S	S	S	S
S. Sao (3,19: e,h: e,n,z15)	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
S. Typhimurium (4,5; i; 1,2)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S