



**An investigation on the prevalence and drug susceptibility of *Salmonella enterica* and
Shigella dysenteriae pathogens in Beitbridge town**

BY

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Abstract

Salmonella enterica and *Shigella dysenteriae* are different groups of Gram-negative bacteria. *Salmonella* infection affects the small and large intestine (enterocolitis) whereas *Shigella* infection affects the colon (colitis). Diarrhea, abdominal pain and fever are the main symptoms in both diseases. The study was conducted among Beitbridge residents who visited Beitbridge District Hospital suffering from diarrhea in the months December 2017 and January 2018. The aim of the study was to determine the prevalence of *Salmonella enterica* and *Shigella dysenteriae* in Beitbridge residents. Determining the most effective antibiotic in the treatment of the patients with *Salmonella enterica* and *Shigella dysenteriae* was also of great concern. After culturing 154 stool samples, twenty-seven (7.1%) were found to be infected with *Salmonella enterica* and *Shigella dysenteriae* or both. Children below the age of 12 years (8.4%) and female (9.7%) were mostly infected with *Salmonella enterica* and *Shigella dysenteriae* for they are the most active groups in the society. *Salmonella enterica* and *Shigella dysenteriae* were resistant to at least one of the six antibiotics used. The most effective antibiotics were ceftriaxone and ciproflacillin (were both susceptible to 100% of *Salmonella enterica* and *Shigella dysenteriae*) and this showed that these two antibiotics are the most effective drugs for treatment of *Salmonella enterica* and *Shigella dysenteriae*. It was concluded that *Salmonella enterica* and *Shigella dysenteriae* prevalence is high in the high-density area (14.9%) than in the low-density area (2.6%) of Beitbridge town. *Salmonella* and *Shigella* infections are reported urgently after isolation according to Ministry of Health. Measures by the local authority and Ministry of Health should be put in place to reduce prevalence of the two infectious bacteria.

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Dedication

To the Lord Almighty.

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1. CHAPTER 1: Introduction

Beitbridge is situated on the border of Zimbabwe and South Africa, spanning the Limpopo River. It is a port of entry and immigration post, handling millions of goods and traffic between Zimbabwe and the Southern African Region. The town was established in 1929, and was named after Alfred Beit, a British South African financier (Montreal Gazette, 1929.)

Beitbridge town lies north of the Limpopo River about one kilometre from the Alfred Beit Road Bridge. The main roads lead from the border 321 km north-west to Bulawayo and 585 km north-east to Harare via Masvingo. According to the 2002 population census, the town had a population of 22,387 dominated by the local Venda people, who are also found across the international border in the Vhembe District of the Republic of South Africa and it is now home to over 26,459 people and the population comprises of different nationalities although the majority are local Zimbabweans (Matebeleland South Zimstat, 2012). The Beitbridge border post is the busiest road border post in Southern Africa. It acts as the corridor which links South African ports to the rest of the sub region. Cargo going as far as the Democratic Republic of Congo, Angola and Malawi passes through Beitbridge Border Post making it a hub for international trade within Southern Africa (Zimbabwe Revenue Authority, 2014).

According to TipTopGlobe.com, Beitbridge has an estimated 2,570 houses in formal settlements primarily for government officials and mid-level private sector staff and 3,000 in informal settlements. Average house occupancy in the low-income and informal settlements varies considerably, as many people do not bring their families to Beitbridge but includes at least four people. Squatter settlements are also very high in Beitbridge town (The Chronicle, November 2017). Recreational facilities are limited in low-income areas, consisting largely of bars and soccer pitches. (TipTopGlobe.com)

Formal employment in Beitbridge is on the decline. Civil service, agriculture, construction, retail, freight forwarding and customs, are the few industry sectors that provide formal employment. Informal sector activities, such as vending, cross-border trading, “briefcase” clearing agents are now a major source of employment and most of these illegal trade operations are showing positive growth.

1.1 *Salmonella enterica*

Salmonellae are gram-negative motile bacilli (Buckle *et al.*, 2012). The genus *Salmonella*, which belongs to the family Enterobacteriaceae, was named after Daniel E. Salmon, an American veterinarian who first isolated *Salmonella choleraesuis* from pigs with hog cholera in 1884 (Cowden *et al.*, 2009). They cause a wide range of human diseases such as enteric fever, gastroenteritis, endocarditis and bacteraemia (Ozumba *et al.*, 2005). *Salmonella* associated infections do not persist with distinct clinical features, other bacterial, viral and even protozoans may mimic its presentations. More than 2 300 *Salmonella enterica* serotypes have been described. Only *Salmonella enterica serovar Typhi*, *Samonella paratyphi*, *Salmonella typhimurium*, *Salmonella enteridis*, *Salmonella cholerasuis*, *Samonella hadar*, *Salmonella virchow* and *Salmonella dublin*, among others, play important epidemiological and spizotiological roles (John, 1996).

Human infections with *Salmonella enteritidis* have been increasing worldwide since 1980 (Cowden *et al.*, 2009). *Salmonella enteritidis* is an important serotype of *Salmonella* comprising 37% of *Salmonella* isolates in Maryland since 1990 (Morris *et al.*, 2012) and 28% in the north-eastern United States in 1986 (St Louis *et al.*, 1988). It is normally found in the intestinal tracts of humans and of animals such as chickens and domestic livestock and remains an important cause of gastroenteritis in humans worldwide. It is usually acquired by ingestion of contaminated water and food, mainly poultry, eggs and egg products (St Louis *et*

al., 1988; Cowden *et al.*, 2009). Eggs are the most probable primary source of contamination in many outbreaks of *Salmonella enterica* infection in developed countries (St Louis *et al.*, 1988).

An estimated 21.6 million new cases of enteric fever with about 216, 510 deaths occurred globally in 2000 (Su and Chiu, 2001) Salmonellosis (disease caused by *Salmonella* infection) caused about 5.4 million illnesses in the same year. *Salmonella enterica* causes 1.2 million infections, 24,000 hospitalizations, and 450 deaths in USA very year. Although more than 2,500 serotypes of *Salmonella* exist, only 50 serotypes are regularly isolated from humans (Scallan *et al.*, 2011). Antimicrobial drug-resistant strains of *Salmonella* are associated with more severe illness and are more likely to result in bloodstream infection, hospitalization, and death than are illnesses caused by drug-susceptible strains (Folster *et al.*, 2012). Most cases of *Salmonella* infections in the world occur in the middle-income and low-income countries where sanitation is poor and water supply is inadequate. Endemic enteric fever is common in the Indian subcontinent, South-East and Far East Asia, Africa, Central and South America, and the Mediterranean region (Werner *et al.*, 2016). Enteric fever was reported to be endemic in Far East Asia, Middle East, Central and South America (Kotloff *et al.*, 2013). It remains a serious problem in Zimbabwe (World Health Organization, 2017). Reports by World Health Organisation (2017) on pathogenesis of enteric fever in Harare, the capital of Zimbabwe, experienced several enteric infection epidemics in recent years: in 2008 and 2009, and again in 2010, epidemics of cholera affected large parts of the country. These epidemics have been linked to chronic underinvestment in the maintenance of water and sanitation infrastructure, leading to irregular water supplies, difficulties in protecting drinking-water supplies, and the breakdown of sanitation systems. The Zimbabwean health ministry published that at least 10 people died and more than 1,800 were infected with enteric fever since the outbreak began in October 2016 (World Health Organization, 2017).

1.2 *Shigella dysenteriae*

Shigella is a group of gram-negative, facultative intracellular pathogens (Lesser and Miller, 2001). The genus is divided into four species or groups: *Shigella dysenteriae* (Group A), *Shigella flexneri* (Group B), *Shigella boydii* (Group C), and *Shigella sonnei* (Group D). Group A, B, and C can be further divided into multiple serotypes: *Shigella dysenteriae* (15 serotypes), *Shigella flexneri* (16 serotypes), and *Shigella boydii* (20 serotypes) (Mounier *et al.*, 2012). Group A, B, and D are the major disease-causing species: *Shigella flexneri* is the most common species and accounts for 60% of shigellosis cases in the developing world; *Shigella sonnei* causes 77% cases in the developed world, and most cases are associated with foreign travelers; *Shigella dysenteriae* is usually the cause of epidemics of dysentery, particularly in confined populations such as refugee camp (Thiem *et al.*, 2014).

Shigellosis, also known as bacillary dysentery or *Shigella* dysentery, is an enteric bacterial infection caused by a group of *Shigella* bacteria (Gaston, 2003). Shigellosis is still a major health problem in many parts of the world, especially in the developing countries (Kotloff *et al.*, 2013). The World Health Organization (WHO) pointed out that the global number of shigellosis was about 165 million per year, of which 163 million were in underdeveloped and developing countries (World Health Organisation, 2015). A world survey by WHO in 2013 revealed that shigellosis was estimated to have caused the death of 34,000 children under the age of 5 in 2013, and 40,000 deaths in people over 5 years of age. *Shigella dysenteriae* has been responsible for large-scale regional outbreaks of dysentery in Africa, Central America, and south Asia (Bowen, 2016). *Shigella dysenteriae* epidemics have been reported in Africa since the 1800s and overall, more than 250 million people in the African region are at risk and subject to a case fatality rate of possibly 1-10%. In January 2012, more than 36 people in Harare western suburbs including Dzivarasekwa were hospitalised after succumbing to diarrhoea called shigellosis (World Health Organization, 2012).

1.3 Problem statement

Beitbridge boarder post is the busiest boarder post in Southern African Development Community (SADC) and this has led Beitbridge town into being over populated with lots of activities happening. An increase in population also means an increase in problems associated with population (Bhunia, 2008). These problems include water shortages, poor sanitation, sewage burst, opening of unregistered food outlets with poor hygiene, opening of a lot of vendors leading to people buying and consuming contaminated poultry, eggs, milk and meat with *Shigella dysenteria* and *Salmonella enterica*. These bacteria cause a wide range of human diseases such as enteric fever, gastroenteritis, endocarditis and bacteraemia. In Beitbridge, hospital records show that every month at least 10 people ranging from neonates to old age are infected by *Salmonella* and *Shigella* related diarrhea (Beitbridge District Hospital Statistics, 2016). The local authority has spent millions of dollars in trying to reduce the problems being faced by the Beitbridge town council. Progress in reducing these problems is also being hindered by the economic situation where people are failing to pay rates to the council so that it earns money for purchasing of chemicals for water treating, purchasing of water from ZINWA to reduces water problems (The Chronicles, 2017).

1.4 Justification

Contaminated food is the major mode of transmission for *Salmonellae enterica* because salmonellosis is a zoonosis and has an enormous animal reservoir (Tassios *et al.*, 2016). The most common animal reservoirs are chickens, turkeys, pigs, and cows. Dozens of domestic and wild animals also harbour these organisms. Due to the ability of *Salmonella* and *Shigella* to survive in meats and animal products that are not thoroughly cooked, animal products are the main vehicle of the transmission. In addition, changes in food handling and consumption, the growing movement of people, animals, and food products across boards, rapid

urbanisation in developing countries and the emergence of new or antibiotic resistant pathogens all contribute to increasing food safety risks (Unnevehr, 2015).

Antimicrobials like ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were first line drugs before the 1980s. However multi-drug resistance with rates more than 50% have been reported in most parts of the world. An extended spectrum of cephalosporins and flouroquinolones was reported after 1991 (Chiu. Su. Chu, 2014). The emergence of strains that are resistant to commonly used antimicrobials should be particularly noted by clinicians, microbiologists and those who are responsible for control of communicable diseases as should producers.

Food contamination with antibiotic-resistant bacteria can be a major threat to public health since the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. Antibiotic resistance poses a challenge to health of people and control of the bacteria (Threlfall, 2012).

This study will help the social community and the local authority to help fight to stop the prevalence of the bacteria by engaging in good hygienic activities, sanitation, maintenance of sewage works, improving water availability and quality. The government will also have to put strict measures on reducing unlawful entry of poultry, eggs, meat from the neighbouring South Africa for they are increasing the prevalence of *Salmonella enterica* and *Shigella dysenteria* in Beitbridge town. The emergence of typhoid out breaks in Zimbabwe in areas that include Harare, Masvingo, Chegutu in 2017 was a motive to my study.

1.5 Objective

1.5.1 Main objectives

The purpose of the study was to investigate on the prevalence and drug susceptibility of *Shigella dysenteria* and *Salmonella enterica* pathogens in Beitbridge town.

1.5.2 Specific objectives

- To positively identify *Salmonella enterica* and *Shigella dysenteria*
- To determine the prevalence of *Salmonella enterica* and *Shigella dysenteria* in the high and low-density suburbs of Beitbridge.
- To determine the distribution of *Salmonella enterica* and *Shigella dysenteria* by age and gender.
- To assess the most effective drug for treatment of the people infected with *Salmonella enterica* and *Shigella dysenteria* infection

2. CHAPTER 2: LITERATURE REVIEW

2.1 *Shigella* spp

Shigella organisms are a group of gram-negative, facultative intracellular pathogens, nonspore-forming, nonmotile, rod-shaped bacteria genetically closely related to *E. coli*. They were recognized as the etiologic agents of bacillary dysentery or shigellosis in the 1890s for whom the genus is named. *Shigella* was adopted as a genus in the 1950s. The genus is named after Kiyoshi Shiga, who first discovered it in 1897. These organisms are members of the family Enterobacteriaceae and tribe Escherichieae. They are grouped into 4 species: *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*, also known as groups A, B, C, and D, respectively (Warren *et al.*, 2006).

2.1.1 *Shigella* Diversity

Shigella remains a leading cause of childhood morbidity and mortality. The recently conducted case-controlled Global Enteric Multicenter Study (GEMS) provided a solid update on the incidence of *Shigella* among severe forms of diarrhea, and convincingly demonstrated that in the sites considered (Sub-Saharan Africa, and Asia), *Shigella* appeared amongst the top ranking pathogens identified (Kotloff *et al.*, 2013).

2.1.2 Geographical Distribution

Geographic distribution and antimicrobial susceptibility varies with different species. *Shigella dysenteriae* serotype 1 causes deadly epidemics, *S boydii* is restricted to the Indian subcontinent, and *S flexneri* and *S sonnei* are prevalent in developing and developed countries, respectively. *S flexneri*, enteroinvasive gram-negative bacteria, is responsible for the worldwide endemic form of bacillary dysentery.

Shigella is one of the leading bacterial causes of diarrhea worldwide, causing an estimated 80–165 million cases. The number of deaths it causes each year is estimated at between

74,000 and 600,000. It is in the top four pathogens that cause moderate-to-severe diarrhea in African and South Asian children.

Many observations have concluded that *Shigella* species are geographically stratified based on the level of economic development in a given country. *S. flexneri* is the primary infectious species in the developing world whereas *S. sonnei* rates increase with economic development. *S. boydii* is most commonly restricted to Bangladesh and South-East Asia and rarely occurs outside of these regions. *S. dysenteriae* type 1 (Sd1) occurs sporadically in outbreak settings with striking examples occurring in refugee camps during the civil war in Rwanda between November 1993 and February 1995 in which more than 180 thousand cases and significant mortality from Sd1 were recorded (Kerneys *et al.*, 2009). The last major Sd1 outbreak occurred in 1999 during the civil war in Sierra Leone resulting in over 4000 cases (Guerin *et al.*, 2004), and the cyclic occurrence of Sd1 in Bangladesh every 10 years is clearly discontinued for unknown reasons, illustrating major remaining uncertainties on changes of epidemiological patterns of these infections.

Outside of outbreak settings, *S. flexneri* and *S. sonnei* account for the majority of Shigellosis cases. Recent epidemiological studies conducted around the world have discovered a rise in the proportion of *S. sonnei* isolates compared to *S. flexneri*. The expansion of *S. sonnei* can clearly be observed from clinical surveillance studies conducted in China which show the proportion of *S. sonnei* isolates increasing from 17.4% in 2003–2004 to 58.2% less than a decade later, closely following the rapid industrialization in China (Mao *et al.*, 2013; Qiu *et al.*, 2015). Noticeably, regions that had undergone significant industrialization reported decreases in *S. flexneri* and increasing cases of *S. sonnei* compared to under developed areas where flexneri levels remain high (Qiu *et al.*, 2015). Rising cases of *S. sonnei* have also been detected in Bangladesh, which has historically been affected by all four species of *Shigella*. Between 2001 and 2011 the proportion of *S. sonnei* infections rose from 7 to 25% of reported

cases in Bangladesh, which also corresponds with enhanced sanitation and clean water efforts throughout the country (Das *et al.*, 2013; Hulland *et al.*, 2013). The reasons for the counter-intuitive increase of *S. sonnei* in the face of better sanitation have not been determined, however several hypotheses have been put forward (Thompson *et al.*, 2015). *S. sonnei* and *Plesiomonas shigelloides* share a common O-antigen that may lead to natural cross-protective immunity in populations that encounter high levels of *P. shigelloides* due to contaminated water supplies (Sack *et al.*, 1994). Other avenues of interest include observations of increased survival and replication of *S. sonnei* in *Acanthamoeba* which may act as a reservoir and increased ability to acquire antibiotic resistances (Saeed *et al.*, 2012). As more countries increase their level of development and sanitation it is likely that *S. sonnei* will become even more of a global public health concern which could have important impacts on vaccine development efforts.

2.1.3 Emerging Antibiotic Resistance

A major concern surrounding *Shigella* is its capacity to rapidly acquire antibiotic resistances. Development of resistance to antibiotics is common in all *Shigella* species, particularly in *S. sonnei*, which can acquire resistance genes directly from *E. coli* through horizontal gene transfer. Several recent reports have suggested that *S. sonnei* is capable of sharing resistance plasmids through conjugation with commensal *E. coli* (Qu *et al.*, 2014). Recently, *S. sonnei* resistance to the frontline antibiotic, ciprofloxacin, has been associated with travel to Asia and India and has been imported to the United States (Bowen *et al.*, 2015; De Lappe *et al.*, 2015; Kim *et al.*, 2015). Preventing the global spread of resistant *S. sonnei* strains presents a significant public health challenge as intercontinental travel has become common.

Antibiotic resistance in *S. flexneri* is also well-documented with many studies finding high rates of resistance to at least one common antibiotic such as ampicillin, tetracycline, and chloramphenicol (Khaghani *et al.*, 2014; Cui *et al.*, 2015). These studies show that without

access to proper testing facilities or multiple different antibiotics, patients affected by *S. flexneri* in developing countries have a high chance of not being successfully treated.

2.1.4 Virulence

The bacteria are gram-negative bacilli and are non-motile. They are able to resist the low pH environment of the stomach, pass through and target the epithelial cells of the colon for infection. Unlike *V. cholerae* in the small intestine, *Shigella* spp. actually invade the epithelial cells of the lower intestine, which is facilitated by a lipoprotein antigen in the cell wall of the bacterium and the production of the Shiga toxin. The organisms invade and multiply in the epithelial cells producing ulcers in the epithelium of the colon and subsequent dysentery, for which shigellosis is known. Disseminated *Shigella* infection is quite rare as the organisms typically remain localized and do not cause bacteremia. Tissue damage in the colon is most pronounced in infections with *S. dysenteriae*. The mechanism by which the organisms invade the epithelial cells is quite interesting and indirect.

The *Shigella* bacteria cannot invade the epithelial cells of the large intestine directly. Rather, they are taken up by microfold cells (M-cells) and delivered to macrophages. The bacteria are able to lyse the phagosome by which it was taken up in the macrophage. Following apoptosis of the macrophage, the *Shigella* survive and can now invade the epithelial cell by way of the Type III secretion system, which acts as a syringe for the infecting bacteria. The bacteria then infect neighboring epithelial cells by way of paracytophagy (Warren *et al.*, 2006).

2.1.5 Intestinal adherence factor

Intestinal adherence factor favours colonization in vivo and in animal models. This is 97-kD outer-membrane protein (OMP) encoded by each gene on chromosomes. This codes for intimin protein, and an anti-intimin response is observed in children with HUS.

2.2 *Salmonella enterica*

2.2.1 Classification and nomenclature

Salmonella was first discovered and isolated from the intestines of pigs infected with classical swine fever, by Theobald Smith in 1855. The bacterial strain was named after Dr Daniel Elmer Salmon, an American pathologist who worked with Smith. The nomenclature of *Salmonella* is controversial and still evolving. Currently, the Centers for Disease Control and Prevention (CDC) uses the nomenclatural system of *Salmonella* recommended by the World Health Organization (WHO) Collaborating Centre (Popoff *et al.*, 2003). The genus *Salmonella* is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis. The type species, *S. enterica*, can be further classified into six subspecies based on their genomic relatedness and biochemical properties (Reeves *et al.*, 1989). The nomenclature and classification of *Salmonella* species have been changed and restructured multiple times. Traditionally, *Salmonella* species were named in accordance with the Kaufmann-White typing system, defined by different combinations of somatic O, surface Vi, and flagellar H antigens. In 2005, *Salmonella enterica* finally gained official approval as the type species of the genus *Salmonella*. The genus *Salmonella* also contains the species *Salmonella bongori* and *Salmonella subterranean*, which was recognized in 2005.

Currently, *Salmonella* species have the serologically defined names appended as serovars or serotypes. For instance, the current nomenclature of *S typhi* is *S enterica* serovar Typhi. *S enterica* is preferred over confusing name *S choleraesuis*, which is also the name of a commonly isolated serotype. To date, more than 2500 serovars of *S enterica* have been described. Certain serovars are host-restricted, while others have a broad host range.

2.2.2 Pathophysiology

The transmission of *Salmonellae* to a susceptible host usually occurs via consumption of contaminated foods. The most common sources of *Salmonellae* include beef, poultry, and eggs. In one recent estimate, consumption of egg shell fragments contaminated with *S enteritidis* was responsible for approximately 182,060 cases of enteritis in the United States in the year 2000. Improperly prepared fruits, vegetables, dairy products, and shellfish have also been implicated as sources of *Salmonella*.

In the spring of 2008, 1442 persons across 43 states developed infection with *S enterica* serotype Saintpaul, with the same genetic fingerprint linking contaminated jalapeno and serrano peppers as a source of infection. Almost any type food product could serve a source for infection, including peanut butter, as seen during a recent outbreak of more than 600 cases. Powdered infant formula has been implicated in two consecutive large outbreaks of *S enterica* serotype Agona among infants in France.

In addition, human to human and animal to animal transmissions can occur. For example, amphibian and reptile exposures are associated with approximately 74,000 *Salmonella* infections annually in the United States. Salmonellosis outbreaks have also been associated with handling chicks, ducklings, kittens, and hedgehogs. Recently, a study of 28 cases of *Styphimurium* identified pet rodents as a previously unrecognised source of human *Salmonella* infection.

Virulence factors of *Salmonellae* are complex and encoded both on the organism's chromosome and on large (34-120 kd) plasmids. Some areas of active investigation include the means by which *Salmonellae* attach to and invade the intestine, survive within phagosomes, effect a massive efflux of electrolytes and water into the intestinal lumen, and

develop drug resistance (Parry, 2006). Several *Salmonella* pathogenicity islands have been identified that mediate uptake of the bacteria into epithelial cells (type III secretion system [TTSS]), nonphagocytic cell invasion (*Salmonella* pathogenicity-island 1 [SPI-1]), and survival and replication within macrophages (*Salmonella* pathogenicity-island 2 [SPI-2], *phoP/phoQ*).

2.2.3 Clinical manifestation

In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal salmonellosis as well as chronic carrier state.

2.2.3.1 Enteric fever

Salmonella Typhi causes typhoid fever whereas Paratyphi A, B and C cause ratyphoid fever with symptoms which are milder and a mortality rate that is lower for the latter. Both serotypes are solely human pathogens. Infection typically occurs due to ingestion of food or water contaminated with human waste. In recent years, antibiotic-resistant strains have been isolated in most endemic areas, particularly Southeast Asia, India, Pakistan and Middle East (Scherer and Miller, 2001). Roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Relapse is the most common occurrence probably due to persisting organisms within reticuloendothelial system (RES). Typhoid encephalopathy, often accompanied by shock, is associated with high mortality. Slight gastrointestinal bleeding can be resolved without blood transfusion but in 1 to 2% of cases can be fatal if a large vessel is involved. Intestinal perforation may present with abdominal pain, rising pulse and falling blood

pressure in sick people. Hence, it is very serious in 1 to 3% of hospitalized patients (Hu and Kopecko, 2003).

2.2.3.2 Gastroenteritis

Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella* Typhimurium and *Salmonella* Enteritidis being the most common serotypes in the United States. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug-resistant *S.* Typhimurium DT104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (Gray and Fedorka-Cray, 2002; Yousef and Carlstrom, 2003). Ciprofloxacin is often administered at the first sign of severe gastroenteritis whereas ceftriaxone is given to children with systemic salmonellosis. In production animals like swine, treatment is usually contraindicated but, when necessary, can be given via injection with several treatment alternatives based on considerations such as withdrawal time. Antibiotic treatment is usually not advised except for rare cases because it can prolong the presence of bacteria in the stool (Gray and Fedorka-Cray, 2002; Yousef and Carlstrom, 2003).

2.2.4 Epidemiology

Typhoid cases are stable with low numbers in developed countries, but nontyphoidal salmonellosis has increased worldwide. Typhoid fever usually causes mortality in 5 to 30% of typhoid-infected individual in the developing world. The World Health Organization (WHO) estimates 16 to 17 million cases occur annually, resulting in about 600,000 deaths. The mortality rates differ from region to region but can be as high as 5 to 7% despite the use of appropriate antibiotic treatment. On the other hand, nontyphoidal cases account for 1.3

billion cases with 3 million deaths. In the United States, approximately 2 to 4 million cases of *Salmonella* gastroenteritis occur with about 500 deaths per year. A more accurate figure of salmonellosis is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported. Data on salmonellosis are scarce in many countries of Asia, Africa and South and Central America where only 1 to 10% of cases are reported (Portillo, 2000; Hanes, 2003; Hu and Kopecko, 2003). Some of the incidence, notification and isolation rate of salmonellosis in different part of the world varies.

Typhoid fever is endemic throughout Africa and Asia as well as persists in the Middle East, some eastern and southern European countries and central and South America. In the US and most of Europe, typhoid is predominantly a disease of the returning traveller. Typhoid incidence in endemic areas is typically low in the first few years of life, peaking in school-aged children and young adults and then falling in middle age. Most infections occur in childhood especially in Mekong Delta region of Vietnam and are recognizable although often mild. The most famous outbreak of enteric fever is Typhoid Mary. Mary Mallon, a New York City hired household cook, transmitted typhoid fever to at least 22 individuals causing 3 deaths between 1900 and 1907. After being apprehended by public health officials in 1907, she was isolated for 3 years. Even though she was released with the stipulation that she never cook again, she broke the promise and consequently caused at least 25 more cases of typhoid fever at Manhattan maternity hospital when she was employed as a cook in 1915. She was finally isolated until her death in 1938 (Scherer and Miller, 2001; Parry, 2006). The infectious dose of *Salmonella* depends upon the serovar, bacteria strain, growth condition and host susceptibility. On the other hand, host factors controlling susceptibility to infection include the condition of the intestinal tract, age and underlying illnesses or immune

deficiencies. The infectious dose of *Salmonella* is broad varying from 1 to 10⁹ cfu/g. However, single-food-source outbreaks indicate that as little as 1 to 10 cells can cause salmonellosis with more susceptibility to infection by YOPI groups (Yousef and Carlstrom, 2003; Bhunia, 2008).

2.2.5 Transmission vehicles

Salmonella are widely distributed in nature and they survive well in a variety of foods. Poultry, eggs and dairy products are the most common vehicles of salmonellosis. In recent years, fresh produce like fruits and vegetables have gained concern as vehicles of transmission where contamination can occur at multiple steps along the food chain (Bouchrif *et al.*, 2009). First, environment contaminated with *Salmonella* serves as the infection source because *Salmonella* can survive in the environment for a long time. After that, *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can shed in their faeces for weeks and even months. Following the direct transmission, moving animals such as swines, cows and chickens act as the important risk factor for infection. These animal reservoirs are infected orally because *Salmonella* normally originates from the contaminated environment and also contaminated feed.

Human get infected when eating the food or drinking the water that is contaminated with *Salmonella* through animal reservoirs. However, *Salmonella* Typhi and *Salmonella* Paratyphi A do not have animal reservoir, therefore infection can happen by eating the improperly handled food by infected individuals (Newell *et al.*, 2010). Besides, transmission of *Salmonella* to the food processing plants and equipment for food preparation are also of great importance. Once carried by vectors or transferred to food, consumption by human can result in the risk of salmonellosis. The *Salmonella* cells can attach to food contact surfaces such as plastic cutting board which may develop into biofilm once attached and hence cause cross-contamination. Consequently, *Salmonella* can enter the food chain at any point from livestock

feed, through food manufacturing, processing and retailing as well as catering and food preparation in the home (Wong *et al.*, 2002).

Disease surveillance reports frequently identify poultry (chickens, turkeys, geese and ducks) as the main vehicles in the salmonellosis outbreak. *Salmonella Pullorum* and *Salmonella Gallinarum* usually cause disease in poultry. Cox and Pavic (2010) provided extensive overview on poultry meat production associated with *Salmonella* and discussed the approaches for the control of this pathogen throughout the whole production chain as poultry can be contaminated from breeder flocks, hatchery environment, feed and litter as well as water troughs in the pens. In Malaysia, Arumugaswamy *et al.*, (1995) reported 39.4% chicken portions, 35.3% chicken liver and 44.4% chicken gizzard were contaminated with *Salmonella* spp. Apart from that, *Salmonella* can enter eggs from the oviduct (particularly ducks). Penetration into the egg is increased when the cuticle is damaged, outside of the egg is wet, temperature is decreased and specific gravity of the shell is low. Contamination of eggs and particularly egg contents by *S. Enteritidis* are believed to be a cause of the large outbreak in Europe and North America since 1980s (Jay *et al.*, 1997; Bhunia, 2008). Recently, the US Centers for Disease Control and Prevention (CDC) mentioned that there were approximately 1469 illnesses associated with eggs infected by *Salmonella* Enteritidis reported in California, Colorado and Minnesota from May 1 to August 31, 2010. On the other hand, *Salmonella* Infantis was the predominant serotype in Australian egg industry (Cox *et al.*, 2002). Spread of *Salmonella* may be facilitated in water storage tanks in a building, from wild animal faeces or even from carcasses. Poor sanitation, improper sewage disposal and lack of clean water system cause the transmission of typhoid fever. In areas where typhoid fever is endemic, water from lakes or rivers which are used for public consumption and are sometimes contaminated by raw sewage are the main sources of infection. The consumption of unboiled water during 1997 typhoid outbreak in Dushanbe, Tajikistan caused 2200 cases of illness and

95 deaths. *Salmonella* contamination of fresh produce could be due to the entry of *Salmonella* through scar tissues, entrapment during embryogenesis of produce, natural uptake through root systems and transfer onto edible plant tissues during slicing. The human health risk is increased further by *Salmonella* preference to grow on fresh produce during retail display at ambient temperature. In 2000, cantaloupe from Mexico resulted in a *Salmonella* Poona outbreak in USA (Penteado and Leitão, 2004; Bordini *et al.*, 2007).

3 CHAPTER 3 Methods and Materials

3.1 Study area

The study was conducted at Beitbridge District Hospital which is located in Beitbridge town high density suburb of Dulibadzimu. The town is located in the southern part of Zimbabwe. It has only two residential areas which are Dulibadzimu, a high density suburb in the western part of the town and the low density suburb located in the eastern part of the town. Dulibadzimu suburb is a container of four-fifth of the town's population of about 26 459 people (Matebeleland South Zimstat, 2012). Samples were collected from patients who visited the outpatient department of Beitbridge District Hospital from December 2017 to January 2018 for complaints of diarrheal disease. The patient flow of the hospital ranges from high to low density dwellers thus having diverse socioeconomic and ethnic backgrounds.

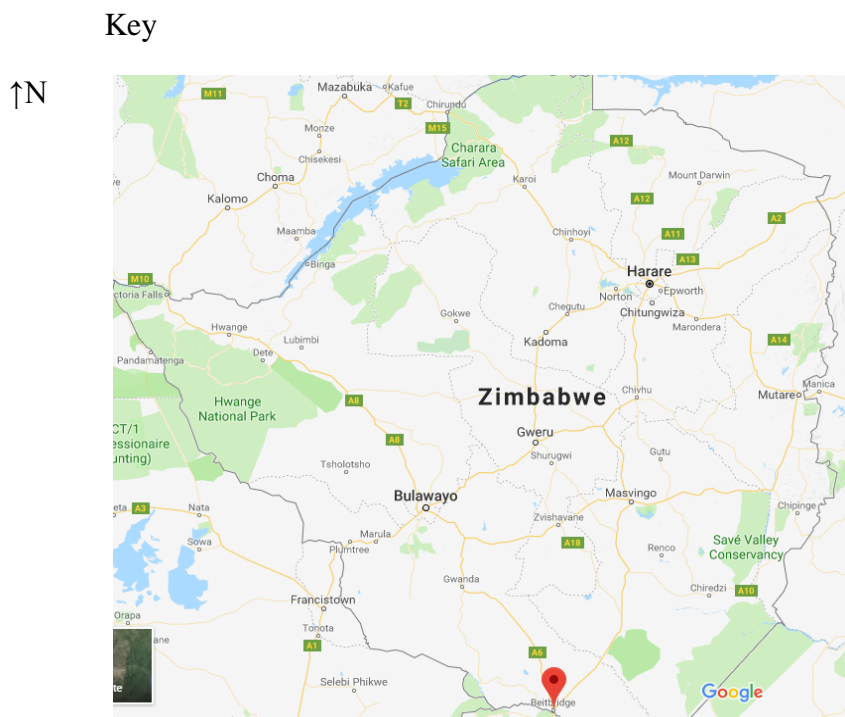


Fig 3.1 Location of Beitbridge town in Zimbabwe. Accessed 30/05/2018 on Google Maps



Fig 3.2 Beitbridge town map in Zimbabwe. (Google Maps Accessed 30/05/2018)

3.2 Specimen collection

Stool specimens were collected from all patients that presented with diarrhea defined as bloody, mucoid, watery, mucoid and bloody using dry, clean, leak proof, and wide mouth stool containers (sterilized containers). A total of 154 samples were collected. Participants who took antibiotics for the diarrheal attack were excluded from the study. Every sample that was collected was labelled by being given a laboratory number and details of each patient recorded in the laboratory which include name, surname, address, sex and age. Ages were divided into five groups, A (0-12 years), B (13-24years), C (25-40years), D (41-60years) and E (above 60 years). Handling of all specimen was done using gloves to avoid contamination.

3.3 Isolation and identification of bacteria

For detection of *Salmonella enterica* and *Shigella dysenteria* isolates, two grams of faeces were collected. Using sterile inoculation loops, the samples were directly inoculated onto xylose lysine deoxycholate (XLD) agar using the streak plate technique, then incubated

aerobically at 37°C for 24 h. After inoculation, one to three colonies suspected to be *Salmonella enterica* (pink colonies with a black dot on centre) or *Shigella dysenteria* (small pink colonies) were isolated. Nutrient agar was used to purify the colonies. Characterization was done using Gram staining technique and biochemical tests which were the citrate, urease and indole tests.

3.3.1 Gram Stain

The colonies suspected to be *Salmonella enterica* and *Shigella dysenteria* were subjected to gram staining and microscopy. Results were noted.

3.3.2 Biochemical tests

3.3.2.1 Kligler Iron Agar

Colonies with typical characteristics of *Salmonella enterica* and *Shigella dysenteria* species from primary media were further cultured on Kligler Iron Agar (KIA) medium by stabbing the butt and streaking the slant and then incubated at 37°C for 24 hrs to determine their glucose and lactose fermentation abilities and production of hydrogen sulfide. The KIA tubes were examined for specific growth and appearance of *Salmonella enterica* and *Shigella dysenteria* species and results were noted

3.3.2.2 Citrate test

Pure colonies of gram negative lactose fermenting of the isolates were tested for citrate utilization ability. A sterilized inoculating loop was used for stabbing the butt and streaking the slant of Simmons Citrate Agar and this was then incubated at 37°C for 24 hrs. Samples were observed for a blue or green colour and checked against the positive and negative controls available in the laboratory and results were noted.

3.3.3.2 Indole test

Indole productions were tested by adding 5 drops of Kovac's reagent into the test tubes containing peptone water. Gram negative lactose fermenting colonies were picked from the XLD agar plates using a sterilised inoculating loop and a suspension was made in the peptone water. These were then incubated at 37°C for 24 hours. Samples were observed for the presence of a red ring or a brown ring and checked against positive and negative controls available in the laboratory then recorded.

3.3.3.3 Urease test

Urease production ability of the suspected colony of *Salmonella enterica* and *Shigella dysenteria* was tested. Suspected colonies were picked from the XLD agar plates using a sterilised inoculating loop. They were then heavily inoculated over the entire surface of the slant urease medium, the caps were loosened and incubated for 24 hours at 37°C.

3.4 Antimicrobial susceptibility testing

The disc diffusion test was performed to test susceptibility of *Salmonella enterica* and *Shigella dysenteria* isolates using standard procedures (Bauer *et al.*, 1966). A sterilised swab was used to collect *Salmonella enterica* and *Shigella dysenteria* colonies on the XLD media. This was then inoculated on the entire surface of Mueller Hinton agar (Oxoid). A ring of disks of each (Mast Diagnostics, UK) containing single concentrations of each antimicrobial agent were used for susceptibility testing. Sterile forceps were used to pick a single ring from the container. The ring was centrally placed on the plate and the lid was placed. The plates were inverted and incubated for 24 hours at 37°C. After incubation, results were noted.

3.4.1 Measurement of zone sizes

The plates were held just above a non-reflecting surface. Clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest millimetre using a

straightline ruler. Zone sizes were recorded and growths up to the edge of the disc were reported as a zone of 0mm. The zones of inhibition were uniformly circular. For the susceptibility testing, the following six antimicrobial drugs were used: ciproflacillin (5ug), chloramphenicol (30ug), gentamycin (10ug), ceftriaxone (30ug), ampicillin (10ug) and tetracycline (30ug), (Oxoid Ltd, UK). Results of antibiotic resistance testing were recorded as susceptible, intermediate, and resistant (National Committee for Clinical Laboratory Standards, 2004). The thresholds for the antibiotics are shown in **Appendix 1**

3.5 Quality control

Quality control was set up using an *Escherichia coli* strain (ATCC 25922) which was susceptible to all the tested drugs (ciproflacillin, chloramphenicol, gentamycin, ceftriaxone, ampicillin and tetracycline).

3.6 Data analysis

Tables and percentages were used to describe findings. Cross tabulations were used to examine prevalence of *Salmonella enterica* and *Shigella dysenteria* and the levels of drug resistance to the respective antibiotics. Data analysis was performed with SPSS version 21 using Chi-square for homogeneity of variance at 95% significance level.

CHAPTER 4 RESULTS

In the study period, a total of 154 diarrheal stool samples were collected for culture and antibiotic susceptibility testing. Twenty-seven (17.5%) samples were found to have either *Shigella*, *Salmonella* and or both *Salmonella* and *Shigella* microbes.

4.1 Identification of bacteria

4.1.1 Macroscopic results

On the xylose lysine deoxycholate (XLD), 19 isolates with *Salmonella enterica* colonies appeared as yellow colonies and eight isolates with *Shigella dysenteria* colonies appeared as red colonies on the agar.

4.1.2 Microscopic results

4.1.2.1 Gram testing

Of the 27 suspected colonies of *Salmonella enterica* and *Shigella dysenteria*, there were all gram negative and cocci for they were observed as red in colour and circle in morphology under the light microscope.

4.2 Biochemical test

4.2.1 Indole test.

Salmonella enterica and *Shigella dysenteria* were both negative in the indole test for they was no colour change from yellow.



Fig 4.1 Picture of results of *Salmonella enterica* and *Shigella dysenteria* on indole

4.2.2 Citrate test.

Salmonella enterica was positive in Citrate test for the agar turned from green to a blue colour and *Shigella dysenteria* was negative for the media remained green.



Fig 4.2 *Shigella dysenteria* identification on citrate agar



Fig 4.3 *Salmonella enterica* identification on citrate agar

4.2.3 Urease test.

Both *Salmonella enterica* and *Shigella dysenteriae* were negative on Urease tests and was detected by no colour change of phenol red which is light orange to pink after 24 hours.



Fig 4.4. *Salmonella enterica* and *Shigella dysenteriae* in Urease test.

4.1.1 Klingler Iron Agar

On the Klingler Iron Agar, the colonies were identified as *Salmonella enterica* and *Shigella dysenteriae*. *Salmonella enterica* were detected present by producing a pink slope, yellow butt and blackening of media. *Shigella dysenteriae* was detected by a pink slope and yellow butt on the media.



Fig 4.5. Detection of *Salmonella enterica* in Klingler Iron Agar



Fig 4.6. Detection of *Shigella dysenteriae* using Klingler Iron Agar.

Table 4.1: Biochemical tests for *Salmonella enterica* and *Shigella dysenteriae*.

Test	<i>Salmonella enterica</i>	<i>Shigella dysenteriae</i>
Indole test	-	-
Citrate	+	-
Urease	-	-
Key	+ positive	- negative

4.3 Prevalence *Salmonella enterica* and *Shigella dysenteriae*

Out of the 154 samples, nineteen (12.3%) were positive *Salmonella enterica* microbes and eight (5.2%) were positive *Shigella dysenteriae*. **Table 4.2**

Table 4. 2 Prevalence of *Salmonella enterica* and *Shigella dysenteria* in Beitbridge town

Bacteria isolated	Frequency (N)	(%)
<i>Salmonella enterica</i>	19	12.3
<i>Shigella dysenteria</i>	8	5.2

Key: N = number of isolate, %=percentage

4.3.1 Prevalence of *Salmonella enterica* and *Shigella dysenteria* with respect to gender

Amongst the people infected, fifteen (9.7%) were females and twelve (7.8%) were males. Of the males infected, nine (5.8%) were positive for *Salmonella enterica* and three (1.9%) had *Shigella dysenteria*. Of the females infected, ten (6.4%) had *Salmonella enterica* and five (5.2%) had *Shigella dysenteria* microbes as shown in **Table 4.3**

Table 4.3 Prevalence of *Salmonella enterica* and *Shigella dysenteria* with respect to gender

Bacteria isolated	Male (n)	(%)	Female (n)	(%)
<i>Salmonella enterica</i>	9	(5.8)	10	(6.4)
<i>Shigella dysenteria</i>	3	(1.9)	5	(5.2)
Total	12	(7.8)	15	(9.7)

Key: n – number of isolates, % - percentage

Chi-square test show that there is no association between bacteria isolated and gender. (P=0.607)

4.3.2 Prevalence of *Salmonella enterica* and *Shigella dysenteria* according to age.

Prevalence with respect to age showed that group A (0-12 years) had the largest number of people infected (8.4%) with *Salmonella enterica* and *Shigella dysenteria* followed by group B (13-24 years) with 4.5 percent The group with the least people infected was E (above 60

years), which had only one (0.6%) person infected with *Shigella dysenteria*. Group results are presented in **Table 4.4**

Table 4.4 Prevalence of *Salmonella enterica* and *Shigella dysenteria* according to age groups

Age groups	<i>Salmonella enterica</i> N, (%)	<i>Shigella dysenteria</i> N, (%)	Total
A (0-12years)	9 (5.8)	4 (2.6)	13 (8.4)
B (13-24years)	6 (3.9)	1 (0.6)	7 (4.5)
C (25-40years)	2 (1.3)	2 (1.3)	4 (2.6)
D (40-60years)	2 (1.3)	0 (0.0)	2 (1.3)
E (Above 60 years)	0 (0.0)	1 (0.6)	1 (0.6)

Key: N-number of isolates, %- percentage

Chi- square tests show that there is no association between age groups and bacteria isolated (p=0.307)

4.3.3 Prevalence of *Salmonella enterica* and *Shigella dysenteria* according to location

In high-density suburb (Dulibadzimu), twenty-three (14.9%) people were infected with microbes of which sixteen (10.4%) people were infected with *Salmonella* microbes and seven (4.5%) with *Shigella* microbes. In low-density suburb, four (2.6%) people were infected, three (1.9%) people were infected by only *Salmonella* microbes and one (0.6%) had *Shigella* microbes, as shown in the graph in **Fig 4.7**

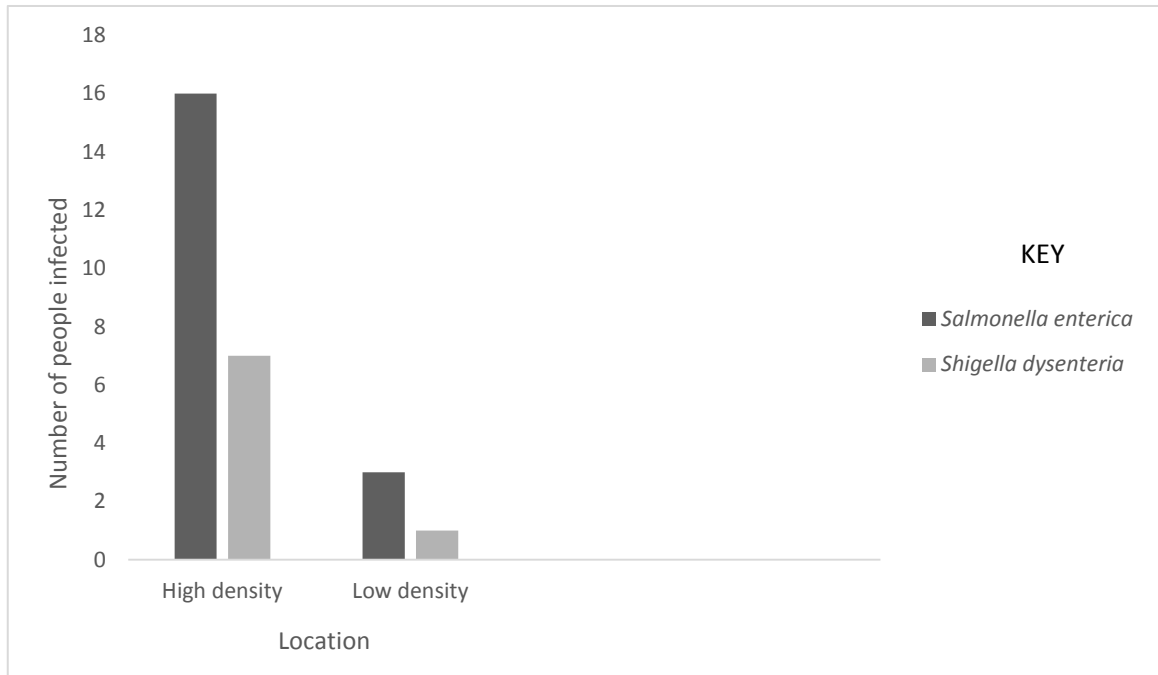


Fig 4.7. Prevalence of *Salmonella enterica* and *Shigella dysenteriae* according to residential area

The Chi-square showed that location is not associated with the people infected ($p=0.826$)

4.4 Antimicrobial resistance

In *Salmonella enterica* isolates, the highest level of resistance was detected for ampicillin ($\leq 13\text{mm}$) and chloramphenicol ($\leq 12\text{mm}$) in which all (100%) isolates were found to be resistant. The highest level of susceptibility was detected for ceftriaxone ($\geq 22\text{mm}$) and ciproflacillin ($\geq 17\text{mm}$) in which 100% of the isolates were susceptible. Out of the 19 *Salmonella enterica* isolates, Gentamycin was susceptible to sixteen (84.2%) of them. For *Shigella dysenteriae* isolates, the highest level of resistance was detected for ampicillin ($\leq 13\text{mm}$) which all four (100%) isolates were found to be resistant. The highest level of susceptibility was detected for gentamycin ($\geq 15\text{mm}$) and ciproflacillin ($\geq 17\text{mm}$), where, 100% (8) isolates were susceptible as presented in **Table 5 and Appendix 1**

Table 5: Antibiotic susceptibility of *Salmonella enterica* and *Shigella dysenteria*.

Organism isolated	Resistant N, (%)	Intermediate N, (%)	Susceptible N, (%)
<i>Salmonella enterica</i> (N=19)			
Ampicillin	19 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	0 (0.0)	0 (0.0)	19 (100.0)
Chloramphenicol	19 (100.0)	0 (0.0)	0 (0.0)
Ciproflacillin	0 (0.0)	0 (0.0)	19 (100.0)
Gentamycin	1 (5.2)	2 (10.5)	16(84.2)
Tetracycline	16 (84.2)	1 (5.2)	2 (10.5)
<i>Shigella dysenteria</i> (N=8)			
Ampicillin	8 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	0 (0.0)	2 (25.0)	6 (75.0)
Chloramphenicol	4 (50.0)	1 (12.5)	3 (37.5)
Ciproflacillin	0 (0.0)	0 (0.00)	8 (100.0)
Gentamycin	0 (0.0)	0 (0.0)	8 (100.0)
Tetracycline	5 (62.5)	1 (12.5)	2 (25.0)

Key. N (number of isolates). % (percentage)

CHAPTER 5 DISCUSSION

5.1 Sample collection

In the study, out of the 154 stool samples, twenty-seven were found to be having *Salmonella enterica* and *Shigella dysenteria* bacteria. In the 154 samples, all the stool samples showed diarrheic appearance associated with *Salmonella enterica* and *Shigella dysenteria* which are bloody, watery, mucoid and mucoid and bloody though only 27 were having *Salmonella enterica* and *Shigella dysenteria*. These diarrhoeic appearances on the rest of the other were caused by other factors other than *Salmonella enterica* and *Shigella dysenteria*. Bloody diarrhea can be caused by proctitis which means inflammation of the rectum. Ulcerative colitis, ulcerative proctitis, and Crohn's colitis are chronic inflammatory diseases of the colon due to overactivity of the body's immune system. These diseases can cause abdominal pain, diarrhea, and bloody diarrhea (Bhunja, 2008). Watery diarrhea is also caused by rotavirus, norovirus, and other kinds of viral gastroenteritis, commonly referred to as stomach flu, are among the viruses that can cause explosive diarrhea. Bloody mucus in stool, or mucus accompanied by abdominal pain, can represent more serious conditions known as the Crohn's disease, ulcerative colitis and even cancer. Irritable bowel syndrome is another causative of mucoid diarrhea (Warren, 2006).

5.2 Prevalence

Prevalence was high in the high-density suburb (Dulibadzimu) (14.9%) than in the low-density suburb (2.6%) and this was shown to be significant by the p -value, (0.826) This is due to differences in the living conditions between the people in these two localities. Low density residents earn high wages as compared to those that reside in the high density. This determines the type of food consumed, quality of the food and source of the food. Most of those who live in the low density afford buying food and meat from supermarkets and well established butcheries. These shops and butcheries have much high prizes than the open

market. In the open market, most of the meat and poultry is not assessed by health workers for any contamination. This leads in having high prevalence of *Salmonella Enterica* and *Shigella dysenteria* in the high density than in the low density. In the high density, most of the people survive by the informal sector, whereby they mostly do cross boarder activities. During their work they smuggle in chicken cuts, poultry, off layers (known as mkokorochi in Beitbridge). These are not allowed in the country due to the 'buy Zimbabwe campaign'. In bringing in these stuff, this increases the prevalence of the bacteria in Beitbridge town.

In Beitbridge, there is high water regulations by the local council due to people failing to pay water rates and bills to the council. This mostly affects the high-density residents. In the low density, most of the resident have reserve tanks and boreholes at their homes to supply them with safe water for consumption. When there is no water supply from the council, they depend on their reserves unlike high density residents who end up using contaminated water due to very few boreholes as compared to the population. The council is also failing to purify the water. This is due to lack of money to buy chemicals for water purification for rate payers are failing to pay their bills. This is leading to supply of sub standard water for human consumption. In the high-density suburb, most of the people are ignorant because most of them are high school drop outs and fail to use chemicals which they are provided with by the health ministry for home purification of water. This is also leading to an increased *Salmonella enterica* and *Shigella dysenteria* occurrence.

Beitbridge town is overpopulated with over 26.459 people (Matebeleland South Zimstat, 2012) with the majority of the people living in the high-density suburb with an average of 3 families per house. This has led to poor sanitation and lack of hygienic practices. The high population and less water supply has also led to more sewage burst in the high density suburb. This has increased the prevalence of the bacteria in the high density. Most of the young children do not know the health risks of playing in these contaminated waters and this

is due to the fact that children in the high density suburbs mostly play outdoors whilst those in the low density play indoors where they have games, Wi-Fi, computers etc This has also influenced the high prevalence of *Salmonella enterica* and *Shigella dysenteria* in the high-density suburbs.

Prevalence of *Salmonella enterica* and *Shigella dysenteria* isolates by age groups was shown to be significant ($p=0.307$). The age group that is most playful is from 0-12 years. This is where most of the people infected are found (48.1%). This is because children have no limits in the area they play. They end up playing in contaminated water. Infants can not bath on their own, and if they do so, they end up not as clean as the older ones, this also increases the prevalence of *Salmonella* and *Shigella* microbes in the young children. Children do not take seriously the necessity of washing hands before and after eating, after visiting the toilet, this is how the bacteria is quickly spread and affects mostly children below the age of 12years. The group from 25- 40 years has people who are engaged in many activities for most of the people are the bread winners. As they work, they end up buying food from many restaurants in Beitbridge for lunch. Most of the fast food outlets lack hygiene and prepare cheap food smuggled from South Africa. This has also led to the isolation of the bacteria in this age range. There is no significant difference between the prevalence due to gender whereby males have 48.1% and females 51.8%. This is due to the fact that every capable individual is in the business of looking for money for living without gender differences. The young people are also engaged in the same activities regardless of gender.

5.3 Antibiotic Susceptibility

The findings show that except for gentamicin, ciproflacin and ceftriaxane the organisms in this study have a high level of resistance to ampicillin, tetracycline and chloramphenicol. There seems to be complete resistance to ampicillin and chloraphenicol by *Salmonella*

organisms in the study which is in disagreement with reports from other parts of the region (Mache *et al.*, 2002; Asrat, 2008). This is a sharp increase from earlier reports indicating the increasing problem of drug resistance by these microbes over the years. A high level of susceptibility to ceftriaxone was detected. Excepting reports by Tiruneh, (2009) and Gedebo *et al.* (2012) However, there seems to be a similar pattern of high resistance to these drugs in the studies in the rest of the country, even though lower in extent than my findings. This could be due to the fact that ampicillin and chloramphenicol have been used in the country for a long time and because of their easy availability and potential for misuse. Even though a reduced level of resistance was detected for tetracycline, compared to ampicillin and chloramphenicol, a relatively similar pattern of resistance was reported from other parts of the country (Roma *et al.*, 2013). The organisms seem to also have increased their resistance to the drugs from lower levels to levels of more than 90% in reports by Asrat, (2008) and Tiruneh, (2009). This is similar to the pattern across the globe where the organisms are consistently increasing their resistance to these commonly used first line drugs (Karuiki *et al.*, 2001; Sharma *et al.*, 2005). Resistance is a natural biological response of microbes to antimicrobials and is currently a worrisome scenario affecting many parts of the world (Sharma *et al.*, 2005; Khatun *et al.*, 2011). Apart from intrinsic resistance, gene transfer and mutation are among the underlying mechanisms involved in the development of antimicrobial resistance by microbes (Sharma *et al.*, 2005). Several factors contribute to resistance by pathogens causing gastroenteritis in the setting of a developing country like Zimbabwe. These include frequent overuse, misuse and factors related to the potency and quality of antimicrobials and the distribution of resistant strains (Sharma *et al.*, 2005; Asrat, 2010). In addition, syndromic diagnosis and diagnostic imprecision usually force physicians to opt for broad spectrum antibiotics such as amoxicillin and tetracycline, over prescribing; and less antibiotic diversity which lead to the emergence and spread of antimicrobial resistance. For

instance, since nontyphoidal gastro-enteritis is usually self-resolving, antibiotic treatment is not commonly recommended (Kasper *et al.*, 2005).

One of the limitations of this study was that due to lack of facilities, it was not possible to conduct identification of strain groups and susceptibility to multiple antibiotics which would have provided us with further insight into the distribution of strains and the extent of antibiotic susceptibility patterns in the area. However, this study is a pragmatic one, given that in the study area in particular, and in Zimbabwe in general, antibiotics are prescribed on empirical bases without implementing the commonly recommended strain isolation and susceptibility testing procedures (Kasper *et al.*, 2005). The small sample size of the current study may also be a limitation. As the study is an *in vitro* one, it may not necessarily reflect *in vivo* resistance patterns and patient outcomes. Unlike previous studies in other parts of the country that assessed *Salmonella* or *Shigella* only (Gedebou *et al.*, 1982; Mache *et al.*, 2002; Roma *et al.*, 2013; Tiruneh, 2009). The fact that the current study examined antimicrobial susceptibility tests for both *Salmonella* and *Shigella* is one of its strengths.

6. CHAPTER 6. Conclusions and recommendations

With the significant prevalence of *Salmonella enterica* and *Shigella dysenteria* in Beitbridge town in this study, it can therefore be concluded that conditions in Beitbridge town are highly influencing this high prevalence. The local council should take action on upping the living conditions of the Beitbridge residents. This include intercepted supply of clean fresh water for the consumption of the people. Garbage should also be collected at regular basis and also repairing of old sewage pipes. Health workers should also inspect the quality of meat being sold by the local people. The government should tighten security at the border post to ensure that they is entry of only allowed goods. Public health awareness should be developed to reduce the incidence of Salmonellosis and Shigellosis among the people in order to avoid contamination and spread of the bacteria.

In conclusion, except for gentamicin, ciproflacillin and ceftriaxone for which both *Salmonella enterica* and *Shigella dysenteria* isolates were highly susceptible meaning that they are the most effective antibiotics for treatment of *Salmonella enterica* and *Shigella dysenteria*, a high level of antimicrobial resistance was detected. Notably, the organisms seem to have developed complete resistance to ampicillin and tetracycline. We assert that gentamicin, ciproflacillin and ceftriaxone may be drugs of choice for treating diarrheal attacks by these organisms in the study area. It is recommended that a more rigorous study of the prevalence, antimicrobial susceptibility pattern and underlying mechanisms of drug resistance by *Salmonella enterica* and *Shigella dysenteria* isolates be conducted. In addition, treatment needs to be based on species identification and susceptibility testing rather than the currently practiced empirical treatment.

Decreasing unnecessary or imprudent antibiotic use will decrease the pressure on organisms which are exposed to them to become resistant. Efforts in human and veterinary medicine are needed to decrease the misuse and overuse of antibiotics. This will preserve the efficacy of

antibiotics for as long as possible. To archive this, medical and veterinary professional organisations may at least issue recommendations to appropriate therapeutic use of antibiotics by physicians and veterinarians.

The development and use of antibiotics has been one of the most important steps towards controlling of infectious bacterial diseases in the 21st century. However, the subsequent appearance and spread of antibiotic resistance in pathogenic organisms have made many currently available antibiotics ineffective (Kam *et al.*, 2007). To successfully fight the increasing number of drug resistant and multidrug resistant *Salmonella* and *Shigella*, extensive knowledge of the molecular mechanisms of acquiring antibiotic resistant and updated information is required.

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APPENDICES

Appendix 1. threshold of antibiotics used for treatment of *Salmonella* and *Shigella* microbes

Antibiotic	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Ampicillin	≤ 13	14-16	$17 \geq$
Ceftriaxone	≤ 17	19-21	$22 \geq$
Chloramphenicol	≤ 12	13-17	$18 \geq$
Ciproflacillin	≤ 15	16-20	$21 \geq$
Gentamycin	≤ 12	13-14	$15 \geq$
Tetracycline	≤ 14	15-18	$19 \geq$

Key: mm (millimetre). measurements to the nearest millimeter.

Appendix 2. Antibiotic susceptibility measurements

Organism	CIPRO	CHLO	GENT	CEFT	AMP	TETRA
1. <i>Salmonella</i>	17	12	14	22	10	13
2. <i>Shigella</i>	18	18	16	24	10	20
3. <i>Salmonella</i>	19	11	12	23	11	14
4. <i>Shigella</i>	19	11	15	20	12	16
5. <i>Salmonella</i>	17	12	15	22	12	19
6. <i>Salmonella</i>	18	12	13	23	12	12
7. <i>Shigella</i>	17	19	16	23	11	12
8. <i>Salmonella</i>	19	11	16	23	13	14
9. <i>Salmonella</i>	19	11	15	24	12	11
10. <i>Salmonella</i>	18	12	15	24	10	15
11. <i>Salmonella</i>	17	10	16	23	13	13
12. <i>Salmonella</i>	18	10	16	22	12	13
13. <i>Shigella</i>	18	12	17	23	12	14
14. <i>Salmonella</i>	17	11	16	23	12	12
15. <i>Shigella</i>	17	11	15	22	13	13

16. <i>Shigella</i>	18	12	15	23	12	12
17. <i>Salmonella</i>	18	10	15	23	12	14
18. <i>Salmonella</i>	19	10	17	24	11	14
19. <i>Salmonella</i>	17	11	16	22	13	13
20. <i>Salmonella</i>	18	12	16	22	12	14
21. <i>Salmonella</i>	17	11	16	23	13	12
22. <i>Salmonella</i>	17	12	15	24	12	13
23. <i>Salmonella</i>	18	11	15	24	11	13
24. <i>Shigella</i>	18	19	17	19	12	19
25. <i>Salmonella</i>	17	12	15	22	11	14
26. <i>Salmonella</i>	19	12	16	22	11	14
27. <i>Shigella</i>	18	17	16	23	13	12

Key: Chlora (chloramphenicol), Cipro (Ciproflacillin), Gent (gentamycin), Tetra (Tetracyclin), Amp (Ampicillin), Ceft (Certrioxone). Table in millimetres.

Appendix 3. Antibiotic cross tabulation

antibiotic ^ susceptibility ^ bacteria Crosstabulation

Count

bacteria			susceptibility			Total
			susceptible	intermediate	resistant	
salmonella	antibiotic	AMPICILLIN	0	0	19	19
		CEFTRIAXONE	19	0	0	19
		CHLORAMPHEMICO	0	0	19	19
		CIPROFLACILLIN	19	0	0	19
		GENTAMYCIN	16	2	1	19
		TETRACYCLINE	2	1	16	19
	Total		56	3	55	114
shigella	antibiotic	AMPICILLIN	0	0	8	8
		CEFTRIAXONE	6	2	0	8
		CHLORAMPHEMICO	3	1	4	8
		CIPROFLACILLIN	8	0	0	8
		GENTAMYCIN	8	0	0	8
		TETRACYCLINE	2	1	5	8
	Total		27	4	17	48
Total	antibiotic	AMPICILLIN	0	0	27	27
		CEFTRIAXONE	25	2	0	27
		CHLORAMPHEMICO	3	1	23	27
		CIPROFLACILLIN	27	0	0	27
		GENTAMYCIN	24	2	1	27
		TETRACYCLINE	4	2	21	27
	Total		83	7	72	162

Appendix 4. Chi square test for bacteria against gender

gender * bacteria Crosstabulation

Count

		bacteria		Total
		salmonella	shigella	
gender	female	10	5	15
	male	9	3	12
Total		19	8	27

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.222 ^a	1	.637		
Continuity Correction ^b	.002	1	.962		
Likelihood Ratio	.224	1	.636		
Fisher's Exact Test				.696	.484
N of Valid Cases	27				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 3.56.

b. Computed only for a 2x2 table

Appendix 5. Chi square test for age groups against isolated bacteria

bacteria ^ age Crosstabulation

Count

		age					Total
		group A (0-12years)	group B (13-24years)	group C (25-40years)	group D (41-60years)	group E (above 60years)	
bacteria	salmonella	9	6	2	2	0	19
	shigella	4	1	2	0	1	8
Total		13	7	4	2	1	27

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.812 ^a	4	.307
Likelihood Ratio	5.480	4	.241
N of Valid Cases	27		

a. 9 cells (90.0%) have expected count less than 5. The minimum expected count is .30.

Appendix 6. Chi square test for isolated bacteria against location

location * bacteria Crosstabulation

Count

		bacteria		Total
		salmonella	shigella	
location	high density	16	7	23
	low density	3	1	4
Total		19	8	27

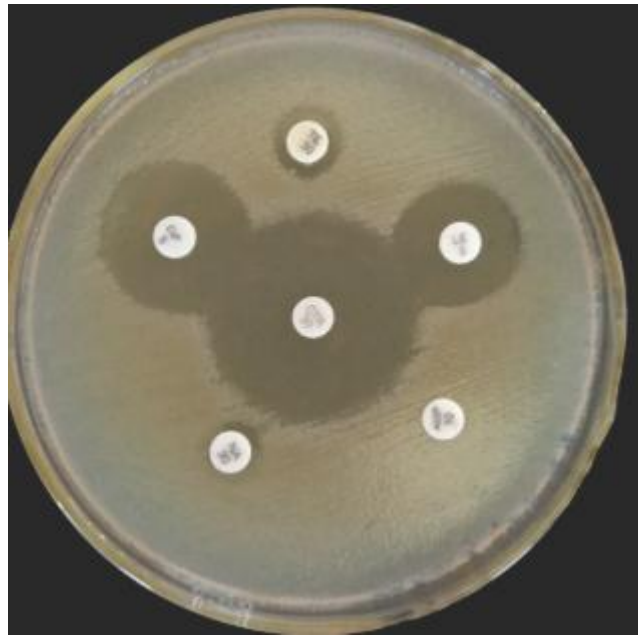
Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.048 ^a	1	.826		
Continuity Correction ^b	.000	1	1.000		
Likelihood Ratio	.050	1	.824		
Fisher's Exact Test				1.000	.663
N of Valid Cases	27				

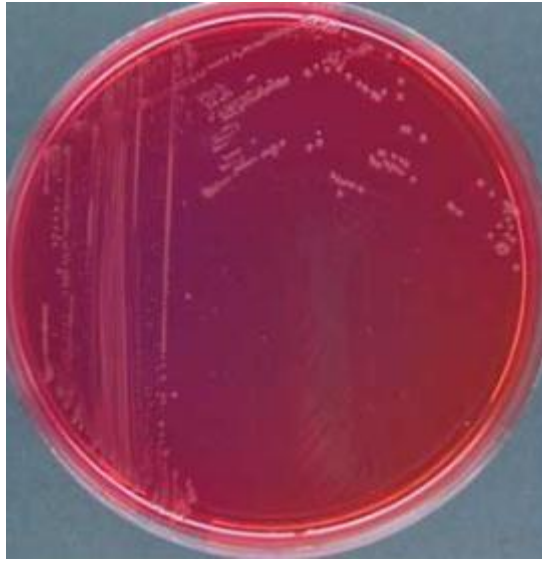
a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.19.

b. Computed only for a 2x2 table

Appendix 7. antibiotic susceptibility testing on Muller Hinton Agar



Appendix 8. Isolated *Shigella dysenteriae* on XLD



Appendix 9. Isolated *Salmonella*

