Phenotypic And Genotypic Characterization Of *Salmonella* Species Isolated From Treated Wastewater Effluents And Receiving Rivers In Durban

By

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Submitted in fulfilment of the academic requirements for the degree of Master of Science (MSc) in the Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville Campus).

As the supervisor of the candidate, I approve this dissertation for submission

Signed: ____________________ **Name:** ____________________ **Date** ______________

PREFACE

The experimental work described in this dissertation was carried out in the Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville Campus), Durban, South Africa from March 2012 to December 2013, under the supervision of Prof. A.O. Olaniran.

These studies represent original work of the author and have not been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation (include publications in preparation, submitted, in press and published and give details of the contributions of each authors to the experimental work and writing of each publication).

Publication 1

Publication 2

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ABSTRACT

Salmonella and *Shigella* spp. are major pathogens of humans and they cause diseases ranging from mild food poisoning to chronic diarrhea, especially in children under the age of 5. They are commonly found in the gastrointestinal tract of animals and humans and contaminate water surfaces through fecal pollution. Discharge of inadequately treated wastewater has been known to be conduits of these pathogens to surface waters. Emergence of antibiotic resistant bacteria is a public health concern worldwide especially in developing countries where disease burden is high. This study investigated the efficiency of two Wastewater Treatment Plants (WWTPs) in Durban for wastewater treatment, and assessed the impact of treated effluent discharge on the receiving surface water. The genotypic characteristics and antibiogram profile of *Salmonella* spp. recovered from the treated effluent samples of theWWTPs and the receiving river was also determined. Water samples were collected from the WWTPs over a 12 month period and analyzed for physico-chemical parameters including temperature, pH, turbidity, BOD and COD using standard methods; while presumptive *Salmonella* and *Shigella* spp. were enumerated on Salmonella-Shigella and xylose-lysine-desoxycholate agar, repectively, via membrane filtration technique. Isolation of *Salmonella* spp. was done by enrichment of samples in Rappaort Vassiliadis soy broth followed by spread plating on Salmonella chromogenic agar and aerobic incubation at 37°C for 18 to 24 h. Presumptive isolates were biochemically characterized and confirmed via PCR amplification of the *inv*A gene. Isolates were tested against 20 selected antibiotics to determine their antibiotic resistance profile. Presence of virulence markers; *spi*C, *mis*L, *orf*L and *pip*D genes were also determined using PCR. Unacceptably high levels of turbidity (5.52-37.58 NTU), BOD (2.19-9.1 mg/l) and COD (67.67-294 mg/l) were observed in the water samples, while temperature (14°C-25°C) and pH (6.72-7.3) fell within the recommended maximum of 25°C and

7.5, respectively, for treated wastewater effluent. Significant positive correlation ($p < 0.05$) was observed between pH and BOD, temperature and COD, and between turbidity and presumptive *Salmonella* count. Presumptive *Salmonella and Shigella* spp. were prevalent at all sampling points, with population ranging from 8.5×10^{2} to 1.59×10^{5} CFU/ml and 0.1×10^{2} to 7.5×10^{3} CFU/ml, respectively. The isolates were highly susceptible to β-lactams, Chloramphenicol, Tetracycline, Quinolones and Trimethoprim-Sulfamethoxazole (99% to 100%). Complete antibiotics resistance was observed against Sulfamethoxazole (100%), Nalidixic acid (27%) and Streptomycin (14%). Intermediate resistance was observed against Streptomycin (74%), Nalidixic acid (44%) and Fosfomycin (8.5%). Of the 200 isolates tested, 93% harbored the *spi*C gene, 84% harbored the *misL* gene, while 87.5% and 87 % of the isolates harbored the *orf*L and *pip*D gene, respectively. Results from this study indicate the inefficiency of the WWTPs investigated to totally eradicate *Salmonella* spp. from the final effluent and discharge of such effluent. Discharge of these effluent to surface water resources could pose health threat to the end-users of the surface water for daily domestic and recreational activities . Thus, appropriate intervention by the regulatory agencies is required to ensure compliance of WWTPs to the stipulated guidelines for safe disposal of treated effluent.

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CHAPTER ONE

LITERATURE REVIEW

1.0 Introduction

Water is indispensable to all forms of life and is needed for almost all human activities such as drinking, washing, farming etc. Access to safe freshwater is now regarded as a universal human right and is one of the main Millennium Development Goals (UNDP, 2006). Domestic and industrial uses of water generate wastes which need to be treated before discharge into surface waters such as rivers, lakes and lagoons. Disposal of raw or inadequately treated wastewater has been identified as the main source of contamination of natural water bodies with pathogenic microorganisms because raw or inadequately treated wastewater contains pathogens that are excreted by disease carrying humans and animals (Kistemann *et al.*, 2008; Ntengwe 2005). Domestic wastewater treatment may be centralized plants, pit latrines, septic tanks or are disposed of in unmanaged lagoons or surface waters via open or closed sewers (Okoh *et al.*, 2007). Globally, wastewater treatment plants (WWTP) are primarily designed to reduce pollution of natural water bodies with suspended solids, nitrogen, phosphorus, organic matter and microorganisms (Kistemann *et al.*, 2008). However, the infrastructural and operational state of most municipal wastewater treatment plant in South Africa is poor and requires maintenance and upgrade especially in poor provinces and rural areas thus, leading to pollution of water bodies depended on by rural communities (Momba *et al.*, 2006). The potential health threat posed by waterborne microbial pathogens has attracted renewed attention to microorganisms once thought to be under control. These are often referred to as “emerging or re-emerging” pathogens.

Emerging infectious diseases have been defined as infectious diseases that have newly appeared in a population or have previously existed but are rapidly increasing in incidence or geographical range (Theron and Cloete, 2002).

Known bacterial pathogens associated with wastewater include *E. coli*, *Salmonella*, and *Shigella, Vibrio* species, fecal and total coliform and *fecal streptococcus*. *Salmonella and Shigella spp.* cause severe diarrhoea in children and adults leading to morbidity and mortality. Invasive non-typhoidal *Salmonella* is endemic to rural and urban Sub-Saharan Africa and is thought to be higher than the incidence of typhoid fever which is estimated at 50 cases per 100, 000 persons per year (Morpheth *et al.,* 2009). Salmonellosis and Shigellosis are water and food-borne diseases caused by *Salmonella* and *Shigella* spp. respectively. Morbidity and mortality rate is highest in developing countries in children under the age of 5 especially in communities without access to proper sanitation and adequate drinking water supplies. In most countries, the microbial quality of final treated effluent is estimated based on the level of indicator organisms present (Bitton, 2005; Savichtcheva and Okabe, 2006). However, several studies have shown that the presence of indicator organisms does not always correlate with the presence of pathogens especially those of viral origins (Godinho *et al.,* 2010; Levantesi *et al.,* 2010). Previous studies have implicated wastewater treatment plants as sources of contamination of rivers with pathogenic microorganisms in South Africa (Odjadjare *et al.*, 2010; Olaniran *et al.*, 2012). However, there is little information on the incidence, prevalence and characteristics of *Salmonella* and *Shigella* spp. in treated wastewater and receiving surface water in Durban, KwaZulu-Natal province of South Africa. This review evaluates the impact of wastewater treatment plants as sources of contamination of receiving surface waters with *Salmonella* and *Shigella* species.

1.1 Overview of water in South Africa

Pitman (2011) describes water as South Africa's most precious natural resource because it is one of the water stressed countries in the world. Water stress is defined as a situation whereby there is not enough water for all uses whether domestic, industrial and agricultural (Mukheibir, 2010). However, defining threshold of water stress in terms of available water use per capita is more complex often entailing assumptions about water use and its efficiency. Nonetheless, it has been proposed that when annual freshwater availability falls below 1,700 cubic meters per annum countries begin to experience regular or periodic water stress, and at levels below 1,000 cubic meters per annum, water scarcity begins to hamper economic development and public health (FAO, 2003).

South Africa is characterized by low and highly variable rainfall and high evaporation rates that subjects large parts of the country to extreme droughts and flood (Duah and Xu, 2013). About two-third of the country is arid or semi-arid with few and relatively small rivers compared to other African countries (Adewumi *et al.,* 2010). Annual rainfall in the country is estimated at an average of 450 mm to 500 mm per annum which is 60% of the global average of 860 mm (Pitman, 2011). Domestic, Rural and Urban sector uses up 54 % of the water resource in South Africa thus generating wastewater, which has to be treated before discharge into water bodies such as rivers while, agriculture uses up 62 % (Table 1.1). Water withdrawals are expected to increase due to development and rapid urbanization causing severe physical water shortage in developing countries (Mukheibir, 2005). Since rainfall displays strong seasonality, the natural availability of water across the country is variable; while stream flow in South African rivers is relatively low level for most of the year (Pitman, 2011). This limits the proportion of stream flow that can be relied upon for use. Moreover, as a result of the excessive extraction of water by extensive forests and sugar cane plantations in the relatively wetter areas of the country, only 9% of the rainfall reaches the rivers, compared to a world average of 31% (DWAF, 2004).

Table 1.1 Percentage of water use by various sectors in South Africa

Water User/Sector	**Proportion of Allocation (%)**
Agriculture	62
Domestic	27
Urban	23
Rural	4
Industrial	3.5
Afforestation	3
Mining	2.5
Power Generation	2
Total	100

Source: DWAF, (2004).

Already 3.7 million people in South Africa are without access to any form of water supply infrastructure and an additional 5.4 million with access had to be brought up to a basic level of service (Adewumi *et al.*, 2010). These people mostly in rural areas rely on surface water for social economic activities thus; access to clean water is the most significant resource for reducing poverty and disease, and improving the lives of poor South Africans. Despite the uneven distribution of fresh or surface water, scarcity, and heavy reliance on surface water to meet the ever-growing demand for water, it is alarming to note the increasing degradation of surface water quality due to pollution (Chong *et al.*, 2010; FAO, 2003; George *et al.*, 2001; Pitman, 2011).

During the past few decades, human development, population growth, extreme weather events, natural calamities, and climate change have exerted many diverse pressures on both the quality and quantity of water resources which in turn impact conditions fostering water-associated diseases (Yang *et al.*, 2012).

1.2 Wastewater effluent as a source of pathogenic microorganisms

Recognizing the need to protect surface water from degradation and destabilization of aquatic ecosystem, and contamination with pathogenic microorganisms, most countries makes it mandatory that municipal waste consisting of industrial and domestic waste be collected and treated prior to release into the environment. However, treated wastewater effluents are still a major source of bacterial pathogens both in developed and developing countries due to inadequate treatment (Table 1.2). Common and emerging bacteria attributed to wastewater effluent include *Salmonella*, *Shigella*, *Listeria*, *Vibrio*, *Pseudomonas* species etc. Various studies in South Africa have reported the prevalence of these bacterial pathogens in final treated effluent and discharge point of various wastewater treatment plants suggesting that treated wastewater is a source of contamination of receiving surface water with pathogens (Igbinosa *et al.*, 2012 a, b; Igbinosa and Okoh 2013; Martone-Rocha *et al.*, 2010; Odjadjare and Okoh, 2010; Okoh *et al.*, 2012; Okoh and Igbinosa 2010; Ye and Zhang, 2011).

Table 1.2 Reported number of selected pathogens associated with wastewater. These references are just a few of the hundreds of references existing.

Pathogen	Counts/L	Country	Reference
Bacteria			
Listeria	2.0×10^{4}–3.5×10^{7}	South Africa	Okoh *et al.*, 2012
Vibrio	0 to 3.45×10^{4}	South Africa	Igbinosa *et al.*, 2009
Campylobacter	500-4.4×10^{6}	Germany	Jones, 2001
Salmonella spp.	2.9×10^{4}	South Africa	Olaniran *et al.*, 2012
Shigella spp.	54.1×10^{5}	South Africa	Olaniran *et al.*, 2012
Enteric Viruses			
Enterovirus	7.81×10^{4}	Switzerland	Masclaux *et al.*, 2013
Rotavirus	<11−10 000	Netherlands	Lodder *et al.*, 1999
Norovirus	<1 000−1.6×10^{6}	Germany	Pusch *et al.*, 2005
Adenovirus	1.15×10^{6}	USA	Fong *et al.*, 2010
Protozoa			
Giardia cysts	1566−2254	Germany	Ajonina *et al.*, 2013
Cryptosporidium	1−560	Canada	Payment *et al.*, 2001

1.2.1 Prevalence of *Salmonella* and *Shigella* species in treated wastewater effluents

The prevalence of *Salmonella* and *Shigella* spp. in sewage and wastewater effluents varies according to the decontamination or treatment process applied (Bonadonna *et al.*, 1999; Jolivet-Gougeon *et al.*, 2006). *Salmonella* and *Shigella* species have been reported to be prevalent at all stages of treatment in conventional wastewater treatment plants including the final effluents indicating the inefficiency of wastewater treatment plants in totally eliminating these pathogens from wastewater (Pant and Mittal, 2007). Olaniran *et al.* (2012) reported high levels of *Shigella* (5.41×10^3 CFU/ml) and *Salmonella* spp at (2.9×10^1 CFU/ml) at the discharge point of a wastewater treatment plant in South Africa. In India, counts of 280 and 37 MPN/100 ml for *Salmonella* and *Shigella* spp. respectively were reported by Pant and Mittal, (2007) at the influent point of a plant investigated. However, the presence of *Salmonella* and *Shigella* species was detected at all points of the wastewater treatment plant including the final effluent. Samie *et al.*, (2009) frequently detected the presence of *Salmonella* and *Shigella* species amongst other pathogens at all stages of treatment from 14 different wastewater treatment plants in South Africa. They described *Salmonella* spp. as one of the most resistant organism to elimination by conventional treatment processes compared to other microorganisms recovered such as *E. coli*, *Shigella* spp. and *Pseudomonas* spp. This finding is also in agreement with previous findings in Nigeria where *Salmonella* was also isolated at all stages of the treatment process sampled including the final effluent (Doughari *et al.*, 2007). Over the years, conventional treatment processes in wastewater treatment plants have failed to completely eliminate *Salmonella* and other pathogens from wastewater. This may be due to the fact that these organisms are not specifically targeted for removal but are assumed eliminated if the treatment process for indicator organisms is efficient (Lermachand and Lebron, 2003). Water quality monitoring that has successfully relied on *E. coli* and *coliforms* as indicator organisms may no longer reflect accurately the presence of bacteria, viruses and protozoa due to reported lack of evidence of correlation with indicator organisms and transition of some bacteria into the viable but not culturable state (Levantesi *et al.,* 2010; Song *et al.,* 2010). In a study by Godinho *et al.* (2010), 85 - 99% reduction of *E. coli* present in a wastewater treatment plant was recoreded however, *Salmonella enterica* subsp. *enterica* was detected at all sampling points using polymerase chain reaction. Koivunen *et al*. (2002) also observed that conventional treatment processes removed enteric organisms quite efficiently but some *Salmonella* and high number of fecal indicator organisms survived the treatment processes and were discharged into the receiving natural waters. *Salmonella* species have been found to be persistent if not better survivors in the environment than *E. coli* depending on the availability of nutrients (Savichtcheva and Okabe, 2006). Though the prevalence of *Shigella* spp. in treated wastewater and surface water is very low compared to other pathogenic organisms such as *Salmonella*, *E. coli* and *Vibrio*, counts greater than 0.01 to 10 cfu/ml is of serious concern due to the low infective dose of the organism estimated at 10–100 cells per ml (Wen *et al.,* 2009). The infective dose of *Salmonella* is estimated at 10^3 – 10^4 cells/ml (Sant'Ana, *et al.,* 2011).

1.2.2 Implication of release of *Salmonella* and *Shigella* species on receiving water bodies

Wastewater treatment plants are usually designed to efficiently remove biological oxygen demanding compounds and nutrients. The removal efficiency of pathogenic and indicator microorganisms in wastewater treatment plants vary according to the quality of influent, type of treatment process, retention time, other biological flora present in activated sludge, oxygen concentration, pH, temperature and the efficiency in removing suspended solids (Jamwal and

Mittal, 2010). Conventional wastewater treatment plants reduces the numbers of enteric microbes, but treatment processes can vary extensively resulting in wastewater effluents that still contain high numbers of fecal microorganisms (Igbinosa *et al.*, 2009).

Discharge of inadequately treated wastewater containing *Salmonella* and *Shigella* spp. can have negative impact on receiving surface water and in turn public health. This is because natural water bodies in Africa and other developing countries are relied upon for socioeconomic activities such as bathing, drinking, farming, and recreational purposes especially in areas without access to potable water (Musyoki *et al.*, 2013). The presence of *Salmonella* and *Shigella* has been reported in river water worldwide and in Africa with municipal wastewater discharge implicated as the major source of pollution (Abraham *et al.*, 2007; Dick *et al.*, 2013; Doughari *et al.*, 2007; Economou *et al.*, 2012; Le Roux *et al.*, 2012; Wahid and Tanaka, 2012, Walters *et al.*, 2013,). Use of river water as well as wastewater containing *Salmonella* and *Shigella* spp. or other pathogens for agricultural purposes, could constitute an important source of contamination of crops and infection of livestock and poultry with these pathogens (Melloul *et al.*, 2002; Srikanth and Naik, 2004). *Salmonella* spp. is commonly found in birds and studies have confirmed their presence in other animals including pigs, cattle, and fish posing a potential health threat to consumers (David *et al.*, 2009; De Busser *et al.*, 2011; Mannion *et al.*, 2012; Van *et al.*, 2012). In a recent study, non-typhoidal *Salmonella* was described as the second leading cause of food-borne illness (11%) after norovirus (58%) and was the leading cause of hospitalization (35%) and death (28%) in the United States (Scallan *et al.*, 2011). However, there is no comparative values for incidence of non typhoidal *Salmonella* in South Africa. The occurrence of these pathogenic bacteria in surface water could result in the outbreak of water-borne diseases (Musyoki *et al.*, 2013).

1.3 Epidemiology of *Salmonella* and *Shigella* spp. in developing countries

Salmonella spp. causes non typhoidal gastroenteritis which results in an estimated 94 million cases and 155,000 deaths (Majowicz *et al.,* 2010) while *S. typhi* the causative agent of typhoid fever is responsible for an estimated 16 million cases of illness and 580,000 deaths annually (Okeke *et al.*, 2005). Typhoid fever is endemic in developing countries particularly rural areas without access to potable water (Smith *et al.,* 2011). The incidence of enteric fever in developed countries is low compared to developing countries and is usually associated with travel to developing countries. In the US, an estimated 400 cases of infections are reported annually while less than 10 cases per 100 000 per year was reported in Europe, Australia and New Zealand and North America (Sánchez-Vargas *et al.,* 2011). An epidemiological survey in Spain reported hospitalization rate of 0.31 cases per 100 000 population for typhoid with higher risks to those travelling to developing countries such as Africa and the Indian subcontinent (Gil *et al.,* 2009).

In Pakistan, incidence was estimated at 451 cases per 100 000 per year (Khan *et al.,* 2012). These values are higher than estimates from Vietnam and China estimated at 21.3 and 15.3 per 100 000 per year respectively. In Africa, the epidemiology of enteric fever is poorly characterized due to limited availability of resources for diagnosis, surveillance tools and consequently epidemiological data making it difficult to estimate the rate of incidence (Crump and Mintz, 2010). However, incidence is estimated at 50 cases per 100 000 people per year, though this estimate is debated because the study was based on reports from Egypt and South Africa in the 1970s and 1980s and may have been over estimated due to outbreaks of the disease in those countries (Feasey *et al.,* 2012; Reddy *et al.,* 2010). Nevertheless, invasive nontyphoidal

Salmonella are leading cause of bacteremia in children and immunocompromised adults with an associated case fatality of 20–25% (Sánchez-Vargas *et al.,* 2011).

Epidemiological report show that 140 million people suffer from shigellosis and an estimated 600,000 deaths occur every year worldwide (Iwalokun *et al.*, 2011). Between 1996 and 2006, a survey in South Africa reported 50 cases of shigellosis affecting mostly children and immunocompromised patients (Davies and Karstaedt, 2008). Another survey in Egypt, between 1995 and 1998, reported 101 cases of shigellosis mostly in children under the age of 3 (Abu-Elyazeed *et al.,* 2004). While in Lagos, Nigeria 62 cases was reported between 1999–2000 in children and young adults with *S. flexneri*, *S. dysenteriae*, *S. boydii* and *S. sonnei* accounting for for 51.6%, 17.7%, and 13% respectively (Iwalokun *et al.,* 2011). In Africa and Nepal, *S. flexneri* was reported as the dominant etiological agent of shigellosis in contrast to Taiwan where *S. sonnei* is reported to have replaced *S. flexneri* as the dominant etiological agent of Shigellosis (Khan *et al.,* 2014; Wei *et al.,* 2007).

1.4 Pathogenicity of *Salmonella* and *Shigella* species

Virulence genes encodes factors such as toxins and adhesins, necessary for pathogenesis in pathogenic microorganisms. These virulence genes may be located on plasmids, transposons or bacteriophages (Hacker *et al.,* 1997) or may be part of certains regions of the bacterial chromosome known as "pathogenicity Islands" (Scmidt and Hensel, 2004) (Table 1.3). Genetic analysis of *Salmonella* genome indicates that each clinical syndrome requires distinct sets of virulence genes (Guiney and Fierer, 2011). Virulence plasmid vary in size (50 - 90 Kb) but have a common 7.8 kb region and are required to trigger systemic disease (Rotger and Casadesú, 1999). Pathogenesis of *Salmonella* spp. begins with the invasion of the host intestinal epithelial cells.

This is done by inducing their uptake in a complex active process involving the type III transport secretion system (TSS3) (Suez *et al.,* 2013). TSS3 is coded for by virulence genes clustered in large DNA regions known as *Salmonella* pathogenicity islands (SPI). The TSS3 creates a channel across both the bacterial and epithelial cell periplasm leading to a translocation of bacterial effectors into the cell cytoplasm (Coburn *et al.,* 2007). The secreted effectors interact with eukaryotic proteins to activate signal transduction pathways and rearrange the actin cytoskeleton leading to membrane ruffling and engulfment (Zou *et al.,* 2011). Once inside the host cell, the effector is capable of altering host cellular functions such as membrane trafficking, signal transduction and cytokine gene expression resulting in the intracellular survival and colonization of the bacteria (Lopez *et al.,* 2012). Clinical presentation and complication of *S. typhi* and *S. paratyphi* are similar with an incubation period of 7–14 days and includes fever, headache, loss of appetite and diarrhoea in immune-compromised people (Sánchez-Vargas *et al.,* 2011).

The pathogenesis of *Shigella* is similar to that of *Salmonella* and also begins with invasion, replication and dissemination within of the human colonic epithelial cells causing rupture and inflammatory destruction of these cells (Sasakawa, 2011). Invasion and colonization is achieved using the TSS3 and effector proteins in a similar manner to *Salmonella* spp. (Phalipon and Sansonetti, 2007). The TSS3 and effector proteins are encoded on genes present on a 213 kb virulence plasmid. Following cell invasion, *Shigella* lyses the phagocytic vacuole to replicate intracellularly and moves by polymerizing actin at one bacterial pole, forming actin comet tails which allows the formation of bacteria-containing protrusions at the cell plasma membrane that invade adjacent cells. After lysis of the donor and recipient cell membranes, the bacteria reinitiate intracellular replication to disseminate into the epithelium. Bacterial intracellular replication occurs at a doubling time estimated at 10–15 min causing death of infected cells a few hours following infection (Carayol and Tran Van Nhieu, 2013).

Table 1.3 Some of the known virulence genes present in *Salmonella* and *Shigella* spp. and their associated functions.

Organism	Virulence gene	Description	Function	References
Salmonella spp.	*inv*A	Type III secretion system apparatus	Encodes a needlelike complex export protein necessary for invasion of host cells	Gassama-Sow *et al.* (2006)
	*sop*B	Type III secreted effector proteins	Cell invasion	Fookes *et al.* (2011)
	*spi*C	Type III secretion system	Required for macrophage survival	Niedergang *et al.* (2000)
	*or*L	Autotransporter and Adhesin	Survival in macrophages and colonization	Dione *et al.,* (2011)
	*mis*L	Required for survival in macrophages	Autotransporter protein involved in intestinal colonization	Dorsey *et al.*, (2005)
Shigella spp.	*Stx* A, B	Verocytotoxin produced by several enteric pathogens, most importantly *Shigella dysenteriae* (serotype 1 only)	Important factors in disease pathogenesis and are responsible for some of the severe complications, such as haemorrhagic colitis and the haemolytic uremic syndrome (HUS)	Cherla *et al.* (2003)
	Ipa B, C, D	TTSS secreted effector proteins	Required for cell invasion and phagosome escape as well as macrophage apoptosis	Guichon *et al.* (2001)

1.5 Antibiotic resistance development in *Salmonella* and *Shigella* species

Antibiotics resistance of microorganisms is a worldwide problem that stirs cause for concern especially in developing countries where antibiotics are used excessively and sometimes inadequately. In the US, data shows that 4.1% of *Salmonella* isolates exhibited decreased susceptibility to cephalosporins and 84% showed multidrug resistance phenotypes (Sjölund-Karlsson *et al.,* 2010). A review in Asia, also shows high increase in resistance to commonly used antibiotics such as ampicillin (23–100%), sulfamethoxazole (44–79%), streptomycin (32–85%) and tetracycline (47–90%) in countries like Malaysia, Thailand and Vietnam (Van *et al.,* 2012). Similar trend of increasing resistance to commonly used antibiotics have been reported in some African countries including the emergence and spread of extended spectrum β-lactamases (ESBL) in *Salmonella* spp. (Bisi-Johnson *et al.*, 2012; Feasey *et al.*, 2012; Harrois *et al.*, 2013). High resistance against tetracycline (65%), streptomycin (77%), and trimethoprim/sulfamethoxazole (93%) was reported in Uganda (Mahero *et al.*, 2013). There have been reports of increasing resistance of *Shigella* spp. to antibiotics including tetracycline, streptomycin, ampicillin to which they were once susceptible leading to the inefficacy of treatment or prophylactic regimes in developing countries. Iwalokun *et al.* (2011) reported increased resistance to ampicillin, streptomycin, co-trimazole and tetracycline between 1990 and 2000. This trend is similar to reports from Kenya, Brazil, India and Vietnam (Feasey *et al.*, 2012). Emergence of resistance to nalidixic acid usually used to treat resistant cases was reported in Taiwan which may suggest decrease in susceptibility to more potent but expensive fluoroquinolones such as ciprofloxacin and norfloxacin (Wei *et al.,* 2007). Besides excessive use of antibiotics in the emergence of resistant *Salmonella* and *Shigella* spp., wastewater treatment plants may also be a source of antibiotic resistant *Salmonella* and *Shigella* spp. in developing countries. There is evidence that wastewater treatment plants are a reservoir for antibiotic resistant bacteria and genetic materials in the environment and may facilitate the emergence of resistant phenotypes through the transfer of genetic materials that confer resistance to an otherwise susceptible bacteria (Gao *et al.*, 2012; Munir *et al.*, 2011; Rizzo *et al.*, 2013). Previous reports have suggested that resistant bacteria may become susceptible once more to an antibiotic following a period of withdrawal of that antibiotic from health care settings (Kariuki *et al.*, 2006, Rahman *et al.*, 2002).

1.6 Conclusion

Water, an important and scarce resource is a route of transmission of *Salmonella* and *Shigella* spp. Wastewater treatment plants in developing countries are inefficient at removing *Salmonella* and *Shigella* spp. from wastewater leading to contamination of receiving surface waters relied on for day to day activities in rural areas. *Salmonella* causes typhoid fever and gastroenteritis while *Shigella* causes dysentery and diarrhoea. Treatment of these diseases is by administration of antibiotics. However, resistance and emerging resistance to commonly used antibiotics worldwide renders empirical treatment ineffective and present a cause for concern. Since vaccines development is still at the research stage, Salmonellosis and Shigellosis can be best controlled by ensuring the discharge of high quality wastewater effluent free from *Salmonella* and *Shigella* spp. into surface water resources being utilized in rural communities.

1.7 Scope of the study

Shortage of water supply is a problem faced worldwide especially in developing countries and arid regions of the world (Wen et al., 2009). Disposal of inadequately treated waste water effluents are a major source of fecal and chemical contamination of aquatic ecosystem causing severe disturbance in water ecology and is a major barrier to water reclamation and reuse (and Zhang and Farahbakhsh, 2007). Previous reports have indicated that wastewater treatment plants in South Africa discharge effluents containing pathogens (Igbinosa and Okoh, 2013; Olaniran *et al.*, 2012). However, there is little information on the prevalence and characteristics of *Salmonella* and *Shigella* spp. in treated wastewater and receiving surface water in Durban, KwaZulu-Natal province of South Africa. The scope of this study was to evaluate the treatment processes of 2 wastewater treatment plants in Durban and to determine the prevalence of *Salmonella* and *Shigella* spp. based on their phenotypic characteristics on selective media. Isolate, purify and confirm the identity of isolates using biochemical and molecular tests. The antibiotics resistance profile as well as virulence gene signatures were also studied.

1.7.1 Hypothesis

It was hypothesized that the wastewater treatment plant in Durban is not efficient in removing microbial load especially pathogenic organisms such as *Salmonella* and *Shigella spp.* and that effluent from these plants are a major source of contamination of natural water bodies with pathogenic *Salmonella* species. It was further hypothesized that wastewater effluents are a reservoir for antibiotic resistant and virulent *Salmonella* and *Shigella* species.

1.7.2 Objectives

1.7.2.1 The proposed study aims to investigate the efficiency of some wastewater treatment plants in Durban in removing *Salmonella* and *Shigella spp.*

1.7.2.2 Characterization of Salmonella species recovered from wastewater effluents and receiving surface waters in Durban.

1.7.3 Aims

To validate the hypothesis, the following objectives were established:

1.7.3.1 Evaluation of selected physicochemical parameters of the wastewater for twelve (12) months.

1.7.3.2 Enumeration of *Salmonella* and *Shigella* species for twelve (12) months by membrane filtration on *Salmonella*-*Shigella* (SS) agar and Xylose Lysine Desoxycholate (XLD) agar, respectively.

1.7.3.3 Statistical analysis of physicochemical parameters and counts of presumptive isolates.

1.7.3.4 Identification and confirmation of *Salmonella* and *Shigella* species isolated via biochemical tests.

1.7.3.5 To elucidate the antibiotic susceptibility profiles of the isolates via the Kirby-Bauer disk diffusion test.

1.7.3.6 Investigation of the absence or presence of virulence genes in the isolates via PCR

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CHAPTER TWO

IMPACT OF TREATED WASTEWATER EFFLUENT ON RECEIVING SURFACE WATERS AND AS A SOURCE OF PRESUMPTIVE *SALMONELLA* AND *SHIGELLA* SPP. IN DURBAN

2.1 Introduction

South Africa is a water stressed country due to low average rainfall (465 mm) received which is below the global average of 860 mm (Pitman, 2011). Demand for this important scarce resource is expected to increase due to rapid industrial development, increasing human population, per capita consumption increase and the resulting impact of human activities on the environment (Adewumi *et al.,* 2010; Ngwa *et al.,* 2013). High water demand and consumption also leads to increases in the volume of wastewater generated (Agrafioti and Diamadopoulos, 2012). The availability of good quality water is of paramount importance bringing to the fore the consequence of contamination of water bodies with pathogenic microorganisms (Levantesi *et al.,* 2012). Natural water bodies such as rivers are subject to dramatic changes in microbial and physico-chemical qualities as a result of a variety of anthropogenic activities on the watershed. These changes are caused by discharges of municipal raw waters or treated effluent at a specific point-source into the receiving surface waters (Igbinosa and Okoh 2008; Igbinosa and Okoh 2009; Momba *et al.*, 2006; Petala *et al.,* 2009). Point-source pollution problems will not only increase treatment costs considerably, but may also introduce a wide range of pathogens and harmful chemicals to surface waters that may be supplied to many rural and urban communities (Petala *et al.,* 2009, Ratola *et al.,* 2012), thus resulting in incidences of waterborne diseases. Although a vast majority of microorganisms present in wastewater are not pathogenic (George *et al.*, 2002), some pathogenic bacteria possibly originating from discharge of inadequately treated wastewater effluent have been implicated in the outbreak of waterborne diseases over the years (Bertuzzo *et al.*, 2008). Conventional biological treatment processes have been recognized as a powerful technology and are widely used in industrial and sewage treatment plants worldwide, for the removal of organic content, nutrients and microorganisms from wastewater (Koivunen *et al.*, 2003, Wen *et al.*, 2009). Conventional treatment process without any tertiary form of treatment has been found to be inefficient in totally eradicating some pathogenic microorganisms and ensuring high physicochemical quality of treated effluents discharge (Baršienė *et al.*, 2009; Igbinosa *et al.*, 2009; Petala *et al.*, 2009, Singh *et al.*, 2004). Development of tertiary treatment processes to remove pathogenic bacteria in wastewater effluent have attracted great interest from researchers with much research focused on processes such as biological filtration and membrane bioreactor (Meng *et al.*, 2012). These processes are associated with high operational and capital cost and are therefore, out of reach of most developing countries (Wen *et al.*, 2009).

Salmonella spp. are ubiquitous enteric pathogens distributed worldwide and comprises a large number of serovars characterized by different host specificity and distribution and are one of the leading cause of acute enterocolitis as well as the etiological agents of more severe systemic diseases such as typhoid and paratyphoid fevers (Levantesi *et al.*, 2012; Touron *et al.*, 2005). *Salmonella* spp. are frequently found in environmental samples. They are usually present in large numbers in raw sewage and can still be present in wastewater effluent after advanced secondary treatment (Koivunen *et al.*, 2003). Bacillary dysentery caused by *Shigella* spp. is endemic throughout the world and is among the most common cause of bacterial diarrheal diseases (Sharma *et al.*, 2010). It is responsible for approximately 140 million casses of shigellosis annually resulting in the death of approximately 600,000 deaths worldwide in developing countries (Iwalokun *et al.*, 2011). Contaminated food and water are known to be the source of epidemic spread of diarrheal diseases caused by pathogenic microorganisms such as *Salmonella* and *Shigella* (Abbassi-Ghozzi *et al.*, 2012). Recently, an outbreak of acute gastroenteritis in KwaZulu-Natal resulting in the hospitalization of 216 people was linked to food contaminated with *Salmonella enterica serovar Enteritidis* (Niehaus *et al.*, 2011). The report also suggested a point source outbreak with a possibility of continued transmission. The role of treated wastewater effluent in the contamination of surface waters with pathogenic microorganisms is well documented (Arvanitidou *et al.*, 2005; Fukushi *et al.*, 2003; Hench *et al.*, 2003; Igbinosa *et al.*, 2009; Igbinosa *et al.*, 2011; Michael *et al.*, 2013;; Ottoson *et al.*, 2006; Touron *et al.*, 2005). Also, previous reports from some provinces in South Africa have implicated treated wastewater effluent as a point source of contamination of receiving watershed with pathogenic and emerging pathogenic microorganisms (Odjadjare *et al.*, 2012). However, there is a dearth of information on the prevalence of *Salmonella* and *Shigella* spp. in treated wastewater effluent discharged by wastewater treatment plants in Durban, South Africa. This study, thus aims to investigate the prevalence of presumptive *Salmonella* and *Shigella* spp. in treated wastewater effluents and evaluate the impact of treated wastewater effluent on receiving surface waters.

2.2 Materials and Methods

2.2.1 Description of wastewater treatment plant investigated in this study

Two wastewater treatment plants namely Northern wastewater treatment works (NWWTW) and the New Germany wastewater treatment plant (NGWTP) previously described by Olaniran *et al.* (2012) were sampled and studied. The NWWTW is located at geographical coordinates 29°48′45.62″ S and 30° 59′ 45.62″ E and processes 70 megalitres per day (ML/day) of industrial and domestic wastewater. Treated effluent from this plant is discharged into the Umgeni River after tertiary treatment by disinfection with chlorine. The NGWTP is located at geographical coordinates 29°48′ 21.68″S and 30°53′ 50.44″E and treats mostly domestic wastewater but sometimes receive industrial wastewater as well. It processes 15% industrial wastewater and 85% domestic wastewater and has a maximum capacity of 7 ML. Treated effluents from this plant is discharged into the Aller River after disinfection with chlorine. On the opposite side of the river is an informal settlement with poor sanitation and inadequate sewage disposal system and residents use the water from the river for day-to-day activities.

2.2.2 Sample collection

Water samples were collected monthly from both wastewater treatment plants at the clarifier before chlorination (B.C), discharge point after chlorination (D.P), 500 meters upstream (U.S) and 500 meters downstream (D.S) of the discharge point between March 2012 and February 2013. Samples were collected in 5L plastic container sterilized 24 hours prior to collection by soaking in 70% ethanol and rinsing with deionized water. During collection of samples, the containers were rinsed with the sampled water before filling (at a depth of approximately one metre at each sampling point) to three-quarter of the container leaving space to allow for proper mixing. The collected samples were placed in ice packs, transported to the laboratory of the Department of Microbiology, University of KwaZulu-Natal (Westville) and processed within 24 hours of collection. During processing, the water samples were not dechlorinated.

2.2.3 Physico-chemical analysis

Temperature of the water samples was measured on site with a mercury thermometer; the pH was determined using Beckman pH meter; while turbidity was measured with a turbidimeter (HACH 21000P). Biochemical oxygen demand (BOD) was determined according to standard protocol using the LDC 101 probe with an HQ40d multimeter (HACH) after incubation for a period of 5 days (APHA, 1992). Predetermined volumes of sample were transferred into BOD bottles and 2 shots of processor nitrate inhibitor (HACH) was added to prevent the oxidation of nitrogen compounds. The bottles were then topped up with dilution water, inverted several times to ensure proper mixing and the initial dissoved oxygen (DO_1) was measured using a HACH probe (LD101). The bottles were incubated at 20°C ± 1°C for 5 days after which the DO_5 was measured. The BOD was calculated as

BOD5 (mg/l)= D_1-D_2/P

Where D_1 = DO of the diluted sample immediately after preparation (mg/l)

D_2 = DO of the diluted sample after 5 days incubation at 20°C (mg/l)

P = Decimal volumetric fraction of sampled used (volume of used sample / total volume)

Chemical oxygen demand (COD) was measured with a Nova 60 spectroquant (Merck, USA) according to manufacturer’s instructions. Sample (3ml) was added to COD test cell (Merck), mixed vigorously and heated at 148°C for 2 hours in a TR420 spectroquant thermo-reactor and cooled to room temperature. The COD test cells were vortexed and cooled for another 10 minutes and read using the Nova 60 spectroquant.

2.2.4 Microbial analysis

Enumeration of presumptive *Salmonella* and *Shigella* spp. present in the water sample was done by standard membrane filtration technique as previously described by Ngwa *et al.* (2013). Serial dilutions of the water samples were made and standard membrane filtration using 0.45µm pore and 47mm diameter filter (Pall Corporation, USA) was used to concentrate 50 ml of appropriately diluted water sample. The membrane filter was then placed on the surface of xylose lysine desoxycholate (XLD) agar and *Salmonella*-*Shigella* (SS) agar and incubated aerobically at 37°C for 18 to 24h to enumerate *Shigella* and *Salmonella* spp., respectively. Colonies on SS agar exhibiting colourless with or without black center depending on the production of hydrogen sulphide were enumerated as presumptive *Salmonella* spp while colonies exhibiting red or colorless and transparent morphologies on XLD agar were enumerated as presumptive *Shigella* spp (Stecchini and Domenis, 1994; Govindarajan *et al.*, 2012). Random isolates from each sampled point was isolated and purified onto fresh nutrient agar plates for biochemical test.

2.2.5 Biochemical test of selected presumptive isolates

Triple sugar iron (TSI), Simmons citrate, lysine iron agar (LIA) and urea agar (Oxoid, UK) slants prepared according to manufacturer’s instructions. and inoculated with a 24 h nutrient agar-grown culture of the presumptive *Salmonella* and *Shigella* isolates. The surface of the agar slant was inoculated using a sterile inoculating loop while a stab was made at the center of the slant using a sterile inoculating needle. The tubes were then incubated under aerobic conditions at 37°C for 24 to 48 h. Tubes exhibiting alkaline slant and acidic butt with H_2S production on TSI slants, purple colour in butt of LIA tube, blue colour development on slant of citrate agar and no colour change on urea indicated positive results for *Salmonella* spp. While TSI tubes exhibiting alkaline slant and acidic butt, without the production of H_2S, LIA tubes exhibiting alkaline slants and purple tubes, no colour change on citrate and urea agar slant indicated positive result for *Shigella* spp.

2.2.6 Statistical Analysis

Mean values of results and standard deviation were calculated using Microsoft excel 2010 edition. Pearson's correlation was determined using the SPSS 21.0 software for windows program (SPSS, Inc. USA) and correlations were considered statistically significant at P values of < 0.05

2.3 RESULTS

2.3.1 Physicochemical parameters of treated wastewater effluent and receiving surface waters

The physicochemical parameters of treated effluent from the wastewater treatment palnts and their receiving surface waters are shown in Table 2.1 and 2.2. Temperature was stable across all sampled points in each month but highly varied across seasons at the NWWTW. The lowest temperature recorded was 12°C at the D.P in June while the highest temperature of 27°C was recorded at the D.S point in the summer month of February. The temperature at the NGWTP was stable across each sampled point in each month and ranged from 12°C at the D.P in August to 26°C at all sampled points in March. However it varied throughout the study period depending on the season.

A the NWWTW, the pH was stable at all sampled points in each month but varied throughout the duration of the study. It ranged from 6.41 (at the U.S in September) to 7.88 (at the D.P in February). While at the NGWTP, the pH ranged from 6.30 at the U.S in July to 8.00 at the D.S in February but was stable across all sampled points in each month.

At the NWWTW, turbidity values recorded varied across all sampled points and months with no significant decrease in turbidity obtained at the D.P (Table 2.1). The values ranged from 6.37 NTU obtained U.S in the month of February, 2013 to 65.553 NTU obtained at the D.P in the month of August 2012. While at the NGWTP, High variability in turbidity was recorded throughout the study ranging from 1.42 NTU at D.P in April to 40.40 NTU at U.S in August, 2012.

At the NWWTWW, COD values varied highly throughout the study and ranged from <10 mg/l in March and May (at the D.P) to 312.44 mg/l in July (at the D.S) while, at the NGWTP, the COD values varied highly throughout the study period ranging from 22.33 mg/l at U.S in July to 313 at U.S in March. In the months of April, May and June, reduction in COD of 36.5%, 21.11% and 55.6% respectively, were observed at the D.P compared to COD values before chlorination.

At the NWWTW, BOD_5 was stable across each sampled point in each month ranging from 1.03 mg/l to 9.42 mg/l throughout the study period. A significant 2.3-fold and 3.16- fold increase in BOD_5 was observed at the D.P in the months of March and May after treatment. The values of BOD_5 at the D.S were higher than values recorded at other sampled points and ranged from 3.58 mg/l to 7.74 mg/l. While at the NGWTP, the BOD_5 was stable across all sampled points in each month but varied throughout the study period ranging from 2.20 mg/l to 11.04 mg/l. BOD values increased at the D.P from after treatment during most of the study period but some level of decrease was recorded in September (17%) and October (9%).

Table 2.1: Physicochemical parameters of wastewater effluent from Northern wastewater treatment works and the receiving river.

MONTH		Temp (°C)	pH	T (NTU)	COD (mg/l)	BOD (mg/l)
	U.S	26	7.25±0.09	16.67±0.38	161.33±4.37	5.62±1.01
MARCH	B.C	26	7.11±0.05	7.91±0.33	104.78±13.73	2.23±0.36
2012	D.P	25	7.36±0.07	23.40±12.13	<10±0.00	5.13±0.18
	D.S	26	7.24±0.06	15.27±0.12	309.33±0.58	5.62±0.24
	U.S	21	7.43±0.12	19.7-±0.00	304.33±2.08	8.49±0.47
	B.C	22	7.67±0.06	56.53±0.12	229.33±9.71	3.30±0.97
APRIL	D.P	22	7.40±0.10	76.43±0.29	311.11±2.01	3.44±0.67
2012	D.S	21	7.63±0.06	14.80±0.00	151.00±0.00	6.33±0.21
	U.S	21	6.91±0.04	12.80±0.00	20.22±1.71	4.29±0.79
	B.C	22	7.08±0.03	19.60±0.00	38.22±11.55	1.03±0.19
MAY	D.P	21	7.28±0.02	13.80±0.17	<10±0.00	3.25±0.17
2012	D.S	22	7.13±0.03	12.90±0.00	309.11±1.71	5.68±0.30
	U.S	13	7.65±0.01	9.57±0.01	112.89±3.02	9.42±0.15
	B.C	13	7.37±0.00	11.27±0.31	300.00±8.65	4.36±0.16
JUNE	D.P	12	7.35±0.01	8.92±0.06	110.00±3.06	4.54±0.12
2012	D.S	14	7.84±0.01	14.37±0.21	88.78±2.41	7.74±0.31
	U.S	15	7.54±0.01	13.27±0.15	311.00±1.00	5.76±1.03
	B.C	16	7.48±0.01	19.33±0.06	114.78±11.65	2.56±0.58
JULY	D.P	15	7.70±0.00	23.07±0.38	290.67±0.88	3.12±0.62
2012	D.S	15	7.87±0.02	22.87±0.12	312.44±0.38	4.69±0.23
	U.S	20	7.12±0.03	28.73±0.06	105.89±3.86	2.80±0.57
	B.C	21	6.85±0.11	56.37±0.35	310.11±0.69	1.51±1.09
AUGUST	D.P	19	7.09±0.4	65.53±0.57	182.78±2.27	1.59±0.84
2012	D.S	20	7.26±0.02	20.77±0.06	309.56±2.14	3.98±0.65

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Table 2.1 continued

MONTH		Temp (°C)	pH	T (NTU)	COD (mg/l)	BOD (mg/l)
	U.S	20	6.41±0.05	10.67±0.06	55.56±0.51	3.73±0.53
	B.C	22	6.76±0.02	20.73±0.06	308.67±0.88	1.51±0.76
SEPTEMBER	D.P	20	6.82±0.04	19.27±0.21	308.44±1.26	2.38±1.10
2012	D.S	20	6.52±0.02	11.50±0.10	139.67±1.73	3.92±0.78
	U.S	24	7.02±0.01	17.07±0.12	195.22±3.98	3.32±0.78
	B.C	22	6.60±0.06	30.53±0.23	306.89±1.84	3.23±1.40
OCTOBER	D.P	23	6.75±0.05	28.50±0.00	109.89±2.80	3.88±0.75
2012	D.S	24	6.91±0.01	29.03±0.06	148.00±0.33	3.58±0.98
	U.S	21	6.86±0.01	21.33±0.76	241.78±21.56	4.24±0.98
NOVEMBER	B.C	22	6.79±0.01	39.13±0.40	123.78±6.91	3.56±0.92
2012	D.P	23	6.68±0.03	48.53±0.55	287.22±14.25	3.26±0.88
	D.S	23	6.72±0.05	14.10±0.46	246.11±14.84	3.87±0.81
	U.S	22	6.85±0.01	12.20±0.26	274.33±4.41	3.65±0.78
	B.C	25	6.78±0.03	36.13±0.40	170.78±3.79	3.29±0.96
DECEMBER	D.P	21	6.69±0.01	31.77±0.23	153.89±0.19	3.52±0.77
2012	D.S	22	6.64±0.02	10.33±0.41	205.33±4.98	4.01±0.79
	U.S	24	7.04±0.01	11.40±0.26	299.22±1.07	3.76±0.67
JANUARY	B.C	24	6.84±0.01	12.67±0.15	<10±0.00	3.68±0.94
2012	D.P	23	6.87±0.03	32.67±0.81	303.67±0.33	3.72±0.95
	D.S	24	6.92±0.02	8.72±0.04	150.11±3.56	3.74±0.66
	U.S	25	7.41±0.01	6.37±0.02	308.89±1.02	3.26±0.39
FEBRUARY	B.C	25	7.80±0.01	40.37±0.21	295.67±4.73	1.82±1.12
2012	D.P	25	7.88±0.01	44.07±0.25	309.33±0.58	2.81±0.86
	D.S	27	7.77±0.01	5.94±0.10	254.78±5.39	4.01±0.80

Values are averages of three replicates ± standard deviation

U.S - Upstream, B.C - Before chlorination, D.P - Discharge point, D.S - Downstream

Temp - Temperature, T - Turbidity, COD - Chemical oxygen demand, BOD5 - Biochemical oxygen demand

Table 2.2: Physico-chemical parameters of wastewater effluent from New Germany wastewater treatment works and the receiving river.

MONTH		Temp (°C)	pH	T (NTU)	COD (mg/l)	BOD (mg/l)
	U.S	26	7.52±0.09	5.15±0.05	313.89±0.19	7.79±0.83
MARCH	B.C	26	7.12±0.21	6.65±0.23	153.67±11.46	2.20±0.13
2012	D.P	26	7.18±0.12	5.71±0.59	239.00±10.00	3.12±0.27
	D.S	26	7.51±0.09	7.32±0.33	141.33±11.06	4.97±0.59
	U.S	18	7.08±0.02	8.23±0.00	104.22±2.83	8.49±0.47
	B.C	20	7.04±0.04	1.52±0.00	202.78±9.10	3.30±0.97
APRIL	D.P	19	6.82±0.01	1.42±0.02	179.67±1.20	3.44±0.67
2012	D.S	20	7.05±0.04	17.00±0.00	114.00±1.73	6.33±0.21
	U.S	16	6.42±0.05	3.18±0.00	298.67±0.33	11.04±0.97
	B.C	19	6.91±0.09	28.70±.00	312.22±0.69	3.15±0.25
MAY	D.P	14	7.02±0.01	30.30±0.00	246.33±3.06	4.72±0.16
2012	D.S	19	7.10±0.00	17.80±0.00	311.89±2.22	9.67±0.55
	U.S	16	7.93±0.01	9.02±0.12	22.33±3.79	10.80±0.41
	B.C	18	7.62±0.01	9.63±0.03	310.00±1.73	4.19±0.11
JUNE	D.P	14	7.55±0.01	10.63±0.51	137.67±9.87	5.03±0.07
2012	D.S	18	7.83±0.00	14.07±0.12	73.33±4.16	7.16±1.57
	U.S	14	6.30±0.01	2.44±0.01	309.67±2.19	5.27±0.41
	B.C	17	6.53±0.10	20.07±0.12	193.67±3.67	2.12±0.17
JULY	D.P	15	6.89±0.01	20.73±0.15	308.67±0.58	3.95±0.34
2012	D.S	17	6.98±0.02	16.10±0.00	299.56±4.62	9.42±0.55
	U.S	15	7.12±0.03	40.400.36	207.56±1.07	3.27±1.02
	B.C	17	6.85±0.11	19.73±0.15	139.56±1.02	3.62±1.02
AUGUST	D.P	12	7.26±0.02	16.80±0.17	309.00±1.33	4.68±0.80
2012	D.S	17	7.09±0.04	14.10±0.10	311.78±0.84	5.86±1.57

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Table 2.2 continued

MONTH		Temp (°C)	pH	T (NTU)	COD (mg/l)	BOD (mg/l)
	U.S	20	6.48±0.02	15.83±0.15	310.33±0.58	4.06±0.91
	B.C	22	6.75±0.08	5.84±0.01	98.22±2.41	4.66±0.84
SEPTEMBER	D.P	20	6.37±0.03	16.33±0.06	310.44±1.17	4.49±1.08
2012	D.S	20	6.59±0.00	6.98±0.02	189.89±2.59	3.42±0.47
	U.S	17	6.97±0.02	3.68±0.01	311.89±0.84	4.46±0.67
	B.C	20	6.91±0.09	20.00±0.10	35.67±3.61	4.51±0.84
OCTOBER	D.P	19	6.85±0.14	6.48±0.04	54.11±3.15	4.42±0.72
2012	D.S	20	6.98±0.03	5.10±0.01	239.22±4.81	4.79±0.79
	U.S	17	7.12±0.01	8.11±0.06	306.78±2.46	4.31±0.78
NOVEMBER	B.C	20	6.82±0.03	5.51±0.08	69.11±1.39	4.14±0.61
2012	D.P	18	7.14±0.04	29.43±0.06	108.56±3.24	4.22±0.71
	D.S	20	7.16±0.01	16.53±0.23	257.56±14.55	4.49±0.81
	U.S	20	6.47±0.01	32.10±0.10	24.33±2.08	3.38±1.46
	B.C	22	6.55±0.28	4.41±0.30	93.33±5.93	2.87±0.81
DECEMBER	D.P	20	6.45±0.01	29.43±0.06	300.44±4.22	3.36±0.92
2012	D.S	22	6.51±0.04	28.10±0.10	35.33±3.84	4.17±0.83
	U.S	22	6.71±0.01	10.80±0.00	80.33±5.13	3.92±0.69
JANUARY	B.C	23	6.59±0.02	9.43±0.04	111.11±0.84	3.43±1.09
2013	D.P	22	6.61±0.02	9.29±0.04	240.78±3.75	3.75±0.79
	D.S	23	6.73±0.00	10.60±0.00	44.56±3.42	4.50±1.27
	U.S	21	7.50±0.05	8.83±0.04	305.33±0.58	3.04±0.80
FEBRUARY	B.C	24	7.72±0.03	3.93±0.01	158.89±4.72	3.97±1.10
2013	D.P	23	7.87±0.02	4.02±0.03	283.44±3.79	4.08±1.04
	D.S	24	8.08±0.00	5.80±0.02	272.89±14.82	4.85±0.85

Values are averages of three replicates ± standard deviation

U.S - Upstream, B.C - Before chlorination, D.P - Discharge point, D.S - Downstream

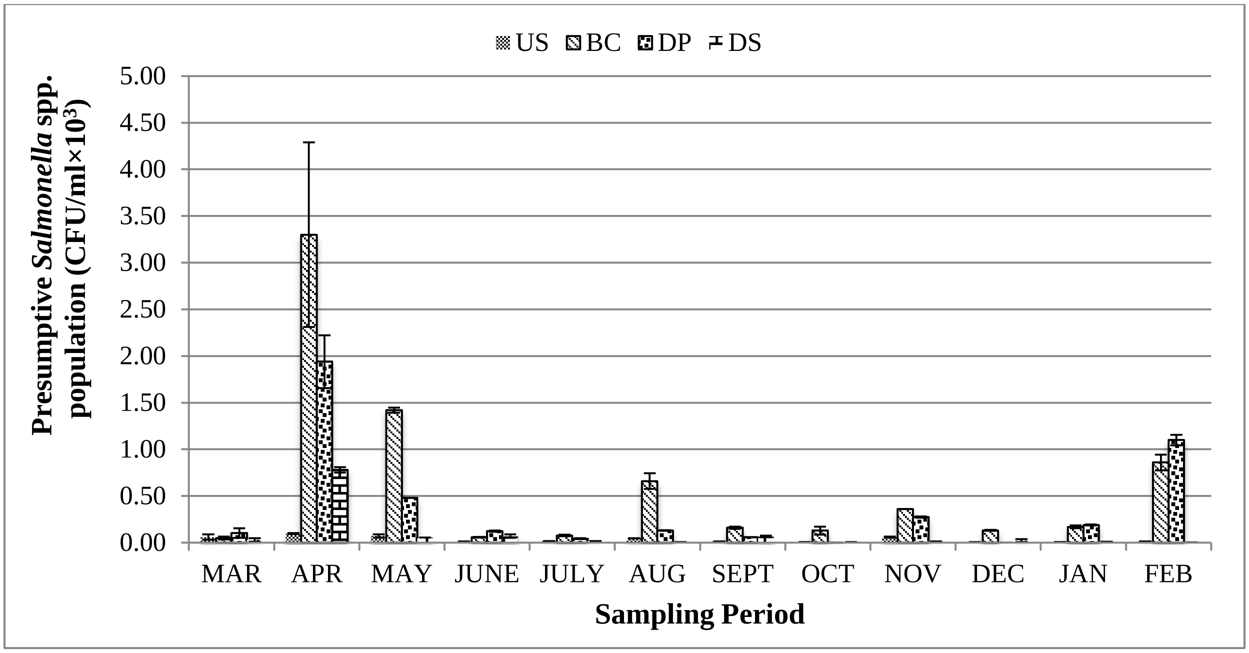
Temp - Temperature, T - Turbidity, COD - Chemical oxygen demand, BOD5 - Biochemical oxygen demand

2.3.2 Microbial profile of treated wastewater effluent and receiving river

Biochemical tests of randomly selected isolates of presumptive *Salmonella* and *Shigella* spp. indicated that the presumptive *Salmonella* isolates were indeed *Salmonella* spp. but not *Shigella* spp. Since the presumptive *Shigella* possessed morphological and phenotypic characteristics on the selective agar consistent with *Shigella* species, these are subsequently referred to as presumptive *Shigella* or *Shigella* like organisms (Stecchini and Domenis, 1994; Govindarajan *et al.*, 2012)

Figure 2.1 shows the monthly variation of presumptive *Salmonella* spp. population in NWWTW and receiving Umgeni River. At U.S, counts for presumptive *Salmonella* spp. ranged from 10 −100 CFU/ml, at the B.C, counts ranged from $5-3.30\times10^3$ CFU/ml. The counts ranged from $0-1.94\times10^3$ CFU/ml and $0-7.8\times10^2$ CFU/ml at the D.P and D.S respectively. Some levels of reduction in presumptive *Salmonella* count at the D.P after chlorination were recorded in April (41.2%), May (66%), August (80%), September (62.5%) and October (100%). Counts for presumptive *Shigella* varied throughout the study period and ranged from $0-17.2\times10^2$ CFU/ml at the U.S, $11-18.2\times10^3$ CFU/ml at the B.C, $30-13.4\times10^3$ CFU/ml at D.P and $0-12.5\times10^3$ CFU/ml at the D.S. Figure 2.2a shows the monthly variation of *Salmonella* spp. at the NGWTP and receiving Aller River. At the U.S, counts ranged from 10 CFU/ml (May and July) to 13.9×10^3 CFU/ml (December), at the B.C counts ranged from $10-14.8\times10^2$ CFU/ml, at the D.P counts ranged from 0−17 CFU/ml while a range of $0-10.5\times10^3$ CFU/ml was recorded at D.S. Monthly variation of presumptive *Shigella* spp. recovered from the NGWTP and its receiving water shed is shown in Figure 2.2b. At the U.S, presumptive counts ranged from $0.00-43.2\times10^2$ CFU/ml, at the B.C counts ranged from 0 CFU/ml (February) to 5×10^2 CFU/ml (January). At the D.P, counts ranged from $0.00-5.5\times10^2$ CFU/ml and $0.00-2.5\times10^2$ CFU/ml at D.S.

(a)



(b)

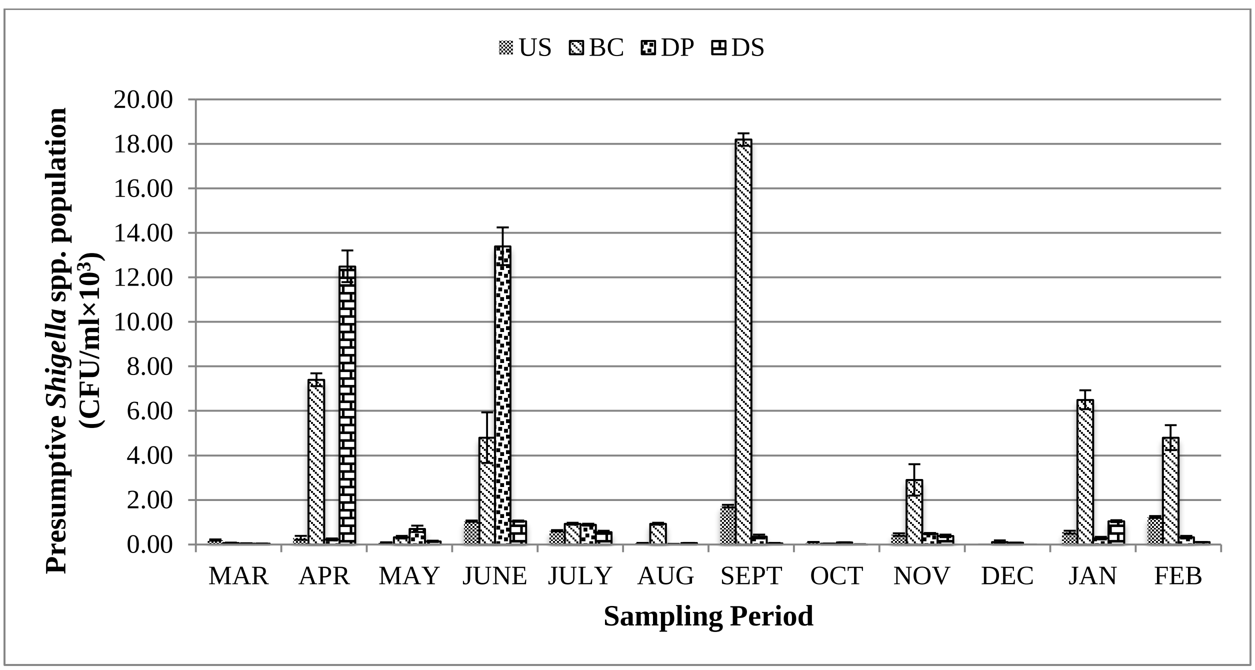
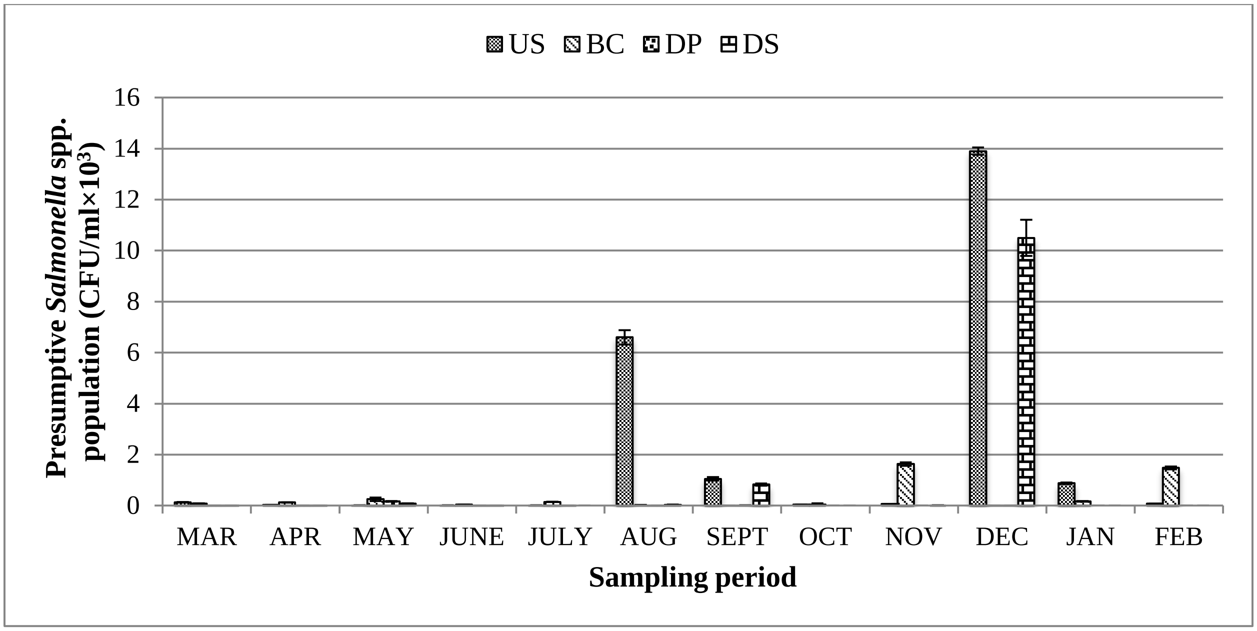


Figure 2.1: Monthly variation of (a) presumptive *Salmonella* spp. and (b) presumptive *Shigella* spp. population in Northern wastewater treatment plant and receiving Umgeni River.

U.S- Upstream, B.C- Before chlorination, D.P- Discharge point, D.S- Downstream

(a)



(b)

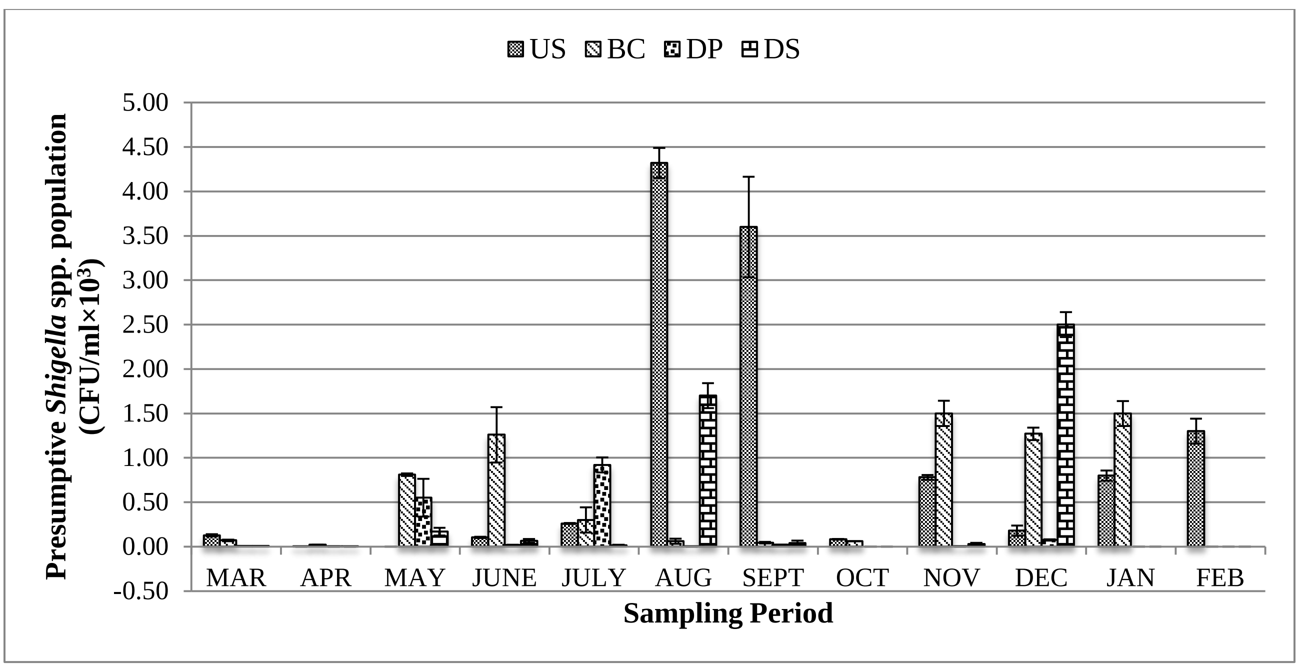


Figure 2.2: Monthly variation of (a) presumptive *Salmonella* spp. and (b) presumptive *Shigella* spp. population at the New Germany wastewater treatment plant and receiving Aller River.

U.S- Upstream, B.C- Before chlorination, D.P- Discharge point, D.S- Downstream

2.3.3 Statistical analysis

Table 2.3 shows correlation matrices of selected physico-chemical parameters with microbial counts from NWWTW. In this study, pH positively correlated with BOD ($r = 0.600$; $p < 0.05$) at D.S but negatively ($r = -0.652$; $p < 0.05$,) correlated at U.S. Turbidity positively correlated with presumptive *Salmonella* spp. at the U.S ($r = 0.613$; $p < 0.05$,) and B.C points ($r = 0.622$; $p < 0.05$,) but correlated negatively with presumptive *Shigella* spp. at U.S ($r = -0.648$; $p < 0.05$,) and D.P ($p < 0.05$; $r = -0.667$). At the D.S, a strong positive correlation was recorded between presumptive *Salmonella* and *Shigella* spp. count ($r = 0.931$; $p < 0.01$,). At the NGWTP (Table 2.4), turbidity strongly correlated with presumptive *Salmonella* spp ($r = 0.839$; $p < 0.01$,) and positively correlated with presumptive *Shigella* spp. count ($p < 0.05$, $r = 0.622$) at U.S but negatively correlated with temperature at B.C ($r = -0.577$; $p < 0.05$,). BOD negatively correlated with presumptive *Shigella* spp. count ($r = -0.628$; $p < 0.05$) at U.S and with temperature ($r = -0.671$; $p < 0.05$) at D.P. At D.S, there was a strong correlation between presumptive *Salmonella* and *Shigella* spp. count ($r = 0.731$; $p < 0.01$) while COD positively correlated with temperature ($p < 0.05$, $r = 0.643$).

Table 2.3: Correlation matrices of selected physicochemical parameters with microbial load at the Northern wastewater treatment plant and receiving Umgeni River

Upstream

Parameters	**pH**	**Turbidity**	**BOD**	**COD**	**Temperature**	***Salmonella***	***Shigella***
pH	1						
Turbidity	.174	1					
BOD	-.652*	-.053	1				
COD	.485	.291	-.128	1			
Temperature	-.165	.084	.332	.002	1		
Salmonella	-.050	.613*	.332	.110	-.045	1	
Shigella	-.123	-.648*	.165	-.193	.187	-.440	1

Before chlorination

Parameters	**pH**	**Turbidity**	**BOD**	**COD**	**Temperature**	***Salmonella***	***Shigella***
pH	1						
Turbidity	.123	1					
BOD	-.027	-.104	1				
COD	.095	.428	-.045	1			
Temperature	.090	.037	.219	.254	1		
Salmonella	.483	.622*	-.240	.031	.031	1	
Shigella	.253	.053	.121	.243	.234	.204	1

Discharge point

Parameters	pH	BOD	COD	Turbidity	Temperature	Salmonella	Shigella
pH	1						
BOD	0.042	1					
COD	0.052	-0.495	1				
Turbidity	-0.066	-0.456	0.510	1			
Temperature	-0.113	-0.249	0.041	-0.356	1		
Salmonella	0.487	-0.115	0.344	0.471	-0.313	1	
Shigella	0.237	0.340	-0.163	-.667*	0.556	-0.105	1

Downstream

Parameters	pH	COD	Turbidity	BOD	Temperature	Salmonella	Shigella
pH	1						
COD	0.142	1					
Turbidity	0.060	0.093	1				
BOD	.600*	-0.237	0.076	1			
Temperature	0.053	-0.070	0.508	0.329	1		
Salmonella	0.272	-0.287	0.050	0.485	0.194	1	
Shigella	0.390	-0.328	0.005	0.491	0.244	.931**	1

*Correlation significant at 0.05 level (2 tailed); **Correlation significant at 0.01 level (2-tailed)

Table 2.4: Correlation matrices of selected physicochemical parameters with microbial load at the New Germany wastewater treatment plant and receiving Aller River

Upstream

Parameters	**pH**	**Turbidity**	**BOD**	**COD**	**Temperature**	***Salmonella***	***Shigella***
pH	1						
Turbidity	0.085	1					
BOD	0.207	-0.529	1				
COD	0.056	0.561	0.029	1			
Temperature	0.219	0.155	-0.150	-0.180	1		
Salmonella	-0.278	.839**	-0.539	0.466	0.046	1	
Shigella	-0.047	.622*	-.628*	-0.028	-0.014	0.394	1

Before chlorination point

Parameters	**pH**	**Turbidity**	**BOD**	**COD**	**Temperature**	***Shigella***	***Salmonella***
pH	1						
Turbidity	-0.241	1					
BOD	0.310	-0.134	1				
COD	0.430	0.300	-0.262	1			
Temperature	0.175	-.577*	-0.094	-0.339	1		
Shigella	-0.262	-0.060	-0.022	0.078	-0.046	1	
Salmonella	0.343	-0.269	0.218	-0.141	0.197	0.110	1

Discharge point

	pH	Turbidity	BOD	COD	Temperature	Salmonella	Shigella
pH	1						
Turbidity	-0.421	1					
BOD	0.307	0.246	1				
COD	0.297	-0.399	-0.063	1			
Temperature	0.054	-0.383	-.671*	0.269	1		
Salmonella	-0.036	0.541	0.319	0.059	-0.139	1	
Shigella	-0.104	0.569	0.091	-0.303	-0.434	0.477	1

Downstream

Parameters	**pH**	**Turbidity**	**BOD**	**COD**	**Temperature**	***Salmonella***	***Shigella***
pH	1						
Turbidity	-0.328	1					
BOD	0.403	0.298	1				
COD	-0.069	-0.155	-0.480	1			
Temperature	0.063	-0.497	-0.499	.643*	1		
Salmonella	-0.528	0.452	-0.334	0.281	0.126	1	
Shigella	-0.369	0.533	-0.038	-0.215	-0.280	.731**	1

*. Correlation significant at 0.05 level (2 tailed); ** Correlation significant at 0.01 level (2-tailed)

2.4 Discussions

Physicochemical analysis of the wastewater gives an indication of the quality of effluent being discharge into the environment. The impact of sub-standard effluent quality or untreated wastewater discharged into receiving water bodies can be detrimental making water quality a primary and direct threat to water availability and security. Wastewater management is the first barrier in a multi-barrier system to ensure safe drinking water, public health and environmental sustainability (Davies and Mazumder, 2003). During the study period, the temperature regime varied depending on season but was still within the acceptable limit of 25°C (DWAF, 1984) and did not pose any threat to the receiving watershed. The temperature of wastewater is a very important parameter because of its effect on the chemical reaction and reaction rates, aquatic life and suitability of the water for beneficial uses (Alan *et al.,* 2000). High temperatures can result in high mortality and encourage the growth of undesirable algae and wastewater fungus (Ntengwe, 2005).

The pH values recorded was stable across all sampling points in each month but varied throughout the duration of the study period in each plant (Tables 2.1 and 2.2). At both plants, the pH ranged between 6.41-7.88 and 6.30-8.08 in Northern wastewater treatment works and New Germany wastewater treatment plant respectively. The neutral to alkaline pH recorded in this study is similar to previous reports (Igbinosa and Okoh, 2009;Momba *et al.,* 2006; Morrison *et al.,* 2001). The pH of water can provide important information about many chemical and biological processes and provides indirect correlations to a number of different impairments in the wastewater treatment processes (Annalakshmi and Amsath, 2012). Changes in pH can be indicative of industrial pollution, photosynthesis or the decomposition of organic matter by microorganisms (Irenosen *et al.,* 2012). Most ecosystems are sensitive to changes in pH and the monitoring of pH has been incorporated into the environmental laws of most industrialized countries. Very low or high pH is toxic to aquatic life and alters the solubility of chemicals in water (Odjadjare and Okoh, 2010). The pH of most natural waters is in the range of 4−9 and the target limit set by the South African Department of Water Affairs is between 5.5 and 9.5 (DWAF 1984). Hence, the pH values recorded in this study fell within the acceptable range indicating that discharge of the treated wastewater may have no negative impact on the river water with respect to pH.

The turbidity of the water samples in this study ranged between 1.42 NTU to 76.43 NTU and varied seasonally (Tables 2.1 and 2.2). There is no standard set by the department of water affairs, South Africa on the limit of turbidity of final effluent discharged into surface waters. However, the World Health Organization (WHO) guidelines stipulates a turbidity of <5 NTU for effluent discharged into the environment (WHO, 2004). The turbidity at the discharge point, upstream and downstream at both plants exceeded the guideline (Table 2.1 and 2.2). Also, the turbidity could be due to storm runoff or anthropogenic activities occurring upstream. High turbidity values recorded in some months at the discharge point could be the result of poor settling in the secondary clarifer. This high variation has been reported in previous studies in the Eastern Cape province of South Africa (Igbinosa *et al.*, 2009; Odjadjare and Okoh 2010). Turbidity is caused by small particles which may be organic or inorganic and can provide food and shelter for microorganisms. If not removed, turbidity can promote the regrowth of pathogens in the final effluent of receiving water body into which the effluent is discharged (Altaher and Alghamdi, 2011). Turbidity also limits the bactericidal effect of chlorine in the wastewater during disinfection (Odjadjare and Okoh, 2010) and may react with organic compounds in the water to form micro-contaminants such as trihalomethane (Baršienė *et al.*, 2009; Ratola *et al.*, 2012).

Biochemical oxygen demand (BOD_5), the amount of oxygen needed by bacteria to oxidize the organic matter present in the water is a basic means of measuring the degree of water pollution (Allan *et al.*, 2000). The BOD_5 values recorded was stable across each sampling point in each month but the values varied in the course of the study ranging from 1.03 mg/l to 11.04 mg/l. There is no South African guideline for BOD in the final effluent of wastewater; however, the European union (EU) recommends a discharge limit of 3 to 6 mg/l for aquatic ecosystems (Momba *et al.*, 2006). On most occasions the recorded BOD_5 values at the D.P were within the recommended EU limit. Discharge of effluent high in BOD into natural water bodies such as rivers and lakes could result in rapid depletion of dissolved oxygen, which may lead to anoxic conditions, and consequent disruption of balance of the aquatic ecosystem (Islam and Tanaka, 2004).

The chemical oxygen demand of the water samples varied remarkably throughout the study period. High COD values were recorded at the upstream while, the average recorded values (212 mg/l) at the D.P greatly exceeded the South African limit of 30 mg/l (DWAF, 1984) suggesting it may have a negative impact on the receiving surface water since it is a measure of the amount of oxygen required to oxidize both organic and inorganic compounds present in the water. High levels of COD observed upstream could be attributed to runoff, agricultural activities and anthropogenic activities upstream (Igbinosa *et al.*, 2009). Igbinosa and Okoh (2009), reported a similar observation and attributed the increase in COD to addition of organic and inorganic substances from the environment and as well as organic contaminants entering the system from municipal sewage treatment plants or other non-point sources of pollution. Higher averages of COD values varying from 512 to 698.11 mg/l was reported in a study on river quality in India and was attributed to the presence of inorganic chemicals in the wastewater of a nearby chemical industry (Singh *et al.*, 2012).

Though pH, temperature and BOD_5 were within South African and International recommended guidelines, Turbidity and COD were not. This suggests that the quality of the final effluent is not fit for discharge because increased turbidity and COD from the final effluent coupled with storm runoff, anthropogenic activites and other environmental factors might increases the possiblity of eutrophication and oxygen depletion in the river downstream as well as possible introduction of toxic chemicals.

Tertiary treatment of final sewage effluent with chlorine at the wastewater treatment plants under investigation reduced the number of viable presumptive *Salmonella* and presumptive *Shigella* spp. at the discharge point during the sampling period but failed to totally eliminate them (Figures 2.1and 2.2). Presumptive *Salmonella* and *Shigella* were also recovered downstream and this could be as a result of discharge of the final effluent, contamination of the river downstream with animal or human feces as well as storm runoffs.

At the NWWTW, recorded counts ranged from $0-1.94\times10^3$ CFU/ml for presumptive *Salmonella* spp. and $30-13.4\times10^3$ CFU/ml for presumptive *Shigella* spp. at the discharge point while at the NGWTP, low presumptive *Salmonella* counts were recorded (0–17 CFU/ml) at the discharge point but higher counts ranging from $0–5.5x10^3$ CFU/ml was recorded for presumptive *Shigella* spp. This indicates that treated wastewater effluent discharged from these treatment plants are a posssible source of contamination of the receiving surface water with presumptive *Salmonella* and presumptive *Shigella* spp. Upstream of the river at the NGWTP is an informal settlement with poor sanitation and inadequate sewage disposal system which contaminate the river with human and animal wastes while the bank of the Umgeni River downstream is littered with feces. Storm runoff from this informal settlement and discharge of inadequately treated wastewater explains the high count of presumptive *Salmonella* and presumptive *Shigella* spp. observed upstream and downstream. Morphological and phenotyic characteristics of the orgainsms on the selective agar plates were consistent with *Shigella* spp., however, biochemical tests of randomly selected isolates of the presumptive *Shigella* were negative. Thus, results of the presumptive count of *Shigella* spp. should be interpreted with caution as some of these isolates may belong to other genera of *Enterobacteriaceae* family. Previous studies have indicated that although *Shigella* species are not as resilient as *Salmonella* to treatment processes, it is still a cause for concern due to its high transmissibility and very low infective dose estimated at 10 - 100 cells per ml (Barnoy *et al.*, 2011).

Olaniran *et al.* (2012) reported low counts of *Salmonella* and *Shigella* spp. from treated wastewater of same plants under investigation which may be due to the short duration of the study or influence of season prevalent during sampling. However, in comparison to this study, they also detected these organisms at all points of the treatment processes sampled. Furthermore, various factors such as environmental stress may cause microorganisms to go into the viable but not culturable state (VBNC) state resulting in possible inaccurate estimation of these organisms (Godinho *et al.*, 2010). The implication therefore is that wastewater effluent containing deadly pathogens are released into receiving surface water and could potentially result in outbreaks of waterborne diseases. Elsewhere, Momba *et al.* (2006) reported recovery of microorganisms including *Salmonella* and *Shigella* spp. in the final effluents of four wastewater treatment plants in the Eastern Cape province of South Africa and concluded that wastewater treatment plants serve as a point source of microbial pollution of natural water bodies.

Recent reports have also suggested that most wastewater treatment plants in South Africa are either dysfunctional or non-functional (Bateman, 2010) and inefficient in removing microbial pathogens from wastewater and producing wastewater effluent of acceptable standard that meet discharge guidelines set by the Department of Water Affairs, South Africa (Dungeni and Momba, 2010; Igbinosa and Okoh 2008; Igbinosa *et al.,* 2009; Odjadjare *et al.,* 2012; Samie *et al.,* 2009). Wastewater treatment efficiency is dependent on the variation in quality of raw water and the dynamics of plant processes (Kistemann *et al.,* 2008; Rose *et al.,* 1996). Wide variation in treatment processes can lead to significant amounts of pathogens passing through the process for various time periods. Inconsistencies in treatment processes were also observed during the study period. For example, In the months of March and April, 2012, at the Northern wastewater tratment plant, there was consistent treatment of wastewater due to the infrastructural upgrade taking place. At the New Germany wastewater treatment plant, during sampling in the month of May, 2012, it was observed that due to mechanical fault with the chlorine pump, the final effluent of the wastewater was not chlorinated while being discharged. However, for the remainder of the study period, the wastewater was chlorinated.

The issue of treatment efficiency is of major importance if the reclaimed water is intended for recreational or potable reuse or is to be discharged into natural water bodies because disposal of inadequately treated wastewater into surface water recipient is one of the major sources of pathogens in the environment (Odjadjare *et al.,* 2012; Ottoson *et al.,* 2006; Touron *et al.,* 2005). Swimming or other recreational activities in sewage contaminated surface water may cause *Salmonella* and *Shigella* infections or other gastroenteritis while ingestion, exposed mucous membrane and breaks in protective skin barrier may serve as a port of entry to pathogenic microorganisms (Schoen and Ashbolt, 2010). Though *Salmonella* is isolated from water in lower numbers than indicator bacteria such as fecal coliform, fecal streptococci and enterococci; counts in the range of 15−1000 CFU/ml may pose public health risks (Girones *et al.,* 2010). Bacillary dysentery caused by *Shigella* is a scourge on developing countries with a reported case of 163 million infections annually occurring mostly in children under the age of 5 (Emch *et al.,* 2008; Gu *et al.,* 2012; Wen *et al.,* 2009). *Shigella* infections are primarily transmitted via contaminated food and water (Hench *et al.,* 2003; Momba *et al.,* 2006; Samie *et al.,* 2009) thus, the presence of *Salmonella* and *Shigella* like organisms in the final effluent of wastewater and receiving surface water is a serious cause for concern where the contaminated water is depended on for irrigation and rural socio-economic activities.

In conclusion, unpolluted water represents an important health-enhancing recreational resource underscoring the importance of regular microbial examination and epidemiological monitoring. The wastewater treatment plants investigated in this study produced low quality final effluent and serve as a source of contamination of receiving watershed with presumptive *Salmonella* and *Shigella* like organisms. This is probably due to various factors including inadequate and poorly maintained infrastructure, shortage of skilled personnel and inadequate training of staff at the treatment plants. Hence urgent intervention is needed by the regulatory authorities in order to insure compliance of these treatment plants with set guidelines.

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CHAPTER THREE

ISOLATION AND GENOTYPIC CHARACTERIZATION OF *SALMONELLA* FROM TREATED WASTEWATER EFFLUENT AND RECEIVING SURFACE WATERS IN DURBAN, SOUTH AFRICA

3.1 Introduction

Salmonella spp. are important Gram-negative bacilli which infect both human and animals causing a wide range of diseases such as diarrhea, typhoid fever, osteomyelitis, septicemia and meningitis (Hansen-Wester and Hensel 2001; Scherer and Miller 2001). This genus comprises of over 2000 recognized serotypes and is divided into two species namely *S. bongori* and *S. enterica*. *Salmonella enterica* consist of six subspecies namely *enterica, arizonae, salamae, diarizonae, houtenae and indica* (Soyer *et al.,* 2009; Fookes *et al.,* 2011). It is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* spp. occur globally each year, with 155,000 deaths (Majowicz *et al.,* 2010). This high number of infections emphasizes the importance of this intracellular pathogen and represents a considerable burden in both developing and developed countries. Mortality rate of *Salmonella* infections is a problem mainly in developing countries (Kotloff *et al.,* 2012) while morbidity due to acute *Salmonella* infection can also have an impact in developed countries (O'Brien, 2013). The mechanism of *Salmonella* invasion and intracellular replication is complex but the knowledge of the whole genome sequence has enabled identification and characterization of many genes involved in its pathogenesis and indicates that *Salmonella* has undergone horizontal gene transfer acquiring certain pathogenicity islands (Lahiri *et al.,* 2010). These pathogenicity islands contains genes which help the bacteria invade, replicate and spread inside the stringent host environment (Dorsey et al., 2005).

Reflecting a complex set of interactions with its host, *Salmonella* spp. require multiple genes for full virulence (Marcus *et al.*, 2000). Many of these genes are found in 'pathogenicity islands' in the chromosome. *Salmonella typhimurium* possesses at least five such pathogenicity islands (SPI), which confer specific virulence traits and may have been acquired by horizontal transfer from other organisms.

The SPI-1 and 2 contains the *inv*A and *spi*C genes are essential for systemic pathogenesis because they encodes a type III secretion system (T3SS) that is required for invasion (Hensel 2004, Miki *et al.,* 2004). The T3SS system is used by the pathogen to deliver virulence factors to the host cell and interfere with or subvert normal host cell signalling pathways (Marcus *et al.,* 2000). The major virulence functions encoded by SPI-3 are the high affinity Mg^{2+} uptake system that is required for the adaptation to the nutritional limitations of the intraphagosomal habitat and the *mis*L, an autotransporter protein involved in intestinal colonization and essential for survival in macrophages (Dorsey *et al.,* 2005; Gassama-Sow *et al.,* 2006; Sánchez-Jiménez *et al.,* 2010). SPI-4 is a 25 kb pathogenicity island containing the *orf*L gene thought to encode a type 1 secretion system (an autotransporter protein) that mediate the secretion of toxins and is necessary for macrophage survival (Gassama-sow *et al.,* 2006; Sánchez-Jiménez *et al.,* 2010). The SPI-5 is a 7.6 kb gene which contains the *pip*D gene and encodes effector proteins for both, the T3SS encoded by SPI-1 and SPI-2 (Dione *et al.,* 2011, Hensel, 2004) and is mainly associated with enteropathogenesis (Marcus *et al.,* 2000).

Added to this disease burden are the complications arising from the inefficacy and failures of antimicrobial chemotherapies applied in clinical practice to remedy these diseases. Bacterial resistance to antibiotics have increased in recent years, worldwide and resistance to antimicrobials in human pathogens such as *Salmonella* spp. poses a great threat to human health (Oluyege *et al.,* 2009). Antimicrobial resistance in *Salmonella* has been associated with an increase in the number of adverse events following infection such as higher levels of hospitalization, longer illness, and higher risk of invasive illness as well as treatment failures (Duffy *et al.*, 2012).

Salmonella spp. have been isolated in different environment contaminated by human and animal feces particularly in rivers, estuarine and sea waters (Touron *et al.*, 2005). Inadequately treated wastewater discharged into rivers and surface waters is a major source of contamination of these natural water bodies with pathogenic microorganisms (Wen *et al.*, 2009) and could result in outbreaks of waterborne diseases (Bertuzzo *et al.*, 2008; Momba *et al.*, 2006). In South Africa, several reports have implicated wastewater effluents as a point source pollution of surfaces water with pathogenic microorganisms, including *Vibrio* spp. (Igbinosa *et al.*, 2011), *Listeria* spp. (Odjadjare and Okoh, 2010), *Pseudomonas* spp. (Odjadjare *et al.*, 2012), and *Salmonella* and *Shigella* spp. (Olaniran *et al.*, 2012) leading to public health risks to those who rely on these waters for socioeconomic activities.

There is a dearth of information on the genotypic characteristics of *Salmonella* spp. in wastewater and receiving surface water in Durban, KwaZulu-Natal. The purpose of this study was to identify and characterize the antibiotic resistance profile and virulence gene signatures of *Salmonella* spp. recovered from treated wastewater effluent and receiving water surfaces in Durban, KwaZulu-Natal province of South Africa.

3.2 Materials and Methods

3.2.1 Sample collection

Samples were collected as per section 2.2.2 of Chapter two (page 33) at different points from two wastewater treatment plants, the Northern wastewater treatment plant (NWWTW) and the New Germany wastewater treatment plant (NGWTP) between March 2012 and February 2013. Samples were collected

3.2.2 Microbial analysis

Isolation of *Salmonella* spp. from the water samples was done by enrichment method previously described by Espigares *et al.* (2006) with modifications. Thoroughly mixed water sample (25 ml) was added to 250 ml of sterile buffered peptone water and incubated at 37°C for 18 to 24 h with shaking at 230 rpm. Thereafter, 1 ml of the pre-enrichment was appropriately diluted in 9 ml of sterile Rappaport-Vassiliadis soy broth (RVS) (Oxoid, UK) depending on the turbidity of the water sample used in the pre-enrichment and incubated at 42°C for 24 to 48 h with shaking at 230 rpm. One hundred microliters (100 µl) of the appropriately diluted RVS broth was spread-plated on *Salmonella* chromogenic agar (Oxoid, UK) in duplicates and incubated aerobically at 37°C for 18 to 24 h. Presumptive *Salmonella* spp. with purple colonies were purified on fresh nutrient agar plates and subjected to further identification using biochemical tests and molecular methods.

3.2.3 Biochemical confirmation of presumptive *Salmonella* spp.

Biochemical tests was carried out as per section 2.2.5 of Chapter 2 (page 35). Biochemically positive isolates were transferred to fresh nutrient agar plates stored for molecular confirmation.

3.2.4 Molecular confirmation of presumptive *Salmonella* spp.

Template DNA was prepared from freshly grown cultures of the isolates on nutrient agar using the boiling method as previously described (Akinbowale *et al.,* 2007) with modifications. Well isolated colonies (3 to 5) were suspended in 70 µl of sterile deionized water, boiled in a water bath at 100°C for 10 min and cooled on ice for a further 5 min. Thereafter, the suspension was centrifuged at 13000 rpm in a micro-centrifuge (eppendorf) for 5 min. The supernatant (50 µl) was carefully transferred to a sterile eppendorf tube and used as a template in the PCR assay. *Salmonella* spp. were confirmed by the amplification of the *inv*A gene as previously described (Gassama-sow *et al.,* 2006) using the primers F−5′-TGC CTA CAA GCA TGA AAT GG-3′ and R−5′-AAA CTG GAC CAC GGT TGA CAA-3′. The PCR mixture contained: 1× PCR reaction buffer, 1mM of $MgCl_2$, 200 µM of each dNTPs, 0.5 µM of each primer, 2 U of *Taq* polymerase (Supertherm) and 2 µl of template DNA in a final volume of 25 µl. Amplification was performed in a thermocycler (Bio-Rad T100, Singapore) with a temperature regime of 2 min at 94°C for initial denaturation followed by 35 cycles of 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min with a final extension step at 72°C for 5 min. The amplification products were examined by electrophoresis in a 1.5% (w/v) agarose gel at 60V for 90 min in 1% TAE buffer. The products were visualized by UV illumination (Syngene, UK) after staining in 1 mg/ml ethidium bromide solution for 15 min. *Salmonella typhimurium* ATCC 13317 was used as positive control.

3.2.5 Virulence gene detection

The isolates were evaluated for the presence of virulence genes in *Salmonella* pathogenicity island (SPI) using the primers shown in Table 3.1 as previously described (Dione *et al.*, 2011) with modifications. The presence of *mis*L and *orf*L virulence genes was confirmed in a duplex reaction; while that of *spi*C and *pip*D were done in a monoplex reaction. The reaction was done in a 25 µl reaction volume consisting of 2.5 µl 10 × buffers, 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 2 µl of template DNA and 2.5 U of *Taq* polymerase (Supertherm). Amplification was carried out in a thermocycler (Bio-Rad T100, Singapore) using a temperature program consisting of initial denaturation of 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 1 min at the respective annealing temperature of various primers (Table 3.1), 72°C for 1 min with a final extension at 72°C for 5 min. The amplicons were examined by electrophoresis in a 1.5% agarose gel at 60V for 90 min, stained in 1 mg/ml ethidium bromide solution for 15 min and viewed under UV light (Syngene, UK). *Salmonella typhimurium* ATCC 13317 was used as positive control.

Table 3.1: Primers used for detection of virulence genes in *Salmonella* spp. recovered from treated wastewater effluent and receiving surface waters (Dione *et al.,* 2011)

Gene Target	Oligonucleotide sequence (5´-3´)	Amplicon size	Annealing temperatures (°C)
*spi*C	CCTGGATAATGACTATTGAT	309bp	54
	AGTTTATGGTGATTGCGTAT		
*pip*D	CGGCGATTCATGACTTTGAT	400bp	56
	CGTTATCATTCGGATCGTAA		
*mis*L	GTCGGCGAATGCCGCGAATA	550bp	60
	GCGCTGTTAACGCTAATAGT		
*orf*L	GGAGTATCGATAAAGATGTT	350bp	60
	CGTTATCATTCGGATCGTAA		

3.2.6 Antibiotics susceptibility test

Antibiotics susceptibility of the isolates was determined using the Kirby-Bauer disk diffusion method described by Tao *et al.* (2010). The isolates were screened against a predetermined and commercially available panel of 20 antibiotics (Oxoid), belonging to 6 classes. Fresh culture were grown overnight in Mueller-Hinton broth and standardized to 0.5 McFarland by diluting with sterile Mueller-Hinton broth until a photometric reading of 0.08 to 0.1 was obtained on a spectrophotometer (Biochrom, Libra S12) at wavelength of 625 nm. The standardized culture of the isolates were inoculated onto Mueller-Hinton agar using sterile swabs for confluence growth and allowed to dry for 10 min. Thereafter, appropriate antibiotic disks were placed at equidistance on the surface of the agar plates with a sterile forceps and incubated at 37°C for 18 to 24 h. The diameter of the zone of inhibition was measured to the nearest millimeter and recorded as recommended by the Clinical and Laboratory Standards Institute (2007).

The following antibiotics and concentrations were used Cephalothin (30 μg), Imipenem (10 μg), Cefoxitin (30 μg), Cefuroxime (30 μg), Piperacillin (100 μg), Ampicillin (10 μg), Cefixime (5 μg), Ceftazidime (30 μg), Aztreonam (30 μg), Gentamycin (10 μg), Amikacin (30 μg), Streptomycin (10 μg), Chloramphenicol (30 μg), Tetracycline (30 μg), Ciprofloxacin (5 μg), Norflaoxacin (10 μg), Nalidixic acid (30 μg), Nitrofurantoin (30 μg), Trimethorprim/Sulfamethoxazole (1.25/23.75 μg) and Sulfamethoxazole (5 μg)

3.3 Results

3.3.1 Distribution and Confirmation of presumptive Salmonella spp. recovered from treated wastewater and receiving surface waters

Two hundred, presumptive *Salmonella* isolates were recovered from the treated wastewater effluent and receiving surface waters. These were confirmed as *Salmonella* spp. both biochemically and by the detection of the *invA* gene (Figure 3.1).

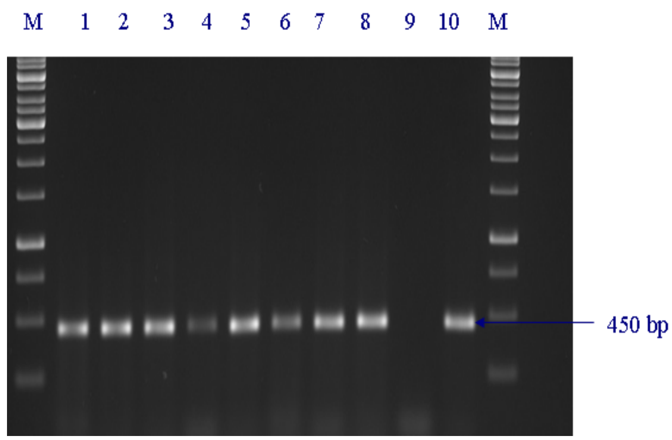

Figure 3.1: Agarose gel showing the expected amplicon size (450bp) of the *inv*A gene in *Salmonella* spp. Lane M contains the marker. Lane 1 to 8 contains representative *Salmonella* isolates; lane 9 contains negative control and lane 10 contains *Salmonella typhimurium* ATCC 13317 used as positive control.

The distribution of confirmed *Salmonella* isolates is given in Table 3.2. The NGTWP and receiving surface water has the highest prevalence (93.5%) of *Salmonella* spp while only 13 (6.5%) isolates were recovered in treated effluent before chlorination at the NWWTW compared to fifty three (26.5%) of the isolates recovered at the NGWTP before chlorination. Also fifty five isolates (27.5%) were recovered at the discharge point of the NGWWTP with additional 27% and 12.5% recovered upstream and downstream of the receiving river of the treated final effluent from the NGTWP respectively.

3.3.2 Antibiogram profile of *Salmonella* spp. in treated effluent and receiving surface water

Antibiogram profile of the confirmed isolates from New Germany wastewater treatment plant is shown in Table 3.2. Complete resistance to Nalidixic acid (100%), Cefixime (2%) was recorded at the upstream while complete resistance to Streptomycin was observed in 13% and 72% of isolates recovered from the discharge point and downstream respectively. Complete resistance to Sulfamethoxazole was recorded at all points sampled. At the discharge point, 71% of the isolates exhibited intermediate resistance to Nalidixic acid compared to 64% of the isolates recovered from downstream. While 84% of the isolates showed intermediate resistance to Streptomycin compared 28% from downstream. Intermediate resistance was recorded against Ciprofloxacin (4%) at the downstream but No resistance to Norfloxacin was observed at all sampled points. Most isolates recovered were susceptible to Amikacin, Ceftazidime, Cefuroxime,Gentamycin, Ampicillin, Ciprofloxacin, Chloramphenicol, Pipracillin, Cephalothin, Norfloxacin and Tetracycline.

Table 3.2 Antibiotics resistance profile of *Salmonella* spp. isolated from New Germany wastewater treatment plants and receiving surface waters N = 187.

	U.S (N = 54)			B.C (N = 53)			D.P (N = 55)			D.S (N = 25)		
	No. of Isolates (%)											
Antibiotics	R	I	S	R	I	S	R	I	S	R	I	S
SXT	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
CFM	1 (2)	0	53 (98)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
FOX	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
S	0	45 (78)	12 (22)	0	40 (75)	12 (23)	7 (13)	48 (87)	0	18 (72)	7 (28)	0
ATM	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
NA	54 (100)	0	0	0	28 (53)	25 (47)	0	39 (71)	16 (29)	0	16 (64)	9 (36)
AK	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	1 (4)	24 (96)
CAZ	0	0	54 (100)	0	0	53 (100)	0	1 (2)	54 (98)	0	1 (4)	24 (96)
CN	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
CXM	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	2 (8)	23 (92)
AMP	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	2 (8)	23 (92)
CIP	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	1 (4)	24 (96)
C	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
PRL	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	1 (4)	2 (8)	22 (88)
KF	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
NOR	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
TE	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	25 (100)
RL	54 (100)	0	0	53 (100)	0	0	55 (100)	0	0	26 (100)	0	0
IPM	0	0	54 (100)	0	0	53 (100)	0	0	55 (100)	0	1 (4)	24 (96)
F	0	0	54 (100)	0	2 (4)	51 (96)	0	4 (7)	51 (93)	0	7 (28)	18 (72)

SXT- Trimethoprim-Sulfamthoxazole, CFM- Cefixime, FOX- Cefoxitin, S-Streptomycin, ATM- Aztreonam, NA- Nalixidic acid, AK-Amikacin, CN- Gentamycin, CAZ- Ceftazidime, CXM-Cefuroxime, AMP- Ampicillin, CIP- Ciprofloxacin, C- Chloramphenicol, PRL-Pipracillin, KF- Cephalothin, NOR- Norfloxacin, TE- Tetracycline, RL- Sulfamethoxazole, IPM- Imipenem, F- Nitrofurantoin.

U.S - Upstream, B.C - Before chlorination, D.P - Discharge point, D.S - Downstream

3.3.3 Distribution of virulence signatures in *Salmonella* spp. recovered from treated wastewater and receiving surface waters.

Figure 3.2 to 3.4 show representative gels of isolates positive for the different virulence genes detected. Of the 200 isolates tested in this study for the presence of virulence genes, 93% harboured the *spi*C gene, 84% harboured the *misL* gene, and 87.5% harboured the *orf*L gene while 87 % harboured *pip*D gene (Table 3.4). All 54 *Salmonella* spp. isolates recovered upstream at the NGWTP contained all four virulence genes The *pip*D gene was present in 51 (96.23%) of the isolates recovered from the B.C point at the NGTWP compared with 2 (15.38%) from the same point at the NWWTW. All 13 isolates at the NWWTP possessed the *spi*C gene compared with 94 % of the isolates at NGWTP at the B.C point. At the D.S of the NGWTP, 96% of the isolates contained the *spi*C gene while only 56% were positive for the *mis*L. The *orf*L gene was present in 100% and 96% of the isolates at the U.S and D.P respectively compared to 80% of the isolates at the D.S (Table 3.5). All isolate possessed more than one virulence gene.

Table 3.3: Distribution of virulence genes in *Salmonella* spp. isolated from treated wastewater effluent and receiving surface water.

Virulence gene	**Location on Pathogenicity island (SPI)**	**No. of positive isolates (%)**
*spi*C	SPI-2	186 (93)
*mis*L	SPI-3	168 (84)
*orf*L	SPI-4	175 (87.5)
*pip*D	SPI-5	174 (87)

Table 3.4: Distribution of Virulence genes in *Salmonella* spp. recovered from treated wastewater effluent from Northern wastewater treatment works (NWWTW) and the New Germany wastewater treatment plant (NGWTP) and receiving surface waters.

Sampling	**Virulence genes**	**NWWTW** **No. of isolate (%)**	**NGWTP** **No. of isolates (%)**
	*pip*D	-	54 (100)
US	*spi*C	-	54 (100)
	*mis*L	-	54 (100)
	*orf*L	-	54 (100)
	*pip*D	2 (15)	51 (96)
BC	*spi*C	13 (100)	50 (94)
	*mis*L	12 (92)	43 (81)
	*orf*L	13 (100)	51 (96)
	*pip*D	-	51 (93)
DP	*spi*C	-	45 (82)
	*mis*L	-	46 (84)
	*orf*L	-	38 (69)
	*pip*D	-	15 (60)
DS	*spi*C	-	24 (96)
	*mis*L	-	14 (56)
	*orf*L	-	20 (80)

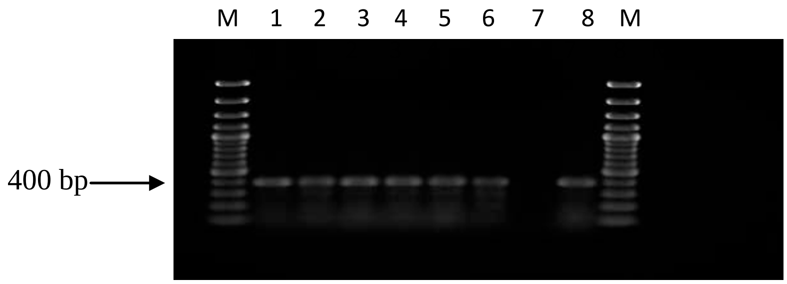

Figure 3.2: Agarose gel showing the expected amplicon size (400 bp) of *pip*D virulence gene in *Salmonella* spp. recovered from wastewater and receiving water surfaces. Lane M contains 100bp marker, lane 1 to 6 contains environmental isolates, lane 7 contains negative control, and lane 8 contains *Salmonella typhimurium* ATCC 13317 as positive control.

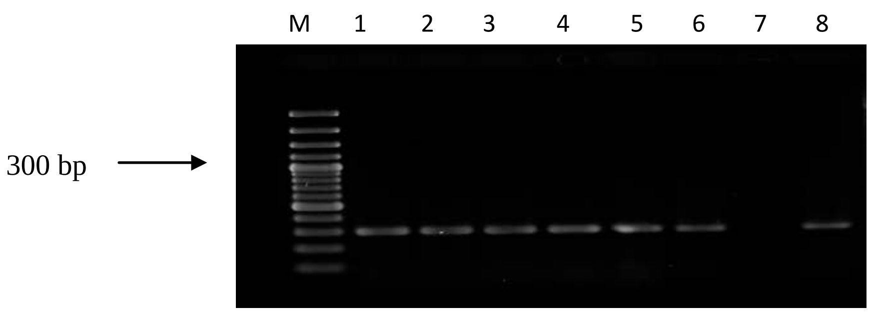

Figure 3.3: Agarose gel showing the expected amplicon size (309 bp) of spiC virulence gene in Salmonella spp. recovered from wastewater and receiving water surfaces. Lane M contains 100 bp marker, lane 1 to 6 contains environmental isolates, lane 7contains negative control and lane 8 contains *Salmonella typhimurium* ATCC 13317 as positive control.

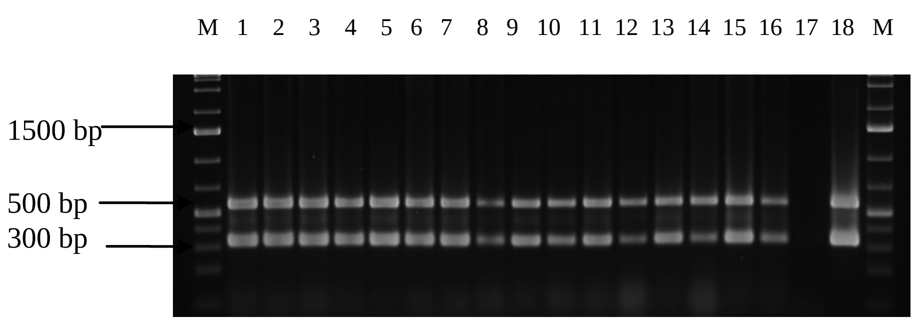

Figure 3.4: Figure 2: Agarose gel showing expected amplicon size of misL (550bp) and orfL (350bp) virulence genes in *Salmonella* spp. recovered from wastewater and receiving water surfaces. Lane M contains marker, lane 1 to 16 contains environmental isolates, lane 17 contains negative control and lane 18 contains *Salmonella tyhimurium* as positive control.

3.4 DISCUSSION

Discharge of inadequately treated wastewater effluent has been known to contaminate surface waters with pathogenic microorganisms such as *Salmonella* and *Shigella* spp. especially in developing countries such as South Africa (Baudart *et al.*, 2000; Chigor *et al.*, 2012). This study thus isolated and characterized *Salmonella* spp. in treated wastewater effluent of two wastewater treatment plants and the receiving surface waters in KwaZulu-Natal province of South Africa. In this study, 200 *Salmonella* spp. were recovered from two wastewater treatment plants and receiving surface waters in Durban, KwaZulu-Natal province of South Africa. Biochemical tests were consistent with *Salmonella* spp. and PCR confirmed the presence of the *inv*A gene in each isolate (Figure 3.1) indicating they are indeed *Salmonella* spp. (Deekshit *et al.*, 2013; Turki *et al.*, 2012). The *inv*A gene is conserved in all *Salmonella* spp. and encodes for a protein in the inner and outer membrane essential for virulence and is thought to trigger the internalization required for invasion into deeper tissues (Dione *et al.*, 2011; Lee *et al.*, 2007).

At the NWWTW, *Salmonella* spp. (6.5%) was only recovered in treated effluent before chlorination compared to fifty three (26.5%) of the isolates recovered at the NGWTP before chlorination. Also fifty five (27.5%) of the isolates were recovered at the discharge point of the NGWWTP with additional 27% and 12.5% recovered upstream and downstream of the receiving river of the treated final effluent from the NGTW respectively. This results suggests that at the NGWTP, the final effluent may be a source of contamination of the river due to the presence of *Salmonella* spp. downstream. Previous studies have reported the detection of *Salmonella* species in final effluent of treated wastewater (Samie *et al.*, 2009). Other possible sources of contamination of the river downstream at the NGWTP include human and animal contamination occuring upstream because *Salmonella* spp. were also isolated and confirmed upstream.

At the NWWTW, *Salmonella* spp. were only recovered at the B.C point (6.5%) but not at the D.P indicating the plant was efficient at removing *Salmonella* spp. from the wastewater during the sampling period. No *Salmonella* spp. were recovered from the Umgeni River samples into which the NWWTW discharges its final effluents indicating that discharge of the final effluent has no negative impact on the microbial quality of the river with respect to *Salmonella* spp. Contrarily, a total 187 (93.5%) isolates were recovered at the NGWTP from every point sampled indicating its inefficiency at removing *Salmonella* spp and contamination of the river upstream.

The inefficiency of wastewater treatment plants in developing countries in removing pathogenic microorganisms has been previously reported (Dungeni and Momba, 2010; Igbinosa and Okoh, 2009; Odjadjare *et al.*, 2012). In the Eastern Cape province of South Africa, Momba *et al.* (2006) observed the presence of *Salmonella* spp. in 50% of final wastewater effluent and 35% in the receiving river samples. Another study in South Africa also recorded the presence of *Salmonella* spp. from wastewater (Samie *et al.*, 2009). The high prevalence of *Salmonella* spp. observed upstream of the Aller River at the NGWTP could be attributed to runoff from the rural settlement located around the river bank which lack proper sewage disposal system and sanitation (Lemarchand and Lebaron, 2003). Poor sanitation, lack of access to proper sewage disposal systems, malnutrition and poverty have been described as some of the leading factors contributing to the high prevalence of salmonellosis and other diarrheal diseases in developing countries (Eisenberg *et al.*, 2007; Fewtrell *et al.*, 2005; Ijaz and Rubino, 2012; Lopez *et al.*, 2006; Wake and Tolessa 2012; Woldemicael 2011).

Antibiogram profile of the confirmed *Salmonella* spp. isolates is shown in Table 3.2. The isolates were susceptible to β-lactams such as Cefuroxime, Pipracillin, Cephalothin, Ceftazidime, and Aztreonam. Susceptibility to Chloramphenicol, Tetracycline, Norfloxacin and Trimethoprim-Sulfamethoxazole (99% to 100%) was also observed. Resistance to Pipracillin was observed in 1 isolate downstream (Table 3.2). Complete resistance was observed against Sulfamethoxazole (100%), Streptomycin (14%) and Nalidixic acid (100%). Resistance to Nalidixic acid suggests possible resistance or decreased susceptibility to more potent quinolones such as Norfloxacin and Ciprofloxacin (CLSI, 2007). In this study, though all isolates recovered from the upstream point were completely resistant to Nalidixic acid, they were completely susceptible to Ciprofloxacin and Norfloxacin (Table 3.3). At the downstream, intermediate resistance to Nalidixic acid was observed against 64% of the isolates but only 4% showed intermediate resistant to Ciprofloxacin. Previous studies have suggested that quinolones should not be used in the treatment of invasive *Salmonellosis* due to strains with decreased sensitivity to fluoroquinolones and possible risk of treatment failure (Lee *et al.,* 2007; Tajbakhsh *et al.*, 2012). The results obtained in this study further emphasises the need for prudent use of fluoroquinolones and other commonly used antibiotics to prevent the emergence of resistant phenotypes (Jin *et al.*, 2012). Consistent with this study, *Salmonella* spp. were reported to be highly sensitive to third generation β- lactams (Micallef *et al.*, 2012; Xia *et al.*, 2009) but resistant to Sulfamethoxazole, Nalidixic acid and Streptomycin (Dahshan *et al.*, 2006; Tajbaksh *et al.*, 2012). Campoini *et al.* (2012) reported that all 128 strainsof *Salmonella* obtained om food and humans over a 24 year period were susceptible to the antimicrobials Tetracycline, Cephalothin, Ampicillin, Amikacin, Ceftriaxone, Chloramphenicol, Trimethoprim-Sulfamethoxazole. In another study Oliveira *et al.* (2006) reported resistance to Nalidixic acid in 21.5% of strains isolated between 2001 and 2002 while, Campioni *et al.* (2012) reported that 21.12% of the isolates in the study were completely resistant to Nalidixic acid. The observation in this study is also contrary to previous report which suggests that *Salmonella* spp. were resistant to third generation β-lactams, Tetracycline, Chloramphenicol and Ciprofloxacin (Economou *et al.,* 2013; Ellerbroek *et al.*, 2010). In Europe and the United States, resistance to Chloramphenicol and other quionlones has been atributed to excessive use of these antibiotics especially as growth promoters in animal production (Hughes and Heritage 2004) which led to their ban in poultry farming (Jin *et al.,* 2012; Petkov *et al.,* 2010) however, no such report has been made in South Africa. Antibioitic resistant microorganisms are on the rise worldwide and pose serious health threats. Data from the National Antimicrobial Resistance Monitoring Systems (NARMS) in the USA from 1996 to 2004 showed increase in resistance of clinical isolates of *Salmonella* against antibiotics (CDC, 2007). The upsurge in antibiotics resistant strains of *Salmonella* over the past decade is threatening successful treatment of diseases caused by this organism especially in developing countries where disease burden is high (Wellington *et al.,* 2013).

Of the 200 isolates of *Salmonella* spp. tested for the presence of virulence genes, 93% harbored the *spi*C gene, 84% harbored the *misL* gene while, 87.5% and 87 % of the isolates harbored the *orf*L and *pip*D gene respectively (Table 3.4). Pathogenicity islands which contain the virulence genes are found on genomes of pathogenic bacteria but are absent in non-pathogenic strains of the same or related species (Dobrindt and Reidl, 2000). All recovered isolates contained one or more virulence genes present in the *Salmonella* pathogenicity island (SPI) indicating that the isolates are pathogenic thus, could pose serious health threats to consumers who depend on the river water for daily activities. This study concur with a previous study in Colombia where the presence of all four virulence genes were reported to be present in 87.2% of *Salmonella* isolated from patients with systemic infection (Sánchez-Jiménez *et al.*, 2010) while 12.8% of *Salmonella* spp. isolated from stool samples lacked the *mis*L and *orf*L gene. Gassama-Sow *et al.* (2006) reported the presence of *inv*A, *spi*C, *mis*L and *pip*D gene in *S. keurmassar* but lacked the *orf*L gene. It also worth noting that the *inv*A gene used to positively confirm the identity of the isolates is a virulence gene located on the SP-1 (Dione *et al.,* 2011). The SPI-1 is a 40 kb gene that encodes a T3SS that mediates the contact-dependent translocation of a complex set of effector proteins into eukaryotic host cells hence, it is essential for invasion of host cells (Gassama-Sow *et al.,* 2006). The presence of these virulence genes in *Salmonella* spp. isolated from treated wastewater effluent and receiving surface water indicate their potential capabilities in causing infections in susceptible hosts. Recently, there was report of an outbreak of acute gastroenteritis in KwaZulu-Natal, which was linked to food, contaminated with *Salmonella enterica serovar Enteritidis* resulting in the hospitalization of 216 people (Niehaus *et al.*, 2011). The report suggested a point source outbreak with a possibility of continued transmission. The true burden of *Salmonella* disease in Africa is unclear thus a comprehensive epidemiological study is needed to elucidate it.

In conclusion, this study shows that NWWTW was more effective in removing *Salmonella* spp from treated effluent compared to the NGWTP. The isolates were susceptible to most of the antibiotics used in this study, however, resistance to other antibiotics were also recoreded. The presence of virulence genes is indicative of possible health threat posed by these organisms if exposed to them. Thus, appropriate intervention is required by the regulatory agencies to ensure compliance of the wastewater treatment plants to the stipulated guidelines for safe disposal of treated effluent to surface water resources.

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CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSION

4.1 Research in perspective

Recognition of the importance of wastewater treatment prior to discharge into receiving natural water bodies has dramatically reduced incidence of waterborne disease outbreaks worldwide. However, in developing countries, wastewater treatment facilities are either scarce or in poor infrastructural conditions and discharge inadequately treated wastewater into receiving surface waters (Massoud *et al.*, 2009). This and lack of proper sanitation has led to a high morbidity and mortality due to waterborne diarrheal disease outbreaks especially in children under the age of 5 in developing countries. In South Africa, typhoid, dysentery, cholera and rotavirus infections are the most common diarrheal disease that results in high morbidity and mortality (Mudzanani *et al.,* 2004).

The physicochemical qualities of the water samples in some instance did not meet the target limit set by the Department of Water Affairs, South Africa. The temperature, pH and BOD were observed to be within the target limit, however, unacceptably high turbidity (>5 NTU) and COD at all points sampled was recorded during the study indicating the unsuitability of the water for discharge into the environment. Statistical analysis indicates there is a positive correlation between turbidity and presence of presumptive *Salmonella* and *Shigella* spp. (Table 2.3).

The prevalence of presumptive *Salmonella* spp. at the NWWTW ranged between $0-1.94\times10^3$ CFU/ml while presumptive *Shigella* spp. ranged between $30-13.4\times10^3$ CFU/ml. At the NGWTP, low *Salmonella* counts were recorded (0–17 CFU/ml) at the discharge point (D.P) but higher counts ranging from 0–5.5×10^3 CFU/ml were recorded for presumptive *Shigella*. This indicates that discharge of treated wastewater from these treatment plants could result in the contamination of the receiving surface water with *Salmonella* and presumptive *Shigella spp*. Biochemical and molecular tests revealed that none of the presumptive *Shigella* were indeed *Shigella* spp. However, due to their similar morphological and phenotypic characteristics on the selective agar plates,these organisms might be other types of *Enterobateriacea*

Antibiogram profile of the confirmed *Salmonella* spp. isolates is shown in Table 3.2. The isolates were highly susceptible to β-lactams such as Cefuroxime, Pipracillin, Cephalothin, Ceftazidime, and Aztreonam. High susceptibility to Chloramphenicol, Tetracycline, Norfloxacin and Trimethoprim-Sulfamethoxazole (99% to 100%) was also observed. Resistance to Pipracillin was observed in 1 isolate downstream (Table 3.2). Complete resistance was observed against Sulfamethoxazole (100%), Streptomycin (14%) and Nalidixic acid (100%). Resistance to Nalidixic acid suggests possible resistance or decreased susceptibility to more potent quinolones such as Norfloxacin and Ciprofloxacin (CLSI, 2007). All all isolates resistant to Nalidixic acid, were completely susceptible to Ciprofloxacin and Norfloxacin (Table 3.3).

Molecular test for the presence of virulence signatures revealed that of the 200 isolates tested in this study, 93% harboured the *spi*C gene, 84% harbored the *misL* gene, and 87.5% harbored the *orf*L gene while 87 % harbored *pip*D gene. All recovered isolates contained one or more virulence genes present in the *Salmonella* pathogenicity island (SPI) thus, posing serious health threats to consumers who depend on the river water for socioeconomic activities (Table 3.2). The presence of these virulence genes indicates the potential of recovered microorganisms to cause diseases in humans. Results from this study indicates that treated wastewater effluent are potential source of virulent and antibiotics resistant *Salmonella* spp. and contaminate receiving surface water. It is therefore imperative that appropriate intervention measures be taken by the regulatory authorities in South Africa to ensure the compliance of wastewater treatment works with the regulatory guidelines.

4.2 Potential for future development of the study

Microbial source tracking can be used to determine the source of these pathogens because human sources could indicate an on-going epidemic or disease outbreak though there was no such report during the study period. Animals can also serve as reservoirs for a variety of enteric pathogens including different serotypes of *Salmonella*, *Escherichia coli*, and *Cryptosporidium* spp (Tyagi *et al.*, 2007). Understanding the origin of fecal pollution is paramount in assessing associated health risks as well as the actions necessary to remedy the problem while it still exists (Scott *et al.*, 2002). Since non-typhoidal Salmonellosis is usually self-limiting, it is less frequently reported and might explain why there has been no report on any of disease outbreak in the province during the study period.

Molecular subtyping methods for the characterization and grouping of organisms based on their genotypic characteristics has become popular in most research studies (Hunter *et al.*, 2005). Of the many molecular methods currently available, macro-restriction analysis by pulsed-field gel electrophoresis (PFGE) has been shown to be particularly useful for the clustering and differentiation of many bacterial pathogens (Chenal-Francisque *et al.*, 2013; Goering, 2010; Scott *et al.*, 2002). Although the sensitivity and discriminatory power of PFGE depends on the organism being subtyped and the restriction enzyme used, its high epidemiologic relevance has made it the primary technique for molecular subtyping of bacterial pathogens (Halpin *et al.*, 2010; Pichel *et al.*, 2012; Sandt *et al.*, 2013; Swaminathan *et al.*, 2001). Hence it is recommended that pulse field gel electrophoresis be used for further molecular analysis and genotyping of the recovered isolates to determine their specie and subtypes.

Bacteria are known to possess and transfer genes which confer resistance to certain class of antibiotics as well as virulence. Though the isolates were susceptible to most antibiotics, they showed resistance to Sulfamethoxazole, Nalidixic acid and Streptomycin with decreased susceptibility to Fosfomycin. To understand the mechanisms and epidemiology of antimicrobial resistance, the genetic elements responsible for the observed resistance must be identified. Due to the myriad of possible genes, DNA microarray techniques can be used for detection of these genes (Ma *et al.*, 2007; Frye *et al.*, 2010). Future studies should also determine the mechanism of pathogenicity and antibiotics resistance as well as the ability of the isolates to obtain and transfer virulence and resistance genes in order to remedy the public health threats posed by these pathogens.

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APPENDIX 1

Enumeration of Salmonella spp and Shigella spp for the month of March at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	51	TMTC	77	75
CFU/ml	0.0102	N/A	0.0154	0.015
10^1	39	TMTC	80	75
CFU/ml	0.0078	N/A	0.016	0.015
10^2	39	30	30	42
CFU/ml	0.078	0.06	0.06	0.084
10^2	40	36	34	22
CFU/ml	0.08	0.072	0.068	0.044
10^3	2	2	10	0
CFU/ml	0.04	0.04	0.2	0
10^3	2	2	7	0
CFU/ml	0.04	0.04	0.14	0
10^4	0	0	1	0
CFU/ml	0	0	0.2	0
10^4	0	0	1	0
CFU/ml	0	0	0.2	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	TMTC	245	TMTC	137
CFU/ml	N/A	0.049	N/A	0.0274
10^1	TMTC	245	TMTC	N/A
CFU/ml	N/A	0.049	N/A	N/A
10^2	TMTC	36	25	9
CFU/ml	N/A	0.072	0.05	0.018
10^2	TMTC	N/A	4	6
CFU/ml	N/A	N/A	0.008	0.012
10^3	11	3	1	2
CFU/ml	0.22	0.06	0.02	0.04
10^3	9	5	2	0
CFU/ml	0.18	0.1	0.04	0
10^4		0	4	0
CFU/ml	0	0	0.8	0
10^4		1	4	1
CFU/ml	0	0.2	0.8	0.2

Enumeration of Salmonella spp and Shigella spp for the month of April at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	13	0
CFU/ml		0	0.0026	0
10^{1}	0	0	7	0
CFU/ml		0	0.0014	0
10^{2}	TNTC	0	0	1
CFU/ml		0	0	0.002
10^{2}	TNTC	0	0	2
CFU/ml		0	0	0.004
10^{3}	12	0	13	65
CFU/ml	0.24	0	0.26	1.3
10^{3}	18	0	15	60
CFU/ml	0.36	0	0.3	1.2
10^{4}	0	36	1	30
CFU/ml	0	7.2	0.2	6
10^{4}	0	38	0	47
CFU/ml	0	7.6	0	9.4

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	A.C	D.S
10^{1}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{1}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{2}	198	250	270	TNTC
CFU/ml	198	250	270	N/A
10^{2}	211	246	290	TNTC
CFU/ml	211	246	290	N/A
10^{3}	23	125	220	221
CFU/ml	230	1250	2200	2210
10^{3}	25	122	228	235
CFU/ml	250	1220	2280	2350
10^{4}	28	32	25	13
CFU/ml	2800	3200	2500	1300
10^{4}	36	0	21	7
CFU/ml	3600	0	2100	700

<u>Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of May at the NWWTW</u>

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	TNTC	108
CFU/ml	N/A	N/A	N/A	0.0216
10^{1}	TNTC	TNTC	TNTC	81
CFU/ml	N/A	N/A	N/A	0.0162
10^{2}	58	TNTC	TNTC	28
CFU/ml	0.116	N/A	N/A	0.056
10^{2}	42	TNTC	TNTC	27
CFU/ml	0.084	N/A	N/A	0.054
10^{3}	3	70	24	2
CFU/ml	0.06	1.4	0.48	0.04
10^{3}	5	72	24	1
CFU/ml	0.1	1.44	0.48	0.02
10^{4}	0	10	1	0
CFU/ml	0	2	0.2	0
10^{4}	0	27	1	0
CFU/ml	0	5.4	0.2	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	1	0	4
CFU/ml	0	0.0002	0	0.0008
10^{2}	44	0	0	27
CFU/ml	0.088	0	0	0.054
10^{2}	36	0	0	10
CFU/ml	0.072	0	0	0.02
10^{3}	0	14	10	8
CFU/ml	0	0.28	0.2	0.16
10^{3}	0	18	15	6
CFU/ml	0	0.36	0.3	0.12
10^{4}	0	3	2	0
CFU/ml	0	0.6	0.4	0
10^{4}	0	4	3	5
CFU/ml	0	0.8	0.6	1

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of June at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	60	53	TNTC	67
CFU/ml	0.012	0.0106	N/A	0.0134
10^{1}	68	60	TNTC	51
CFU/ml	0.0136	0.012	N/A	0.0102
10^{2}	20	28	60	42
CFU/ml	0.04	0.056	0.12	0.084
10^{2}	16	25	64	28
CFU/ml	0.032	0.05	0.128	0.056
10^{3}	5	6	20	13
CFU/ml	0.1	0.12	0.4	0.26
10^{3}	8	10	8	11
CFU/ml	0.16	0.2	0.16	0.22
10^{4}	6	3	9	13
CFU/ml	1.2	0.6	1.8	2.6
10^{4}	3	3	12	14
CFU/ml	0.06	0.06	0.24	0.28

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0		0	0
10^{1}	0	50	0	0
CFU/ml	0	0.01	0	0
10^{2}	TNTC	20	0	0
CFU/ml	N/A	0.04	0	0
10^{2}	TNTC	3	0	0
CFU/ml	N/A	0.006	0	0
10^{3}	51	81	TNTC	53
CFU/ml	1.02	1.62	N/A	1.06
10^{3}	53	82	TNTC	51
CFU/ml	1.06	1.64	N/A	1.02
10^{4}	27	20	70	27
CFU/ml	5.4	4	14	5.4
10^{4}	15	28	64	15
CFU/ml	3	5.6	12.8	3

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of July at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	90	TNTC	TNTC	84
CFU/ml	0.018	N/A	N/A	0.0168
10^{1}	87	TNTC	TNTC	88
CFU/ml	0.0174	N/A	N/A	0.0176
10^{2}	8	35	14	17
CFU/ml	0.016	0.07	0.028	0.034
10^{2}	25	41	23	17
CFU/ml	0.05	0.082	0.046	0.034
10^{3}	0	11	2	0
CFU/ml	0	0.22	0.04	0
10^{3}	2	10	5	1
CFU/ml	0.04	0.2	0.1	0.02
10^{4}	1	0	0	0
CFU/ml	0.2	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{1}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{2}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{2}	TNTC	TNTC	TNTC	TNTC
CFU/ml	N/A	N/A	N/A	N/A
10^{3}	30	45	43	25
CFU/ml	0.6	0.9	0.86	0.5
10^{3}	32	48	46	30
CFU/ml	0.64	0.96	0.92	0.6
10^{4}	8	10	6	3
CFU/ml	1.6	2	1.2	0.6
10^{4}	6	9	5	2
CFU/ml	1.2	1.8	1	0.4

Enumeration of Salmonella spp. and Shigella spp. for the month of August at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	225	TNTC	TNTC	44
CFU/ml	0.045	N/A	N/A	0.0088
10^1	262	TNTC	TNTC	43
CFU/ml	0.0524	N/A	N/A	0.0086
10^2	21	TNTC	64	3
CFU/ml	0.042	N/A	0.128	0.006
10^2	24	TNTC	66	6
CFU/ml	0.048	N/A	0.132	0.012
10^3	2	30	13	0
CFU/ml	0.04	0.6	0.26	0
10^3	3	36	16	0
CFU/ml	0.06	0.72	0.32	0
10^4	1	7	2	0
CFU/ml	0.2	1.4	0.4	0
10^4	0	7	3	0
CFU/ml	0	1.4	0.6	0

Enumeration of *Shigella* spp.

vb	U.S	B.C	D.P	D.S
10^1	0	0	0	0
CFU/ml	0	0	0	0
10^1	0	0	0	3
CFU/ml	0	0	0	
10^2	30	0	14	29
CFU/ml	0.06	0	0.028	0.058
10^2	33	3	12	32
CFU/ml	0.066	0.006	0.024	0.064
10^3	24	48	8	16
CFU/ml	0.48	0.96	0.16	0.32
10^3	12	45	7	20
CFU/ml	0.24	0.9	0.14	0.4
10^4	13	23	10	4
CFU/ml	2.6	4.6	2	0.8
10^4	12	20	9	2
CFU/ml	2.4	4	1.8	0.4

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of September at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	60	TNTC	TNTC	143
CFU/ml	0.012	N/A	N/A	0.0286
10^{1}	68	TNC	TNTC	140
CFU/ml	0.0136	N/A	N/A	0.028
10^{2}	7	76	30	30
CFU/ml	0.014	0.152	0.06	0.06
10^{2}	6	84	35	36
CFU/ml	0.012	0.168	0.07	0.072
10^{3}	0	5	3	0
CFU/ml	0	0.1	0.06	0
10^{3}	0	9	1	0
CFU/ml	0	0.18	0.02	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	2
CFU/ml	0	0	0	0.4

Enumeration of *Shigella* spp. .

DIL.	U.S	B.C	D.P	D.S
10^{1}	12	0	0	0
CFU/ml	0.0024	0	0	0
10^{1}	15	8	0	0
CFU/ml	0.003	0.0016	0	0
10^{2}	TNTC	9	0	4
CFU/ml	N/A	0.018	0	0.008
10^{2}	TNTC	5	0	8
CFU/ml	N/A	0.01	0	0.016
10^{3}	88	50	21	3
CFU/ml	1.76	1	0.42	0.06
10^{3}	84	48	15	2
CFU/ml	1.68	0.96	0.3	0.04
10^{4}	12	90	0	0
CFU/ml	2.4	18	0	0
10^{4}	9	92	0	0
CFU/ml	1.8	18.4	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of October at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	21	TNTC	1	36
CFU/ml	0.0042	N/A	0.0002	0.0072
10^1	30	TNTC	4	30
CFU/ml	0.006	N/A	0.0008	0.006
10^2	2	48	1	4
CFU/ml	0.004	0.096	0.002	0.008
10^2	5	53	1	3
CFU/ml	0.01	0.106	0.002	0.006
10^3	0	5	0	1
CFU/ml	0	0.1	0	0.02
10^3	0	10	0	0
CFU/ml	0	0.2	0	0
10^4	1	0	0	0
CFU/ml	0.2	0	0	0
10^4	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	0	16	17	38
CFU/ml	0	0.0032	0.0034	0.0076
10^1	0	16	20	40
CFU/ml	0	0.0032	0.004	0.008
10^2	50	20	48	25
CFU/ml	0.1	0.04	0.096	0.05
10^2	52	28	50	34
CFU/ml	0.104	0.056	0.1	0.068
10^3	20	2	6	7
CFU/ml	0.4	0.04	0.12	0.14
10^3	19	1	12	6
CFU/ml	0.38	0.02	0.24	0.12
10^4	1	1	4	1
CFU/ml	0.2	0.2	0.8	0.2
10^4	0	4	6	1
CFU/ml	0	0.8	1.2	0.2

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of November at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	246	TNTC	TNTC	63
CFU/ml	0.0492	N/A	N/A	0.0126
10^{1}	250	TNTC	TNTC	66
CFU/ml	0.05	N/A	N/A	0.0132
10^{2}	32	180	140	6
CFU/ml	0.064	0.36	0.28	0.012
10^{2}	29	189	135	3
CFU/ml	0.058	0.378	0.27	0.006
10^{3}	1	20	23	1
CFU/ml	0.02	0.4	0.46	0.02
10^{3}	2	18	21	0
CFU/ml	0.04	0.36	0.42	0
10^{4}	0	14	2	0
CFU/ml	0	2.8	0.4	0
10^{4}	0	5	5	0
CFU/ml	0	1	1	0

Enumeration of *Shigella* spp .

DIL.	U.S	B.C	D.P	D.S
10^{1}	1	0	0	0
CFU/ml	0.0002	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	2	0	1	23
CFU/ml	0.004	0	0.002	0.046
10^{2}	3	0	0	27
CFU/ml	0.006	0	0	0.054
10^{3}	24	29	25	17
CFU/ml	0.48	0.58	0.5	0.34
10^{3}	16	30	23	21
CFU/ml	0.32	0.6	0.46	0.42
10^{4}	2	17	5	1
CFU/ml	0.4	3.4	1	0.2
10^{4}	0	12	6	1
CFU/ml	0	2.4	1.2	0.2

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of December at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	40		1	50
CFU/ml	0.008	0	0.0002	0.01
10^{1}	41		0	52
CFU/ml	0.0082	0	0	0.0104
10^{2}	5	68	0	16
CFU/ml	0.01	0.136	0	0.032
10^{2}	4	64	0	14
CFU/ml	0.008	0.128	0	0.028
10^{3}	0	5	0	0
CFU/ml	0	0.1	0	0
10^{3}	0	4	0	0
CFU/ml	0	0.08	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	0	0	0	0
CFU/ml	0	0	0	0
10^{3}	0	8	4	0
CFU/ml	0	0.16	0.08	0
10^{3}	0	3	4	0
CFU/ml	0	0.06	0.08	0
10^{4}	0	0	9	0
CFU/ml	0	0	1.8	0
10^{4}	0	0	11	0
CFU/ml	0	0	2.2	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of January at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	30	TNTC	TNTC	47
CFU/ml	0.006	N/A	N/A	0.0094
10^{1}	25	TNTC	TNTC	43
CFU/ml	0.005	N/A	N/A	0.0086
10^{2}	10	TNTC	97	9
CFU/ml	0.02	N/A	0.194	0.018
10^{2}	4	TNTC	93	4
CFU/ml	0.008	N/A	0.186	0.008
10^{3}	0	9	12	0
CFU/ml	0	0.18	0.24	0
10^{3}	0	8	10	0
CFU/ml	0	0.16	0.2	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	11	0	0	TNTC
CFU/ml	0.022	0	0	N/A
10^{2}	26	0	0	TNTC
CFU/ml	0.052	0	0	N/A
10^{3}	25	0	16	54
CFU/ml	0.5	0	0.32	1.08
10^{3}	30	0	12	51
CFU/ml	0.6	0	0.24	1.02
10^{4}	15	31	20	1
CFU/ml	3	6.2	4	0.2
10^{4}	10	34	32	5
CFU/ml	2	6.8	6.4	1

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of February at the NWWTW

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	62	TNTC	TNTC	18
CFU/ml	0.0124	N/A	N/A	0.0036
10^{1}	60	TNTC	TNTC	23
CFU/ml	0.012	N/A	N/A	0.0046
10^{2}	13	TNTC	TNTC	1
CFU/ml	0.026	N/A	N/A	0.002
10^{2}	14	TNTC	TNTC	3
CFU/ml	0.028	N/A	N/A	0.006
10^{3}	4	46	53	0
CFU/ml	0.08	0.92	1.06	0
10^{3}	1	40	57	0
CFU/ml	0.02	0.8	1.14	0
10^{4}	0	2	3	0
CFU/ml	0	0.4	0.6	0
10^{4}	14	6	5	0
CFU/ml	2.8	1.2	1	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	17
CFU/ml	0	0	0	0.0034
10^{1}	0	0	0	20
CFU/ml	0	0	0	0.004
10^{2}	0	0	0	56
CFU/ml	0	0	0	0.112
10^{2}	0	0	0	52
CFU/ml	0	0	0	0.104
10^{3}	60	20	14	3
CFU/ml	1.2	0.4	0.28	0.06
10^{3}	63	16	18	4
CFU/ml	1.26	0.32	0.36	0.08
10^{4}	31	26	32	2
CFU/ml	6.2	5.2	6.4	0.4
10^{4}	32	22	34	2
CFU/ml	6.4	4.4	6.8	0.4

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of March at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TMTC	TMTC	22	19
CFU/ml	N/A	N/A	0.0044	0.0038
10^{1}	TMTC	TMTC	41	25
CFU/ml	N/A	N/A	0.0082	0.005
10^{2}	59	46	3	3
CFU/ml	0.118	0.092	0.006	0.006
10^{2}	68	40	4	2
CFU/ml	0.136	0.08	0.008	0.004
10^{3}	3	15	1	0
CFU/ml	0.06	0.3	0.02	0
10^{3}	13	17	0	0
CFU/ml	0.26	0.34	0	0
10^{4}	6	3	0	0
CFU/ml	1.2	0.6	0	0
10^{4}	3	5	0	0
CFU/ml	0.6	1	0	0

Enumeration of *Shigella* Spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TMTC	0	22	19
CFU/ml	N/A	0	0.0044	0.0038
10^{1}	TMTC	4	41	25
CFU/ml	N/A	0.0008	0.0082	0.005
10^{2}	59	0	3	3
CFU/ml	0.118	0	0.006	0.006
10^{2}	68	0	4	2
CFU/ml	0.136	0	0.008	0.004
10^{3}	3	0	1	0
CFU/ml	0.06	0	0.02	0
10^{3}	13	7	0	0
CFU/ml	0.26	0.14	0	0
10^{4}	6	0	0	0
CFU/ml	1.2	0	0	0
10^{4}	3	0	0	0
CFU/ml	0.6	0	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of April at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	14	22
CFU/ml	N/A	N/A	0.0028	0.0044
10^{1}	TNTC	TNTC	7	16
CFU/ml			0.0014	0.0032
10^{2}	17	67	2	3
CFU/ml	0.034	0.134	0.004	0.006
10^{2}	16	60	0	5
CFU/ml	0.032	0.12	0	0.01
10^{3}	6	34	0	2
CFU/ml	0.12	0.68	0	0.04
10^{3}	7	35	0	1
CFU/ml	0.14	0.7	0	0.02
10^{4}	1	1	0	1
CFU/ml	0.2	0.2	0	0.2
10^{4}	5	1	0	1
CFU/ml	1	0.2	0	0.2

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	1	0	1
CFU/ml	0	0.2	0	0.0002
10^{1}	2	0	1	0
CFU/ml	0.0004	0	0.0002	0
10^{2}	0	1	1	0
CFU/ml	0	0.002	0.002	0
10^{2}	0	0	0	0
CFU/ml	0	0	0	0
10^{3}	0	1	0	0
CFU/ml	0	0.02	0	0
10^{3}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of May at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	TNTC	TNTC	70
CFU/ml	0	N/A	N/A	0.014
10^{1}	0	TNTC	TNTC	TNTC
CFU/ml	0	N/A	N/A	N/A
10^{2}	0	79	78	45
CFU/ml	0	0.158	0.156	0.09
10^{2}	0	80	80	44
CFU/ml	0	0.16	0.16	0.088
10^{3}	0	15	19	2
CFU/ml	0	0.3	0.38	0.04
10^{3}	0	10	9	4
CFU/ml	0	0.2	0.18	0.08
10^{4}	0	0	1	0
CFU/ml	0	0	0.2	0
10^{4}	0	1	4	0
CFU/ml	0	0.2	0.8	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	0	57	TNTC	TNTC
CFU/ml	0	0.114	N/A	N/A
10^{2}	0	82	TNTC	TNTC
CFU/ml	0	0.164	N/A	N/A
10^{3}	0	30	35	10
CFU/ml	0	0.6	0.7	0.2
10^{3}	0	41	20	7
CFU/ml	0	0.82	0.4	0.14
10^{4}	0	4	8	0
CFU/ml	0	0.8	1.6	0
10^{4}	0	5	10	0
CFU/ml	0	1	2	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of June at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	93	TNTC	2	0
CFU/ml	0.0186	N/A	0.0004	0
10^{1}	80	TNTC	2	0
CFU/ml	0.016	N/A	0.0004	0
10^{2}	3	20	0	2
CFU/ml	0.006	0.04	0	0.004
10^{2}	5	22	0	2
CFU/ml	0.01	0.044	0	0.004
10^{3}	0	1	0	0
CFU/ml	0	0.02	0	0
10^{3}	1	0	0	0
CFU/ml	0.02	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp .

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	40	TNTC
CFU/ml	N/A	N/A	0.008	N/A
10^{1}	TNTC	TNTC	36	TNTC
CFU/ml	N/A	N/A	0.0072	N/A
10^{2}	50	TNTC	7	25
CFU/ml	0.1	N/A	0.014	0.05
10^{2}	55	TNTC	3	40
CFU/ml	0.11	N/A	0.006	0.08
10^{3}	30	74	1	16
CFU/ml	0.6	1.48	0.02	0.32
10^{3}	26	52	0	9
CFU/ml	0.52	1.04	0	0.18
10^{4}	8	8	0	1
CFU/ml	0.16	0.16	0	0.02
10^{4}	9	17	0	1
CFU/ml	0.18	0.34	0	0.02

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of July at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	63	TNTC	1	0
CFU/ml	0.0126	n/a	0.0002	0
10^{1}	70	TNTC	2	0
CFU/ml	0.014	n/a	0.0004	0
10^{2}	11	81	0	0
CFU/ml	0.022	0.162	0	0
10^{2}	14	76	0	0
CFU/ml	0.028	0.152	0	0
10^{3}	1	7	0	0
CFU/ml	0.02	0.14	0	0
10^{3}	0	5	0	0
CFU/ml	0	0.1	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp..

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	1	0
CFU/ml	N/A	N/A	0.2	0
10^{1}	TNTC	TNTC	2	0
CFU/ml	N/A	N/A	0.4	0
10^{2}	128	TNTC	0	3
CFU/ml	0.256	N/A	0	0.006
10^{2}	132	TNTC	0	0
CFU/ml	0.264	N/A	0	0
10^{3}	13	49	0	1
CFU/ml	0.26	0.98	0	0.02
10^{3}	12	43	0	0
CFU/ml	0.24	0.86	0	0
10^{4}	2	15	0	0
CFU/ml	0.4	3	0	0
10^{4}	0	18	0	0
CFU/ml	0	3.6	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of August at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	45	2	0
CFU/ml	N/A	0.009	0.0004	0
10^{1}	TNTC	50	1	0
CFU/ml	N/A	0.01	0.0002	0
10^{2}	TNTC	11	0	0
CFU/ml	N/A	0.022	0	0
10^{2}	TNTC	13	0	0
CFU/ml	N/A	0.026	0	0
10^{3}	50	1	0	1
CFU/ml	1	0.02	0	0.02
10^{3}	47	0	0	1
CFU/ml	0.94	0	0	0.02
10^{4}	34	0	0	8
CFU/ml	6.8	0	0	1.6
10^{4}	32	0	0	10
CFU/ml	6.4	0	0	2

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	2	2
CFU/ml	0	0	0.0004	0.0004
10^{1}	0	0	3	1
CFU/ml	0	0	0.0006	0.0002
10^{2}	0	4	0	0
CFU/ml	0	0.008	0	0
10^{2}	0	6	0	1
CFU/ml	0	0.012	0	0.002
10^{3}	4	2	0	0
CFU/ml	0.08	0.04	0	0
10^{3}	9	4	0	0
CFU/ml	0.18	0.08	0	0
10^{4}	22	7	0	8
CFU/ml	4.4	1.4	0	1.6
10^{4}	21	2	1	9
CFU/ml	4.2	0.4	0.2	1.8

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of September at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	50	82	TNTC
CFU/ml	N/A	0.01	0.0164	N/A
10^{1}	TNTC	52	85	TNTC
CFU/ml	N/A	0.0104	0.017	N/A
10^{2}	TNTC	4	10	TNTC
CFU/ml	N/A	0.008	0.02	N/A
10^{2}	TNTC	5	40	TNTC
CFU/ml	N/A	0.01	0.08	N/A
10^{3}	50	0	0	43
CFU/ml	1	0	0	0.86
10^{3}	55	0	0	40
CFU/ml	1.1	0	0	0.8
10^{4}	16	0	0	16
CFU/ml	3.2	0	0	3.2
10^{4}	9	0	0	15
CFU/ml	1.8	0	0	3

Enumeration of *Shigella* spp. .

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	0	25	12	0
CFU/ml	0	0.05	0.024	0
10^{2}	0	19	11	0
CFU/ml	0	0.038	0.022	0
10^{3}	0	0	4	1
CFU/ml	0	0	0.08	0.02
10^{3}	0	2	4	3
CFU/ml	0	0.04	0.08	0.06
10^{4}	16	0	2	12
CFU/ml	3.2	0	0.4	2.4
10^{4}	20	0	1	15
CFU/ml	4	0	0.2	3

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of October at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	76	0	0
CFU/ml	N/A	0.0152	0	0
10^{1}	TNTC	80	0	0
CFU/ml	N/A	0.016	0	0
10^{2}	12	42	0	0
CFU/ml	0.024	0.084	0	0
10^{2}	17	40	0	0
CFU/ml	0.034	0.08	0	0
10^{3}	2	13	0	0
CFU/ml	0.04	0.26	0	0
10^{3}	2	16	0	0
CFU/ml	0.04	0.32	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{1}	0	0	0	0
CFU/ml	0	0	0	0
10^{2}	48	0	0	0
CFU/ml	0.096	0	0	0
10^{2}	43	0	0	0
CFU/ml	0.086	0	0	0
10^{3}	5	3	0	0
CFU/ml	0.1	0.06	0	0
10^{3}	4	3	0	0
CFU/ml	0.08	0.06	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0
10^{4}	0	0	0	0
CFU/ml	0	0	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of November at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	128	TNTC	0	100
CFU/ml	0.0256	N/A	0	0.02
10^{1}	120	TNTC	0	86
CFU/ml	0.024	N/A	0	0.0172
10^{2}	33	TNTC	0	10
CFU/ml	0.066	N/A	0	0.02
10^{2}	31	TNTC	0	6
CFU/ml	0.062	N/A	0	0.012
10^{3}	2	79	0	0
CFU/ml	0.04	1.58	0	0
10^{3}	5	84	0	0
CFU/ml	0.1	1.68	0	0
10^{4}	0	12	0	0
CFU/ml	0	2.4	0	0
10^{4}	0	13	0	0
CFU/ml	0	2.6	0	0

Enumeration of *Shigella* spp .

DIL.	U.S	B.C	D.P	D.S
10^{1}	0	0	1	0
CFU/ml	0	0	0.0002	0
10^{1}	0	0	6	0
CFU/ml	0	0	0.0012	0
10^{2}	0	0	2	7
CFU/ml	0	0	0.004	0.014
10^{2}	0	0	1	10
CFU/ml	0	0	0.002	0.02
10^{3}	38	0	0	3
CFU/ml	0.76	0	0	0.06
10^{3}	40	0	0	2
CFU/ml	0.8	0	0	0.04
10^{4}	3	8	0	0
CFU/ml	0.6	1.6	0	0
10^{4}	0	7	0	0
CFU/ml	0	1.4	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of December at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1		48	3	
CFU/ml	0	0.0096	0.0006	0
10^1		52	4	
CFU/ml	0	0.0104	0.0008	0
10^2		9	0	
CFU/ml	0	0.018	0	0
10^2		12	0	
CFU/ml	0	0.024	0	0
10^3	85	0	0	99
CFU/ml	1.7	0	0	1.98
10^3	93	0	0	95
CFU/ml	1.86	0	0	1.9
10^4	69	0	0	50
CFU/ml	13.8	0	0	10
10^4	70	0	0	55
CFU/ml	14	0	0	11

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	4		67	0
CFU/ml	0.0008	0	0.0134	0
10^1	11	0	73	0
CFU/ml	0.0022	0	0.0146	0
10^2	0	0	35	0
CFU/ml	0	0	0.07	0
10^2	11	TNTC	41	0
CFU/ml	0.022	N/A	0.082	0
10^3	7	66	4	0
CFU/ml	0.14	1.32	0.08	0
10^3	11	61	3	0
CFU/ml	0.22	1.22	0.06	0
10^4	5	12	29	12
CFU/ml	1	2.4	5.8	2.4
10^4	4	4	25	13
CFU/ml	0.8	1	5	2.6

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of January at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}	TNTC	TNTC	0	0
CFU/ml	N/A	N/A	0	0
10^{1}	TNTC	TNTC	0	0
CFU/ml	N/A	N/A	0	0
10^{2}	88	80	0	0
CFU/ml	0.176	0.16	0	0
10^{2}	90	89	0	0
CFU/ml	0.18	0.178	0	0
10^{3}	45	13	0	0
CFU/ml	0.9	0.26	0	0
10^{3}	43	5	0	0
CFU/ml	0.86	0.1	0	0
10^{4}	12	0	0	0
CFU/ml	2.4	0	0	0
10^{4}	13	0	0	0
CFU/ml	2.6	0	0	0

Enumeration of *Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^{1}			0	0
CFU/ml	0	0	0	0
10^{1}	4		0	0
CFU/ml	0.0008	0	0	0
10^{2}	0		0	0
CFU/ml	0	0	0	0
10^{2}	0		0	0
CFU/ml	0	0	0	0
10^{3}	38	5	0	0
CFU/ml	0.76	0.1	0	0
10^{3}	42	8	0	0
CFU/ml	0.84	0.16	0	0
10^{4}	0	7	0	0
CFU/ml	0	1.4	0	0
10^{4}	0	18	0	0
CFU/ml	0	3.6	0	0

Enumeration of *Salmonella* spp. and *Shigella* spp. for the month of February at the NGWTP

Enumeration of *Salmonella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1	TNTC	TNTC	0	0
CFU/ml	#VALUE!	N/A	0	0
10^1	TNTC	TNTC	0	0
CFU/ml	#VALUE!	N/A	0	0
10^2	43	TNTC	0	0
CFU/ml	0.086	N/A	0	0
10^2	42	TNTC	0	0
CFU/ml	0.084	N/A	0	0
10^3	7	76	0	0
CFU/ml	0.14	1.52	0	0
10^3	7	72	0	0
CFU/ml	0.14	1.44	0	0
10^4	0	5	5	6
CFU/ml	0	1	1	1.2
10^4	0	6	0	0
CFU/ml	0	1.2	0	0

Enumeration of*Shigella* spp.

DIL.	U.S	B.C	D.P	D.S
10^1		0	0	0
CFU/ml	0	0	0	0
10^1		0	0	0
CFU/ml	0	0	0	0
10^2		0	0	0
CFU/ml	0	0	0	0
10^2		0		0
CFU/ml	0	0	0	0
10^3	1	4	0	0
CFU/ml	0.02	0.08	0	0
10^3	8	0	0	0
CFU/ml	0.16	0	0	0
10^4	7	0	0	4
CFU/ml	1.4	0	0	0.8
10^4	6	0	4	3
CFU/ml	1.2	0	0.8	0.6

Table 2: Turbidity and pH values for NWWTW over a 12 month period

		Turbidity					pH				
		1	2	3	Avg	Sd	1	2	3	Avg	Sd
March	US	17.1	16.4	16.5	16.67	0.38	7.29	7.15	7.31	7.25	0.09
	BC	8.08	8.12	7.53	7.91	0.33	7.15	7.05	7.12	7.11	0.05
	DP	37.4	16.6	16.2	23.40	12.13	7.31	7.44	7.34	7.36	0.07
	DS	15.2	15.4	15.2	15.27	0.12	7.23	7.3	7.19	7.24	0.06
April	US	19.7	19.7	19.7	19.70	0.00	7.5	7.3	7.5	7.43	0.12
	BC	56.6	56.6	56.4	56.53	0.12	7.7	7.6	7.7	7.67	0.06
	DP	76.6	76.6	76.1	76.43	0.29	7.5	7.3	7.4	7.40	0.10
	DS	14.8	14.8	14.8	14.80	0.00	7.7	7.6	7.6	7.63	0.06
May	US	12.8	12.8	12.8	12.80	0.00	6.88	6.95	6.91	6.91	0.04
	BC	19.6	19.6	19.6	19.60	0.00	7.05	7.07	7.11	7.08	0.03
	DP	13.6	13.9	13.9	13.80	0.17	7.26	7.29	7.28	7.28	0.02
	DS	12.9	12.9	12.9	12.90	0.00	7.15	7.1	7.13	7.13	0.03
June	US	9.56	9.57	9.57	9.57	0.01	7.64	7.65	7.65	7.65	0.01
	BC	11.6	11.2	11	11.27	0.31	7.37	7.37	7.37	7.37	0.00
	DP	8.87	8.99	8.91	8.92	0.06	7.34	7.35	7.35	7.35	0.01
	DS	14.2	14.6	14.3	14.37	0.21	7.84	7.85	7.84	7.84	0.01
July	US	13.3	13.1	13.4	13.27	0.15	7.54	7.55	7.54	7.54	0.01
	BC	19.4	19.3	19.3	19.33	0.06	7.47	7.49	7.48	7.48	0.01
	DP	23.5	22.8	22.9	23.07	0.38	7.7	7.7	7.7	7.70	0.00
	DS	22.8	22.8	23	22.87	0.12	7.85	7.88	7.87	7.87	0.02
August	US	28.7	28.7	28.8	28.73	0.06	7.1	7.11	7.15	7.12	0.03
	BC	56.7	56	56.4	56.37	0.35	6.73	6.86	6.95	6.85	0.11
	DP	68.7	67.9	69	68.53	0.57	7.05	7.09	7.13	7.09	0.04
	DS	20.7	20.8	20.8	20.77	0.06	7.24	7.26	7.28	7.26	0.02
Sept.	US	10.7	10.6	10.7	10.67	0.06	6.46	6.37	6.39	6.41	0.05
	BC	20.8	20.7	20.7	20.73	0.06	6.74	6.75	6.78	6.76	0.02
	DP	19.5	19.1	19.2	19.27	0.21	6.82	6.85	6.78	6.82	0.04
	DS	11.4	11.6	11.5	11.50	0.10	6.5	6.51	6.54	6.52	0.02
October	US	17	17.2	17	17.07	0.12	7.02	7.01	7.02	7.02	0.01
	BC	30.8	30.4	30.4	30.53	0.23	6.63	6.54	6.64	6.60	0.06
	DP	28.5	28.5	28.5	28.50	0.00	6.7	6.74	6.8	6.75	0.05
	DS	29	29.1	29	29.03	0.06	6.9	6.92	6.92	6.91	0.01
Novem.	US	20.5	22	21.5	21.33	0.76	6.87	6.85	6.85	6.86	0.01
	BC	40.1	39.9	39.9	39.97	0.12	6.8	6.78	6.78	6.79	0.01
	DP	48.9	47.9	48.8	48.53	0.55	6.65	6.68	6.7	6.68	0.03
	DS	14.2	14.5	13.6	14.10	0.46	6.67	6.72	6.76	6.72	0.05
Decemb.	US	12	12.5	12.1	12.20	0.26	6.86	6.85	6.84	6.85	0.01
	BC	36.5	36.2	35.7	36.13	0.40	6.77	6.82	6.76	6.78	0.03
	DP	31.5	31.9	31.9	31.77	0.23	6.68	6.69	6.7	6.69	0.01
	DS	10.7	9.88	10.4	10.33	0.41	6.64	6.63	6.66	6.64	0.02
January	US	11.7	11.3	11.2	11.40	0.26	7.05	7.04	7.04	7.04	0.01
	BC	12.8	12.5	12.7	12.67	0.15	6.83	6.85	6.84	6.84	0.01
	DP	32.2	32.2	33.6	32.67	0.81	6.89	6.84	6.88	6.87	0.03
	DS	8.77	8.7	8.7	8.72	0.04	6.9	6.93	6.93	6.92	0.02
February	US	6.37	6.36	6.39	6.37	0.02	7.42	7.41	7.41	7.41	0.01
	BC	40.3	40.6	40.2	40.37	0.21	7.81	7.79	7.79	7.80	0.01
	DP	44.3	44.1	43.8	44.07	0.25	7.88	7.89	7.88	7.88	0.01
	DS	5.94	5.85	6.04	5.94	0.10	7.78	7.77	7.77	7.77	0.01

Table 2.1: Temperature and COD values for NWWTW over a 12 month period

		Temperature (°C)					COD (mg/l)				
		1	2	3	Avg	Sd	1	2	3	Avg	Sd
March	US	26	26	26	26.00	0.00	165.33	156.67	162.00	161.33	4.37
	BC	26	26	26	26.00	0.00	89.00	114.00	111.33	104.78	13.73
	DP	25	25	25	25.00	0.00	<10	<11	<12	<10	n/a
	DS	26	26	26	26.00	0.00	116.00	309.33	152.00	192.44	102.82
April	US	21	21	21	21.00	0.00	306.00	305.00	302.00	304.33	2.08
	BC	22	22	22	22.00	0.00	221.00	227.00	240.00	229.33	9.71
	DP	22	22	22	22.00	0.00	309.00	313.00	311.33	311.11	2.01
	DS	21	21	21	21.00	0.00	151.00	151.00	151.00	151.00	0.00
May	US	21	21	21	21.00	0.00	18.33	20.67	21.67	20.22	1.71
	BC	22	22	22	22.00	0.00	50.33	37.00	27.33	38.22	11.55
	DP	21	21	21	21.00	0.00	>10	>10	>10	>10	N/A
	DS	22	22	22	22.00	0.00	308.67	307.67	311.00	309.11	1.71
June	US	13	12.5	12.5	12.67	0.29	109.67	115.67	113.33	112.89	3.02
	BC	13	13	13	13.00	0.00	290.33	302.67	307.00	300.00	8.65
	DP	12	12	12	12.00	0.00	106.67	112.67	110.67	110.00	3.06
	DS	13.5	13.5	13.5	13.50	0.00	86.00	90.00	90.33	88.78	2.41
July	US	15	15	15	15.00	0.00	312.00	311.00	310.00	311.00	1.00
	BC	16	16	16	16.00	0.00	122.00	101.33	121.00	114.78	11.65
	DP	15	15	15	15.00	0.00	291.33	291.00	289.67	290.67	0.88
	DS	15	15	15	15.00	0.00	312.67	312.00	312.67	312.44	0.38
August	US	20	20	20	20.00	0.00	110.00	105.33	102.33	105.89	3.86
	BC	21	21	21	21.00	0.00	310.33	309.33	310.67	310.11	0.69
	DP	19	19	19	19.00	0.00	185.33	181.00	182.00	182.78	2.27
	DS	20	20	20	20.00	0.00	308.00	312.00	308.67	309.56	2.14
Sept.	US	20	20	20	20.00	0.00	56.00	55.00	55.67	55.56	0.51
	BC	22	22	22	22.00	0.00	309.33	307.67	309.00	308.67	0.88
	DP	20	20	20	20.00	0.00	309.00	309.33	307.00	308.44	1.26
	DS	20	20	20	20.00	0.00	138.67	141.67	138.67	139.67	1.73
October	US	24	24	24	24.00	0.00	197.00	198.00	190.67	195.22	3.98
	BC	22	22	22	22.00	0.00	309.00	306.00	305.67	306.89	1.84
	DP	23	23	23	23.00	0.00	106.67	111.67	111.33	109.89	2.80
	DS	24	24	24	24.00	0.00	148.33	148.00	147.67	148.00	0.33
Novem.	US	21	21	21	21.00	0.00	230.00	266.67	228.67	241.78	21.56
	BC	22	22	22	22.00	0.00	116.33	130.00	125.00	123.78	6.91
	DP	22.5	22.5	22.5	22.50	0.00	278.67	303.67	279.33	287.22	14.25
	DS	23	23	23	23.00	0.00	262.67	241.67	234.00	246.11	14.84
Decemb.	US	22	22	22	22.00	0.00	279.33	271.00	272.67	274.33	4.41
	BC	25	25	25	25.00	0.00	175.00	167.67	169.67	170.78	3.79
	DP	21	21	21	21.00	0.00	154.00	154.00	153.67	153.89	0.19
	DS	22	22	22	22.00	0.00	211.00	203.33	201.67	205.33	4.98
January	US	24	24	24	24.00	0.00	299.67	298.00	300.00	299.22	1.07
	BC	24	24	24	24.00	0.00	<10	<10	<10	N/A	N/A
	DP	23	23	23	23.00	0.00	303.33	304.00	303.67	303.67	0.33
	DS	24	24	24	24.00	0.00	152.33	146.00	152.00	150.11	3.56
February	US	25	25	25	25.00	0.00	308.67	310.00	308.00	308.89	1.02
	BC	25	25	25	25.00	0.00	292.00	301.00	294.00	295.67	4.73
	DP	25	25	25	25.00	0.00	261.00	251.67	251.67	254.78	5.39
	DS	27	27	27	27.00	0.00	309.00	310.00	309.00	309.33	0.58

BOD values of treated wastewater effluent and receiving surface waters at the Norther wastewater treatment plant

		BOD DAY 0				BOD DAY 5			
March	SAMPLE	1	2	3	AVG	1	2	3	AVG
	NW BC 200	7.63	7.63	7.70	7.65	6.89	6.41	6.03	6.44
	NW BC 225	7.36	7.43	7.43	7.41	6.79	6.45	6.50	6.58
	NW BC 275	7.34	7.50	7.46	7.43	5.68	5.63	5.42	5.58
	NW BC 300	7.69	7.56	7.63	7.63	3.51	3.66	3.86	3.68
	NW AC 200	7.80	7.98	7.99	7.92	4.04	3.96	4.00	4.00
	NW AC 225	8.14	8.20	8.26	8.20	3.14	3.37	3.58	3.36
	NW AC 275	8.36	8.35	8.37	8.36	4.96	5.23	4.94	5.04
	NW AC 300	8.09	8.04	8.11	8.08	3.71	3.62	3.88	3.74
	NW US 60	8.09	8.16	8.15	8.13	6.20	5.02	6.00	5.74
	NW US 150	8.38	8.36	8.34	8.36	6.61	6.67	6.44	6.57
	NW US 200	7.97	7.91	7.94	7.94	5.98	5.80	5.61	5.80
	NW US 300	8.14	8.13	8.11	8.13	4.66	4.07	4.40	4.38
	NW DS 60	8.16	8.10	8.15	8.14	5.58	5.57	5.69	5.61
	NW DS150	7.90	7.92	7.91	7.91	5.42	5.52	5.63	5.52
	NW DS 200	7.72	8.02	8.03	7.92	5.75	5.81	5.80	5.79
	NW DS 300	7.81	7.71	7.80	7.77	5.99	5.96	5.61	5.85
	CONTROL	8.37	8.32	8.36	8.35	6.68	6.69	6.79	6.72
	NW BC 200	7.68	7.82	7.71	7.74	5.86	4.54	4.84	5.08
April	NW BC 225	8.17	7.31	7.70	7.73	3.95	4.99	4.30	4.41
	NW BC 275	7.34	7.08	7.29	7.24	5.50	3.43	4.79	4.57
	NW BC 300	7.87	7.58	7.59	7.68	6.60	5.58	5.60	5.93
	NW AC 200	7.69	7.45	7.62	7.59	5.19	5.62	5.01	5.27
	NW AC 225	7.36	7.78	7.90	7.68	4.77	4.21	4.92	4.63
	NW AC 275	7.14	7.61	7.63	7.46	4.28	4.13	4.78	4.40
	NW AC 300	7.95	7.92	7.95	7.94	6.28	4.83	4.45	5.19
	NW US 60	8.52	8.53	8.52	8.52	4.17	4.37	4.49	4.34
	NW US 150	8.48	8.50	8.50	8.49	5.63	5.55	5.83	5.67
	NW US 200	8.51	8.53	8.51	8.52	5.45	5.96	5.29	5.57
	NW US 300	8.49	8.47	8.48	8.48	5.54	5.19	5.73	5.49
	NW DS 60	8.31	8.31	8.28	8.30	5.15	5.14	5.05	5.11
	NW DS150	8.05	8.05	7.98	8.03	5.89	5.97	5.92	5.93
	NW DS 200	7.76	7.74	7.89	7.80	5.78	5.80	5.43	5.67

	NW DS 300	7.23	7.27	7.45	7.32	5.24	5.13	5.49	5.29
	CONTROL	8.37	8.32	8.36	8.35	6.68	6.69	6.79	6.72
	NW BC 200	6.78	6.75	6.72	6.75	6.69	6.21	6.22	6.37
	NW BC 225	6.78	6.90	6.81	6.83	5.25	5.18	5.27	5.23
May	NW BC 275	6.23	6.28	6.11	6.21	6.21	6.49	6.36	6.35
	NW BC 300	6.86	6.93	6.98	6.92	5.35	5.53	5.42	5.43
	NW AC 200	8.00	8.02	7.99	8.00	5.45	5.09	5.10	5.21
	NW AC 225	7.95	7.94	7.95	7.95	5.12	5.05	5.02	5.06
	NW AC 275	8.09	8.05	8.07	8.07	5.42	5.81	5.49	5.57
	NW AC 300	7.94	7.95	7.97	7.95	5.80	5.78	5.90	5.83
	NW US 60	8.12	8.11	8.09	8.11	6.25	6.45	6.90	6.53
	NW US 150	8.13	8.11	8.14	8.13	6.14	6.30	6.61	6.35
	NW US 300	8.01	8.02	7.98	8.00	6.49	6.75	6.40	6.55
	NW DS 60	8.17	8.16	8.15	8.16	5.61	5.53	5.39	5.51
	NW DS150	8.09	8.10	8.15	8.11	5.99	6.10	6.09	6.06
	NW DS 200	8.14	8.17	8.19	8.17	5.76	5.40	5.95	5.70
	NW DS 300	8.11	8.14	8.10	8.12	6.54	6.22	6.49	6.42
	CONTROL	8.13	8.16	8.14	8.14	7.92	8.03	8.07	8.01
	NW BC 200	8.00	7.96	7.96	7.97	4.76	4.77	4.50	4.68
	NW BC 225	8.09	8.10	7.90	8.03	4.33	4.29	4.39	4.34
June	NW BC 275	7.78	7.77	7.79	7.78	3.88	3.85	3.63	3.79
	NW BC 300	7.59	7.59	7.60	7.59	4.46	4.53	4.59	4.53
	NW AC 200	8.45	8.42	8.44	8.44	4.45	4.42	4.32	4.40
	NW AC 225	8.43	8.41	8.42	8.42	5.11	5.27	5.35	5.24
	NW AC 275	8.56	8.57	8.57	8.57	4.69	4.78	4.65	4.71
	NW AC 300	8.69	8.66	8.68	8.68	5.07	5.32	5.13	5.17
	NW US 200	8.10	8.12	8.14	8.12	4.72	4.79	4.79	4.77
	NW US 225	8.31	8.30	8.32	8.31	4.45	4.24	4.31	4.33
	NW US 275	8.34	8.34	8.35		4.40	4.33	4.26	
	NW US 300	8.21	8.16	8.21	8.19	4.69	4.75	4.56	4.67
	NW DS 200	8.41	8.48	8.48	8.46	4.60	4.46	4.49	4.52
	NW DS 225	8.56	8.57	8.52	8.55	4.87	4.50	4.79	4.72
	NW DS 300	8.44	8.48	8.50	8.47	5.04	4.97	4.60	4.87
	CONTROL	8.42	8.43	8.43	8.43	8.14	8.13	8.15	8.14
	NW BC 200	6.61	6.92	6.80	6.78	4.07	4.91	4.27	4.42
	NW BC 225	6.17	6.68	6.67	6.51	5.46	5.10	5.20	5.25
	NW BC 300	6.48	5.74	6.18	6.13	4.84	4.64	4.31	4.60

	NW AC 200	7.73	7.71	7.66	7.70	4.76	4.93	4.72	4.80
July	NW AC 225	7.71	7.13	7.40	7.41	5.44	5.23	5.10	5.26
	NW AC 275	7.70	7.21	7.35	7.42	5.46	5.05	5.00	5.17
	NW AC 300	7.27	7.36	7.42	7.35	4.92	4.85	4.72	4.83
	NW US 60	8.35	8.45	8.32	8.37	4.85	4.05	4.96	4.62
	NW US 150	8.22	8.25	8.24	8.24	4.51	4.34	4.93	4.59
	NW US 200	8.21	8.18	8.35	8.25	4.96	4.27	4.16	
	NW US 300	8.23	8.17	8.16	8.19	4.90	4.83	4.41	4.71
	NW DS 60	8.51	8.37	8.33	8.40	4.64	4.52	4.84	4.67
	NW DS150	8.31	8.26	8.21	8.26	4.81	4.81	4.93	4.85
	NW DS 200	8.30	8.21	8.51	8.34	4.80	4.77	3.94	4.50
	NW DS 300	8.34	8.28	8.31	8.31	4.70	4.20	4.07	4.32
	CONTROL	8.51	8.45	8.39	8.45	7.35	7.18	7.29	7.27
	US 200	7.52	7.43	7.52	7.49	5.25	5.61	5.11	5.32
	US 225	7.26	7.33	7.27	7.29	4.80	4.76	5.01	4.86
August	US 275	7.20	7.27	7.24	7.24	4.77	4.83	4.80	4.80
	US 300	7.11	7.10	7.14	7.12	4.86	5.17	5.19	5.07
	BC 200	6.17	6.18	6.07	6.14	4.23	4.20	4.24	4.22
	BC 225	5.82	5.81	5.91	5.85	4.60	4.96	4.81	4.79
	BC 275	5.74	5.70	5.96	5.80	4.37	4.40	4.38	4.38
	BC 300	5.27	5.68	5.66	5.54	5.35	5.29	5.31	5.32
	DP 200	6.64	6.62	6.79	6.68	5.07	5.02	5.04	5.04
	DP 225	6.40	6.32	6.56	6.43	4.78	4.75	5.00	4.84
	DP 275	5.66	5.98	5.40	5.68	4.78	4.66	4.62	4.69
	DP 300	5.47	5.61	5.70	5.59	4.59	4.96	5.17	4.91
	DS 200	8.00	8.06	8.02	8.03	5.15	5.03	4.81	5.00
	DS 225	7.84	7.98	7.97	7.93	4.65	5.04	4.79	4.83
	DS 300	7.97	7.98	8.01	7.99	4.70	4.65	4.81	4.72
	CONTROL	8.04	8.02	8.00	8.02	7.02	7.07	7.13	7.07
	US 200	8.14	8.18	8.18	8.17	4.13	4.76	7.87	5.59
September	US 225	8.35	8.43	8.43	8.40	5.55	5.03	5.07	5.22
	US 275	8.11	8.21	8.24	8.19	4.60	4.74	4.75	4.70
	US 300	7.50	7.71	7.67	7.63	4.30	4.83	4.80	4.64
	BC 200	6.93	6.72	6.87	6.84	5.00	4.69	5.69	5.13
	BC 225	5.24	6.36	6.38	5.99	5.27	5.21	5.65	5.38
	BC 275	6.01	6.01	6.30	6.11	4.73	5.50	4.89	5.04
	BC 300	5.64	6.08	6.37	6.03	4.57	4.52	4.52	4.54

	DP 200	7.30	6.58	7.36	7.08	4.33	4.49	4.52	4.45
	DP 225	7.08	6.51	6.53	6.71	5.15	4.82	5.04	5.00
	DP 275	5.30	6.41	6.54	6.08	4.36	4.87	4.83	4.69
	DP 300	6.07	6.17	6.11	6.12	3.51	4.67	4.90	4.36
	DS 200	7.92	7.93	7.95	7.93	5.76	4.82	4.56	5.05
	DS 225	7.39	7.58	7.58	7.52	4.69	4.28	4.36	4.44
	DS 275	7.50	7.64	7.68	7.61	4.82	4.11	4.69	4.54
	DS 300	7.22	7.26	7.25	7.24	4.53	4.81	4.55	4.63
	CONTROL	8.36	8.37	8.40	8.38	5.98	6.51	6.59	6.36
October	US 200	7.75	7.98	7.85	7.86	9.98	3.89	4.65	6.17
	US 225	7.68	7.84	7.86	7.79	3.83	4.92	4.93	4.56
	US 275	7.65	7.65	7.65	7.65	4.35	4.45	4.42	4.41
	US 300	7.42	7.44	7.45	7.44	3.97	4.85	4.80	4.54
	BC 200	7.59	7.73	7.78	7.70	3.71	5.02	4.71	4.48
	BC 225	7.44	7.49	7.21	7.38	4.50	4.84	4.75	4.70
	BC 275	7.03	7.13	7.00	7.05	4.63	4.73	7.84	5.73
	BC 300	8.06	8.04	8.04	8.05	5.04	4.90	5.00	4.98
	DP 200	8.06	8.30	8.05	8.14	4.75	5.03	4.90	4.89
	DP 225	7.96	7.97	7.85	7.93	5.05	4.94	5.00	5.00
	DP 275	7.79	7.88	7.98	7.88	4.15	4.54	4.84	4.51
	DP 300	7.86	7.90	7.85	7.87	4.63	4.84	4.97	4.81
	DS 200	7.99	7.80	7.85	7.88	4.16	4.99	4.79	4.65
	DS 225	7.81	7.79	7.80	7.80	4.91	5.07	5.02	5.00
	DS 275	7.65	7.56	7.52	7.58	4.15	4.96	4.81	4.64
	DS 300	7.39	7.39	7.39	7.39	4.19	5.20	5.19	4.86
	CONTROL	8.13	8.03	8.13	8.10	6.75	7.33	7.43	7.17
November	US 200	7.72	7.73	7.69	7.71	3.93	4.27	4.27	4.16
	US 225	7.61	7.69	7.66	7.65	3.97	4.15	4.15	4.09
	US 275	7.50	7.54	7.56	7.53	4.19	4.22	4.12	4.18
	US 300	7.44	7.52	7.59	7.52	4.32	4.31	4.34	4.32
	BC 200	7.08	7.19	7.25	7.17	4.50	3.63	4.46	4.20
	BC 225	7.03	6.96	7.02	7.00	3.42	4.03	4.03	3.83
	BC 275	6.82	6.81	6.99	6.87	4.32	4.21	4.21	4.25
	BC 300	6.51	6.56	6.75	6.61	3.70	4.04	4.02	3.92
	DP 200	7.04	7.14	7.05	7.08	4.30	4.45	4.25	4.33
	DP 225	7.04	7.01	7.03	7.03	4.23	4.17	4.25	4.22
	DP 275	6.80	6.97	6.76	6.84	3.48	4.21	4.38	4.02

	DP 300	6.49	6.50	6.41	6.47	3.95	4.68	4.46	4.36
	DS 200	7.55	7.37	7.46	7.46	4.32	4.33	4.35	4.33
	DS 225	7.46	7.45	7.51	7.47	4.24	4.21	4.28	4.24
	DS 275	7.25	7.31	7.30	7.29	4.64	4.84	4.59	4.69
	DS 300	7.47	7.49	7.52	7.49	3.89	3.55	4.04	3.83
	CONTROL	7.82	7.83	7.86	7.84	4.86	5.66	4.94	5.15
	US 200	7.56	7.66	7.66	7.63	4.76	4.47	4.38	4.54
	US 225	7.36	7.37	7.46	7.40	4.55	4.52	4.51	4.53
	US 275	7.57	7.66	7.64	7.62	4.40	4.63	4.66	4.56
	US 300	7.38	7.37	7.33	7.36	4.39	4.66	4.60	4.55
December	BC 200	7.17	7.20	7.23	7.20	4.13	4.24	4.20	4.19
	BC 225	7.09	7.06	7.03	7.06	4.50	4.46	4.52	4.49
	BC 275	6.86	6.90	6.88	6.88	4.05	4.15	4.12	4.11
	BC 300	6.50	6.63	6.98	6.70	4.48	4.64	4.41	4.51
	DP 200	7.75	7.78	7.58	7.70	5.89	5.41	5.72	5.67
	DP 225	7.62	7.66	7.62	7.63	4.03	4.23	4.21	4.16
	DP 275	7.52	7.51	7.56	7.53	4.32	4.44	4.30	4.35
	DP 300	7.47	7.48	7.89	7.61	4.92	4.55	4.51	4.66
	DS 200	7.56	7.61	7.58	7.58	4.30	4.51	4.52	4.44
	DS 225	7.63	7.60	7.68	7.64	4.72	4.57	4.55	4.61
	DS 275	7.63	7.67	7.38	7.56	4.44	4.50	4.68	4.54
	DS 300	7.52	7.62	7.52	7.55	4.48	4.56	4.78	4.61
	CONTROL	7.90	7.76	7.85	7.84	7.02	7.10	7.05	7.06
	US 200	7.64	7.71	7.05	7.47	4.46	4.63	4.44	4.51
	US 225	7.60	7.67	7.62	7.63	4.40	4.47	4.54	4.47
	US 275	7.56	7.62	7.58	7.59	4.25	4.59	4.59	4.48
	US 300	7.50	7.57	7.58	7.55	4.62	4.39	4.61	4.54
	BC 200	7.73	7.73	7.73	7.73	4.63	4.45	4.56	4.55
	BC 225	7.78	7.76	7.72	7.75	4.65	4.59	4.79	4.68
January	BC 275	7.54	7.51	7.56	7.54	4.65	4.40	4.72	4.59
	BC 300	7.31	7.42	7.59	7.44	5.01	4.63	4.75	4.80
	DP 200	7.74	7.77	7.58	7.70	4.63	4.55	4.49	4.56
	DP 225	7.76	7.74	7.71	7.74	4.41	4.58	4.55	4.51
	DP 275	7.64	7.56	7.52	7.57	4.86	4.67	4.42	4.65
	DP 300	7.55	7.53	7.55	7.54	4.80	4.99	4.81	4.87
	DS 200	7.74	7.76	7.66	7.72	4.64	4.99	4.92	4.85
	DS 225	7.58	7.65	7.85	7.69	4.86	4.96	4.81	4.88

	DS 275	7.75	7.78	7.75	7.76	4.93	4.85	4.83	4.87
	DS 300	7.47	7.55	7.35	7.46	4.80	4.70	4.45	4.65
	CONTROL	7.98	7.95	7.94	7.96	6.48	6.40	6.04	6.31
	US 200	7.60	7.59	7.59	7.59	4.46	4.63	4.44	4.51
	US 225	7.57	7.59	7.62	7.59	4.40	4.47	4.54	4.47
	US 275	7.44	7.40	7.46	7.43	4.25	4.59	4.59	4.48
February	US 300	7.37	7.40	7.37	7.38	4.62	4.39	4.61	4.54
	BC 200	6.61	6.56	6.53	6.57	4.63	4.45	4.56	4.55
	BC 225	6.21	6.14	6.12	6.16	4.65	4.59	4.79	4.68
	BC 275	5.80	5.70	5.73	5.74	4.65	4.40	4.72	4.59
	BC 300	5.41	5.10	5.10	5.20	5.01	4.63	4.75	4.80
	DP 200	7.23	7.28	7.22	7.24	4.63	4.55	4.49	4.56
	DP 225	7.08	6.95	7.11	7.05	4.41	4.58	4.55	4.51
	DP 275	6.77	6.79	6.68	6.75	4.86	4.67	4.42	4.65
	DP 300	6.45	6.41	6.57	6.48	4.80	4.99	4.81	4.87
	DS 200	7.62	7.64	7.65	7.64	4.64	4.99	4.92	4.85
	DS 225	7.58	7.61	7.63	7.61	4.86	4.96	4.81	4.88
	DS 275	7.43	7.50	7.46	7.46	4.93	4.85	4.83	4.87
	DS 300	7.37	7.29	7.30	7.32	4.80	4.70	4.45	4.65
	CONTROL	7.85	7.85	7.85	7.85	6.48	6.40	6.04	6.31

BOD values of treated wastewater effluent and receiving surface waters at the New Germany wastewater treatment plant.

		BOD DAY 0				BOD DAY 5			
March	SAMPLE	1	2	3	AVG	1	2	3	AVG
	BC 200	8.37	8.17	8.25	8.26	7.15	7.05	7.23	7.143333
	BC 225	8.06	8.08	8.03	8.06	6.18	6.36	6.15	6.23
	BC 275	8.24	8.15	8.04	8.14	5.89	5.73	5.76	5.793333
	BC 300	8.27	8.12	8.15	8.18	6.12	6.04	6.31	6.156667
	DP 200	8.37	8.27	8.15	8.26	5.64	5.86	5.43	5.643333
	DP 225	8.23	8.25	8.26	8.25	6.69	6.41	6.33	6.476667
	DP 275	8.15	8	8	8.05	6.06	5.83	5.93	5.94
	DP 300	7.97	8.18	8.08	8.08	5.99	5.73	5.96	5.893333
	US 60	8.47	8.56	8.56	8.53	5.57	5.21	5.05	5.276667
	US 150	8.64	8.59	8.59	8.61	6.59	6.38	6.93	6.633333
	US 200	8.61	8.58	8.58	8.59	4.24	4.12	4.72	4.36
	US 300	8.63	8.62	8.6	8.62	5.83	5.15	5.36	5.446667
	DS 60	8.69	8.63	8.62	8.65	6.68	6.62	6.18	6.493333
	DS150	8.59	8.58	8.57	8.58	6.76	6.25	6.86	6.623333
	DS 200	8.65	8.66	8.64	8.65	6.41	6.1	6.62	6.376667
	DS 300	8.66	8.61	8.59	8.62	6.86	6.72	6.82	6.8
	CONTROL	8.71	8.7	8.69	8.70	6.84	6.19	6.19	6.41
	BC 200	8.49	8.45	8.45	8.46	5.73	5.33	5.49	5.52
April	BC 225	8.37	8.36	8.35	8.36	5.31	5.52	5.86	5.56
	BC 300	8.35	8.38	8.38	8.37	5.17	5.59	5.46	5.41
	DP 200	8.49	8.49	8.46	8.48	6.33	6.50	6.30	6.38
	DP 225	8.5	8.49	8.5	8.50	5.31	5.37	5.41	5.36
	DP 275	8.52	8.52	8.54	8.53	5.38	5.39	5.09	5.29

	DP 300	8.56	8.52	8.5	8.53	4.74	4.47	4.71	4.64
	US 60	8.51	8.47	8.46	8.48	4.93	4.98	4.83	4.91
	US 150	8.64	8.68	8.68	8.67	4.22	4.22	4.35	4.26
	US 200	8.73	8.78	8.78	8.76	4.72	4.25	4.34	4.44
	US 300	8.83	8.86	8.89	8.86	4.97	4.95	4.88	4.93
	DS 60	8.54	8.52	8.56	8.54	4.25	4.95	4.20	4.47
	DS150	8.64	8.64	8.63	8.64	4.63	4.35	4.01	4.33
	DS 200	8.71	8.72	8.75	8.73	5.69	5.86	5.32	5.62
	DS 300	8.85	8.85	8.81	8.84	5.07	5.05	5.13	5.08
	CONTROL	8.38	8.38	8.35	8.37	5.13	5.80	5.64	5.52
	BC 200	8.34	8.36	8.47	8.39	5.99	5.00	5.10	5.36
	BC 225	8.38	8.34	8.35	8.36	4.96	4.88	4.81	4.88
May	BC 275	8.17	8.16	8.08	8.14	4.95	4.88	4.81	4.88
	BC 300	8.08	8.16	8.10	8.11	4.44	4.35	4.27	4.35
	DP 200	8.41	8.45	8.35	8.40	4.39	4.61	4.24	4.41
	DP 225	8.09	8.00	8.10	8.06	4.41	4.67	4.40	4.49
	DP 275	8.25	8.13	8.14	8.17	4.43	4.24	4.36	4.34
	DP 300	8.79	8.73	8.70	8.74	4.88	4.87	4.24	4.66
	US 60	8.61	8.65	8.68	8.65	4.84	4.09	4.44	4.46
	US 150	8.79	8.87	8.89	8.85	4.92	4.38	4.64	4.65
	US 300	8.83	8.87	8.89	8.86	4.94	4.07	4.52	4.51
	DS 60	8.49	8.56	8.59	8.55	4.35	4.07	4.24	4.22
	DS150	8.64	8.64	8.61	8.63	4.95	4.21	4.56	4.57
	DS 200	8.68	8.65	8.65	8.66	4.30	4.87	4.62	4.60
	DS 300	8.57	8.49	8.51	8.52	5.84	5.53	5.55	5.64
	CONTROL	8.65	8.63	8.60	8.63	7.66	7.67	7.69	7.67
	BC 200	7.31	7.43	7.37	7.37	4.21	4.18	4.11	4.17
	BC 225	7.53	7.55	7.51	7.53	3.97	3.91	3.85	3.91

June	BC 275	7.48	7.49	7.46	7.48	4.06	4.24	4.19	4.16
	BC 300	7.47	7.43	7.43	7.44	4.19	3.90	4.16	4.08
	DP 200	8.04	8.01	8.03	8.03	3.55	3.71	3.68	3.65
	DP 225	8.25	8.23	8.23	8.24	4.27	4.24	4.22	4.24
	DP 275	8.18	8.17	8.15		3.72	3.67	3.84	
	DP 300	8.23	8.23	8.21	8.22	4.00	4.04	3.92	3.99
	US 60	8.31	8.32	8.32	8.32	4.34	4.32	4.04	4.23
	US 150	8.37	8.37	8.36	8.37	4.41	4.54	4.71	4.55
	US 300	8.50	8.51	8.52		4.45	4.44	4.37	
	DS 60	8.68	8.71	8.73	8.71	4.40	4.31	4.31	4.34
	DS150	8.23	8.25	8.23	8.24	4.59	4.71	4.51	4.60
	DS 200	8.46	8.45	8.45	8.45	4.10	4.04	8.85	5.66
	DS 300	8.65	8.67	8.68	8.67	5.29	5.62	5.24	5.38
	CONTROL	7.75	7.72	7.65	7.71	7.74	7.90	7.81	7.82
	BC 200	7.75	7.67	7.41	7.61	4.82	4.80	5.09	4.90
	BC 225	7.54	7.34	7.33	7.40	4.15	4.60	4.35	4.37
	BC 275	6.52	6.80	6.39	6.57	4.59	4.21	4.06	4.29
	BC 300	8.62	8.59	8.65	8.62	4.94	4.49	4.59	4.67
July	DP 200	8.66	8.66	8.66	8.66	4.22	4.38	4.60	4.40
	DP 225	8.68	8.69	8.72	8.70	4.26	4.52	4.97	4.58
	DP 275	8.80	8.89	8.88	8.86	4.46	4.22	4.35	4.34
	DP 300	8.47	8.36	8.31	8.38	4.12	4.31	4.59	4.34
	US 60	8.61	8.58	8.58	8.59	4.15	4.19	4.39	4.24
	US 150	8.37	8.42	8.53	8.44	4.98	4.27	4.82	4.69
	US 300	8.69	8.74	8.84	8.76	4.39	4.91	4.57	4.62
	DS 60	8.16	8.25	8.28	8.23	4.92	4.56	4.85	4.78
	DS150	8.32	8.38	8.41	8.37	3.97	3.61	3.62	3.73
	DS 200	8.40	8.59	8.55	8.51	4.59	4.08	4.29	4.32

	DS 300	8.57	8.73	8.82	8.71	3.73	3.89	3.87	3.83
	CONTROL	8.15	8.17	8.17	8.16	7.21	7.26	7.37	7.28
	US 200	7.75	7.92	7.97	7.88	4.50	4.78	4.78	4.69
	US 225	7.35	7.75	7.57	7.56	5.64	5.27	5.30	5.40
August	US 275	7.46	7.54	7.68	7.56	5.24	5.20	4.79	5.08
	US 300	7.43	7.82	7.67	7.64	5.18	4.61	5.00	4.93
	BC 200	7.89	7.77	7.89	7.85	4.39	4.63	4.47	4.50
	BC 225	7.72	7.62	7.40	7.58	4.75	4.90	4.73	4.79
	BC 275	7.31	7.47	7.52	7.43	4.65	5.05	4.46	4.72
	BC 300	7.58	7.51	7.50	7.53	4.72	4.90	4.65	4.76
	DP 200	8.33	8.20	8.17	8.23	4.46	4.58	4.16	4.40
	DP 225	8.20	8.20	8.19	8.20	4.53	4.43	4.76	4.57
	DP 275	8.19	8.15	8.15	8.16	4.55	4.57	4.35	4.49
	DP 300	8.22	8.20	8.19	8.20	4.02	4.17	4.08	4.09
	DS 200	8.49	8.49	8.50	8.49	4.26	4.23	4.03	4.17
	DS 225	8.75	8.62	8.69	8.69	3.34	3.52	3.39	3.42
	DS 300	8.64	8.57	8.68	8.63	4.53	4.51	4.60	4.55
	CONTROL	8.07	8.06	8.04	8.06	6.66	6.84	6.62	6.71
	US 200	7.82	7.82	7.82	7.82	4.15	4.56	4.67	4.46
September	US 225	7.56	7.98	7.83	7.79	4.44	4.30	4.48	4.41
	US 275	7.52	7.65	7.83	7.67	4.07	4.23	4.52	4.27
	US 300	7.65	7.78	7.62	7.68	4.65	4.91	4.56	4.71
	BC 200	8.11	8.32	8.31	8.25	4.58	4.17	4.71	4.49
	BC 225	8.13	8.32	8.30	8.25	4.43	4.45	4.49	4.46
	BC 275	8.23	8.21	8.30	8.25	4.36	4.32	4.85	4.51
	BC 300	8.25	8.23	8.27	8.25	4.50	4.22	4.44	4.39
	DP 200	8.20	8.22	8.25	8.22	4.30	4.30	4.32	4.31
	DP 225	8.23	8.21	8.25	8.23	5.31	4.22	4.39	4.64

	DP 275	8.23	8.38	8.25	8.29	4.99	5.54	4.86	5.13
	DP 300	8.18	8.26	8.08	8.17	4.11	4.35	4.50	4.32
	DS 200	7.42	7.47	7.36	7.42	4.80	4.99	5.02	4.94
	DS 225	7.41	7.46	7.55	7.47	4.39	4.49	4.75	4.54
	DS 275	7.37	7.26	7.39	7.34	5.09	4.10	4.22	4.47
	DS 300	7.03	7.22	7.24	7.16	4.28	4.19	4.25	4.24
	CONTROL	8.48	8.43	8.41	8.44	7.10	7.05	7.15	7.10
October	US 200	8.41	8.42	8.45	8.43	4.44	5.28	5.25	4.99
	US 225	8.43	8.46	8.50	8.46	4.63	4.90	4.85	4.79
	US 275	8.43	8.43	8.43	8.43	4.46	4.90	4.87	4.74
	US 300	8.46	8.51	8.56	8.51	3.69	5.24	5.28	4.74
	BC 200	8.23	8.24	8.35	8.27	4.48	4.69	4.28	4.48
	BC 225	8.19	8.23	8.45	8.29	4.75	5.19	5.00	4.98
	BC 275	8.26	8.36	8.35	8.32	4.15	4.53	4.68	4.45
	BC 300	8.30	8.33	8.36	8.33	4.84	4.79	4.26	4.63
	DP 200	8.38	8.37	8.35	8.37	4.73	4.96	4.85	4.85
	DP 225	8.36	8.40	8.45	8.40	4.25	4.90	5.41	4.85
	DP 275	8.26	8.36	8.41	8.34	4.67	4.54	5.10	4.77
	DP 300	8.30	8.33	8.50	8.38	4.64	4.67	4.54	4.62
	DS 200	8.47	8.46	8.49	8.47	4.70	4.78	4.89	4.79
	DS 225	8.49	8.46	8.47	8.47	4.66	4.62	5.02	4.77
	DS 275	8.52	8.53	8.55	8.53	4.48	5.16	5.23	4.96
	DS 300	8.50	8.55	8.65	8.57	4.46	4.87	4.52	4.62
	CONTROL	8.31	8.33	8.40	8.35	5.23	5.60	5.80	5.54
November	US 200	7.82	7.84	7.87	7.84	4.24	4.35	4.44	4.34
	US 225	7.79	7.89	7.80	7.83	4.33	4.53	4.39	4.42
	US 275	7.85	7.86	7.82	7.84	4.31	4.09	4.11	4.17
	US 300	7.85	7.87	7.88	7.87	4.15	4.72	4.45	4.44

	BC 200	7.52	7.53	7.45	7.50	4.27	4.44	4.38	4.36
	BC 225	7.46	7.71	7.89	7.69	4.05	4.30	4.42	4.26
	BC 275	7.63	7.70	7.84	7.72	4.07	4.37	4.11	4.18
	BC 300	7.61	7.62	7.85	7.69	4.22	4.60	4.02	4.28
	DP 200	7.57	7.48	7.56	7.54	4.12	4.20	4.28	4.20
	DP 225	7.65	7.67	7.86	7.73	4.14	4.27	4.35	4.25
	DP 275	7.65	7.67	7.50	7.61	4.09	4.10	4.21	4.13
	DP 300	7.61	7.62	7.89	7.71	3.95	4.40	4.36	4.24
	DS 200	7.59	7.62	7.54	7.58	3.90	4.24	4.37	4.17
	DS 225	7.65	7.64	7.65	7.65	4.06	4.21	4.21	4.16
	DS 275	7.64	7.59	7.63	7.62	4.03	4.39	4.29	4.24
	DS 300	7.53	7.54	7.54	7.54	4.06	4.19	4.20	4.15
	CONTROL	7.90	8.01	7.87	7.93	7.04	7.20	7.07	7.10
	US 200	7.82	7.85	7.83	7.83	4.62	4.94	5.01	4.86
	US 225	7.88	7.91	7.78	7.86	3.58	5.16	5.14	4.63
	US 275	7.91	7.91	7.91	7.91	4.50	4.95	4.75	4.73
	US 300	7.87	7.89	7.90	7.89	4.79	7.43	7.58	6.60
December	BC 200	7.52	7.53	7.58	7.54	4.76	5.09	5.02	4.96
	BC 225	7.00	6.90	7.01	6.97	4.71	4.41	4.65	4.59
	BC 275	6.93	6.94	6.85	6.91	5.17	4.85	4.92	4.98
	BC 300	6.75	7.01	7.22	6.99	4.64	4.68	4.65	4.66
	DP 200	7.71	7.48	7.52	7.57	4.41	4.33	4.58	4.44
	DP 225	7.37	7.46	7.45	7.43	4.82	5.21	5.20	5.08
	DP 275	7.40	7.42	7.46	7.43	4.63	4.52	4.85	4.67
	DP 300	7.31	7.38	7.32	7.34	4.85	5.07	4.26	4.73
	DS 200	7.67	7.69	7.65	7.67	4.00	4.98	4.56	4.51
	DS 225	7.85	7.86	7.84	7.85	4.47	4.89	4.65	4.67
	DS 275	7.61	7.63	7.66	7.63	4.64	4.25	4.32	4.40

	DS 300	7.62	7.68	7.82	7.71	4.79	4.98	4.87	4.88
	CONTROL	7.88	8.02	8.04	7.98	6.69	6.77	6.89	6.78
	US 200	7.72	7.76	7.74	7.74	4.67	4.03	4.97	4.56
	US 225	7.73	7.78	7.75	7.75	4.31	4.67	4.93	4.64
	US 275	7.62	7.78	7.81	7.74	4.43	4.67	4.52	4.54
	US 300	7.64	7.66	7.78	7.69	4.42	4.45	4.48	4.45
	BC 200	7.49	7.59	7.50	7.53	4.28	4.45	4.47	4.40
	BC 225	7.54	7.45	7.45	7.48	4.36	4.66	4.45	4.49
January	BC 275	7.05	7.00	7.01	7.02	4.46	4.80	4.87	4.71
	BC 300	6.95	6.95	7.00	6.97	4.39	4.43	4.57	4.46
	DP 200	7.79	7.79	7.82	7.80	4.86	4.50	4.78	4.71
	DP 225	7.75	7.81	7.89	7.82	4.85	4.78	4.59	4.74
	DP 275	7.69	7.66	7.85	7.73	4.32	4.56	4.90	4.59
	DP 300	7.47	7.62	7.55	7.55	4.72	4.62	4.81	4.72
	DS 200	8.04	7.79	8.02	7.95	4.68	4.63	4.65	4.65
	DS 225	8.04	8.04	8.04	8.04	3.76	4.91	5.05	4.57
	DS 275	8.02	8.02	8.02	8.02	4.06	4.66	4.52	4.41
	DS 300	7.98	8.05	7.98	8.00	5.01	7.78	4.92	5.90
	CONTROL	7.99	7.98	8.02	8.00	6.24	5.84	6.12	6.07
	US 200	7.44	7.52	7.51	7.49	4.26	4.78	7.28	5.44
	US 225	7.24	7.33	7.37	7.31	4.20	4.21	4.43	4.28
	US 275	6.98	7.20	7.06	7.08	4.15	4.46	4.50	4.37
February	US 300	6.94	6.84	6.88	6.89	4.90	4.61	4.86	4.79
	BC 200	7.67	7.64	7.57	7.63	4.11	4.25	4.22	4.19
	BC 225	7.79	7.67	7.68	7.71	4.12	4.14	4.38	4.21
	BC 275	7.30	7.38	7.38	7.35	4.51	4.60	4.55	4.55
	BC 300	7.49	7.41	7.36	7.42	4.32	4.55	4.32	4.40
	DP 200	7.86	7.87	7.83	7.85	4.21	4.25	4.27	4.24

DP 225	7.86	7.84	7.84	7.85	4.45	4.64	4.59	4.56
DP 275	7.65	7.60	7.70	7.65	4.49	4.58	4.93	4.67
DP 300	7.65	7.65	7.65	7.65	4.33	4.36	4.51	4.40
DS 200	7.98	7.99	7.99	7.99	4.22	4.49	4.47	4.39
DS 225	7.98	7.93	7.97	7.96	4.11	4.21	4.70	4.34
DS 275	7.97	7.91	7.93	7.94	4.20	3.71	3.98	3.96
DS 300	7.84	7.88	7.90	7.87	4.28	4.57	4.62	4.49
CONTROL	8.02	8.02	8.02	8.02	7.26	7.34	7.50	7.37

Table 2.4 Temperature and COD value for NGWTP

		Temperature (°C)					COD (mg/l)				
		1	2	3	Avg	Sd	1	2	3	Avg	Sd
	US	26	26	26	26.00	0.00	314.00	314.00	313.67	313.89	0.19
March	BC	26	26	26	26.00	0.00	149.33	166.67	145.00	153.67	11.46
	DP	26	26	26	26.00	0.00	249.00	239.00	229.00	239.00	10.00
	DS	26	26	26	26.00	0.00	153.00	140.00	131.00	141.33	11.06
	US	18	18	18	18.00	0.00	101.00	105.33	106.33	104.22	2.83
April	BC	20	20	20	20.00	0.00	211.00	193.00	204.33	202.78	9.10
	DP	20	20	20	20.00	0.00	180.67	180.00	178.33	179.67	1.20
	DS	19	19	19	19.00	0.00	113.00	116.00	113.00	114.00	1.73
May	US	16	16	16	16.00	0.00	298.33	298.67	299.00	298.67	0.33
	BC	19	19	19	19.00	0.00	312.00	311.67	313.00	312.22	0.69
	DP	19	19	19	19.00	0.00	247.00	249.00	243.00	246.33	3.06
	DS	14	14	14	14.00	0.00	313.33	313.00	309.33	311.89	2.22
June	US	16	16	16	16.00	0.00	25.00	18.00	24.00	22.33	3.79
	BC	18	18	18	18.00	0.00	312.00	309.00	309.00	310.00	1.73
	DP	18	18	18	18.00	0.00	149.00	133.00	131.00	137.67	9.87
	DS	14	14	14	14.00	0.00	78.00	72.00	70.00	73.33	4.16
July	US	14	14	14	14.00	0.00	311.67	310.00	307.33	309.67	2.19
	BC	17	17	17	17.00	0.00	193.67	190.00	197.33	193.67	3.67
	DP	17	17	17	17.00	0.00	309.00	308.00	309.00	308.67	0.58
	DS	15	15	15	15.00	0.00	298.33	295.67	304.67	299.56	4.62
	US	15	15	15	15	0.00	206.33	208.00	208.33	207.56	1.07
August	BC	17	17	17	17	0.00	138.67	140.67	139.33	139.56	1.02
	DP	17	17	17	17	0.00	307.67	309.00	310.33	309.00	1.33
	DS	12	12	12	12	0.00	312.67	311.67	311.00	311.78	0.84
Sept.	US	20	20	20	20.00	0.00	310.00	310.00	311.00	310.33	0.58
	BC	22	22	22	22.00	0.00	96.67	101.00	97.00	98.22	2.41
	DP	20	20	20	20.00	0.00	309.33	311.67	310.33	310.44	1.17
	DS	20	20	20	20.00	0.00	192.00	187.00	190.67	189.89	2.59
October	US	17	17	17	17.00	0.00	311.00	312.00	312.67	311.89	0.84
	BC	20	20	20	20.00	0.00	32.67	39.67	34.67	35.67	3.61
	DP	20	20	20	20.00	0.00	53.00	57.67	51.67	54.11	3.15
	DS	19	19	19	19.00	0.00	242.00	242.00	233.67	239.22	4.81
Novem.	US	17	17	17	17.00	0.00	304.00	308.67	307.67	306.78	2.46
	BC	20	20	20	20.00	0.00	68.00	70.67	68.67	69.11	1.39
	DP	20	20	20	20.00	0.00	105.00	111.33	109.33	108.56	3.24
	DS	18	18	18	18.00	0.00	248.33	250.00	274.33	257.56	14.55
	US	20	20	20	20.00	0.00	22.00	26.00	25.00	24.33	2.08
Decemb.	BC	22	22	22	22.00	0.00	88.67	91.33	100.00	93.33	5.93
	DP	22	22	22	22.00	0.00	302.00	303.67	295.67	300.44	4.22
	DS	20	20	20	20.00	0.00	39.67	34.00	32.33	35.33	3.84
	US	22	22	22	22.00	0.00	84.67	81.67	74.67	80.33	5.13
January	BC	23	23	23	23.00	0.00	110.33	112.00	111.00	111.11	0.84
	DP	23	23	23	23.00	0.00	236.67	244.00	241.67	240.78	3.75
	DS	22	22	22	22.00	0.00	48.33	43.67	41.67	44.56	3.42
	US	21	21	21	21.00	0.00	305.00	305.00	306.00	305.33	0.58
February	BC	24	24	24	24.00	0.00	156.33	164.33	156.00	158.89	4.72
	DP	24	24	24	24.00	0.00	282.33	287.67	280.33	283.44	3.79
	DS	23	23	23	23.00	0.00	264.00	264.67	290.00	272.89	14.82

Table 2.5: Turbidity and pH value for NGWTP

		Turbidity					pH				
		1	2	3	Avg	Sd	1	2	3	Avg	Sd
	US	5.22	5.15	5.12	5.16	0.05	7.61	7.51	7.44	7.52	0.09
March	BC	6.41	6.86	6.67	6.65	0.23	7.08	6.93	7.35	7.12	0.21
	DP	5.45	5.29	6.38	5.71	0.59	7.29	7.05	7.21	7.18	0.12
	DS	7.7	7.13	7.14	7.32	0.33	7.43	7.6	7.49	7.51	0.09
	US	8.23	8.23	8.23	8.23	0.00	7.06	7.09	7.1	7.08	0.02
April	BC	1.52	1.52	1.52	1.52	0.00	7.08	7.01	7.02	7.04	0.04
	DP	1.43	1.43	1.4	1.42	0.02	6.83	6.82	6.82	6.82	0.01
	DS	17	17	17	17.00	0.00	7.07	7.08	7	7.05	0.04
May	US	3.18	3.18	3.18	3.18	0.00	6.39	6.4	6.48	6.42	0.05
	BC	28.7	28.7	28.7	28.70	0.00	6.97	6.94	6.81	6.91	0.09
	DP	30.3	30.3	30.3	30.30	0.00	7.02	7.02	7.03	7.02	0.01
	DS	17.8	17.8	17.8	17.80	0.00	7.1	7.1	7.1	7.10	0.00
June	US	8.91	9.15	8.99	9.02	0.12	7.94	7.92	7.93	7.93	0.01
	BC	9.65	9.65	9.6	9.63	0.03	7.61	7.63	7.63	7.62	0.01
	DP	10.5	10.2	11.2	10.63	0.51	7.54	7.55	7.55	7.55	0.01
	DS	14	14.2	14	14.07	0.12	7.83	7.83	7.83	7.83	0.00
July	US	2.43	2.45	2.43	2.44	0.01	6.29	6.3	6.31	6.30	0.01
	BC	20	20	20.2	20.07	0.12	6.63	6.43	6.53	6.53	0.10
	DP	20.7	20.6	20.9	20.73	0.15	6.88	6.89	6.9	6.89	0.01
	DS	16.1	16.1	16.1	16.10	0.00	7	6.96	6.99	6.98	0.02
	US	40.1	40.3	40.8	40.4	0.36	7.1	7.11	7.15	7.12	0.03
August	BC	19.6	19.7	19.9	19.73	0.15	6.73	6.86	6.95	6.85	0.11
	DP	16.9	16.6	16.9	16.8	0.17	7.05	7.09	7.13	7.09	0.04
	DS	14	14.1	14.2	14.1	0.10	7.24	7.26	7.28	7.26	0.02
Sept.	US	16	15.7	15.8	15.83	0.15	6.46	6.49	6.48	6.48	0.02
	BC	5.83	5.83	5.85	5.84	0.01	6.83	6.74	6.67	6.75	0.08
	DP	16.5	16.6	16.8	16.63	0.15	6.34	6.38	6.39	6.37	0.03
	DS	6.96	6.98	6.99	6.98	0.02	6.59	6.59	6.59	6.59	0.00
October	US	3.68	3.68	3.69	3.68	0.01	6.98	6.98	6.95	6.97	0.02
	BC	20.1	19.9	20	20.00	0.10	6.92	6.81	6.99	6.91	0.09
	DP	16.3	16.4	16.3	16.33	0.06	6.92	6.95	6.69	6.85	0.14
	DS	5.1	5.1	5.11	5.10	0.01	7.01	6.98	6.96	6.98	0.03
Novem.	US	8.06	8.17	8.09	8.11	0.06	7.12	7.12	7.11	7.12	0.01
	BC	5.48	5.6	5.46	5.51	0.08	6.84	6.83	6.79	6.82	0.03
	DP	6.52	6.48	6.44	6.48	0.04	7.1	7.15	7.18	7.14	0.04
	DS	16.4	16.8	16.4	16.53	0.23	7.15	7.17	7.16	7.16	0.01
	US	32	32.2	32.1	32.10	0.10	6.46	6.47	6.48	6.47	0.01
Decemb.	BC	4.45	4.68	4.09	4.41	0.30	6.4	6.38	6.88	6.55	0.28
	DP	29.4	29.5	29.4	29.43	0.06	6.45	6.46	6.45	6.45	0.01
	DS	28.2	28.1	28	28.10	0.10	6.47	6.51	6.55	6.51	0.04
	US	10.8	10.8	10.8	10.80	0.00	6.71	6.72	6.71	6.71	0.01
January	BC	9.38	9.45	9.45	9.43	0.04	6.6	6.57	6.59	6.59	0.02
	DP	9.26	9.34	9.28	9.29	0.04	6.6	6.59	6.63	6.61	0.02
	DS	10.6	10.6	10.6	10.60	0.00	6.73	6.73	6.73	6.73	0.00
	US	8.87	8.82	8.8	8.83	0.04	7.56	7.48	7.47	7.50	0.05
February	BC	3.92	3.93	3.94	3.93	0.01	7.76	7.7	7.7	7.72	0.03
	DP	4	4.05	4.01	4.02	0.03	7.87	7.88	7.85	7.87	0.02
	DS	5.82	5.79	5.8	5.80	0.02	8.08	8.08	8.08	8.08	0.00

APPENDIX 2

Statistical Analysis of physicochemical parameters and microbial counts at the NWWTW (B.C)

```
CORRELATIONS
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  /PRINT=TWOTAIL NOSIG
  /STATISTICS DESCRIPTIVES
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created		23-JUL-2013 19:02:55
Comments		
Input	Active Dataset	DataSet0
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	12
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=pH Turbidity BOD COD TemperatureR SalmonellaT ShigellaT /PRINT=TWOTAIL NOSIG /STATISTICS DESCRIPTIVES /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.01
	Elapsed Time	00:00:00.00

```
[DataSet0]
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Descriptive Statistics

	Mean	Std. Deviation	N
pH	7.0942	.39617	12
Turbidity	29.2839	16.74611	12
BOD	2.6733	1.04760	12
COD	191.9175	114.31746	12
TemperatureR	.6283	.31881	12
SalmonellaT	.1633	.18691	12
ShigellaT	.4987	.42413	12

Correlations

		pH	Turbidity	BOD	COD	TemperatureR	SalmonellaT	ShigellaT
pH	Pearson Correlation	1	.123	-.027	.095	.090	.483	.253
	Sig. (2-tailed)		.704	.933	.770	.780	.111	.428
	N	12	12	12	12	12	12	12
Turbidity	Pearson Correlation	.123	1	-.104	.428	.037	.622*	.053
	Sig. (2-tailed)	.704		.747	.166	.909	.031	.871
	N	12	12	12	12	12	12	12
BOD	Pearson Correlation	-.027	-.104	1	-.045	.219	-.240	.121
	Sig. (2-tailed)	.933	.747		.890	.494	.453	.709
	N	12	12	12	12	12	12	12
COD	Pearson Correlation	.095	.428	-.045	1	.254	.031	.243
	Sig. (2-tailed)	.770	.166	.890		.425	.923	.447
	N	12	12	12	12	12	12	12
TemperatureR	Pearson Correlation	.090	.037	.219	.254	1	.031	.234
	Sig. (2-tailed)	.780	.909	.494	.425		.924	.464
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	.483	.622*	-.240	.031	.031	1	.204
	Sig. (2-tailed)	.111	.031	.453	.923	.924		.526
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	.253	.053	.121	.243	.234	.204	1
	Sig. (2-tailed)	.428	.871	.709	.447	.464	.526	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

(U.S)

```
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Correlations

Notes

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[DataSet0]

Descriptive Statistics

	Mean	Std. Deviation	N
pHr	.1728	.10123	12
TurbidityT	1.1435	.17524	12
BODt	.6553	.16550	12
CODr	1.6366	.82794	12
TempR	.6837	.32313	12
SalmonellaT	.0148	.01307	12
ShigellaT	.1620	.14111	12

Correlations

		pHr	TurbidityT	BODt	CODr	TempR	SalmonellaT	ShigellaT
pHr	Pearson Correlation	1	.174	-.652*	.485	-.165	-.050	-.123
	Sig. (2-tailed)		.588	.022	.110	.609	.876	.702
	N	12	12	12	12	12	12	12
TurbidityT	Pearson Correlation	.174	1	-.053	.291	.084	.613*	-.648*
	Sig. (2-tailed)	.588		.870	.359	.796	.034	.023
	N	12	12	12	12	12	12	12
BODt	Pearson Correlation	-.652*	-.053	1	-.128	.332	.332	.165
	Sig. (2-tailed)	.022	.870		.692	.291	.291	.609
	N	12	12	12	12	12	12	12
CODr	Pearson Correlation	.485	.291	-.128	1	.002	.110	-.193
	Sig. (2-tailed)	.110	.359	.692		.994	.734	.547
	N	12	12	12	12	12	12	12
TempR	Pearson Correlation	-.165	.084	.332	.002	1	-.045	.187
	Sig. (2-tailed)	.609	.796	.291	.994		.890	.560
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	-.050	.613*	.332	.110	-.045	1	-.440
	Sig. (2-tailed)	.876	.034	.291	.734	.890		.153
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	-.123	-.648*	.165	-.193	.187	-.440	1
	Sig. (2-tailed)	.702	.023	.609	.547	.560	.153	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

(D.P)

```
CORRELATIONS
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Correlations

Notes

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[DataSet0]
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Descriptive Statistics

	Mean	Std. Deviation	N
pH	7.1558	.40248	12
BOD	3.3867	.92561	12
COD	198.9167	118.11472	12
TurbidityT	1.4690	.27298	12
temperatureR	.6035	.35286	12
SalmonellaT	.1108	.14458	12
ShigellaT	.1996	.31297	12

Correlations

		pH	BOD	COD	TurbidityT	temperatureR	SalmonellaT	ShigellaT
pH	Pearson Correlation	1	.042	.052	-.066	-.113	.487	.237
	Sig. (2-tailed)		.897	.871	.839	.727	.109	.459
	N	12	12	12	12	12	12	12
BOD	Pearson Correlation	.042	1	-.495	-.456	-.249	-.115	.340
	Sig. (2-tailed)	.897		.102	.137	.436	.722	.279
	N	12	12	12	12	12	12	12
COD	Pearson Correlation	.052	-.495	1	.510	.041	.344	-.163
	Sig. (2-tailed)	.871	.102		.090	.899	.274	.613
	N	12	12	12	12	12	12	12
TurbidityT	Pearson Correlation	-.066	-.456	.510	1	-.356	.471	-.667*
	Sig. (2-tailed)	.839	.137	.090		.256	.122	.018
	N	12	12	12	12	12	12	12
temperatureR	Pearson Correlation	-.113	-.249	.041	-.356	1	-.313	.556
	Sig. (2-tailed)	.727	.436	.899	.256		.322	.061
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	.487	-.115	.344	.471	-.313	1	-.105
	Sig. (2-tailed)	.109	.722	.274	.122	.322		.746
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	.237	.340	-.163	-.667*	.556	-.105	1
	Sig. (2-tailed)	.459	.279	.613	.018	.061	.746	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

(D.S)

```
CORRELATIONS
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  /STATISTICS DESCRIPTIVES
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Correlations

Notes

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Syntax		CORRELATIONS /VARIABLES=pH COD TurbidityT BODt TempR SalmonellaT ShigellaT /PRINT=TWOTAIL NOSIG /STATISTICS DESCRIPTIVES /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.01
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[DataSet0]

Descriptive Statistics

	Mean	Std. Deviation	N
pH	7.2042	.48120	12
COD	208.9442	76.51935	12
TurbidityT	1.1417	.18681	12
BODt	.6649	.10775	12
TempR	.7226	.32310	12
SalmonellaT	.0318	.06962	12
ShigellaT	.1873	.31795	12

Correlations

		pH	COD	TurbidityT	BODt	TempR	SalmonellaT	ShigellaT
pH	Pearson Correlation	1	.142	.060	.600*	.053	.272	.390
	Sig. (2-tailed)		.660	.854	.039	.870	.392	.210
	N	12	12	12	12	12	12	12
COD	Pearson Correlation	.142	1	.093	-.237	-.070	-.287	-.328
	Sig. (2-tailed)	.660		.774	.459	.829	.366	.298
	N	12	12	12	12	12	12	12
TurbidityT	Pearson Correlation	.060	.093	1	.076	.508	.050	.005
	Sig. (2-tailed)	.854	.774		.814	.092	.879	.988
	N	12	12	12	12	12	12	12
BODt	Pearson Correlation	.600*	-.237	.076	1	.329	.485	.491
	Sig. (2-tailed)	.039	.459	.814		.296	.110	.105
	N	12	12	12	12	12	12	12
TempR	Pearson Correlation	.053	-.070	.508	.329	1	.194	.244
	Sig. (2-tailed)	.870	.829	.092	.296		.547	.444
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	.272	-.287	.050	.485	.194	1	.931**
	Sig. (2-tailed)	.392	.366	.879	.110	.547		.000
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	.390	-.328	.005	.491	.244	.931**	1
	Sig. (2-tailed)	.210	.298	.988	.105	.444	.000	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Statistical Analysis of physicochemical parameters and microbial counts at the NGWTW

(B.C)

```
CORRELATIONS
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Correlations

Notes

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	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
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Correlations

		pH	Turbidity	BOD	COD	Temperature	LogshigellaT	LogSalmonellaT
pH	Pearson Correlation	1	-.241	.310	.430	.175	-.262	.343
	Sig. (2-tailed)		.450	.327	.163	.586	.410	.275
	N	12	12	12	12	12	12	12
Turbidity	Pearson Correlation	-.241	1	-.134	.300	-.577*	-.060	-.269
	Sig. (2-tailed)	.450		.678	.343	.050	.852	.397
	N	12	12	12	12	12	12	12
BOD	Pearson Correlation	.310	-.134	1	-.262	-.094	-.022	.218
	Sig. (2-tailed)	.327	.678		.411	.771	.946	.497
	N	12	12	12	12	12	12	12
COD	Pearson Correlation	.430	.300	-.262	1	-.339	.078	-.141
	Sig. (2-tailed)	.163	.343	.411		.282	.809	.663
	N	12	12	12	12	12	12	12
Temperature	Pearson Correlation	.175	-.577*	-.094	-.339	1	-.046	.197
	Sig. (2-tailed)	.586	.050	.771	.282		.886	.539
	N	12	12	12	12	12	12	12
LogshigellaT	Pearson Correlation	-.262	-.060	-.022	.078	-.046	1	.110
	Sig. (2-tailed)	.410	.852	.946	.809	.886		.734
	N	12	12	12	12	12	12	12
LogSalmonellaT	Pearson Correlation	.343	-.269	.218	-.141	.197	.110	1
	Sig. (2-tailed)	.275	.397	.497	.663	.539	.734	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

(U.S)

```
CORRELATIONS
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  /PRINT=TWOTAIL NOSIG
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Correlations

Notes

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	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
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[DataSet1] /Users/ejovwokekollinz/Documents/New Germany/US/US New Germany.sav
```

Correlations

		pHt	TurbidityT	BODt	CODr	TempT	SalmonellaT	ShigellaT
pHt	Pearson Correlation	1	.085	.207	.056	.219	-.278	-.047
	Sig. (2-tailed)		.793	.518	.862	.494	.381	.885
	N	12	12	12	12	12	12	12
TurbidityT	Pearson Correlation	.085	1	-.529	.561	.155	.839**	.622*
	Sig. (2-tailed)	.793		.077	.058	.629	.001	.031
	N	12	12	12	12	12	12	12
BODt	Pearson Correlation	.207	-.529	1	.029	-.150	-.539	-.628*
	Sig. (2-tailed)	.518	.077		.928	.641	.071	.029
	N	12	12	12	12	12	12	12
CODr	Pearson Correlation	.056	.561	.029	1	-.180	.466	-.028
	Sig. (2-tailed)	.862	.058	.928		.576	.127	.932
	N	12	12	12	12	12	12	12
TempT	Pearson Correlation	.219	.155	-.150	-.180	1	.046	-.014
	Sig. (2-tailed)	.494	.629	.641	.576		.887	.964
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	-.278	.839**	-.539	.466	.046	1	.394
	Sig. (2-tailed)	.381	.001	.071	.127	.887		.206
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	-.047	.622*	-.628*	-.028	-.014	.394	1
	Sig. (2-tailed)	.885	.031	.029	.932	.964	.206	
	N	12	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

(D.P)

```
CORRELATIONS
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Correlations

Notes

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Correlations

		pH	Turbidity	BOD	CODr	Temperature	SalmonellaT	ShigellaT
pH	Pearson Correlation	1	-.421	.307	.297	.054	-.036	-.104
	Sig. (2-tailed)		.173	.332	.348	.869	.912	.749
	N	12	12	12	12	12	12	12
Turbidity	Pearson Correlation	-.421	1	.246	-.399	-.383	.541	.569
	Sig. (2-tailed)	.173		.442	.199	.219	.069	.053
	N	12	12	12	12	12	12	12
BOD	Pearson Correlation	.307	.246	1	-.063	-.671*	.319	.091
	Sig. (2-tailed)	.332	.442		.846	.017	.313	.780
	N	12	12	12	12	12	12	12
CODr	Pearson Correlation	.297	-.399	-.063	1	.269	.059	-.303
	Sig. (2-tailed)	.348	.199	.846		.397	.855	.338
	N	12	12	12	12	12	12	12
Temperature	Pearson Correlation	.054	-.383	-.671*	.269	1	-.139	-.434
	Sig. (2-tailed)	.869	.219	.017	.397		.667	.159
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	-.036	.541	.319	.059	-.139	1	.477
	Sig. (2-tailed)	.912	.069	.313	.855	.667		.117
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	-.104	.569	.091	-.303	-.434	.477	1
	Sig. (2-tailed)	.749	.053	.780	.338	.159	.117	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

(D.S)

```
CORRELATIONS
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  /MISSING=PAIRWISE.
```

Correlations

Notes

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	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=pHt TurbidityT BODt CODr Temperature SalmonellaT ShigellaT /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
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[DataSet1] /Users/ejovwokekollinz/Documents/New Germany/D.S/D.S.sav
```

Correlations

		pHt	TurbidityT	BODt	CODr	Temperature	SalmonellaT	ShigellaT
pHt	Pearson Correlation	1	-.328	.403	-.069	.063	-.528	-.369
	Sig. (2-tailed)		.298	.195	.831	.846	.078	.238
	N	12	12	12	12	12	12	12
TurbidityT	Pearson Correlation	-.328	1	.298	-.155	-.497	.452	.533
	Sig. (2-tailed)	.298		.347	.630	.100	.141	.074
	N	12	12	12	12	12	12	12
BODt	Pearson Correlation	.403	.298	1	-.480	-.499	-.334	-.038
	Sig. (2-tailed)	.195	.347		.114	.098	.289	.908
	N	12	12	12	12	12	12	12
CODr	Pearson Correlation	-.069	-.155	-.480	1	.643*	.281	-.215
	Sig. (2-tailed)	.831	.630	.114		.024	.377	.502
	N	12	12	12	12	12	12	12
Temperature	Pearson Correlation	.063	-.497	-.499	.643*	1	.126	-.280
	Sig. (2-tailed)	.846	.100	.098	.024		.697	.378
	N	12	12	12	12	12	12	12
SalmonellaT	Pearson Correlation	-.528	.452	-.334	.281	.126	1	.731**
	Sig. (2-tailed)	.078	.141	.289	.377	.697		.007
	N	12	12	12	12	12	12	12
ShigellaT	Pearson Correlation	-.369	.533	-.038	-.215	-.280	.731**	1
	Sig. (2-tailed)	.238	.074	.908	.502	.378	.007	
	N	12	12	12	12	12	12	12

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

APPENDIX 3

<u>Antibiotic susceptibility profile of each *Salmonella* spp. isolate recovered from treated wastewater effluent and receiving surface waters.</u>

ID	SXT	CFM	FOX	S	ATM	NA	AK	CAZ	CN	CXM	AMP	CIP	C	PRL	KF	NOR	TE	RL	IPM	F
1	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
2	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
3	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
4	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
5	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
6	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
7	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
8	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
9	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
10	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
11	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
12	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
13	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
14	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
15	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
16	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
17	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
18	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
19	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
20	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
21	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
22	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
23	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
24	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
25	S	R	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
26	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
27	S	R	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
28	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
29	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
30	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
31	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
32	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
33	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
34	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
35	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
36	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S

37	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
38	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
39	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
40	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
41	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
42	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
43	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
44	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
45	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
46	S	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
47	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
48	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
49	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
50	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
51	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
52	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
53	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
54	S	S	S	I	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
55	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
56	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
57	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
58	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
59	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
60	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
61	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
62	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
63	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
64	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
65	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
66	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
67	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
68	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
69	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
70	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
71	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
72	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
73	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
74	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
75	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
76	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
77	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
78	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
79	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
80	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S

81	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
82	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
83	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
84	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
85	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
86	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
87	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
88	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
89	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
90	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
91	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
92	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
93	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
94	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
95	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
96	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
97	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
98	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
99	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
100	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
101	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
102	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
103	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
104	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
105	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
106	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
107	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
108	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
109	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
110	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
111	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
112	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
113	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
114	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
115	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
116	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
117	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
118	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
119	S	S	S	I	S	I	S	S	S	S	S	S	S	I	S	S	S	R	S	S
120	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
121	S	S	S	I	S	I	S	S	S	S	S	S	S	I	S	S	S	R	S	S
122	S	S	S	I	S	I	S	S	S	S	S	S	S	I	S	S	S	R	S	I
123	S	S	S	I	S	I	S	S	S	S	R	S	S	I	S	S	S	R	R	I
124	S	S	S	R	S	I	I	I	S	S	I	I	S	R	S	S	S	R	I	I

125	S	S	S	R	S	I	S	S	S	I	I	S	S	S	S	S	S	R	S	I
126	S	S	S	I	S	I	S	S	S	I	S	S	S	S	S	S	S	R	S	S
127	S	S	S	R	S	I	S	S	S	I	S	S	S	I	S	S	S	R	S	S
128	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
129	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
130	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
131	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
132	S	S	S	R	I	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
133	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
134	S	S	S	R	S	S	S	S	S	S	S	S	S	I	S	S	S	R	S	S
135	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
136	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
137	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
138	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
139	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
140	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
141	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
142	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
143	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
144	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
145	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
146	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	I
147	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
148	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
149	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
150	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
151	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
152	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
153	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
154	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
155	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
156	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
157	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
158	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
159	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
160	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
161	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
162	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
163	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
164	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
165	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
166	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
167	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
168	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	I

169	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
170	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
171	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
172	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
173	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
174	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
175	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
176	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
177	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	I
178	S	S	S	I	S	i	S	S	S	S	S	S	S	S	S	S	S	R	S	S
179	S	S	S	I	S	i	S	S	S	S	S	S	S	S	S	S	S	R	S	S
180	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
181	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
182	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
183	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
184	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
185	S	S	S	R	S	I	S	I	S	S	S	S	S	S	S	S	S	R	S	S
186	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
187	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
188	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
189	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S
190	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
191	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
192	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
193	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
194	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
195	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
196	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
197	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
198	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S
199	S	S	S	I	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	I
200	S	S	S	R	S	I	S	S	S	S	S	S	S	S	S	S	S	R	S	S

KF: Cephalothin; IPM: Imipenem; FOX: Cefoxitin; CXM: Cefuroxime; PRL: Piperacillin; AMP: Ampicillin; CFM: Cefixime; CAZ: Ceftazidime; ATM: Aztreonam CN: Gentamycin; AK: Amikacin; S: Streptomycin; C: Chloramphenicol; TE: Tetracycline; CIP: Ciprofloxacin; NOR: Norfloxacin; NA: Nalidixic acid; F: Nitrofurantoin SXT: Trimethorprim/Sulphamethoxazole; RL: Sulphamethoxazole

APPENDIX 4

Distribution of virulence signatures in *Salmonella* spp. isolated from treated wastewater effluent and receiving surface waters.

Isolate ID	*pip*D	*spi*C	*mis*L	*orf*L
1	Y	Y	Y	Y
2	Y	Y	Y	Y
3	Y	Y	Y	Y
4	Y	Y	Y	Y
5	Y	Y	Y	Y
6	Y	Y	Y	Y
7	Y	Y	Y	Y
8	Y	Y	Y	Y
9	Y	Y	Y	Y
10	Y	Y	Y	Y
11	Y	Y	Y	Y
12	Y	Y	Y	Y
13	Y	Y	Y	Y
14	Y	Y	Y	Y
15	Y	Y	Y	Y
16	Y	Y	Y	Y
17	Y	Y	Y	Y
18	Y	Y	Y	Y
19	Y	Y	Y	Y
20	Y	Y	Y	Y
21	Y	Y	Y	Y
22	Y	Y	Y	Y

23	Y	Y	Y	Y
24	Y	Y	Y	Y
25	Y	Y	Y	Y
26	Y	Y	Y	Y
27	Y	Y	Y	Y
28	Y	Y	Y	Y
29	Y	Y	Y	Y
30	Y	Y	Y	Y
31	Y	Y	Y	Y
32	Y	Y	Y	Y
33	Y	Y	Y	Y
34	Y	Y	Y	Y
35	Y	Y	Y	Y
36	Y	Y	Y	Y
37	Y	Y	Y	Y
38	Y	Y	Y	Y
39	Y	Y	Y	Y
40	Y	Y	Y	Y
41	Y	Y	Y	Y
42	Y	Y	Y	Y
43	Y	Y	Y	Y
44	Y	Y	Y	Y
45	Y	Y	Y	Y
46	Y	Y	Y	Y
47	Y	Y	Y	Y
48	Y	Y	Y	Y
49	Y	Y	Y	Y

50	Y	Y	Y	Y
51	Y	Y	Y	Y
52	Y	Y	Y	Y
53	Y	Y	Y	Y
54	Y	Y	Y	N
55	Y	Y	N	N
56	Y	Y	Y	Y
57	Y	Y	Y	Y
58	Y	Y	Y	Y
59	Y	Y	Y	Y
60	Y	Y	Y	N
61	Y	Y	N	N
62	Y	Y	N	N
63	Y	Y	Y	Y
64	Y	Y	Y	N
65	Y	Y	Y	N
66	Y	Y	Y	N
67	Y	Y	Y	N
68	Y	Y	Y	N
69	Y	Y	Y	N
70	Y	N	Y	N
71	Y	Y	N	N
72	Y	Y	Y	Y
73	Y	N	Y	Y
74	Y	Y	Y	Y
75	Y	Y	N	N
76	Y	N	Y	N

77	Y	N	Y	Y
78	Y	Y	Y	Y
79	Y	N	N	N
80	Y	Y	Y	Y
81	Y	Y	Y	Y
82	Y	Y	Y	Y
83	Y	Y	Y	Y
84	Y	Y	Y	Y
85	Y	Y	Y	Y
86	Y	Y	Y	Y
87	Y	Y	Y	Y
88	Y	Y	Y	Y
89	Y	Y	Y	Y
90	Y	Y	Y	Y
91	Y	Y	Y	Y
92	Y	Y	Y	Y
93	Y	Y	Y	Y
94	Y	Y	Y	Y
95	Y	Y	Y	Y
96	Y	Y	Y	Y
97	Y	Y	Y	Y
98	N	Y	Y	Y
99	N	Y	Y	Y
100	N	Y	Y	Y
101	N	N	N	Y
102	Y	N	Y	Y
103	Y	N	Y	Y

104	Y	Y	N	Y
105	Y	N	N	N
106	Y	Y	Y	Y
107	Y	Y	Y	Y
108	Y	Y	Y	Y
109	Y	Y	N	Y
110	Y	Y	Y	Y
111	N	Y	Y	Y
112	N	Y	Y	Y
113	N	Y	Y	Y
114	N	Y	Y	Y
115	N	Y	Y	Y
116	N	Y	Y	Y
117	N	Y	Y	Y
118	N	Y	Y	Y
119	N	Y	Y	Y
120	N	Y	N	Y
121	Y	Y	Y	Y
122	Y	Y	Y	Y
123	Y	Y	Y	Y
124	Y	N	Y	Y
125	N	Y	N	N
126	N	Y	Y	Y
127	Y	Y	Y	Y
128	Y	Y	N	N
129	N	Y	N	N
130	Y	Y	N	N

131	N	Y	N	Y
132	Y	Y	Y	Y
133	Y	Y	N	Y
134	Y	Y	Y	Y
135	Y	Y	Y	Y
136	N	Y	Y	Y
137	N	Y	N	Y
138	N	Y	N	Y
139	Y	Y	N	Y
140	Y	Y	Y	Y
141	N	Y	N	Y
142	N	Y	N	N
143	Y	Y	Y	Y
144	N	Y	N	Y
145	Y	Y	Y	Y
146	Y	Y	Y	Y
147	N	Y	N	N
148	Y	Y	N	Y
149	Y	Y	N	Y
150	N	Y	N	N
151	Y	Y	Y	Y
152	Y	N	Y	Y
153	Y	Y	Y	Y
154	Y	Y	Y	Y
155	Y	Y	N	Y
156	Y	Y	Y	Y
157	Y	N	Y	Y

158	Y	Y	Y	Y
159	Y	N	Y	Y
160	Y	Y	Y	Y
161	Y	Y	Y	Y
162	Y	Y	N	Y
163	Y	Y	N	Y
164	Y	Y	N	Y
165	Y	Y	N	Y
166	Y	Y	Y	Y
167	Y	Y	Y	Y
168	Y	Y	Y	Y
169	Y	Y	Y	Y
170	Y	Y	Y	Y
171	Y	Y	Y	Y
172	Y	Y	Y	Y
173	Y	Y	Y	Y
174	Y	Y	Y	Y
175	Y	Y	Y	Y
176	Y	Y	Y	Y
177	Y	Y	Y	Y
178	Y	Y	Y	Y
179	Y	Y	Y	Y
180	Y	Y	Y	Y
181	Y	Y	Y	Y
182	Y	Y	Y	Y
183	Y	Y	Y	Y
184	Y	Y	Y	Y

185	Y	Y	Y	Y
186	Y	Y	Y	Y
187	Y	Y	Y	Y
188	Y	Y	Y	Y
189	Y	Y	Y	Y
190	Y	Y	Y	Y
191	Y	Y	Y	Y
192	Y	Y	Y	Y
193	Y	Y	Y	Y
194	Y	Y	Y	Y
195	Y	Y	Y	Y
196	Y	Y	Y	Y
197	Y	Y	Y	Y
198	Y	Y	Y	Y
199	Y	Y	N	N
200	Y	Y	Y	Y

Y = Yes (present)

N = No (No)