PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *Escherichia coli* ISOLATES RECOVERED FROM TREATED WASTEWATER EFFLUENT AND RECEIVING AQUATIC *MILIEU* IN DURBAN, SOUTH AFRICA

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 January 2013 – May 2015, 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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DECLARATION 2– PUBLICATIONS

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: Prevalence of virulent and multiple-antibiotic resistant *Escherichia coli* in treated effluent of two wastewater treatment plants and receiving aquatic *milieu* in Durban, South Africa. *Environmental Monitoring and Assessment* (Submitted).

Publication 2: Phenotypic and Genotypic Characterization of *Escherichia coli* isolates recovered from treated wastewater effluent and receiving aquatic *milieu* in Durban, South Africa. *Science of the Total Environment* (Submitted).

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ACKNOWLEDGEMENTS

Firstly, to GOD, without whom none of this would have been possible.

To my supervisor, Prof. A. O. Olaniran for his endless patience, sound guidance and support for the duration of my studies.

The National Research Foundation for their financial support.

To Harley, the best friend and companion I could ever ask for, thank you mostly for your unconditional love. I will miss you always.

A special thank you to Seelan Kisten for your support, good advice and endless bounds of love and laughter. You motivate me to be better.

To my friends and collegues Joash Govindsamy, Lekita Singh, Ashmita Arjoon, Ejovwokoghene Collins Odajare, Ndumiso Mkize, Noyise Ntoshobeni and Ajit Kanwal, thank you for your overall support and for being my family away from home, I appreciate all that you do.

To the staff and postgraduate students of the Microbiology Department, Westville Campus, UKZN, thank you for your assistance and guidance.

And lastly, to my parents, grandparents and sister, for all your love, support, prayer and guidance throughout the years.

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LIST OF ACRONYMS

Antibiotic Resistance Genes	**ARGs**
Biological Oxygen Demand	**BOD**
Chemical Oxygen Demand	**COD**
Chromosomal Integrons	**CIs**
Department of Water Affairs and Forestry	**DWAF**
Diffusely adherent Escherichia coli	**DAEC**
Electrical Conductivity	**EC**
Enteroadherant Escherichia coli	**EAEC**
Enterohaemorrhagic Escherichia coli	**EHEC**
Enteroinvasive Escherichia coli	**EIEC**
Enteropathogenic Escherichia coli	**EPEC**
Enterotoxigenic Escherichia coli	**ETEC**
Gene Cassettes	**GCs**
Horizontal Gene Transfer	**HGT**
Labile Toxin	**LT**
Mobile Integrons	**MIs**
New Germany Treatment Works	**NGTWs**
Northern Wastewater Treatment Plant	**NWWTP**
Random Amplified Polymorphism DNA	**RAPD**
Random Fragment Length Polymorphism	**RFLP**
Shiga Toxigenic Escherichia coli	**STEC**
South African Water Research Council	**SAWRC**
Stable Toxin	**ST**
Total Dissolved Solids	**TDS**
Total Suspended Solids	**TSS**
World Health Organization	**WHO**

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Water is generally accepted to be a vital natural resource because of its significant roles in the environment, food production, hygiene and industry. Increased urbanisation caused by the rapid increase in global population places strain on already stressed water supplies and sanitation facilities, especially in developing countries (FDIRI, 2014). According to the World Health Organisation (WHO), poor access to safe water and inadequate sanitation continues to be a danger to human health, especially in the African continent where more than 300 million people reside in water scarce environments (Vorosmarty *et al*., 2010). According to WHO (2008) 'safe water' to be consumed should meet the following requirements: it should be free from viral, bacterial and protozoic pathogens; it should meet national or international water quality guidelines for colour, taste and appearance, and chemicals present in the water should be present in a concentration of an acceptable standard. However, improper sanitation facilities, inefficient wastewater treatment processes, political upheaval as well as the insufficient funding contribute significantly to people of developing and developed countries not having access to microbiologically safe water (WHO, 2002a).

The absence of stringent water quality guidelines coupled with the infrequent monitoring of wastewater treatment plants and receiving surface waters are one of the major contributors to unsafe water (Olaniran *et al*., 2012). Water of poor quality

is sometimes discharged into surface waters used for domestic purposes such as drinking, bathing and washing as well as in the preparation of food. Inadequately treated water used for these purposes are often home to a plethora of potentially pathogenic microorganisms such as *Vibrio spp*., *Escherichia coli* and emerging pathogens such as *Legionella spp*. These microorganisms are the causative agents for a variety of gastro-intestinal infections and diarrheal diseases and if untreated, could even lead to death (Nataro and Kaper, 1998). Inadequately treated wastewater has also been identified as a reservoir of antibiotic resistant bacteria (ARB) which could affect the treatment of infections and promote the dissemination of resistance amongst bacteria in aquatic environments (Marti *et al*., 2013). It is therefore imperative that frequent monitoring of wastewater treatment plants (WWTPs) and the receiving water bodies be carried out to investigate the efficiency of these plants used in the treatment of wastewater in comparison with acceptable standards, in order to safeguard the health of the public.

1.1 The current state of water resources in South Africa

According to the South African Water Research Commission (SAWRC) and the Department of Water Affairs and Forestry (DWAF), South Africa is the 30th driest country in the world, having average rainfall levels of approximately 465 mm, and an average annual runoff of less than 50 000 million m^3 (DWAF, 2013). However, this scarcity is not only attributed to the lack of available supply, but also due to failure of infrastructure, environmental changes as well as deteriorating water quality (Hedden and Cilliers, 2014). South Africa is home to approximately 51.77 million people, with an average population

growth rate of 1%. The country has 12 water boards which supply potable water to more than 28 million people, however, this is still below the maximum supply capacity of 39 million (DWAF, 2013). This scarcity of water not only affects food security, the production of energy and environmental integrity but also results in conflict between human and animal populations who share the same water resources (Kusiluka *et al*., 2005).

According to DWAF (2013), approximately 4.9 million people do not have access to basic sanitation. Informal settlement dwellers are therefore forced to rely on untreated water resources such as dams, pools, rivers and springs for drinking and other domestic purposes, and as a result, they are frequently attacked by water-borne diseases (El-Jakee *et al*., 2009; DWAF, 2013). Although sanitary infrastructure has been provided to a number of rural communities, these facilities are often not in working order or are unhealthy for use due to the number of residents far outnumbering the number of facilities available. Therefore, residents are again forced to use available water sources for their daily water needs. Almost all aquatic environments are polluted by a number of microbial pathogens stemming from human use. These pollutants are obtained from point and non-point sources. Point source pollution enters the environment at various and different locations through the direct route of discharge of either treated or untreated domestic sewage, acid mine drainage and industrial effluent. Non-point pollution sources include urban and agricultural waste run-off, sewage and septic tank leakages as well as sewer overflows, and accounts for almost 80% of the pollution of water bodies (Stewart *et al*., 1990).

1.2 Wastewater Treatment Plants

The South African Water Act was established in 1956 and stipulated that wastewater be treated to an acceptable standard prior to discharge into receiving water bodies (Hedden and Cilliers, 2014). However, economic expansion and population explosion coupled with pressure from international authorities to meet certain quality guidelines exerted pressure on water and sanitation authorities in the country, and as a result many wastewater treatment plants fail (Morrison *et al*., 2001; Mema, 2002). Water resources are contaminated largely by wastewater effluent, which is defined as a mixture of raw sewage, primary, secondary and tertiary effluents (Naidoo and Olaniran, 2014).

Currently, there are 1286 municipal and privately owned WWTPs in South Africa, discharging approximately 21.3 km^3 of treated water back into the countries river systems. However, only 14% of river water is re-used by the municipality, and the rest is available as secondary water sources to the public (DWAF, 2013; Hedden and Cilliers, 2014). Many wastewater treatment facilities fail to treat water adequately, and final effluent of poor microbial quality then enter public waters such as rivers and lakes. Such activities affect the quality of water, and serve as a reservoir for a multitude of diseases. Therefore, the DWAF has implemented an incentive-based certification process known as the Green Drop certification process whereby critical performance indicators such as design and capacity of WWTPs, technical skills of employees as well as the adherence of effluent to set standards are assessed. Each WWTP in each of the 9 provinces in South Africa are evaluated and given a score based on set criteria (DWAF, 2013). In 2013, a national Greendrop score of 73.8% was achieved, indicating that majority of WWTPs in South

Africa are functioning adequately (DWAF, 2013). Water quality can be determined by the assessment of three attributes, namely the physical, chemical and biological characteristics of the water, each differing in accordance with its intended purpose (Kregar, 2004). The quality of water varies depending on the area in which it is found, seasonal changes as well as the type of soil and rocks through which it passes. The regular monitoring of water quality is therefore an important step in protecting public health (Franck, 2004). The lack of above-mentioned monitoring procedures together with the declining state of wastewater treatment infrastructure in South Africa has contributed to the incidence of numerous water-borne illnesses as demonstrated by outbreaks of cholera, typhoid fever and diarrheal diseases occurring in the Mpumalanga, Kwa-Zulu Natal and Eastern Cape provinces respectively (Mema, 2002).

1.3 Wastewater treatment in KwaZulu-Natal

The province of KwaZulu-Natal is situated on the east coast of South Africa and it's geography is characterized by a number of rivers, valleys and associated catchments. This province is home to approximately one hundred and fifty WWTPs ranging from small to macro sized plants. An audit conducted by the Department of Water Affairs in 2009 stated that there are concerns regarding the treatment of wastewater and subsequent discharge into receiving water bodies within the province. This audit determined that a majority of WWTPs failed to meet at least three or more of the respective discharge standards as stipulated by the South African water quality guidelines. The main problems which were identified include: plants' operation at a capacity which exceeded their hydraulic design capacity; plants' were operating close to or exceeding their organic design loads; process

control problems; funding constraints; the age of the treatment plant as well as the use of inappropriate technology.

According to Mema (2002), burst sewage pipes resulted in the release of raw sewage into the Durban harbour in 2008. Poor management of wastewater treatment facilities and failure to repair burst pipes by the municipality were identified as the cause of the problem which resulted in the death of numerous fish and other marine life. Reports by Mema (2002) also identified industrial growth, ineffective industrial on-site treatment facilities and the failure to implement proper environmental laws as major contributors to high coliform counts in the water catchments of Kwa-Zulu Natal. This could be seen clearly when evaluating treated effluent at the Northern Wastewater Treatment Plant in Durban, KwaZulu-Natal where COD values ranged between 50.33 mg/L and 276.33mg/L over a 1 year sample period, exceeding the limit of 30 mg/L as stipulated by the DWAF (Naidoo and Olaniran, 2014). Such case studies then necessitate the need for treatment facilities having improved management and infrastructure as well as more frequent monitoring programs, which exploit the use of indicator microorganisms to monitor water quality.

1.4 Microbiological quality of water

According to Grabow (1996), the detection of each pathogenic organism in water is technically difficult and time consuming therefore microbiological indicators have been used for decades as a means to monitor pollution of water sources. An ideal indicator organism should meet the following requirements:

- The concentration of the indicator organism should have a quantitative relationship to the risk of disease associated with exposure;
- The indicator organism should not be pathogenic and be present in higher numbers than that of the pathogen;
- The indicator organism should be easy to quantify;
- The indicator should be present whenever the pathogen is present;
- The indicator organism should not reproduce in water and its growth characteristics should be similar to that of its associated pathogen;
- Tests to determine the concentration of the indicator organism should be easy, rapid and inexpensive;
- The indicator organism should be specific to a source.

Since no single organism meets all of the above-mentioned requirements, it has been advised that several indicator organisms be used for water quality assessments. In addition to microbial analysis, the survival of microorganisms depends on the physico-chemical characteristics of the water. Such characteristics include: Biological Oxygen Demand (BOD); Chemical Oxygen Demand (COD); Total suspended solids (TSS); Total dissolved solids (TDS); pH; temperature; turbidity; salinity, electrical conductivity (EC) and resistivity. These parameters are also known to impact on the efficiency of water treatment processes and could themselves be affected by drastic climatic changes (DWAF, 1996).

1.5 *Escherichia coli*

Escherichia coli (*E. coli*) plays a central role in water bacteriology and has been used as an indicator of fecal pollution originating from human and warm blooded animals. Its presence in water samples can be confirmed through IMViC biochemical testing and testing for the presence of the β-glucuronidase enzyme using chromogenic media. The appearance of pathogenic *E. coli* O157:H7 has been associated with various diseases in humans, and at present, six groups of *E. coli* pathotypes have been identified (Doyle *et al*., 1997). Virulence of these pathogens is dependent on both genetic determinants as well as gene expression (Doyle *et al*., 1997). The detection of specific virulence attributes of any strain of *E. coli* allows for the determination of the potential reservoirs of virulence genes, which also play a key role in the detection of new strains of the organism. Such aspects are useful when determining the origin of emerging disease.

The production of enterotoxins by disease causing bacteria such as *E. coli* is the primary determinant in the establishment of diseases such as diarrhea in humans (Sanchez and Holmgren, 2005). The most prevalent pathotype of *E. coli*, Enterohemorrhagic *E. coli* (EHEC) is characterized by the presence of two Shiga-like toxins, *Stx1* and *Stx2* which increase the harshness of the disease caused by this pathogen. Other pathotypes such as Enterotoxigenic *E. coli* (ETEC) also mediate infection through the production of enterotoxins whilst Enteroinvasive *E. coli* (EIEC) cause infections by attaching to host cells. The principal symptom caused by all six pathotypes of *E. coli* infections involves the onset of diarrhea in the human host (Kothary and Babu, 2001). Diarrheal diseases caused by *E. coli* infections are further enhanced in immuno-compromised patients such as

those infected with HIV/AIDS, and are also involved in the establishment of haemolytic uremic syndrome.

The use of *E. coli* as a microbial indicator for water quality monitoring is a concept that has been widely accepted and used for many years, and the presence of *E. coli* in water indicates that it is unsuitable for consumption (Ahmed *et al*., 2005). *E. coli* is a common inhabitant of the intestinal microflora of both humans and other warm blooded animals. Due to its prevalence in the intestine and faeces of such animals, its presence in water is an indication of faecal contamination (Gleeson and Gray, 1997).

The majority of the identified strains of *E. coli* are harmless, however, over the past decade, a number of strains were identified to be pathogenic, and the cause of numerous gastro-intestinal infections (Umoh *et al*., 2006). *E. coli* O157:H7 has been identified as the pathogenic strain responsible for majority of severe cases of diarrhoea (Galane and le Roux, 2001). It has several characteristics which are uncommon amongst other strains of *E. coli*. These characteristics include: An inability to ferment sorbitol within a 24 hour period; inability to produce β-glucuronidase; contains the *eaeA* gene; cannot grow well or at all at temperatures ≥44.5 ºC; tolerant to acidic environments; increased resistance to streptomycin and tetracycline when compared to normal *E. coli* strains (Padhye and Doyle, 1991; Feng, 1995; Doyle *et al*., 1997).

The differentiation of a pathogenic strain from a harmless one is based on the virulence properties, mechanisms of pathogenicity, clinical syndromes as well as the serotyping of three antigens, namely the somatic ("O"), flagella ("H") and capsule ("K") antigens (Doyle

et al., 1997). All strains of *E. coli,* which are classified as pathogenic are further classified into 6 major virotypes. These virotypes, referred to as diarrheagenic *E. coli,* are are classified as enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC), with each group having a unique virulence factor profile (Doyle *et al*., 1997).

1.5.1 Enteropathogenic *E. coli*

Until the 1970s, serotyping was the only method used to distinguish EPEC strains from normal flora as no biochemical, microbiological and animal tests were available for differentiation at that time. Currently, the presence of specific virulence markers can be used to determine the presence of EPEC (Jafari *et al*., 2012). The mechanisms of pathogenesis of EPEC do not resemble other strains, since they are neither associated with heat labile enterotoxins, heat stable enterotoxins or invasiveness (Jafari *et al*., 2012). EPEC initiates attaching and effacing lesions in the host cell to which they adhere and subsequently invade. Classically, EPEC attaches to Hep-2 cells of the intestinal mucosa to form a pattern of attachment. Chromosomal virulence and EPEC adherence factors are necessary for the full expression of adherence, and the intimate attachment to these epithelial cells is enabled by intimin, an outer membrane protein encoded for by the *eaeA* gene. Affected cells respond by calcium influx, a redisposition of cytoskeletal elements and protein phosphorylation which lead to an increase in intestinal secretions (Wilshaw *et* al., 2000). Zoonotic reservoirs of EPEC have not been encountered therefore the only source of infection to date is the human gastrointestinal tract.

EPEC symptoms are similar to that of other diarrheagenic strains and include fever, vomiting and watery diarrhea. They are amongst the most important pathogens infecting children less than 2 years of age in the developing world who in most cases, contract the disease via a direct faecal-oral route (Mossel *et al*., 1995; Wilshaw *et al*., 2000; Aslani and Bouzari, 2012). Over the last several decades however, the significance of EPEC infections has declined which may be attributed to the promotion of breast feeding or the over-estimation of this organism to cause disease when only serotyping methods were used for detection (Aslani and Bouzari, 2012).

1.5.2 Enterotoxigenic *E. coli*

According to Jafari *et al*. (2012), ETEC is the most important but under recognized bacterial cause of diarrhea and cholera-like diseases in humans living in areas without proper sanitation and clean water. Strains of ETEC are known to adhere specifically to the microvilli of the small intestine epithelial cells and produce site specific enterotoxins, which are defined as extracellular proteins or peptides which affect intestinal epithelial cells (Nagy and Fekete, 1999). They cause watery diarrhea with the absence of blood, mucus or pus in humans globally, with symptoms of fever and vomiting also being reported (Kothary and Babu, 2001). ETEC strains capable of causing disease are characterised by the presence of either a large molecular weight heat-labile enterotoxin (LT) or by small molecular weight heat-stable peptide toxins (ST), which ultimately determine virulence (Sears and Kaper, 1996).

Individuals prone to ETEC infections are young children residing in tropical countries (Brussow *et al*., 1992). Children younger than 2 years of age were reported to be most symptomatic of ETEC infections when compared to infected adults in the area. In addition, ETEC is responsible for "travellers diarrhea", with approximately 20 – 60% of people travelling to low-income areas contracting the disease. An incidence rate of 60% was also observed by Hunter (2003). ETEC infections caused through human contact is rare and occurs most frequently through the interaction between people and contaminated food and water sources. Inadequate chlorination and contamination by *E. coli* in water distribution systems was identified as the cause of an ETEC outbreak amongst 175 Israeli military personnel and 54 civilians according to Huerta *et al*. (2000). ETEC has also been identified in the farming industry where it is known to cause disease in both cattle and young pigs (Jafari *et al*., 2012).

1.5.2.1 Pathogenesis of ETEC infections

According to Nagy and Fekete (1999), the pathogenesis of all ETEC infections involves two steps: intestinal colonisation and the elaboration of the respective enterotoxins. This is done through the adherence of either fimbriae or pili to the epithelial cells of the small intestine within the host. These pili, by which all ETEC strains are characterised, act as a ligand to bind the bacterial cell to carbohydrate receptors on the epithelial cell surface. Pili, together with species-specific fimbriae are termed colonization factor antigens (CFAs), and are often associated with attachment to the gastrointestinal tract. All CFAs are characterised based on their morphology and broadly grouped into three categories: rigid rods, bundle-forming flexible rods and thin flexible wiry structures. The arrangement and

morphology of CFAs within the bacterial cell plays a role in determining host specificity. After attachment to the epithelial cells, toxins are translocated through the cell via trans-Golgi vesicular transport. These toxins can be labile (LTs) or stable (STs) (Nagy and Fekete, 1995).

1.5.2.1.1 Labile Toxins

LTs are large oligomeric toxins that consist of two antigenic types namely LT-I and LT-II (Hunter, 2003). Both types are found in *E. coli* strains but only LT-I is known to be pathogenic to humans and animals. Antigenic type LT-II is not known to cause disease and is frequently found in animals and rarely in humans (Prescott *et al*., 2005). LT-I causes diarrhea by a chain of intracellular reactions by binding to specific gangliocides on the epithelial cells of the small intestine and activating membrane bound adenylate cyclase. This leads to an increase in cyclic adenosine monophosphate (cAMP), and ultimately, an increase in osmoregularity within the lumen of the host, causing water to be drawn into the gut. This, in conjunction with the excretion of chloride into the gut lumen and a decrease in the absorption of sodium by the host cells lead to watery diarrhea (Hunter, 2003).

1.5.2.1.2 Stable Toxins

STs are small monomeric toxins which contain multiple cysteine residues and disulphide bonds which are responsible for its's heat stable properties (Prescott *et al*., 2005). Similar to LTs, STs contain two antigenic types, namely STa and STb (Hunter, 2003). STa is an 18 amino acid peptide which functions by binding to an enzyme, guanylate cyclase C. Binding to this enzyme, which is present in the luminal membrane of enterocytes, results in the increase in intracellular cAMP. This then functions in a similar manner when

compared to LT mechanisms where it results in the increase of chloride excretion. STb, a larger amino-acid peptide functions differently, in which cAMP levels and chloride transport is not affected. This antigenic type results in the net increase of bicarbonate excretion (Hunter, 2003).

1.5.3 Enteroinvasive *E. coli*

EIEC was first identified as the causative agent of diarrheal disease in 1971, however evidence suggests it dates back as early as the second world war (Jafari *et al*., 2012). Symptoms of an infection resemble that of shigellosis caused by *Shigella spp*. Like *Shigella*, EIEC are biochemically anaerogenic, non-lactose fermenting and lysine decarboxylase negative (Doyle *et al*., 1997). They cause disease by invading the epithelial cells, multiplying intracellularly, and moving laterally to penetrate adjacent cells (Hunter, 2003). Their ability to invade cells is associated by the presence of a plasmid encoding numerous outer membrane proteins which are responsible for their invasive abilities (Doyle *et al*., 1997). This process is often the cause of inflammation and results in death of the host cells and non-bloody but watery diarrhea (Flowers *et al*., 1992).

1.5.4 Enterohaemorrhagic *E. coli*

Amongst the six characterized groups of diarrheagenic *E. coli*, EHEC strains are probably the most important emerging pathogen of the past decade, with recent outbreaks occurring in the United States of America and the United Kingdom. They are the causative agents for numerous diarrheal outbreaks globally, with patients often suffering from complications such as haemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HS), with HUS

being identified as the major cause of renal failure amongst children (Leelaporn *et al*., 2003; Khan and Naim, 2011). EHEC strains are subdivided into pathotypes with the most important being the Shiga-toxin producing *E*. *coli* (STEC). STEC refers to those strains of *E*. *coli* which produce one or more members of a class of cytotoxins referred to as shiga toxins (O' Brien *et al*., 1982). The most dominant serotype of STEC, *E*. *coli* O157:H7 is most frequently associated with human disease.

STEC is often found in the intestine of cattle, sheep and goats, which is considered the major reservoir of EHEC/STEC (Figure 1.1) (Raji *et al*., 2006). However, sporadic outbreaks have been reported to occur in chicken, pigs and horses (Beutin *et al*., 1993). Highly sensitive techniques involving enrichment procedures in broth culture followed by immune-magnetic separation and subsequent plating allow for strains of STEC to be detected in animal faeces (Chapman *et al*., 1994). The excretion of several serotypes of STEC in faeces is dependent upon the age and weaning of the animal, with the lowest rate occurring in calves prior to weaning (Mechie *et al*., 1997).

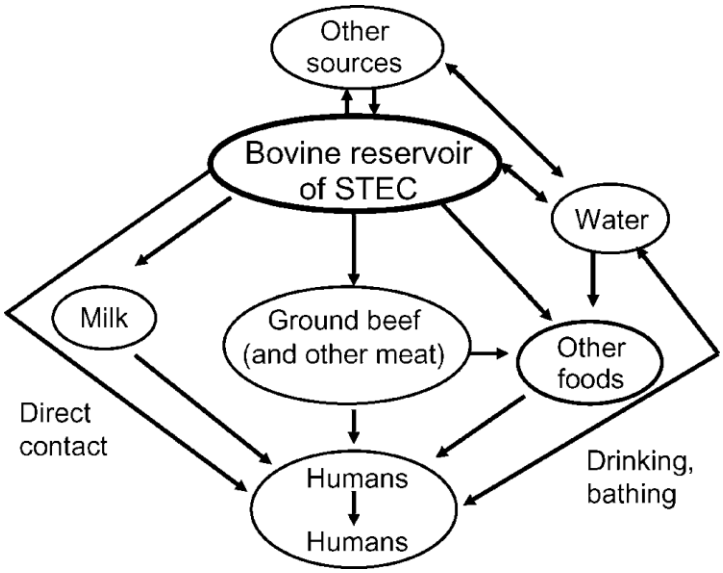


Figure 1.1: The central role of cattle in the transmission of STEC to humans (Gyles, 2006).

1.5.4.1 STEC as a causative agent for human disease

Over 500 serotypes of STEC have been isolated from infected individuals, but less than 10 of these belong to the O serotype responsible for the majority of fatal infections (Blanco *et al*., 2004). Human infection can be due to direct faecal-oral route from an infected animal or human, through contaminated water or most frequently through the consumption of inadequately cooked meat products, raw milk, and unpasteurised drinks (Figure 1.2) (Hunter, 2003). The infectious dose for *E. coli* O157:H7 is significantly low, requiring less than 50 to 100 microorganisms (Tilden *et al*., 1996). The renal system has been identified to be the most frequent target of STEC infections, but cases involving the lungs, pancreas, heart and central nervous system have been reported (Griffin and Tauxe, 1991). All infectious serotypes are characterised by the production of previously mentioned Shiga-like toxins which play a role in disease production.

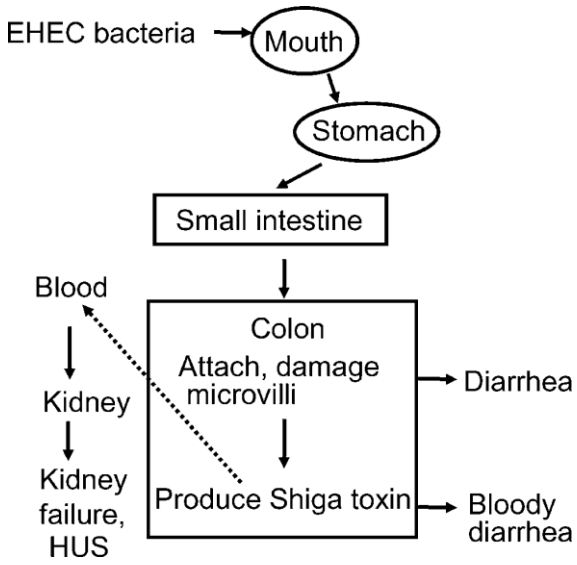


Figure 1.2: An overview of diseases in humans caused by EHEC/STEC (Gyles, 2006).

1.5.4.2 The production of Shiga toxins in EHEC

Shiga toxins are believed to be the principal operator of diseases caused by EHEC (Khan and Naim, 2011). They are prototypes of the Shiga-like toxin family and are produced by *Shigella dysenteriae* serotype 1 and are known to increase the harshness of the disease caused by this bacterium (Gyles, 2006). Shiga-like toxins are similar to Shiga toxins in structure and function but are produced largely by certain strains of *E. coli*. However, it has also been identified in strains of *Citrobacter freundii, Enterobacter cloacae*, *Aeromonas hydrophila* and *Aeromonas caviae* (Paton and Paton, 1998).

Immunocompromised individuals such as young children and the elderly are most susceptible to diseases caused by shiga-like toxin producing bacteria. These bacteria which are commonly found in food products, vegetables and milk are causative agents for HUS

which is characterised by symptoms such as haemolytic anaemia, renal failure and thrombocytopenia (Paton and Paton, 1998). These toxins bind to cells and are subsequently encytocysed. Thereafter, only the enzymatically active part of the molecule enters the host cell cytosol and inhibits protein synthesis, thereby killing the cell (Sandvig and van Deurs, 1996). Two broad types of toxins (stx) have been recognised, stx1 and stx2, with variants of these types existing as well (Zhang *et al*., 2002). Genes encoding these toxins in both *E. coli* and *S. dysenteriae* are usually phage-borne.

1.5.4.3 Types of Shiga toxins

Stx1 is a highly conserved molecule which is almost identical to the toxins produced by *S. dysenteriae* (Khan and Naim, 2011; Gyles, 2006). Two variants of Stx1 have been identified, Stx1c and Stx1d. Stx1c is found frequently in strains originating from sheep either alone or in conjunction with other variants, and rarely causes mild disease in humans (Friedrich *et al*., 2002). When compared to stx1, stx2 has more variants within its group, which include stx2c, stx2c2, stx2d, stx2e, stx2f and stx2g (Khan and Naim, 2011). Although stx2 shares less than 60% homology with the stx1 toxin, stx2 and its variants all differ significantly in their biological activity and its association with disease (Gyles, 2006). Pathogenic strains of STEC can either express one or both of the above-mentioned toxins. It has been reported that the development of critical infections does not entirely depend on the amount of toxin present but rather on the type. Within the stx2 antigenic type, stx2 and stx2c have been reported to be associated with elevated virulence and the ability to cause HUS, whereas other variants within this group cause a milder course of the

disease (Khan and Naim, 2011). Other variants of both stx1 and stx2 have been identified but reports on their clinical significance have not been established (Friedrich *et al*., 2002).

1.5.4.4 Structure of Shiga toxins

Shiga toxins are holotoxins which belong to a family of toxins named the AB protein toxins. Protein toxins are defined as toxins which have one enzymatically active part (A), and another part which binds to the host cell surface (B) (Figure 1.3) (Sandvig, 2001; Tesh and O'Brien, 1991and Fraser *et al*., 1994). The 32kDa A subunit, which is referred to as the functional domain, is non-covalently associated with the 7.7kDa B subunit also known as the recognition pentamer (Khan and Naim, 2011).

1.5.4.4.1 A subunit

The A subunit is a chain like structure composed of two smaller subunits called A1 and A2. These smaller subunits are linked by disulphide bonds and function differently within the A subunit. A2 is responsible for the attachment of the A subunit to the B subunit whereas A1 is an N-glycosidase which utilizes enzyme activity to cleave a specific adenine base from 28 S rRNA which would result in an inhibition of protein synthesis (Khan and Naim, 2011; Gyles, 2006).

1.5.4.4.2 B subunit

The B subunit is responsible for the binding of the Shiga toxin to mammalian cells by interacting with membrane glycolipids (Tesh and O'Brien, 1991). These neutral glycolipid globotriasoylceramide (Gb3) membranes serve as functional receptors for both stx1 and stx2 toxins. Although stx1 and stx2 have the same receptor, they differ in their binding

affinity to Gb3 with stx1 having the greater affinity (Khan and Naim, 2011). Entry of Gb3 into host cells is thought to be mediated by receptor-dependant endocytosis (Tesh and O'Brien, 1991).

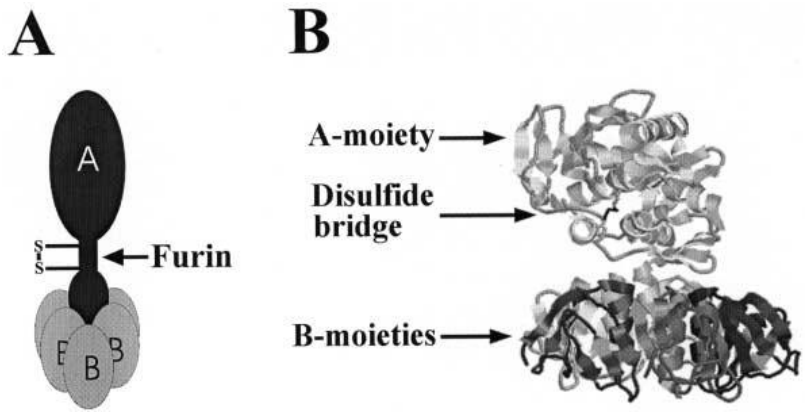


Figure 1.3: (A) A schematic and (B) crystallographic representation of a Shiga toxin (Fraser *et al*., 1994)

1.5.4.5 Entry of Shiga toxins into host cells

1.5.4.5.1 Binding

Shiga toxins bind selectively to cells containing a toxin binding site. The most common cell receptor for toxins is Gb3 where both the carbohydrate and the lipid tail components play an important role in the toxin-receptor interaction (Sandvig and van Deurs, 1996).

1.5.4.5.2 Endocytosis

After binding, the toxin-receptor complex is transported to a clathrin-coated domain in a process mediated by the toxin (Figure 1.4) (Sandvig, 2001). During the transportation process, a coated vesicle produced by the pinching of a fragment of the cell membrane harbours the toxin molecules on its internal surface. These vesicles then fuse with lysosomal vesicles, leading to the destruction of the protein toxin. This then creates a protective barrier for the cell. In cells which are susceptible to this mechanism, Stx is transported to the Golgi apparatus.

The A subunit then enters the cytosol where it is nicked by trypsin to form the two component subunits A1 and A2 as previously mentioned (Gyles, 2006). Reduction of the disulphide bond linking these subunits releases the active subunit A1, which by enzymatic reactions, inactivates the 60S ribosomal subunit of the host cells. Inactivation of this subunit occurs by an irreversible process involving the removal of a single adenine base from 28S rRNA within the subunit (Obrig, 2010). This leads to the inhibition of peptide chain elongation in protein synthesis and cell death.

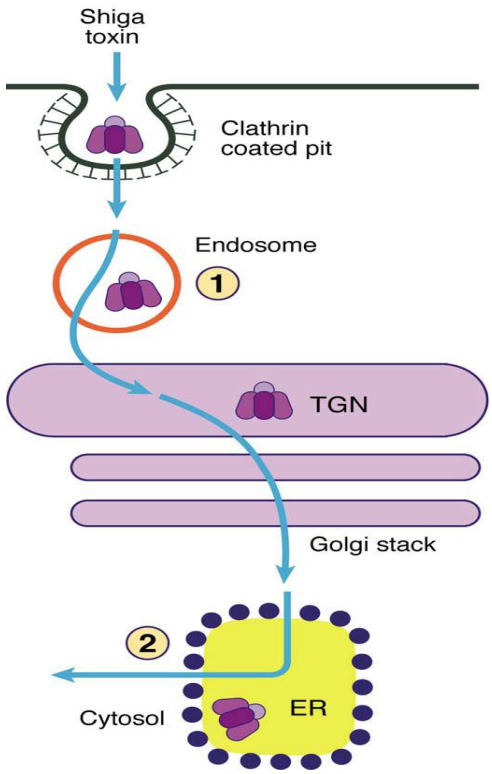


Figure 1.4: Receptor mediated uptake and transport of Shiga toxins from the membrane to the cytosol (Gyles, 2006)

In addition to this, Shiga toxins can exert detrimental effects on eukaryotic cells using two other mechanisms:

1. The generation of depurinated 28S rRNA introduces a signal transduction response which is quite unique. This response is known as a ribotoxic stress response (RSR) and leads to the activation of factors such as cytokines and chemokines which have the potential to cause cell apoptosis.
2. Binding of the toxin or the B- subunit to the receptor potentially initiates a cytoplasmic signal-transduction cascade different to the RSR.

Hence, the expression of Shiga toxins in eukaryotic cells exerts different responses which include death by apoptosis or necrosis and inflammation (Obrig, 2010).

1.5.5 Enteroadherant and Diffusely Adherant *E.coli*

As stated by Lior (1996), Cravioto *et al*. (1979) recognized that most EPEC adhered to HEp-2 cells in culture, and that presented adherence phenotypes could be used to differentiate EPEC strains. However, it was later observed that many non-pathogenic strains also had the ability to adhere to HEp-2 cells but the observed phenotype was different, in which the phenotype was diffuse when compared to the localized phenotype of EPEC strains. These diffusely adherent strains were later divided into two new sub-categories termed: (1) aggregative, and (2) true diffuse (Nataro *et al*., 1987). This later became two independent categories known as: Enteroaggregative *Escherichia coli* (EAEC) and Diffusely Adherant *Escherichia coli* (DAEC) (Vial *et al*., 1988).

1.5.5.1 EAEC

EAEC are currently defined as *E. coli* isolates, which lack the ability to secrete heat labile or heat stable toxins, and which adhere to HEp-2 cells in an aggregative pattern. In addition, pathogenesis is characterized by the ability to produce enterotoxins and cytotoxins and to induce inflammation of cells (Nataro *et al*., 1998) In many regions of the world, EAEC is recognized as the leading cause of sporadic diarrhea, with contaminated food suspected to be the leading source of infection (Cohen *et al*., 2005; Nataro *et al*., 2006). Most individuals infected with EAEC typically have watery, mucoid diarrhea with little to no fever however, reports by Nataro *et al*. (1998) state that up to one third of infected patients have bloody

stools, which is suspected to be strain dependent. In animal models, pathogenic and non-pathogenic EAEC strains adhere to the mucosal epithelium of piglets forming a mucous blanket. It is suspected that this "biofilm" may contribute to the ability to cause diarrhea and to persist in the human gut. Nataro *et al*. (1998) proposed a 3 stage model for pathogenesis of EAEC strains: Stage I: Adherence to intestinal mucosa, mediated by fimbriae; Stage II: The formation of pits in goblet cells leading to mucus hyper-secretion and the formation of a mucus-containing biofilm packed with EAEC; and Stage III: Elaboration of toxins or inflammation resulting in a damaged mucosa. Genes encoding for the aggregative phenotype and dispersin, an antiaggregatve protein used in adhesion are contained in a large plasmid termed pAA.

1.5.5.2 DAEC

Diarrheagenic strains of *E. coli,* which present a diffuse adherence pattern on cultured HEp-2 cells are termed DAEC (Servin, 2005). A majority (75%) of strains contain adhesions belonging to the Afa/Dr family, and since Germani *et al*. (1996) demonstrated that only those strains which were positive to the daaC probe (which recognized conserved regions in Afa/Dr adhesion operons) were found most frequently in infected patients, more attention has been placed on DAEC strains harboring Afa/Dr adhesions (Kaper *et al*., 2004). The Afr/Dr family of adhesions contain adhesions such as Dr, AfaE-I, AfaE-II, AfaEIII, which are implicated in DAEC as well as UPEC strains (le Bouguenec and Servin, 2006). Some of them recognize the decay-accelerating factor CD55 as receptors and upon binding, can promote the dismantling of the actin network in intestinal cells. However, certain studies have reported the isolation of Afr/Dr containing DAEC strains from healthy

children. Therefore, Scaletsky *et al*. (2002), states that the role of DAEC in diarrhea remains controversial, as "pathogenic" strains have been detected in children with and without diarrhea.

1.6 Diarrheal diseases

According to the WHO, approximately 88% of diarrheal diseases worldwide are caused by the consumption and use of unsafe water and improper sanitation and hygiene (WHO, 2002). Diarrheal diseases are responsible for approximately 2.5 million deaths every year. Most affected are children under the age of 5, those suffering from malnutrition, immuno-compromised individuals, the elderly, or people predisposed to other illnesses such as diabetes (Theron and Cloete, 2002). The susceptibility of these individuals to infection is further increased due to the constant use of antimicrobial drugs, which have led to the selection of ARB. Enteric pathogens such as bacteria, viruses and protozoa are responsible for the spread of water-borne diseases via the faecal oral route (Theron and Cloete, 2002). Studies by Theron and Cloete (2002) also stated that the survival of these pathogens depend on numerous factors such as; the infectious dose required to cause disease; the microbiological quality of the water such as the nutrient levels and water temperature; the survival of the microorganism in the water; seasonal changes; the presence or absence of water treatment practices as well as the physico-chemical quality of the water.

It is important to note that the definition of microbiologically safe water is blurred when attempting to assess the impact of water borne diseases on public health, and that water deemed safe for healthy individuals may have a severe impact on infants, immuno-compromised patients and the elderly (Hayes *et al*., 2003). Diarrheal diseases are most

prevalent amongst HIV infected individuals and are also recognised as a common symptom of water-borne diseases (Momba *et al*., 2002). Malabsorption caused by the diarrhea results in drastic weight loss. This, in addition to unhygienic conditions, poor water quality and the lack of antiretroviral drugs further enhance the effects of diarrheal diseases (Carcamo *et al*., 2005). Previous studies have determined that water-borne pathogens were most prevalent amongst HIV/AIDS infected individuals. It was then established that morbidity and mortality rates due to diarrheal disease are further compounded in countries with a high HIV/AIDS prevalence rate (Levine *et al*., 1991).

In a case study conducted by Momba *et al*. (2002), which aimed to determine the prevalence of *E. coli* O157:H7 in drinking water and its association with HIV/AIDS infected individuals in the Eastern Cape, South Africa, it was established that 25.56% in a total of 180 water samples were positive for *E. coli* O157:H7. Of the 360 tested stool samples, 36.39% were positive, and 56.50% of positively identified samples originated from a HIV/AIDS infected individuals suffering from diarrhea. A similar study carried out by Obi *et al*. (2007) aimed to establish the range and incidence rate of human gastro-intestinal disease causing microorganisms from the faeces of both diarrheic and non-diarrheic individuals who tested either HIV-positive or HIV-negative. In addition, drinking water sources were also tested for the range and incidence rate of a number of enteric microorganisms including *Salmonella spp*., *Campylobacter spp*., and *E. coli*. Results from this study show a link between the level of enteric pathogens detected in drinking water, and the rate of diarrheal diseases in HIV-positive individuals. Although the profile of enteric pathogens obtained from HIV-negative individuals were similar, the incidence rate

of these pathogens were higher in infected individuals. Such studies have demonstrated the link between the quality of drinking water and HIV. HIV/AIDS positive individuals were more susceptible to infections caused by water-borne pathogens when compared to their healthy counterparts (Lubeck *et al*., 1993). These pathogens which are responsible for majority of infections are also known to produce extended spectrum β-lactamases and as a result, most variants are resistant to cephalosporins, penicillin and monobactums.

1.7 Integrons

According to Ravi *et al*. (2014) and Gillings (2014), integrons are stable genetic platforms, which assist with the assembly and expression of exogenous mobile genes known as gene cassettes. Integrons were first characterized in the late 1980's, and it was then that it was regarded as the genetic system responsible for the gathering of resistance determinants (Martinez *et al*., 1988). The importance of these structures within clinical and agricultural settings is demonstrated by the continued surveillance of their prevalence and evolution. Integrons themselves are non-mobile but are found on mobile genetic elements (MGEs) such as plasmids and transposons, which act as vehicles for the transfer of integron encoded genes between species. Integrons are widely known for their role in the dissemination of antibiotic resistance genes (ARGs) mainly amongst gram-negative bacterial pathogens. A common promoter ensures the correct expression of gene cassettes, which almost always contain ARGs. The number of gene cassettes within an integron may vary, but the highest number of cassettes observed thus far is eight (Mazel, 2006). Through evaluation of the evolutionary history of chromosomal embedded integrons (chromosomal integrons), it was discovered that integrons have been in the bacterial genome for a long time and that these chromosomal elements are possibly the source of the backbone of integrons embedded

within MGEs i.e mobile integrons (Mazel, 2006).

1.7.1 Structure of integrons

The general structure of an integron (Figure 1.5) consists of 3 core elements whose combined function involved the capture and subsequent expression of genes located on gene cassettes: (1) An integrase gene (*intI*) which encodes an integron integrase (IntI), belonging to the tyrosine recombinase family. This gene is responsible for the integration of the gene cassette to the attachment site through recombination of incoming gene cassettes with the second core feature, an integron associated recombination site *att1* (Mazel, 2006 and Gillings, 2014). (2) One of two types of recombination sites which is dependent on location (a) *att1*: which is located on the integron and is the primary site if attachment of gene cassettss (b) attC: is found on the gene cassette (Fluit *et al*., 2004). According to Ravi *et al* (2014), these sites are essential in the recombination of gene cassettes. An intergron-associated promoter, which comprises of an outward promoter, is responsible for expression and directing transcription of the recombined gene cassettes as they lack the ability of independent expression (Gillings, 2014).

1.7.2 Structure of gene cassettes

According to Cambray *et al*. (2010), successive integration at the *attI* site results in the assembly of different gene cassette arrays. Gene cassettes are compact DNA elements, which have a simple basic structure and are intended to be mobilized by the integrase of the integron. They consists of a single open reading frame bound by the recombination site *attC*, which exhibits significant homology that allows for the production of stable secondary structures, which are important for recognition and recombination by IntI

(Cameron *et al*., 1986; Stokes *et al*., 1997). Recombination between *attI* and *attC* is the most common form of cassette insertion and ensures the integration of circular gene cassettes, which is a reversible process and allows for the excision of circular free DNA elements if needed (Gillings, 2014).

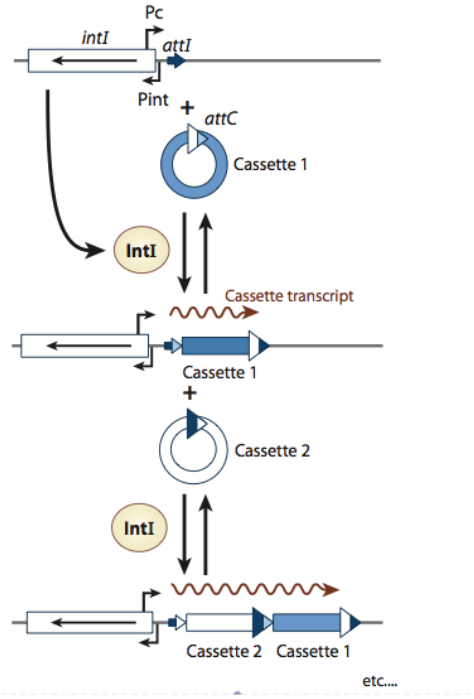


Figure 1.5: Structure of an integron: process by which circular gene cassettes are integrated at the *attI* site downstream of the Pc promoter (Cambray *et al*., 2010)

1.7.2.1 Expression, diversity and function of gene cassettes

As previously mentioned, gene cassettes rely on an external promoter for expression. Most work thus far has been based on the class 1 integron system, where cassette expression is driven by either the Pc1 or Pc2 promoters, located on the IntI gene and *attI* respectively (Levesque *et al*., 1994;Collis and Hall, 1995). Gene cassette expression is governed by the proximity to the promoter. Promoters found on the *IntI* gene drive expression in the associated array but the strength of the expression decreases as cassettes are located further away from the associated promoter (Collis and Hall, 1995;Coyne *et al*., 2010). Promoters responsible for cassette expression have also been identified in the *attI* region of class 2 integrons, and within the *intI* region of class 3 integrons (Levesque *et al*., 1994;Collis and Hall, 1995). It then appears that probably all integrons possess a promoter located within the *attI-intI* region. However, when there are hundreds of gene cassettes, it becomes impossible for a single promoter to drive the expression of all cassettes and it is believed that these cassettes are either silent or that they carry their own internal promoters.

As with integrons, gene cassettes are widely disseminated in environmental samples and are found in riverine sediment, springs, estuaries, biofilms, plant surfaces and a vast array of soils (Stokes *et al*., 2001; Holmes *et al*., 2003; Gillings, 2014). Homology and codon usage analysis has demonstrated that open reading frames within gene cassettes originated in diverse bacterial phyla, further demonstrating the diversity of the gene cassettes themselves (Mazel, 2006; Boucher *et al*., 2007; Koenig *et al*., 2009). Gene cassettes offer an enormous reservoir of originality. A combined analysis of meta-genomic and chromosomal cassettes revealed that approximately only 20% of cassettes have adequate homology to characterized proteins where their function can be predicted (Boucher *et al*.,

2007). However, 65% of cassettes and their encoded polypeptides have no known homologues in any DNA or protein database, further highlighting the novel nature of gene cassettes. This 20 % of gene cassettes are of environmental origin and whose functions include secondary metabolism, plasmid maintenance, virulence, and surface properties.

In addition, a review done by Gillings (2014), reveal that toxin-antitoxin systems are commonly found within or adjacent to integrons, and that the loss of these systems kill the cells which harbor them due to the longer half-life of the toxin as compared to the antitoxin which inactivates it. These systems are also found in integrons where their presence is believed to stabilize chromosomal arrays and maintain integron-bearing plasmids within cells (Szekeres *et al*., 2007; Yuan *et al*., 2011; Guerout *et al*., 2013). Functions associated with virulence and host relationships are also a common trait encoded by gene cassettes. These functions include but are not limited to: lipocalin (Barker and Manning, 1994), capsular polysaccharide (Smith and Siebeling, 2003), enterotoxin (Ogawa and Takeda, 1993) and lipases (Holmes *et al*., 2003). Other inferred functions are vast and span DNA modification; functions related to phage, polysaccharide biosynthesis, amino acid synthesis and efflux systems (Boucher *et al*., 2007; Gillings *et al*., 2009). Gene cassettes are therefore an essential component of bacterial adaptation and play a major role in the generation of adaptive diversity by integrons (Koenig, 2011).

Integrons are now regarded to be at the forefront of genomic innovation as it has two key advantages: (1) The integration of new genetic material into the existing bacterial genome does not disturb existing genes as it is integrated at a specific recombination site. (2) Newly integrated genes are instantly subjected to natural selection due to it being expressed via the Pc promoter. According to Gillings (2014), this natural selection process often occurs

within a population of integron-containing cells, each containing different gene cassettes, and that newly generated variants may confer genes having advantageous properties.

1.7.3 Evolution of integrons

Integrons are said to have evolved from similar structures termed "super-integrons", which unlike integrons, are always chromosomally located (Mazel, 2006). The first super-integrons were discovered in *Vibrio* spp. and although similar in structure, super-integrons can harbor more than 20 gene cassettes due to their larger size. In addition, these gene cassettes are not expressed by a common promoter, but rather through independent expression (Mazel *et al*., 1998). Integrons are defined by the presence of IntI. Integrases within the integron are defined by the presence of a unique 16 amino acid conserved motif, which is essential for activity (Messier and Roy, 2001). When evaluated, Boucher *et al*. (2007) and Cambray *et al*. (2010) determined that more than 15 % of genome sequenced bacteria contained an integron, when based on the presence of the IntI gene.

Integrons have been identified in a variety of natural environments such as forest soils, desert soils, hot springs and deep-sea sediment (Elsaied *et al*., 2007). Over the last decade, hundreds of different integron families have been identified and distinguished based on the relative homology of the *intI* gene. Based on their respective integrase genes, integrons have been divided into three broad groups (1) Clinically important class 1 and 3 integrons; integrons found in proteobacteria in freshwater and soil environments (2) integrons found on the STX integrative conjugative element and pRSV1 plasmid from *Vibrio*; class 2 integrons; gammaproteobacteria from marine environments (3) integrons whose integrase genes are in the reverse order from those belonging to groups one and two.

Gillings *et al*. (2005) and Boucher *et al*. (2011) state that the location of integrons has vital evolutionary and functional consequences as the presence of integrons on mobile elements such as plasmids and transposons allows for dissemination into new taxa, while it's presence on chromosomes allows for the generation of genomic complexity and phenotypic diversity. By comparing phylogenetic trees based on the 16S rRNA gene, it was demonstrated that lateral gene transfer of integrons can occur between bacterial species which reside in similar environments. Based on these comparisons, it can be deduced that the integron system dates back at least hundreds of millions of years and that over this time period, there has been a considerable amount of lateral gene transfer between different bacterial groups (Mazel, 2006;Boucher *et al*., 2007;).

1.7.4 Types of integrons

Integrons are located on MGEs (transposons), on chromosomes or on both. Mobile integrons (MIs), and chromosomal integrons (CIs) have distinct characteristics, which reflect their different evolutionary histories (Cambray *et al*., 2010).

1.7.4.1 Mobile integrons (MIs)

MIs associated with mobile DNA elements can be carried by conjugative plasmids (Cambray *et al*., 2010). These elements act as natural genetic vehicles for the transfer of genes between the same bacterial species and even between distantly related groups. The longest array of gene cassettes is 8 and was reported by Naas *et al*. (2001). Gene cassettes associated with MIs display functional homogeneity, as they are mostly involved in antibiotic resistance (Cambray *et al*., 2010). According to Fluit *et al*. (2004) and Partridge *et al*. (2009), more than 130 gene cassettes containing a variety of ARGs have been

identified. These genes encode for resistance towards numerous classes of antimicrobial agents such as aminoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampicin, erythromycin, quinolones, β-lactams and antiseptics belonging to the family of quaternary ammonium compounds (Mazel, 2006;Fluit *et al*., 2007;Partridge *et al*., 2009; Cambray *et al*., 2010).

1.7.4.1.1 Classification of integrons

to date, five classes of integrons have been discovered based on sequence similarity of encoded integrases (40 - 58% identity). Class I is the most widespread and clinically important and is therefore the most characterized. It is associated with both functional and non-functional transposons derived from Tn402 and is found in approximately 22 to 59% of gram-negative clinical isolates such as *Acinetobacer* spp., *E. coli* and *Salmonella* (Gallego and Towner, 2001; Cambray *et al*., 2010). Class II integrons are the second most common class and are often associated with the Tn7 family of transposons. It is commonly found in *Salmonella* and *Shigella* species (Gonzalez *et al*., 1998;Orman *et al*., 2002). The integrase gene found in class II integrons (*IntII*) generally contains a mutation at codon 179, which results in the production of a non-functional protein (Hansson *et al*., 2002). Class III integrons are highly similar to Classes I and II integrons but are less prevalent. They are related to the Tn402 transposon. Classes IV and V have only been associated with *Vibrio* species through their role in the development of trimethoprim resistance (Mazel, 2006). Gillings *et al*. (2008), Stokes *et al*. (2006) and Van Melderen *et al*. (2009) have reported the discovery of bacterial isolates belonging to classes I and III, whose integrons are not associated with resistance genes. These integrons harbored cassettes encoding genes of unknown functions. Such a discovery infers that MIs are not solely dedicated to

conferring antibiotic resistance but also play a role in mediating bacterial adaptation.

1.7.4.1.2 Class I integrons

Class I integrons have been named so as they were the first to be discovered. In addition, these intrgeons were able to move by lateral DNA transfer to a wide range of other bacteria and had the ability to accumulate ARGs (Gillings *et al*., 2014). Movement by lateral DNA transfer meant that this class of integrons had the following properties: they were located on the chromosome of Betaproteobacteria found as a part of the human food chain; ability to move between locations on the chromosome and to other bacterial species; it was carried by at least 0.002% of bacteria in unaffected soils (Gaze *et al*., 2011; Gillings *et al*., 2014); ability to acquire a wide range of gene cassettes (Biskri *et al*., 2005) and frequent association with *qac* genes which encode for versatility in efflux pumps (Gillings *et al*., 2009).

Upon inspection of metagenomic DNA of bacteria from environmental sources, it was revealed that diverse genes belonging to *intI* can be detected, however, when the metagenomic DNA of clinical isolates were examined, identical DNA sequences of *intI* were discovered. This suggests a common ancestor of class I integrons in clinical isolates which are possibly responsible for the spread of antibiotic resistance amongst gram-negative bacterial pathogens (Gillings *et al*., 2008b; Gillings *et al*., 2014). The origin of a class I integron can be best described as that an integron found in an environmental betaproteobacteria was captured by a transposon belonging to the Tn402 family.

The gene cassette found within this integron contained a resistance gene *qac* which infers resistance to disinfectants. A subsequent acquisition of a sulfonamide resistance gene, *sul1*

resulted in the deletion of the *qac* terminus. The property of Tn402 transposon to target *res* sites on plasmids then allowed for this Tn402-classI integron hybrid to be transposed into a wide variety of plasmids (Kholodi *et al*., 1995; Gillings *et al*., 2008a; Gillings *et al*., 2014; Gillings *et al*., 2014). Such insertion and deletion events have therefore resulted in the generation of extensive internal variations in cassette arrays, which has allowed for the production of over 130 different gene cassettes (Partridge *et al*., 2009).

A recent study conducted by Gillings *et al*. (2014) proposed that class I integron-integrase gene *intI* could serve as a generic marker for anthropogenic pollutants. This idea was supported by independent studies conducted by Gaze *et al*. (2011), Pruden *et al*. (2012) and Jechalke *et al*. (2013b) where correlations were observed between *intI* and associated genetic elements with various measures of human impact. Gillings *et al*. (2014) further lists four characteristics, which promote the use of *intI* as a generic marker as follows:

- *intI* is frequently linked to genes which confer resistance to disinfectants, heavy metals and a vast array of antibiotics (Liebert *et al*., 1999; Partridge *et al*., 2001).
- It has already infiltrated pathogenic and commensal bacteria of both humans and animals (Goldstein *et al*., 2001; Stokes and Gillings, 2011).
- Class I integrons reside in bacteria, which have rapid generation times and is often located on MGEs, which are frequently transferred between bacteria. This allows the abundance of *intI* to rapidly change in response to environmental stress.
- The common forms of *intI* are recently assembled under selection pressure imposed by human activity (Gillings *et al*., 2008a).

1.7.4.2 Chromosomal integrons (CIs)

Upon investigation of the *V*. *cholerae* genome in the late 1900s, a cluster of repeated DNA sequences were discovered. This was later revealed to be a distinct type of integron located on chromosome 2 of the *V*. *cholerae* serotype O1 biotype E1 Tor strain N16961 (Barker *et al*., 1994; Mazel *et al*., 1998). This integron was located on the chromosome only and possessed a distinct integrase which seemed to be related to integrases of MIs, but were not associated with any MGEs (Heidelberg *et al*., 2000). Research conducted in March 2010 on the genomes of chromosomal integrons revealed that approximately 17% of sequenced integrons possessed integron integrases.

Based on these results, phylogenetic relationships were established between integrases and allowed for the distinction of three major groups of CIs (Cambray *et al*., 2010): (1) The proteobacteria group of integrons obtained from soil and freshwater environments (2) The γ-proteobacteria group obtained from marine environments and; (3) The inverted integrase group in which the *attI* site is found at the 3′ end of the integrase.

Gene cassette arrays of CIs can contain up to 217 gene cassettes as observed in *Vibrio vulnificus*, however combinations of fewer cassettes and even no cassettes have been observed. CIs with large cassette arrays (>20) and a high degree of identity with *attC* sites (>80%) have been termed "superintegrons" as previously mentioned. Superintegrons have been identified in most *Vibrio* species genomes, in *Pseudomonas alcaligenes* and in numerous *Xanthomonas* species (Cambray *et al*., 2010; Coleman *et al*., 2004; Rowe-Magnus *et al*., 2001; Vaisvila *et al*., 2001).

1.7.5 Mobility of integrons

According to Guo *et al*. (2011), integrons move through bacterial species and transfer multi-drug resistance properties. SOS response systems in bacterial cells are responsible for controlling the expression of the integrase gene. This response then drives the regulation of gene cassettes within the integron. According to Hocquet *et al*. (2012), the expression of gene cassettes are higher if the cassette is closer to the *att1* site, and that the transfer of integrons through horizontal gene transfer is also likely to occur due to the close proximity of bacterial species. Ravi *et al*. (2014) states that the emergence of multi-drug resistance mainly due to the close proximity of certain bacteria with antimicrobial agents.

Studies by Guo *et al*. (2011) indicated that integrons had the ability to generate new linkages for antibiotic resistance in areas of high exposure to antimicrobial agents, which then further highlights the notion that antimicrobial selective pressure plays an important role in acquiring resistance genes.

1.8 Antibiotic resistance

The development of antibiotics and their use to treat bacterial infections can be regarded as one of the major achievements of the 20th century. Antibiotics have been known for hundreds of years and in some cases thousands of years – although not in pure form. Since the discovery of penicillin and streptomycin in the 1940s, millions of lives have been saved (Marti *et al*., 2013). Antibiotics are not only used to treat infections, they are also components of livestock feed and are indispensable in the medical field during organ transplantation, chemotherapy, surgical procedures and in the care of pre-mature babies

(Marti *et al*., 2013). They are composed of an extensive range of both structural and molecular families whose sole purpose is to inhibit bacterial growth when available at optimal concentrations (Gillings and Stokes, 2012). In addition to bacterial inhibition, antimicrobial agents may act as signaling compounds or regulatory molecules in the environment when produced at sub-inhibitory levels (Aminov, 2009; Martinez *et al*., 2009; Gillings and Stokes, 2012).

While antibiotics are currently obtained by chemical synthesis, the first antibiotics were of natural origin. Now, millions of metric tons of antibiotics are produced and distributed by humans each year (Gillings and Stokes, 2012). Antibiotics are largely used for human and veterinary purposes, however, a large portion of antibiotics are incompletely metabolized in the gut of humans and animals and are released into the environment unchanged, where they persist and may even become mobile (Sarmah *et al*., 2006;Le-Minh *et al*., 2010).

Majority of the antibiotics found in environmental settings today seems to originate from industry, which affects the role antibiotics play in natural ecosystems (Davies and Davies, 2010). Based on chemical structure and mode of action, all antimicrobial agents have been grouped into representative classes, whose targets and modes of action are represented in Table 1.1 (Davies and Davies, 2010).

Table 1.1: Classes of antimicrobial agents, targets and modes of antimicrobial resistance

Antibiotic class	Example(s)	Target	Mode (s) of bacterial resistance
β-Lactams	Penicillins, cephalosporins, penems, monobactams	Peptidoglycan biosynthesis	Hydrolosis, efflux, altered target
Aminoglycosides	Gentamicin streptomycin, spectinomycin	Translation	Phosphorylation, acetylation, nucleotidylation, efflux, altered target
Glycopeptides	Vancomycin, teicoplanin	Peptidoglycan biosynthesis	Reprogramming peptidoglycan biosynthesis
Tetracyclines	Minocycline, tigecycline	Translation	Monooxygenation, efflux, altered target
Macrolides	Erythromycin, azithromicin	Translation	Hydrolysis, glycosylation, phosphorylation, efflux, altered target
Lincosamides	Clindamycin	Translation	Nucleotidylation, efflux, altered target
Streptogramins	Synercid	Translation	C-O lyase, acetylation, efflux, altered target
Oxazolidinones	Linezoid	Translation	Efflux, altered target
Phenicols	Chloramphenicol	Translation	Acetylation, efflux, altered target
Quinolones	Ciprofloxacin	DNA replication	Acetylation, efflux, altered target
Pyrimidines	Trimethoprim	C_1 metabolism	Efflux, altered target
Sulfonamides	Sulfamethoxazole	C_1 metabolism	Efflux, altered target
Rifamycins	Rifampicin	Transcription	ADP-ribosylation, efflux, altered target
Lipopeptides	Daptomycin	Cell membrane	Altered target
Cationic peptides	Colistin	Cell membrane	Altered target, efflux

(Davies and Davies, 2010)

1.8.1 Antibiotic resistance: A global concern

The concept of antibiotic resistance has become a global concern as more disease causing organisms are becoming resistant to commonly prescribed antibiotics. The result of this is prolonged illness and in some cases, death (Marti *et al*., 2013). This problem is heightened by the fact that drug resistance is not only towards a single class of antibiotics, but to many classes which result in multi-drug resistant and extreme drug resistant bacteria. The concept of multi drug resistance is defined as having an acquired non-susceptibility to at least one agent in three or more antimicrobial categories, while extreme drug resistance is defined as the non-susceptibility to at least one agent in all but two or fewer antimicrobial categories.

The idea of antibiotic resistance was not previously recognized as a global issue due to a constant supply of novel antibiotics. However, the overuse of antimicrobial agents has lead to an increased survival pressure on relevant microorganisms and it is now an ever-increasing problem. This problem is further compounded by international travel, trade and immigration.

1.8.2 Origin of ARGs

Allen *et al*. (2009) and D'Costa *et al*. (2011) reported that the analysis of metagenomic DNA from soil and permafrost reveal ancient lineages of resistance genes which confer resistance to aminoglycosides, tetracyclines, glycopeptides, and β-lactams which pre-dates the antibiotic era. This concurs with reports by Gillings (2014) and Gillings and Stokes (2012) which state that antibiotics, resistance genes and their associated DNA vectors have

originated in natural environments from environmental organisms, and that humans have disturbed the dynamics of this system through the overuse of antimicrobial agents. The current database of resistance genes spans over 20 000 genes but it is considered to be only a fraction of the total genes contained in the environment (Liu and Pop, 2009). Therefore, history and current scientific discoveries show that natural environments contain a vast array of potential resistance genes, and that bacteria have the ability to acquire these genes through lateral gene transfer if the need arises.

1.8.3 Acquisition of bacterial resistance

Many bacterial species are inherently resistant to certain antibiotics due the possession of certain characteristics. According to Sayah *et al*. (2005), there exists four general mechanisms of resistance in bacterial populations and each of these mechanisms are controlled by the action of specific genes. These mechanisms include: the modification or inactivation of antimicrobial agents through the action of enzymes; impermeability of the bacterial cell wall or membrane; expulsion of the drug using cellular efflux pumps as well as the alteration of target receptors (Figure 1.6). These resistance mechanisms can be acquired through the uptake of transposons, plasmids and integrons into the cell (Prescott *et al*., 2005). Bacteria who do not have natural resistance properties acquire resistance genes through lateral gene transfer.

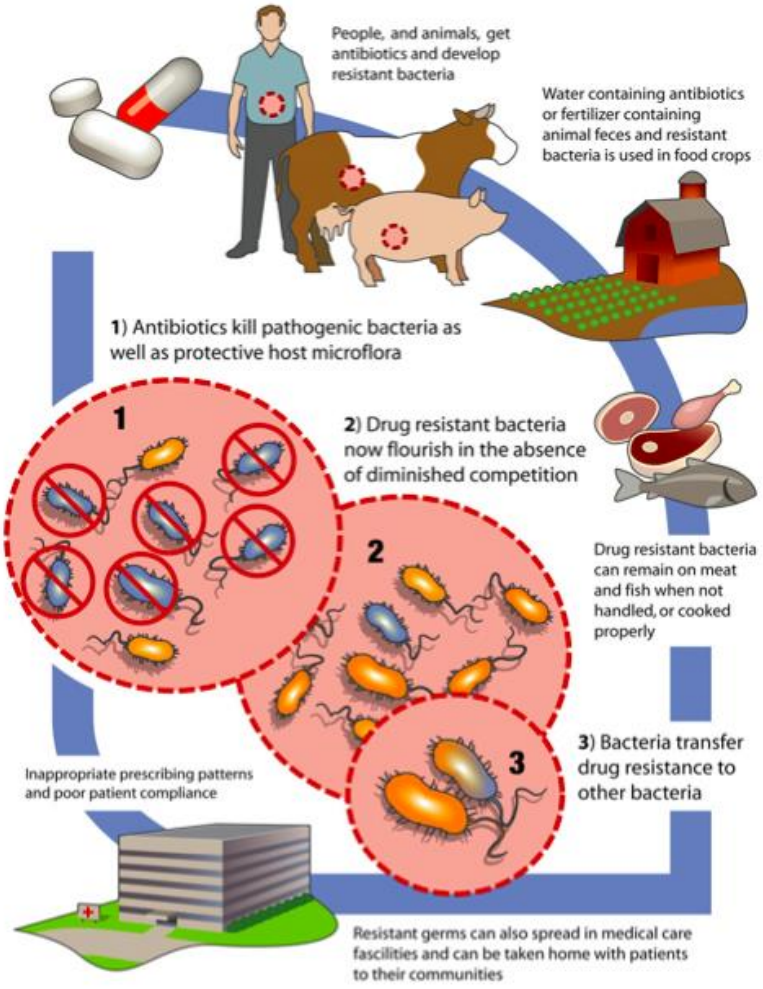


Figure 1.6: Behavioural and molecular mechanisms of resistance (Brooks and Brooks, 2014)

Transformation, transduction and conjugation are some examples of lateral gene transfer which bacteria employ (Stokes and Gillings, 2011). Transformation involves the acquisition of DNA encoding resistance determinants from the environment, while transduction is defined as the movement of DNA between cells through the use of bacteriophages. The process of conjugation is the most studied and widely described and involves the direct transfer of DNA between cells. During conjugation, ARGs found on

MGEs such as plasmids, transposons, integrons and genomic islands are transferred between bacteria (Davies and Davies, 2010; Stokes and Gillings, 2011; Gillings and Stokes, 2012).

1.8.4 The role of WWTPs in the spread of antibiotic resistance

Wastewater treatment plants can be regarded as the interface between human waste and the aquatic and soil environments. Effluent from hospitals, households, industries and animal husbandries are suspected to be the main anthropogenic sources of antibiotics, which converge at WWTPs and contribute to the final ecosystem of the plant. This ecosystem consists of organics, chemicals and microbiological waste (Stalder *et al*., 2012). WWTPs have been identified as hotspots for antibiotic resistance gene transfer because they combine several favorable factors such as a high bacterial cell density, a nutrient rich environment and a constant influx of antibiotics and ARB (Dröge *et* al., 2000). In addition, antibiotics which are potentially present in WWTPs, could select for antibiotic-resistant bacteria as seen in studies involving erythromycin (Louvet *et al*., 2010). According to Stalder *et al*. (2012), this co-existance of bacteria and antibiotics in WWTPs increases the frequency of genetic variations and the possible emergence of novel mechanisms of resistance. Human and animal commensal bacteria have been the main focus in the study of ARB in WWTPs due to their closeness with humans and the ability to isolate and identify them easily (Rizzo *et al*., 2013). Approximately $10^9 – 10^{12}$ colony forming units (CFU) of bacteria per day, per inhabitant are discharged by WWTPs in their final effluent and amongst this, at least $10^7 – 10^{10}$ could have acquired resistance, which highlights the

importance of WWTPs in the accumulation and spread of ARB in the environment (Novo and Manaia, 2010).

Mobile genetic elements such as plasmids carrying ARGs have repeatedly been detected and isolated from WWTPs but their direct involvement mediating ARG transfer in WWTPs has not yet been demonstrated due to technical difficulties experienced when studying bacterial donor/recipient relationships (Sørensen *et al*., 2004;Rizzo *et al*., 2013). To date, three approaches have been investigated to monitor the transfer of plasmids through conjugation namely: 1) Culture based approach involving the inoculation of an environmental sample with a known donor (genetic element of interest) and recipient bacterium with selectable characteristics. Transconjugants are then recovered and enumerated on selective media (Kolwalchuk *et al*., 2004); 2) Fluorescence based approach involving the use of genetically modified plasmid derivatives, which express a fluorescent protein once it leaves the donor cell. It is assumed that all transconjugants should appear fluorescent when viewed under a fluorescent microscope (Sørensen *et al*., 2005); and 3) the molecular-based approach which makes use of q-PCR to monitor the dissemination of a given plasmid in the vastness of microbial community DNA, based on the fact that conjugative transfer of the plasmid is a certain form of DNA replication, and that the plasmid to donor DNA ratio increases when the plasmid of interest disseminates into indigenous populations (Bonot and Merlin, 2010). Genes encoding resistance to all classes of antibiotics are rife in effluent of WWTPs and from the diversity of genes detected, it was concluded that every mechanism available in nature to resist antibiotics has the capability to survive the wastewater treatment process (Rizzo *et al*., 2013).

1.8.4.1 The effect of wastewater treatment on ARB removal

A generalized wastewater treatment process involves three steps namely: primary, secondary and tertiary treatment. Primary treatment involves the removal of solids by physical treatment, while secondary treatment utilizes biological and chemical processes to remove organic matter. During tertiary treatment, all components that were not removed by secondary treatment are eliminated. These additional processes include nutrient removal, the removal of toxic compounds, and the removal of additional organic and suspended solids. The wastewater treatment process also allows for the removal for a portion of antimicrobial agents from wastewater through the sorption to biosolids and degradation during treatment processes such as the secondary and tertiary processes, but not all antibiotics are completely removed (Giger *et al*., 2003;Batt *et al*., 2006). Wagner and Loy (2002), and Guardabassi *et al*. (2002) state that there is a significant reduction in bacterial number as well as a reduction in the total number of resistant bacteria during the wastewater treatment process. However, according to Goni-Urriza *et al*. (2000), the reduction in resistant bacterial populations is insufficient as wastewater and sometimes treated wastewater contain higher proportions of resistant bacterial populations in relation to the respective proportions contained in surface waters. A possible explanation for this is that several factors contribute to WWTPs being a favourable environment for the dissemination and proliferation of ARB, which in turn could transfer resistance genes to non-resistant strains (Davies, 2012).

Researchers have used cultivation and resistance testing, as well as the detection of resistance encoding genes in wastewater and sewage sludge to study the resistance of selected organisms against beta-lactams, quinolones, tetracycline, trimethoprim and other sulfonamides and have concluded that the presence of resistant bacteria and their respective resistance genes do not correspond to the activity and spectrum of antimicrobial compounds found in the environment (Kummerer, 2004; Schluter *et al*., 2007). Studies by Reinthaler *et al*. (2003) on sewage sludge and receiving surface waters of WWTPs in Austria revealed the presence of 767 resistant *E. coli* isolates. Further testing revealed that untreated sludge could serve as a source of resistant microorganisms, which could enter the environment through agricultural reprocessing and by the application of sludge as ground fertilizer.

1.9 Scope of the present study

The absence of strict guidelines for treated wastewater effluent, insufficient funding, infrastructure and skilled staff together with the infrequent monitoring of wastewater treatment plants in Durban, South Africa results in the discharge of improperly treated effluent into receiving surface waters, which is often the only source of water for many South Africans. As a result, potentially pathogenic bacteria such as *E. coli* are released into the environment. These isolates have been identified as causative agents for numerous diarrheal diseases and are fast becoming resistant to commonly prescribed drugs. In addition, these isolates are capable of transferring resistance determinants to non-resistant strains, which further exacerbates the problem. Most studies on water quality conducted in South Africa thus far have focused mainly on drinking water quality. Information regarding

the detection and characterization of *E. coli* isolates recovered from treated wastewater effluent and receiving river sources is scarce. This is especially worrisome considering that between 160 and 200 infants below the age of 5 die per day in South Africa due to diarrheal diseases. This project therefore aimed at evaluating the prevalence of *E. coli* in treated wastewater effluent and receiving rivers of two independent wastewater treatment plants in Durban, South Africa, as well as to establish possible correlations between the physico-chemical profiles of the water samples and the microbial loads. Additionally, the antibiotic resistance profiles and virulence determinants of the *E. coli* isolates were characterized. Isolates were screened for the presence of integrons and gene cassettes and the ability to transfer these resistance determinants to susceptible strains were evaluated. Random Amplified Polymorphism DNA (RAPD) analysis was employed to establish possible correlations between the phenotypic and genotypic attributes of the *E. coli* isolates.

1.9.1 Hypotheses

It is hypothesized that *E. coli* isolates recovered from treated wastewater effluent and receiving aquatic *milieu* are resistant to commonly used antimicrobial agents and/or harbour antibiotic resistance determinants. It is further hypothesized that most of these isolates have diverse phenotypic and genotypic antimicrobial resistance patterns and contain integrons which can be transferred to non-resistant strains.

1.9.2 Objectives

The following objectives were established:

1.9.2.1 To determine the prevalence of *E. coli* in the treated wastewater effluent and respective receiving surface waters of two independent wastewater treatment facilities.

1.9.2.2 To determine the physico-chemical parameters of the water samples and correlate them with levels of microbial contamination.

1.9.2.3 To characterize antimicrobial resistance phenotypes of recovered *E. coli* isolates.

1.9.2.4 To determine the virulence gene signatures of these isolates.

1.9.2.5 To determine the genetic diversity amongst the *E. coli* isolates.

1.9.3 Aims

The following aims were pursued:

1.9.3.1 To enumerate the presumptive *E. coli* population in the water sample using a membrane filtration technique and the appropriate selective media for a six month period.

1.9.3.2 To analyze various physico-chemical parameters such as Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), temperature, turbidity, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Electrical conductivity (EC) and resistivity in the collected water samples.

1.9.3.3 To confirm the identity of the *E. coli* isolates from water samples using biochemical tests and PCR amplification of the Malate dehydrogenase (*mdh*) gene.

1.9.3.4 To determine the relationship between *E. coli* populations and physico-chemical parameters using statistical analysis.

1.9.3.5 To determine the antibiotic resistance profiles of the confirmed bacterial isolates using the standard Kirby-Bauer disc diffusion assay.

1.9.3.6 To determine the presence of classes 1, 2 and 3 integrase genes.

1.9.3.7 To determine the presence of gene cassette arrays within integron positive isolates.

1.9.3.8 To determine the identity of gene cassettes via Restriction Fragment Length Polymorphism (RFLP) and sequence analysis.

1.9.3.9 To determine the location of identified integrons via PCR.

1.9.3.10 To determine if integrons are transferrable from resistant to non-resistant *E. coli* strains using conjugation experiments.

1.9.3.11 To obtain the DNA fingerprint profile of integron positive isolates using RAPD analysis.

1.9.3.12 To determine the presence of specific virulence genes.

CHAPTER TWO

PREVALENCE OF VIRULENT AND MULTIPLE-ANTIBIOTIC RESISTANT *Escherichia coli* IN TREATED EFFLUENT OF TWO WASTEWATER TREATMENT PLANTS AND RECEIVING AQUATIC *MILIEU* IN DURBAN, SOUTH AFRICA

Abstract

The poor operational status of some wastewater treatment plants often result in the discharge of inadequately treated effluent into receiving surface waters. This is of significant public health concern as there are many informal settlement dwellers that rely on these surface waters for their domestic use. This study investigated the treatment efficiency of two independent wastewater treatment plants (WWTPs) in Durban, South Africa and determined the impact of treated effluent discharge on the physico-chemical and microbial quality of the receiving water bodies over a 6-month sampling regime. Presumptive *E. coli* isolates were confirmed using biochemical tests and detection of the *mdh* gene via PCR. Six major virulence genes namely: *eae*, *hly*, *fliC*, *stx1*, *stx2* and *rfbE* were also detected via PCR while antibiotic resistance profiles of the isolates were determined using Kirby-Bauer disc diffusion assay. The physico-chemical parameters of the water samples ranged variously between 9 - 313.33 mg/L, 1.52 – 76.43 NTUs and 6.30 – 7.87 for COD, turbidity and pH respectively, while the *E. coli* counts ranged between 0 and 31.2 x 10^3 cfu/ml. Of the 200 confirmed *E. coli* isolates, the *hly* gene was detected in 28%, *fliC* in 20%, *stx2* in 17%, *eae* in 14%, with *stx1* and *rfbE* in only 4% of the isolates. Notable resistance was observed towards trimethoprim (97%), tetracycline (56%) and ampicillin (52.5%). These results further highlight the poor operational status of these WWTPs and emphasize the need for improved water quality monitoring and enforcement of stringent guidelines.

2.1 Introduction

The absence of stringent water quality guidelines coupled with the infrequent monitoring of wastewater treatment plants (WWTPs) and receiving surface waters are major contributors to unsafe water (WHO, 2002). Water is accepted to be a vital natural resource because of its basic roles in the environment, food production, hygiene and industry. According to the World Health Organisation (WHO), poor access to safe water and inadequate sanitation continues to be a danger to human health, especially in the African continent where more than 300 million people reside in water scarce environments (WHO, 2002). In addition, inefficient wastewater treatment processes, political upheaval as well as insufficient funding contribute significantly to people of developing and developed countries not having access to microbiologically safe water (WHO, 2002). South Africa is defined as a semi-arid country with high water stress (40-60%), as the average rainfall (450 mm per annum) is below the global average (Momba *et al*., 2002; Adewumi *et al*., 2010).

The South African population has undergone major demographic changes since the abolishment of apartheid in 1994, with an annual population growth rate of over 3.34%, however, the level of natural resources have remained unchanged. This has led to a scarcity of water, which not only affects food security, energy production and environmental integrity but also results in conflict between human and animal populations who share the same water resources (Kusiluka *et al*., 2005). The South African Water Act was established in 1956 and aimed to treat wastewater effluent to an acceptable standard prior to discharge into receiving water bodies. However, economic expansion and population growth and the need to meet certain water quality guidelines exerted pressure on water and sanitation

authorities in the country, and as a result many WWTPs did not function optimally (Morrison *et al*., 2001;Mema, 2002). Water resources are then contaminated largely by wastewater effluent, which is defined as a mixture of raw sewage, primary, secondary and tertiary effluents (Toze, 2004). Consequently, informal settlement dwellers who rely on untreated water sources (dams, rivers and springs) for drinking and domestic purposes are exposed to a plethora of potentially pathogenic microorganisms such as *Vibrio* spp., *E. coli* and emerging bacterial pathogens such as *Legionella* spp. which are the causative agents for a variety of gastro-intestinal infections and diarrheal diseases. The inadequate monitoring procedures together with the declining state of wastewater treatment infrastructure in South Africa has hugely contributed to the incidence of numerous water-borne illnesses as demonstrated by outbreaks of cholera, typhoid fever and diarrheal diseases occurring in the Mpumalanga, KwaZulu-Natal and Eastern Cape provinces respectively (Mema, 2002).

Durban, located in KwaZulu-Natal province, is situated on the east coast of South Africa, and is characterized by a number of rivers, valleys and associated catchments. The province is home to approximately one hundred and fifty wastewater treatment plants ranging from small to macro sized plants. The Durban Metropolitan Area produces over 1.8 million tons of solid and liquid waste annually, with approximately 455 000 tons of waste being discharged through marine pipelines (www.environment.gov.za/enviro-info/sote/citysoe/durban/durban.pdf). Following an audit carried out in 2009, majority of the WWTPs in the province failed to meet at least three or more of the respective discharge standards for wastewater as stipulated by the South African water quality guidelines

(DWAF, 1996). The detection of potentially pathogenic organisms in water is technically difficult and time consuming, therefore microbiological indicators have been used for decades as a means to monitor pollution of water sources (Grabow, 1996). No single microorganism meets the requirements of an ideal indicator organism however; *E. coli* is recognized as an indicator of fecal pollution due to its presence in the intestinal tract of humans and other warm-blooded animals.

Pathogenic *E. coli* has been associated with various diseases in humans, and at present, six groups of *E. coli* pathotypes have been identified (Doyle *et al*., 1997). Pathogenic *E. coli* strains are classified into 6 major virotypes: enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC), with each group having a unique virulence factor profile (Doyle *et al*., 1997). Of these, EHEC strains are probably the most important emerging pathogen of the past decade, with recent outbreaks occurring in the United States of America and the United Kingdom. They are the causative agents for numerous diarrheal outbreaks globally, with patients often suffering from complications such as haemolytic-uremic syndrome (HUS) and hemorrhagic colitis, with HUS being identified as the major cause of renal failure amongst children. Enterohemorrhagic *E. coli* strains are further subdivided into pathotypes with the most significant being the Shiga-toxin producing *E. coli* (STEC) strains, which produce one or more Shiga toxins (O' Brien *et al*., 1982). Reservoirs of STEC include the intestine of cattle, sheep and goats; however, sporadic outbreaks have been reported to occur in chicken, pigs and horses (Beutin *et al*., 1993).

Inadequately treated wastewater has been identified as a reservoir of antibiotic resistant bacteria (ARB), worldwide (Rizzo *et al*., 2013). The occurrence of these ARB affects the treatment of infections and promotes the dissemination of resistance amongst bacteria in aquatic environments. In addition to use in human medicine, antibiotics are widely used as a feed additive to prevent livestock diseases. It is poorly absorbed in the gut of animals and is excreted in large amounts, thus leading to the high load found in wastewater influent (Akiyama and Savin, 2010). Even at low concentrations, antibiotics may act as signaling compounds and select for ARB. Resistance to specific antibiotics can be acquired through the uptake of mobile genetic elements into the cell (Akiyama and Savin, 2010).

In addition to microbial analysis, the survival of microorganisms depends on the physico-chemical characteristics of the water, including: Biological Oxygen Demand (BOD); Chemical Oxygen Demand (COD); Total Suspended Solids (TSS); Total Dissolved Solids (TDS); pH; temperature; turbidity; salinity, electrical conductivity and resistivity. These parameters are known to impact on the efficiency of water treatment processes and could themselves be affected by drastic climatic changes (DWAF, 1996). As the clinical impacts of *E. coli* infections are considerable, monitoring of pathogenic strains in environmental reservoirs has become a serious concern, worldwide (Rebello and Regua-Mangia, 2014). Therefore, the current study investigated the efficiency of two independent WWTPs situated in Durban, South Africa over a six month sampling regime and the impacts of the treated effluent discharge from these WWTPs on the receiving surface waters. The correlation between the *E. coli* load and the associated physico-chemical parameters were

equally established. The antimicrobial resistance profiles and virulence gene signatures of positively identified *E. coli* isolates were also determined.

2.2 Materials and Methods

2.2.1 Description of study site

Two independent wastewater treatment plants were selected for this study; the Northern Wastewater Treatment Plant (NWWTP) and the New Germany Treatment Works (NGTWs) situated in the geographical coordinates of 29° 48' 45.62"S, 30°59' 45.22"E and 29°48' 21.68"S, 30°53' 50.44"E, respectively. The NWWTP has a treatment capacity of approximately 70 Ml/d and has been awarded a Greendrop score of 86% for the year 2011 by the eThekwini Municipality. Ninety two percent of influent consists of domestic wastewater whilst the remaining 8% is of industrial origin. The uMgeni River, into which treated effluent is discharged, has a catchment area of 4416 km^2 and is approximately 255 km long from source to mouth. The river receives tributaries from the Klaarkloof, Impolweni and the Umsunduzi Rivers before entering the Indian Ocean. The NGTWs is a smaller plant with a 7 Ml/d treatment capacity and has been awarded a Greendrop score of 87% for the year 2011. It processes approximately 15% industrial wastewater and 85% domestic sewage before discharging into the Aller River, which is a small but significant tributary on the lower uMgeni. It is estimated that close to 2 million people live within the catchment area of the uMgeni River, therefore these rivers are a source of water for majority of the population situated in the Pietermaritzburg and Durban area.

2.2.2 Collection of samples

Duplicate wastewater samples were collected monthly in 5 L plastic containers, over a six month period (March – August 2012) from four pre-determined points. These points are: before chlorination (BC), discharge point (DP), 500 m upstream from the discharge point (US), and 500 m downstream from the discharge point (DS). The containers were washed with de-ionized water and sterilized with 70% (v/v) alcohol prior to use. The alcohol was left in the container to maintain sterility and rinsed using the sample water at the point of collection. In areas of the river where there were no water current, samples were collected using a sampling stick by collecting water and pouring it into the container. If a current did exist, the mouth of the container was placed opposing the water current which permitted the flow of water into the container. The containers were then sealed, stored away from sunlight and transported on ice to the University of KwaZulu-Natal (Westville Campus) and stored at 4 ºC. Samples were analyzed within 48 h of collection.

2.2.3 Determination of physico-chemical parameters

All samples were inverted several times to re-suspend any sediment. The temperatures of the water samples were taken on site using a mercury-in-glass thermometer (Lloyds register quality company). Parameters that were tested in the laboratory include: pH using a Beckman 320 pH meter; turbidity using a 2100 P turbidimeter (HACH); BOD/5, resistivity, salinity, TDS and conductivity using a HQ 40d multi meter and parameter specific probes (HACH), COD using a Spectroquant NOVA 60 (Merck); as well as TSS using standard methods. Nitrate and phosphate analyses were conducted by Aquatico Scientific (Pty) Ltd. (Gauteng, South Africa) using standard methods (Clesceri *et al*., 1998)

2.2.4 Enumeration and presumptive identification of *E. coli*

Wastewater samples were serially diluted with sterile distilled water. Membrane filtration was carried out according to standard methods where 50 ml of the appropriate dilution was filtered through a 0.45 µm pore size cellulose nitrate filter (Pall, USA). Each filter was then placed on a 45 mm petri plate containing Chromocult Coliform Agar (Merck) and incubated at 37 °C for 24 h. Presumptive *E. coli* populations were enumerated and expressed as colony forming units per milliliter (cfu/ml). All presumptive isolates (18-24 h old) were then subjected to IMViC biochemical testing using *E. coli* HB101 as a positive control.

2.2.5 Molecular identification of *E. coli* isolates

Confirmation of the *E. coli* isolates were carried out via PCR amplification of the conserved *mdh* genes (Wose Kinge *et al*., 2012). DNA was isolated from the isolates using the boiling method and used as template in the PCR assay. The 25 µl PCR mixture assay contained 2.5 µl DNA template, 5 µl of 200 µM dNTP, 3 µl of 2.5 mM $MgCl_2$, 2.5 µl of 10 × reaction buffer, 1.25 U Taq DNA Polymerase, 9.25 µl RNAse free water and 1.25 µl each of 10 µM primer (Table 2.1). Amplification of the target gene was performed in a T100 Thermal Cycler (Bio-rad, USA) under the following conditions: Initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 20 sec, annealing at 60 °C for 30 sec and elongation at 72 °C for 30 sec. Amplified DNA was resolved by electrophoresis on a 1.5 % agarose gel (Seakem), and products visualized after staining of gel with ethidium bromide (5µg/ml) using the Chemigenius Bioimaging System (Syngiene). *E. coli* ATCC 25922 was used as a positive control.

2.2.6 Antimicrobial resistance profiling

All isolates confirmed via PCR were inoculated into nutrient broth and incubated at 37 ºC for 24 h. Cultures were then standardized to a 0.5 Mcfarland standard according to CLSI (2013) and swabbed onto Mueller-Hinton agar. Determination of the antibiotic resistance profile of each isolate was done using the Kirby-Bauer disc diffusion assay using the following antibiotics (μg per disc): Penicillins: ampicillin (10), amoxicillin (10); Cephems: cephalothin (30), cefazolin (30), ceftazidime (30); Tetracyclines: tetracycline (30), doxycycline (10); Phenicols: chloramphenicol (30); Aminoglycosides: amikacin (30), gentamicin (10); Quinolones: nalidixic acid (30); Fluoroquinolones: norfloxacin (10), ciprofloxacin (5); Monobactams: aztreonam (30); Fosfomycin: fosfomycin (200). Plates were incubated at 37 ºC for 24 h. The diameters (in millimeters) of the clear zones of growth inhibition around the antimicrobial disc, including the 10 mm disc diameter, were measured. The breakpoints used to categorize isolates as resistant, susceptible or intermediate to each antimicrobial agent were those recommended by the CLSI (2013). *E. coli* ATCC 25922 was used for quality control.

2.2.7 Virulence gene profiling

A multiplex PCR procedure was used to detect the presence of six virulence genes in all confirmed isolates (Bai *et al*., 2010). DNA was extracted according to manufacturer's instructions using a ZR Fungal/Bacterial DNA MiniPrep™ kit (Zymo Research). The 25 µl PCR assay mixture contained 1 µl of each primer (Table 2.1), 0.25 µl of Taq DNA Polymerase, 1.5 µl of 1 mM dNTP, 7.5 µl $MgCl_2$, 2.5 µl of 10 × reaction buffer, 1 µl of DNA and 1.25 µl of RNAse free water. *E. coli* O157:H7 (ATCC 35150) was used as a positive control. Amplification of target genes was performed in a T100 Thermal Cycler (Biorad, USA) under the following conditions: Initial denaturation at 94 ºC for 5 min, 25 cycles of denaturation at 94 ºC for 30 sec, annealing at 65 ºC for 30 sec and elongation at 68 ºC for 75 sec. A final elongation step was done at 68 ºC for 7 min. Amplified DNA was resolved by electrophoresis on a 1.2 % agarose gel (Seakem), and products visualized after staining of gel with ethidium bromide (0.5 µg/ml) using the Chemigenius Bioimaging System (Syngiene) (Bai *et al*., 2010). The prevalence of virulence genes were expressed as a percentage (n=200).

2.2.8 Statistical analysis

The co-efficient of correlation between *E. coli* populations and the physicochemical parameters were calculated by the Pearson correlation test. Statistical significance was set at P values of <0.05 or <0.01.

Table 2.1: Primers used in this study (;)

Target Gene	Sequence(5'→3')	Expected product size (bp)	Reference
mdh-F	CGTTCTGTTCAAATGGCCTCAGG	392	(Wose Kinge *et al*., 2012)
mdh-R	ACTGAAAGGCAAACAGCCAAG		
fliC-F	AGC TGC AAC GGT AAG TGA TTT	949	(Bai *et al*., 2010)
fliC-R	GGC AGC AAG CGG GTT GGT C		
stx1-F	TGT CGC ATA GTG GAA CCT CA	655	
stx1-R	TGC GCA CTG AGA AGA AGA GA		
stx2-F	CCA TGA CAA CGG ACA GCA GTT	477	
stx2-R	TGT CGC CAG TTA TCT GAC ATT C		
eae-F	CAT TAT GGA ACG GCA GAG GT	375	
eae-R	ACG GAT ATC GAA GCC ATT TG		
rfbE-F	CAG GTG AAG GTG GAA TGG TTG TC	296	
rfbE-R	TTA GAA TTG AGA CCA TCC AAT AAG		
hly-F	GCG AGC TAA GCA GCT TGA AT	199	

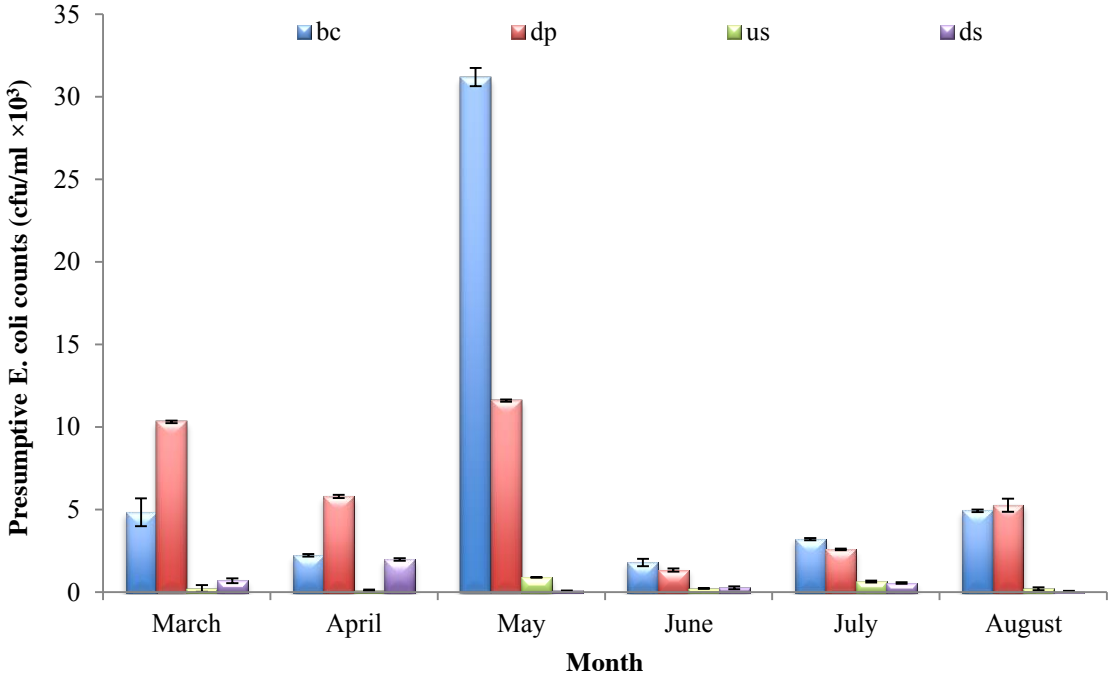
2.3 Results

2.3.1 Enumeration and identification of *E. coli*

Figures 2.1 shows the presumptive *E. coli* populations obtained from treated wastewater effluent and receiving aquatic milieu over a six month period. All values obtained for final effluent at both the NWWTP and NGTWs exceeded the acceptable limit of 0/100 cfu/ml for *E. coli* in water used for domestic purposes (DWAF, 1996). The *E. coli* population obtained at the DP of NWWTP during March, April and August increased by 53.10%, 61.55% and 6.45%, respectively when compared to values at BC (Figure 1a). However, in May, June and July, a decrease in the *E. coli* populations was observed at the DP when

compared to BC, with treatment efficiency rates being 62.82%, 25% and 19.25%, respectively. The highest *E*. *coli* population was obtained at BC in May at both WWTPs. *E*. *coli* was not detected at the DS points for March and April, and at the DP in August at the NGTWs (Figure 1b). Treatment efficiency rates of 98.78%, 95.55%, 8.33%, 99.97% and 93.75% for the month of March, May, June, July and August, were obtained at the NGTWs respectively.

(a)



(b)

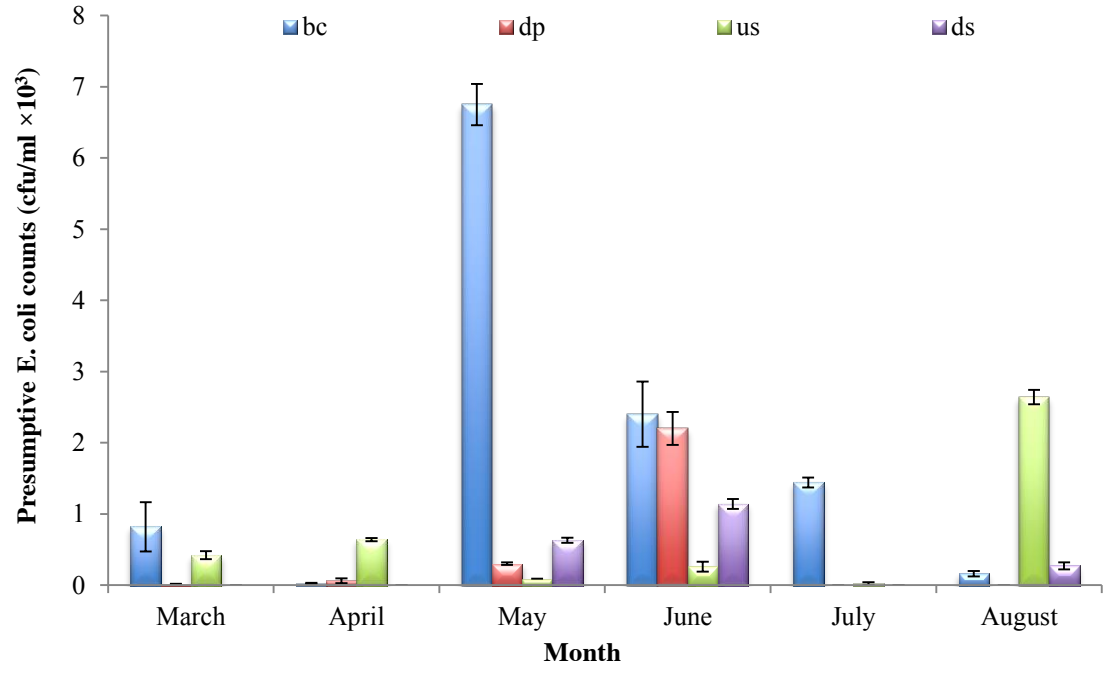


Figure 2.1: Presumptive *E. coli* population obtained at (a) Northern Wastewater Treatment Plant and (b) New Germany Treatment works and their receiving rivers

2.3.2 Physico-chemical parameters

The profiles of the physico-chemical variables of the water samples obtained at the NWWTP and NGTWs are depicted in Tables 2.2 and 2.3, respectively. The temperatures (°C) of the water samples at NWWTP ranged from 13.50 – 26.00 for BC; 12.67 – 25.00 for DP, 13.00 – 26.00 for US and 12.00 – 25.5 for DS while the BOD values (mg/L) obtained ranged between 1.40 – 4.01 for BC; 2.23 – 5.13 for DP; 4.52 – 8.49 for US and 5.06 – 7.34 for DS over the six month period (Table 2.2). COD concentrations (mg/L) ranged between 90.44 – 310.11 for BC; 9 – 291.22 for DP, and 51.00 – 311.72 and 89.39 – 311.72 for US and DS sampling points of uMgeni River, respectively. Conductivity levels ranged between 606.00 – 2115.67 µS/cm across all points during the sampling period, while TDS concentrations ranged between 342.67 – 418.00 mg/L for BC; 368.33 – 475.00 mg/L for DP; 370.00 – 1067.00 mg/L for US and 294.67 – 348.67 mg/L for DS. TSS values obtained at the NWWTP ranged between 0.013 – 0.096 g/L for BC; 0.016 – 0.066 g/L for DP; 0.010 – 1.244 g/L for US and 0.010 – 5.925 g/L for DS. The pH values obtained did not vary significantly between sampling points with values ranging between 6.85 –7.76 across all points. The highest turbidity (71.02 ± 6.25) was recorded at the discharge point in the month of April while nitrate levels (mg/L) (Table 2.4) obtained in the study ranged between <0.057 – 5.980 for BC; <0.057 – 3.920 for DP; 0.785 – 2.400 for US and 0.318 – 1.780 for DS.

The temperature profiles observed at NGTWs were lower when compared to NWWTP with ranges of 13.50 – 26.00 while BOD values (mg/L) for the NGTWs ranged between 2.24 – 4.18 for BC; 3.12 – 4.49 for DP; 7.79 – 11.04 and 4.97 – 9.27 for US and DS,

respectively (Table 2.3). The COD concentrations (mg/L) observed were considerably higher across all sampling points when compared to the NWWTP with ranges of 154.33 – 312.78 for BC; 124.17 – 309.00 for DP; 26.33 – 309.39 for US and 72.28 – 313.61 at the DS point. Conductivity (µS/cm) fell within the ranges 878.33 – 1148.33 for BC; 884.00 – 1253.67 for DP; 321.67 – 706.66 for US and 615.00 – 939.67 for the DS points. Salinity (%) ranged between 0.39 – 0.57 for BC; 0.43 – 0.62 for DP; and 0.15 – 0.34 and 0.30 – 0.4 for the US and DS points respectively, while TDS (mg/L) fell within the ranges of 386.67 – 567.33 for BC; 433.67 – 621.33 for DP; 154.30 – 344.67 for US and 299.00 – 462.00 for the DS sampling point. TSS and pH values obtained were similar for both plants. Turbidity values were observed to be lower for majority of the study period across all sampling points with values ranging 1.34 – 20.78 NTU for BC; 1.44 – 24.58 NTU for DP; and between 3.18 – 8.75 NTU and 7.32 – 19.76 NTU for the US and DS points respectively at the NGWWTWs. The highest values were recorded in August for both plants across all points.

Nitrate levels ranged between <0.057 – 5.980 mg/L for BC; <0.057 – 3.920 mg/L for DP; 0.785 – 2.400 mg/L for US and 0.318 – 1.780 mg/L for DS, while the phosphate concentrations ranged between 1.829 – 12.377 mg/L for BC; 0.751 – 9.021 mg/L for DP; 0.025 – 2.173 mg/L for US and 0.381 – 1.439 mg/L for the DS point at the NWWTP (Table 2.4). Analysis of water samples at the NGWWTWs yielded nitrate levels between <0.057 – 2.809 mg/L for BC; <0.057 – 2.351 mg/L for DP; 0.800 – 2.380 mg/L for US and 0.514 – 2.290 mg/L for DS. Finally, the phosphate concentrations ranged between 0.025 – 1.091 mg/L for BC; <0.025 – 1.467 mg/L for DP; <0.025 – 1.894 mg/L for US and <0.025 – 0.591 mg/L for the DS point.

The correlation matrices of the different physico-chemical parameters measured for the NWWTP and NGTWs is presented in Table 2.5 and 2.6, respectively. COD and pH had no significant correlations with other variables in both treatment plants. Significant positive correlations were observed between and amongst conductivity (0.999 at $p<0.05$) and TDS (1.000 at $p<0.05$) with salinity for the NWWTP. *E. coli* negatively correlated with BOD (-0.719 at $p<0.05$), whilst resistivity had significant positive correlations (0.627, 0.625 and 0.623 at $p<0.05$) with salinity, conductivity and TDS, respectively. At the NGTWs, a significant positive correlation was observed between TDS (0.780 and 0.999 at $p<0.05$) and salinity and conductivity, respectively. BOD (0.661 at $p<0.05$) correlates positively with salinity and negatively (-0.529 at $p<0.05$) with temperature. Negative correlations were also be observed between TSS (-0.657 and -0.554 at $p<0.05$) and salinity and BOD, respectively. Turbidity negatively correlated with temperature (-0.409 at $p<0.01$) whilst resistivity (0.439 at $p<0.01$) and conductivity (0.755 at $p<0.05$) had positive correlations with salinity.

2.3.3 Antimicrobial resistance and virulence gene signatures of confirmed *E. coli* isolates

The antibiogram of 200 *E. coli* isolates confirmed via PCR detection of *mdh* gene (Figure 2.2) is depicted in Figure 2.3. The highest resistance was observed against tetracycline (56%), and this was followed by ampicillin (52.5%), amoxicillin (40%) and doxycycline (31%). Furthermore, 27%, 21.5%, 20.5%, 18% and 16.5% of the isolates were resistant towards nalidixic acid, ciprofloxacin, norfloxacin, chloramphenicol and cephalothin, respectively, while 3.5% of the isolates exhibited resistance to gentamicin and fosfomycin,

and 7.5% of the isolates were resistant to ceftazidime. All isolates tested were susceptible to amikacin and cefazolin. The presence of virulence genes is depicted in Figure 2.4 while the virulence gene distribution among the *E. coli* isolates is depicted in Figure 2.5. The most prevalent gene was the *hly* gene, which was detected in 28% of the isolates. This was followed by the *fliC* gene (20%), *stx2* (17%), and *eae* (14%). The *rfbE* and stx1genes were found in only 4% of the isolates, while 13% of tested isolates did not harbor any of the tested virulence genes.

Table 2.2: Physico-chemical parameters of water samples from the Northern Wastewater Treatment Plant and receiving surface water bodies

Month	Sampling Point	Temp (°C)	Sal (%)	Cond (µs/cm)	Resist (Ω/m)	TDS (mg/L)	Turbidity (NTU)	pH	COD (mg/L)	BOD (mg/L)	TSS (mg/L)
MARCH	bc	26 ± 0.00	0.41 ± 0.00	839.67 ± 0.58	1191.00 ± 1.00	411.33 ± 0.58	7.91 ± 0.33	7.11 ± 0.05	104.77 ± 0.00	2.23 ± 0.36	0.010 ± 0.00
	dp	25 ± 0.00	0.44 ± 0.00	888.00 ± 1.00	1126.00 ± 1.00	436 ± 0.00	23.4 ± 12.13	7.36 ± 0.07	9.00 ± 0.00	5.13 ± 0.18	0.030 ± 0.02
	us	26 ± 0.00	0.37 ± 0.00	757.67 ± 2.52	1320.33 ± 4.51	370 ± 1.00	16.67 ± 0.38	7.25 ± 0.09	161.33 ± 0.00	5.62 ± 1.01	1.240 ± 4.29
	ds	25.5 ± 0.00	0.35 ± 0.00	714.67 ± 2.08	1400.00 ± 4.36	348.67 ± 1.15	15.27 ± 0.12	7.24 ± 0.06	191.94 ± 0.58	5.62 ± 0.24	1.130 ± 1.02
APRIL	bc	22 ± 0.00	0.38 ± 0.00	783.33 ± 2.08	1276.67 ± 2.89	383 ± 1.00	53.98 ± 2.94	7.38 ± 0.33	127.88 ± 0.01	3.30 ± 0.97	0.096 ± 0.01
	dp	22 ± 0.00	0.44 ± 0.01	903.67 ± 2.08	1106.67 ± 2.31	444 ± 1.00	71.02 ± 6.25	7.22 ± 0.21	152.88 ± 0.01	3.44 ± 0.67	0.066 ± 0.02
	us	21 ± 0.00	0.49 ± 0.00	997.67 ± 2.89	1004.00 ± 0.00	491 ± 1.73	18.35 ± 1.56	7.29 ± 0.17	310.11 ± 1.02	8.49 ± 0.47	0.015 ± 0.00
	ds	21 ± 0.00	0.33 ± 0.00	676.33 ± 0.58	1478.67 ± 2.31	329.33 ± 0.58	14.6 ± 0.23	7.4 ± 0.28	303.00 ± 0.00	6.33 ± 0.21	0.012 ± 0.00
MAY	bc	21.93 ± 0.12	0.42 ± 0.00	853.00 ± 0.00	1173.00 ± 0.00	418 ± 0.00	20.65 ± 1.21	7.02 ± 1.21	54.89 ± 0.00	1.41 ± 0.41	0.035 ± 0.00
	dp	21 ± 0.00	0.48 ± 0.00	966.00 ± 0.00	1036.00 ± 1.00	475 ± 0.00	14.22 ± 0.17	7.06 ± 0.17	9.00 ± 0.00	4.31 ± 0.26	0.016 ± 0.00
	us	22 ± 0.00	1.08 ± 0.01	2115.67 ± 0.58	473.00 ± 0.00	1067 ± 3.46	13.58 ± 0.9	6.87 ± 0.9	35.39 ± 18.02	9.61 ± 0.22	0.013 ± 0.00
	ds	22 ± 0.00	0.30 ± 0.00	621.00 ± 2.65	1612.33 ± 3.51	302 ± 1.00	13.58 ± 0.79	6.85 ± 0.79	306.72 ± 2.75	7.34 ± 0.47	5.925 ± 9.12
JUNE	bc	13.5 ± 0.00	0.34 ± 0.00	703.00 ± 1.00	1423.33 ± 2.52	342.67 ± 0.58	7.3 ± 0.08	7.3 ± 0.08	113.33 ± 0.00	4.01 ± 0.41	0.017± 0.00
	dp	12.67 ± 0.29	0.37 ± 0.00	757.33 ± 2.08	1320.33 ± 3.21	368.33 ± 2.08	7.29 ± 0.06	7.29 ± 0.06	300.00 ± 0.00	4.31 ± 0.26	0.020± 0.00
	us	13 ± 0.00	0.54 ± 0.00	1082.33 ± 2.08	924.00 ± 1.73	534.33 ± 1.15	7.59 ± 0.06	7.59 ± 0.06	113.83 ± 4.42	9.61 ± 0.22	0.006 ± 0.00
	ds	12 ± 0.00	0.31 ± 0.00	633.33 ± 0.58	1580.00 ± 1.73	308 ± 0.00	7.8 ± 0.05	7.8 ± 0.05	89.38 ± 0.71	7.34 ± 0.47	0.011 ± 0.00
JULY	bc	14.8 ± 0.00	0.34 ± 0.01	724.67 ± 6.51	1370.67 ± 7.37	348.33 ± 7.37	6.61 ± 3.03	7.2 ± 0.32	116.55 ± 2.05	2.13 ± 0.5	0.030 ± 0.00
	dp	15.3 ± 0.17	0.42 ± 0.00	849.67 ± 1.15	1177.00 ± 7.37	416.33 ± 0.58	6.61 ± 1.94	7 ± 0.81	291.22 ± 0.60	3.10 ± 0.03	0.028 ± 0.00
	us	14.93 ± 0.12	0.72 ± 0.00	1428.67 ± 4.16	700.00 ± 1.73	710 ± 4.36	7.29 ± 3.03	6.96 ± 0.67	311.72 ± 0.80	7.80 ± 2.36	0.007 ± 0.00
	ds	15.83 ± 0.29	0.29 ± 0.00	606.00 ± 1.73	1650.33 ± 4.04	294.67 ± 0.58	10.89 ± 6.94	6.93 ± 1.08	311.72 ± 0.80	6.75 ± 2.37	0.016 ± 0.00
AUGUST	bc	21 ± 0.00	0.37 ± 0.00	754.67 ± 3.79	1319.33 ± 6.81	370.33 ± 2.08	56.37 ± 0.35	6.85 ± 0.11	310.11 ± 0.00	1.54 ± 0.3	0.037 ± 0.11
	dp	19 ± 0.00	0.41 ± 0.00	840.00 ± 1.00	1190.33 ± 1.53	411.67 ± 0.58	68.53 ± 0.57	7.09 ± 0.04	182.78 ± 0.00	2.23 ± 0.28	0.048± 0.00
	us	20 ± 0.00	0.44 ± 0.00	899.00 ± 3.61	1112.33 ± 4.73	441.33 ± 1.53	28.73 ± 0.06	7.12 ± 0.03	105.89 ± 0.00	7.8 ± 0.21	0.023 ± 0.00
	ds	19 ± 0.00	0.33 ± 0.00	676.00 ± 3.00	1479.00 ± 7.55	329.67 ± 1.53	20.77 ± 0.06	7.26 ± 0.02	309.56 ± 0.00	5.74 ± 0.5	0.015 ± 0.00

BC: Before chlorination; DP: Discharge point; US: Upstream; DS: Downstream; Temp: Temperature; Salinity; Cond: Conductivity; Resist: Resistivity; TDS: Total dissolved solids; COD: Chemical oxygen demand; BOD: Biological oxygen demand; TSS: Total suspended solids

Table 2.3: Physico-chemical parameters of water samples from the New Germany Treatment Works and receiving surface water bodies

Month	Sampling Point	Temp (°C)	Sal (%)	Cond (µs/cm)	Resist (Ω/m)	TDS (mg/L)	Turbidity (NTU)	pH	COD (mg/L)	BOD (mg/L)	TSS (mg/L)
MARCH	bc	25.67 ± 0.29	0.44 ± 0.01	878.33 ± 21.22	1125.67 ± 5.51	436 ± 2.00	6.65 ± 0.00	7.12 ± 0.00	154.33 ± 0.38	2.20 ± 0.13	0.038 ± 0.03
	dp	26 ± 0.00	0.48 ± 0.01	970.33 ± 11.59	1023.67 ± 1.53	477.33 ± 5.51	5.71 ± 0.00	7.18 ± 0.00	239.05 ± 1.21	3.12 ± 0.27	0.279 ± 0.27
	us	26 ± 0.00	0.21 ± 0.00	431.33 ± 2.89	2320.00 ± 7.32	207.97 ± 1.70	5.16 ± 0.00	7.52 ± 0.00	141.67 ± 0.25	7.79 ± 0.83	0.003 ± 0.00
	ds	25.5 ± 0.00	0.30 ± 0.00	615.00 ± 1.73	1622.67 ± 7.51	299 ± 1.00	7.32 ± 0.00	7.51 ± 0.00	313.61 ± 0.70	4.97 ± 0.59	0.016 ± 0.01
APRIL	bc	20.17 ± 0.29	0.39 ± 0.01	790.33 ± 3.06	1265.33 ± 4.51	386.67 ± 1.53	1.34 ± 0.21	6.82 ± 0.25	199.28 ± 4.04	3.75 ± 0.29	0.045 ± 0.05
	dp	20.17 ± 0.29	0.43 ± 0.01	884.00 ± 4.58	1131.33 ± 6.03	433.67 ± 2.08	1.44 ± 0.02	6.70 ± 0.15	180.34 ± 0.76	3.72 ± 0.14	0.085 ± 0.10
	us	17.67 ± 0.58	0.16 ± 0.00	328.67 ± 1.53	304.33 ± 1.53	157.67 ± 0.71	8.75 ± 0.60	6.92 ± 0.18	102.27 ± 2.24	9.26 ± 0.24	0.004 ± 0.00
	ds	19 ± 0.00	0.30 ± 0.00	627.33 ± 0.58	1594.33 ± 2.08	305.33 ± 0.58	17.10 ± 0.12	7.01 ± 0.09	115.39 ± 1.60	9.34 ± 0.83	0.012 ± 1.33
MAY	bc	19.00 ± 0.00	0.57 ± 0.00	1148.33 ± 5.51	871.00 ± 4.00	567.33 ± 2.52	25.20 ± 4.04	6.93 ± 0.03	312.76 ± 0.64	3.15 ± 0.25	0.046 ± 0.06
	dp	18.50 ± 0.00	0.62 ± 0.01	1253.67 ± 3.06	797.67 ± 2.52	621.33 ± 1.53	22.30 ± 9.2	6.93 ± 0.11	245.33 ± 1.15	4.72 ± 0.16	0.031 ± 0.04
	us	13.67 ± 0.58	0.17 ± 0.00	363.67 ± 1.15	2.75 ± 0.01	174.67 ± 0.64	8.77 ± 6.46	6.63 ± 0.23	305.61 ± 8.01	11.04 ± 0.97	0.011 ± 0.00
	ds	16.07 ± 0.12	0.46 ± 0.00	939.67 ± 1.15	1064.33 ± 1.15	462 ± 1.00	16.03 ± 2.04	6.83 ± 0.31	302.67 ± 10.64	9.76 ± 0.55	0.057 ± 0.06
JUNE	bc	18.00 ± 0.00	0.53 ± 0.01	1084.33 ± 2.08	925.33 ± 1.15	534.33 ± 1.53	7.68 ± 3.41	7.6 ± 0.03	307.17 ± 3.27	4.17 ± 0.01	0.038 ± 0.00
	dp	17.50 ± 0.00	0.58 ± 0.00	1166.33 ± 2.08	857.33 ± 1.53	577.33 ± 1.15	7.47 ± 1.78	7.61 ± 0.07	110.33 ± 0.38	4.35 ± 0.77	0.027 ± 0.36
	us	16.00 ± 0.00	0.18 ± 0.00	383.67 ± 0.58	2610 ± 0.00	184.53 ± 0.31	6.87 ± 3.19	7.93 ± 0.00	26.33 ± 4.61	9.85 ± 1.09	0.000 ± 0.00
	ds	13.87 ± 0.12	0.40 ± 0.00	826.67 ± 1.53	1209.33 ± 1.15	404.67 ± 0.58	9.13 ± 5.70	7.81 ± 0.03	72.28 ± 1.21	6.94 ± 0.24	0.016 ± 0.00
JULY	bc	16.50 ± 0.00	0.47 ± 0.01	974.67 ± 0.58	1026.67± 0.58	467 ± 3.00	20.67 ± 0.69	6.73 ± 0.23	193.67 ± 0.00	2.37 ± 0.30	0.014 ± 0.00
	dp	17.00 ± 0.00	0.52 ± 0.01	1069 ± 1.00	935.33 ± 0.58	525.67 ± 2.31	24.50 ± 4.35	6.60 ± 0.34	308.67 ± 0.00	4.44 ± 0.56	0.009 ± 0.00
	us	13.50 ± 0.00	0.15 ± 0.00	321.67 ± 0.58	3110 ± 0.00	154.17 ± 0.23	7.40 ± 5.74	6.34 ± 0.05	309.39 ± 0.32	8.14 ± 3.31	0.002 ± 0.00
	ds	14.50 ± 0.00	0.34 ± 0.00	706.67 ± 1.53	1415 ± 2.65	344.67 ± 0.58	19.80 ± 4.27	6.47 ± 0.54	298.06 ± 1.73	6.87 ± 2.94	0.007 ± 0.00
AUGUST	bc	16.80 ± 0.00	0.42 ± 0.01	849.33 ± 1.53	1187 ± 6.64	416.67 ± 0.58	19.73 ± 0.00	6.65 ± 0.00	139.56 ± 0.00	4.10 ± 0.31	0.021 ± 0.00
	dp	16.50 ± 0.00	0.47 ± 0.00	958.00 ± 2.00	1044 ± 2.00	471 ± 1.00	16.80 ± 0.00	7.11 ± 0.00	309.00 ± 0.00	4.49 ± 0.38	0.021 ± 0.00
	us	14.50 ± 0.00	0.34 ± 0.00	706.67 ± 7.51	1419 ± 1.36	344.67 ± 3.51	40.40 ± 0.00	7.12 ± 0.00	207.55 ± 0.00	10.61 ± 0.63	0.000 ± 0.00
	ds	12.00 ± 0.00	0.37 ± 0.00	757.33 ± 2.31	1320.67 ± 4.04	369.67 ± 1.15	14.10 ± 0.00	7.26 ± 0.00	311.78 ± 0.00	8.84 ± 0.60	0.015 ± 0.00

BC: Before chlorination; DP: Discharge point; US: Upstream; DS: Downstream; Temp: Temperature; Salinity; Cond: Conductivity; Resist: Resistivity; TDS: Total dissolved solids; COD: Chemical oxygen demand; BOD: Biological oxygen demand; TSS: Total suspended solids

Table 2.4: Nitrate and phosphate levels in the water samples over the six month period

Month	Sample Point	NWWTP		NGWWTW	
		Nitrate (mg/L)	**Phosphate (mg/L)**	**Nitrate (mg/L)**	**Phosphate (mg/L)**
March	BC	< 0.057	3.509	1.81	0.904
	DP	< 0.057	3.473	1.401	0.797
	US	1.062	0.051	1.062	0.051
	DS	< 0.057	0.157	2.143	0.047
April	BC	< 0.057	2.105	2.809	1.091
	DP	< 0.057	2.097	2.351	0.859
	US	0.856	0.232	1.292	0.168
	DS	0.318	0.381	0.818	0.282
May	BC	0.156	2.72	< 0.057	0.051
	DP	0.202	2.338	< 0.057	< 0.025
	US	1.028	1.08	0.843	< 0.025
	DS	1.716	0.816	0.514	< 0.025
June	BC	5.98	12.377	3.76	1.048
	DP	3.92	9.021	1.99	0.876
	US	2.4	2.173	2.38	0.655
	DS	1.78	1.439	2.15	0.33
July	BC	1	1.829	0.472	0.025
	DP	0.892	0.751	0.573	0.093
	US	0.785	-0.025	1.03	0.025
	DS	1.35	0.092	0.712	0.071
Aug	BC	0.148	1.696	1.42	0.931
	DP	< 0.057	1.994	1.96	1.467
	US	< 0.057	0.115	2.29	0.591
	DS	1.47	0.163	0.8	1.894

BC: Before chlorination; DP: Discharge point; US: Upstream; DS: Downstream

Table 2.5: Correlation coefficient for the different physico-chemical variables and *E. coli* population at the Northern Wastewater Treatment Plant

Variables	Salin.	Cond.	TDS	Turb.	pH	COD	BOD	TSS	EC	Temp.	Resist.
Salin.	1.000	.999**	1.000**	-.024 ns	-.255 ns	-.341 ns	.103 ns	-.353 ns	.063 ns	.024 ns	.627**
Cond.		1.000	1.000**	-.023 ns	-.261 ns	-.337 ns	.095 ns	-.353 ns	.069 ns	.028 ns	.625**
TDS			1.000	-.018 ns	-.263 ns	-.337 ns	.097 ns	-.352 ns	.067 ns	.025 ns	.623**
Turb.				1.000	-.141 ns	-.032 ns	-.300 ns	.249 ns	.226 ns	-.266 ns	.152 ns
pH					1.000	-.105 ns	.412*	-.210 ns	-.325 ns	.097 ns	.083 ns
COD						1.000	.168 ns	.166 ns	-.294 ns	.238 ns	-.319 ns
BOD							1.000	-.065 ns	-.719**	.147 ns	-.190 ns
TSS								1.000	-.153 ns	-.498*	-.172 ns
EC									1.000	.250 ns	.132 ns
Temp.										1.000	-.165 ns
Resist.											1.000

Table 2.6: Correlation coefficient for the different physico-chemical parameters and *E. coli* population at the New Germany Treatment Plant

Variables	Resist.	Temp.	Salin.	Cond.	TDS	pH	BOD	TSS	EC	Turb.	COD
Resist.	1.000	0.012 ns	.439*	0.317 ns	0.329 ns	-0.221 ns	0.240 ns	-0.413 ns	-0.092 ns	-0.147 ns	0.035 ns
Temp.		1.000	-0.166 ns	-0.097 ns	-0.104 ns	-0.158 ns	-.529**	0.364 ns	0.032 ns	-.409*	0.180 ns
Salin.			1.000	.755**	.780**	-0.016 ns	.661**	-.657**	-0.117 ns	-0.223 ns	0.094 ns
Cond.				1.000	.999**	-0.041 ns	0.351 ns	-0.337 ns	-0.108 ns	-0.258 ns	0.011 ns
TDS					1.000	-0.034 ns	0.371 ns	-0.359 ns	-0.114 ns	-0.257 ns	0.019 ns
pH						1.000	-0.116 ns	-0.136 ns	-0.390 ns	0.169 ns	-0.222 ns
BOD							1.000	-.554**	-0.030 ns	0.179 ns	-0.025 ns
TSS								1.000	0.148 ns	-0.235 ns	0.076 ns
EC									1.000	-0.002 ns	0.186 ns
Turb.										1.000	-0.251 ns
COD											1.000

Salin: Salinity; Cond: Conductivity,Turb: Turbidity; COD: Chemical oxygen demand; BOD: Biological oxygen demand; TSS: Total suspended solids; EC: *E. coli*; Temp: Temperature; Resist: Resistivity.

** **Correlation is significant at $p < 0.05$ level (2-tailed)**
* **Correlation is significant at $p < 0.01$ level (2-tailed)**
ns **Not significant**

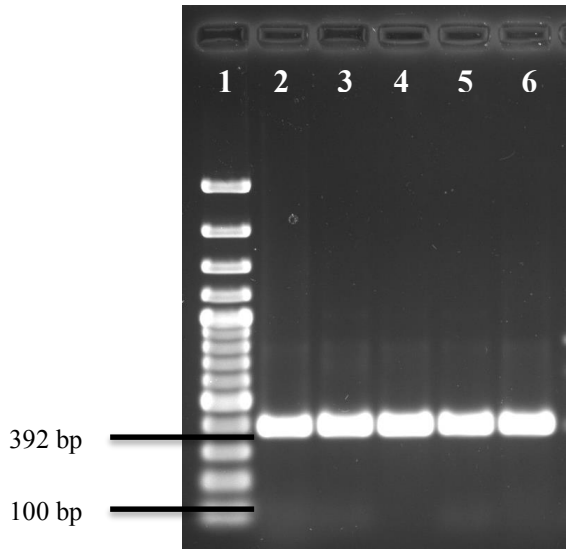


Figure 2.2: Representative gel image depicting the presence of *mdh* (392bp) in *E. coli* isolates

Where, L1: MW DNA Marker and L2-L6: Representative *E. coli* isolates

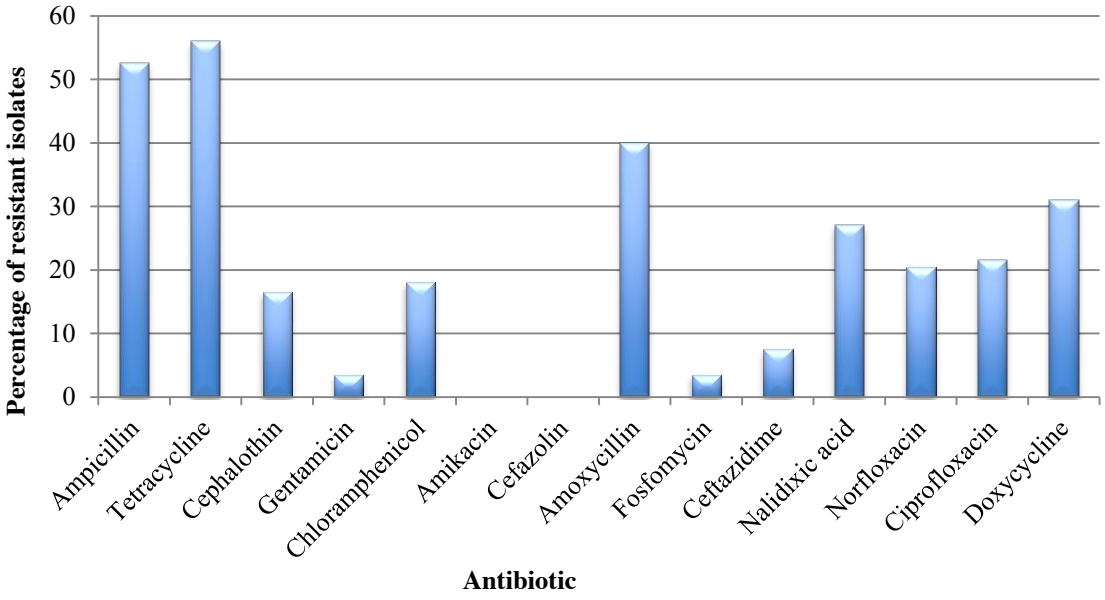


Figure 2.3: Antibiogram of the *E. coli* isolates recovered from treated effluents and receiving rivers to selected antibiotics

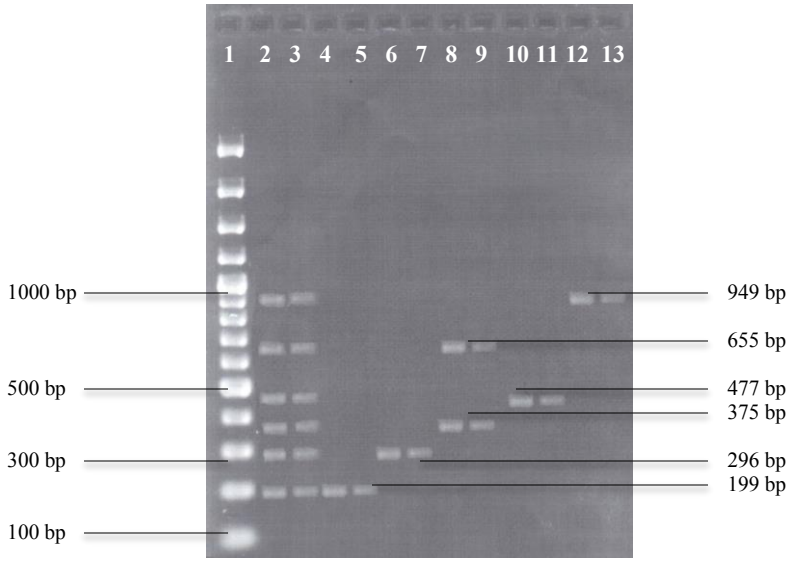


Figure 2.4: Representative gel image depicting the presence of six virulence genes in selected *E. coli* isolates

Where: L1: MW DNA Marker; L2-L3: Positive control; L5-L5: *hly*; L6-L7: *rfbE*; L8-L9: *eae* and *stx1*; L10-L11: *stx2* and L12-L13: *fliC*

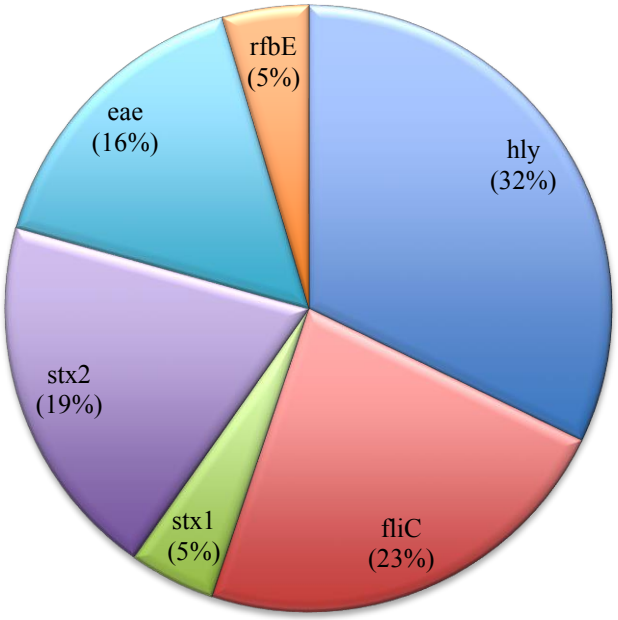


Figure 2.5: Virulence gene distribution among the *E. coli* isolates recovered from treated effluents and receiving rivers

2.4 Discussion

Monitoring of the NWWTP and the respective receiving water bodies over the six-month period revealed dramatic fluctuations in *E. coli* populations as shown in Figure 1a. Increases in the *E. coli* population at the DP during the months of March, April and August could be attributed to the inefficiency of the plant as a result of a plant upgrade. During the months of May, June and July, the NWWTP was efficient in reducing the microbial load at the DP when compared to BC, having treatment efficiency rates of 62.82%, 25.00% and 19.25%, respectively. Although informal settlement dwellers were seen washing clothes and dumping household waste at the US and DS sampling points of the uMgeni River, *E. coli* populations at these points were relatively low compared to populations at the discharge point. In addition, poorly maintained pipes which transport final effluent to the discharge point could be a possible source of contamination of receiving water bodies. *E. coli* populations at the NGTWs as depicted in Figure 1b. Although the treatment plant was efficient in reducing the *E. coli* population at the discharge point during all sampling months, *E. coli* counts did not fall within the recommended guideline. The relatively low counts of *E. coli* obtained at the discharge point could be attributed to the fact that the plant is of a smaller capacity and treated approximately 1% of the total carrying capacity of 7 megalitres per day. According to the Government Gazette (DWAF, 1984), effluent should not contain any substance in a concentration capable of producing any colour, odour or taste. During most months of sampling it was noted that water at the BC and DP sampling points at the NGTWs were amber in colour due to dyes from nearby industries. It may be assumed that the presence of these chemicals may be inhibitory to bacterial growth and may have contributed to low *E. coli* counts at the plant. Various informal settlements, industries, and a school is located alongside the Aller River where the US and DS samples were taken. This however, did not seem to be a contributing

factor to the *E. coli* load at these points as the *E. coli* counts at these points were lower when compared to the discharge point.

Beyond microbial measurements, safe water also has physico-chemical characteristics which are readily affected by climatic events and impact on the survival of microorganisms and the efficiency of treatment processes (DWAF, 1996). The increase or decrease in water temperature depends mainly on climatic conditions, sampling times, the number of sunshine hours and also on characteristics such as turbidity, humidity and plant cover. Seasonal variations was noted in the temperature profiles obtained at both WWTPs, with the lowest temperature values obtained at the DS points in winter, whilst the highest values were obtained in autumn at the BC and DP. With these two exceptions, all values fell within the recommended guideline of 25 °C (DWAF, 1984). BOD is a measure of how much of oxygen is required to completely oxidise the pollutant (Momba *et al*., 2002). The current South African guideline for BOD for final effluent or river sources is 5 mg/L (DWAF, 1999), while the European Union guidelines stipulate that BOD levels should be between 3 – 6 mg/L for the protection of aquatic life and fisheries (Chapman, 1996). By this standard, it can be noted that all BOD values for the BC and DP points at both treatment plants were within specified guidelines. The highest values were obtained for the US points of both rivers, suggesting that the WWTPs were not the only contributors to the organic pollution load. A significant ($r=-0.719$ at $p<0.05$) relationship existed between BOD and *E. coli* at the NWWTP. The highest values for COD (311.72 ± 0.8) at the NWWTP were obtained at the US and DS sample points which indicate the presence of other point sources of pollution. COD concentrations at the DS point were higher than that observed at the DP, implying that WWTP effluent had little contribution to elevated COD concentrations further down the river. The COD values obtained at

the NGTWs were comparatively higher than values obtained at NWWTP probably due to the influent being mainly of industrial origin. The standard for COD of water used for domestic purposes is 30 mg/L (DWAF, 1984). With the exception of samples from the DP point with COD values of 9 mg/L in both March and May at the NWWTP, and the US point of NGTWs with a COD value of 26.33 ± 1.21mg/L during the month of June, all COD values obtained were not within recommended guidelines. A positive correlation ($r=0.755$ at $p<0.05$) is noted between COD and salinity at NGTWs. In addition, results obtained indicate that the NGTWs is a direct source of pollution of receiving water bodies, as the COD concentrations increased at the DP when compared to concentrations at the BC point over the six month period. This is in agreement with studies conducted by Fatoki *et al*. (2001), and Morrison *et al*. (2001), which state that the contribution of COD to effluent and receiving water bodies in South Africa is significant. Elevated COD concentrations have been shown to have a negative impact not only on receiving water sources but on aquatic life present as well.

According to the South African guidelines for water for domestic use, TDS concentrations for final effluent and receiving surface waters should be between 0 and 450 mg/L (DWAF, 1996). Results obtained indicate that both treatment plants investigated in this study were inefficient in reducing the TDS concentration throughout the sampling period as the TDS concentration increased at the DP sampling point by an average of 10.79% and 9.4%, respectively relative to BC. The highest TDS concentration was recorded for the US point (1067.00 ± 0.00) in May, whilst the lowest (294.67 ± 0.58) was found at the DS in July. With the exception of April, all TDS values obtained at the DP sampling point at the NGTWs were not within specified guidelines. The lower TDS values obtained at the US point, and the higher values at the DS sampling point together with

elevated concentrations at the DP sampling point suggests that the NGTWs is a source of contamination of receiving water bodies and contribute to the TDS concentrations at the DS point. It is therefore not surprising that TDS had strong positive correlations with conductivity and salinity at both treatment plants. According to McCulloch *et al*. (1993), elevated TDS concentrations in surface waters are toxic to freshwater animals as they cause osmotic stress and affect the osmoregularity capability of the organisms.

There is no standard for the turbidity of effluent discharge in South Africa. However, water used for domestic purposes should have <1.0 NTU (DWAF, 1996). All values obtained at both treatment plants were not within specified guidelines. For the NWWTP, the turbidity values at the DP were higher when compared to the BC point for most of the sampling period. This could be attributed to the upgrade of the plant infrastructure being undertaken at the plant during the sampling period. A comparison of the six months of sampling across both plants revealed the highest turbidity in August. This may be a result of surface runoff and soil erosion into receiving water bodies caused by the heavy rains experienced during this month. The high turbidity values obtained therefore excludes all water sources for direct domestic use (Fatoki *et al*., 2001). According to Ekholm and Krogenus (1998), highly turbid water is difficult to disinfect and is often associated with the growth of pathogenic organisms.The nutrient analysis of all water samples can be seen in Table 2.4. According to Igbinosa and Okoh (2009), nitrate is the most highly oxidised form of nitrogen compounds and is commonly found in surface and groundwater, as it is an end product of aerobic decomposition of organic nitrogenous matter. Unpolluted water usually only contains minute amounts of nitrate (Jaji et al. 2007). The South African standard for nitrate in sewage effluent is 1.5 mg/L NO_3^- as N (DWAF, 1984). This study showed dramatic fluctuations

in nitrate concentrations across all sampling points at both plants. The NWWTP was efficient in reducing the nitrate concentration for final effluent by 34.5% and 10.80% for the months of June and July, respectively, with an increase in concentration observed at the DP sampling point during April and May. All values, except for those obtained in May at the DS point, and all points during the month of June, did meet the recommended standards. The elevated nitrate concentrations observed at some of the US and DS points when compared to the DP suggest that there are other point sources of nitrate contamination on the surface of the water. On the contrary, NGTWs was not as efficient in reducing nitrate levels in final effluent when compared to the NWWTP, having treatment efficiency rates of 16.30% and 47.1% for the months of April and June, respectively. The nitrate levels increased at the DP sampling point during July and August, suggesting that the plant was responsible for contributing to nitrate contamination of surface waters. All values obtained during the month of June, together with samples from the DP in April and August, as well as the DS point in August did not meet the specified guidelines. It is important to note that the nitrate levels in treated final effluent could serve as a source of eutrophication for receiving water bodies. The nitrate levels at the US and DS points could be a result of diffuse sources from agricultural and settlement runoff (Igbinosa and Okoh, 2009). Nitrate has been identified as a pollutant and could lead to excessive growth and the proliferation of toxic algal blooms when found in elevated concentrations (Santos *et al*., 2008). Orthophosphates are generally a limiting factor in aquatic environments, and at levels above 0.1 mg/L in water, orthophosphates usually result in increased eutrophication (Osode and Okoh, 2009). There is no target quality range for phosphate concentrations in final effluent used for domestic or recreational purposes according to South African guidelines, however, the level of phosphate in water that reduces the likelihood of algal and other plant growth is 0.005 mg/L (DWAF, 1996). By this standard, all values obtained

at both treatment plants and respective receiving surface water sources were not within specified limits. The NGTWs was efficient in reducing the phosphate concentrations in final effluent from April – June, whilst an increased phosphate load was obtained after treatment during July and August. The high values obtained at the DP sampling point at both plants suggest an inadequate removal of phosphate by the wastewater treatment facility. Increased levels along other sampling points may be attributed to a variety of activities such as municipal, agricultural and domestic run-off from surrounding areas.

Chloramphenicol is a drug rarely used in non-life threatening situations and works by inhibiting protein synthesis. Therefore, resistance to this drug is rare. However, higher resistance (18%) to chloramphenicol was observed in this study compared to similar studies. Aminoglycosides are a similar class of antimicrobials that bind to ribosomes and therefore prevent protein synthesis. A similar trend in resistance to gentamicin which belongs to this class was seen in previous studies (Ram *et al*., 2008). Studies by Olaniran *et al*. (2009) showed resistance towards amikacin among *E. coli* isolates recovered from river sources in Durban however, all isolates in this study were susceptible. According to Goni-Urriza *et al*. (2000), the low levels of resistance to this class may be due to its reduced use, as it has been known to be associated with kidney and auditory nerve damage. Due to the low toxicity of β-Lactams and Tetracycline antimicrobials, it is commonly used to treat bacterial infections. This has been a major contributing factor to the increased resistance levels observed in many studies. β-lactams work by inhibiting the final step in cell wall synthesis. Among the *E. coli* isolates tested, the most common resistance was to tetracycline, ampicillin, amoxicillin and doxycycline with a percentage resistance of 56%, 52.5%, 40% and 31%, respectively. Resistance to this group of antimicrobial agents has been attributed mainly to

the acquisition of mobile genetic elements between bacterial strains (WHO, 2014). A similar trend of resistance was also observed in studies by Luczkiewicz *et al*. (2010). An increase in tetracycline, chloramphenicol and ampicillin resistance observed in this study was in agreement with the findings of Olaniran *et al*. (2009). Reports by WHO (2014), name *E*. *coli* as an organism of international concern due to an emerging resistance to fluoroquinolones and cephalosporins. Quinolones are one of the most frequently used drugs for the treatment of urinary tract infections whilst cephalosporins are used in more severe cases (WHO, 2014). Reports based on twenty nine publications in South Africa revealed a higher resistance to fluoroquinolones than cephalosporins. This study indicates that 20.5% and 21.5% of the isolates were resistant to norfloxacin and ciprofloxacin, respectively. In addition, isolates exhibited resistance towards cephalothin (16.5%), ceftazidime (7.5%) and cefazolin (0%), clearly indicating a similar trend as observed across the country. It is then suggested that populations exhibiting these resistance patterns be initiated with broader therapy such as carbapenems when the use of cephalosporins is considered (WHO, 2014). Urban wastewater treatment plants are among the main sources of antibiotics' release into the environment and could be a contributing factor to the dissemination of antibiotic resistance in the environment if effluent is not properly treated (Rizzo *et al*., 2013). In addition, changes in the nutrient composition could also lead to selective pressures which favour antibiotic resistance (Ram *et al*., 2008). According to Olaniran *et al*. (2009), multiple antibiotic resistance among *E*. *coli* may also be due to the spread of genetic elements such as plasmids and integrons amongst the organisms.

The virulence gene profiles of tested isolates revealed a high prevalence of the hly gene similar to that reported by Ram *et al*. (2008). However, the frequency of potentially pathogenic *E*. *coli* observed in this study is lower than those reported by Ram *et al*. (2008) and Obi *et al*. (2004). The

low prevalence of Shiga toxigenic genes obtained was insufficient to make any definite comparisons or draw conclusions, however, it was noted that the *eaeA* gene was found in conjunction with either *stx1* or *stx2* in 14% of the tested isolates. This gene is essential in the attachment stage of pathogenesis where attachment to Hep-2 epithelial cells within a host is enabled by intimin, an outer membrane protein which is encoded for by the *eaeA* gene (Wilshaw *et al*., 2003). The combination of *eaeA* and either *stx1* or *stx2* is characteristic of EHEC. Pathogenic strains of STEC can either express *stx1*, *stx2*, or both. It has been reported that the development of critical infections does not entirely depend on the amount of toxin present but rather on the type, with *stx2* being most pathogenic due to variants which exists within the group (Khan and Naim, 2011). Human infection can be due to direct fecal-oral transmission from an infected animal or human, through contaminated water or most frequently through the consumption of inadequately cooked meat products, raw milk, and unpasteurized drinks (Hunter, 2003). However, it should be noted that the presence of a virulence gene only indicates a potential to cause disease. Even if the virulence factor is active, it may not be sufficient to cause disease, as disease is a complex process which contains various steps which include entry into the body, attachment and survival within the host as well as survival in the environment (Edberg, 2009).

2.5 Conclusion

This study was carried out to evaluate the efficiency of two independent wastewater treatment plants and the impact of discharged effluent from these plants on receiving water bodies. The results revealed that both treatment plants were efficient at some point in reducing the *E. coli* load of the effluent, but not to acceptable standards in water for domestic use. The treatment plant did exhibit final effluent qualities that were of acceptable limits for physico-chemical parameters such as temperature; pH and BOD but fell short for others such as turbidity, nitrate and phosphate. This study therefore shows that inefficiently treated effluent is one of the major contributors to the pollution of surface waters. Pollution from industry and domestic practices further compounds the problem, resulting in surface waters of poor microbiological and physico-chemical quality. The study also revealed that incomplete elimination of bacteria during wastewater treatment resulted in the entry of antibiotic resistant bacteria into receiving streams with effluent input as some of the isolates tested in this study exhibit multiple antimicrobial resistance, which may compromise the treatment of infections caused by these organisms. Although the prevalence of virulence genes amongst *E. coli* isolates investigated in this study is lower when compared to similar studies, their detection in these strains cannot be disregarded as it indicates the potential pathogenic nature of these isolates. The pollution of water sources continues to be a global challenge, especially in rural communities where residents rely on these water sources for daily domestic and recreational use. In addition, the decrease in water quality leads to increased treatment costs of potable and industrial process water as well as agricultural yields (Osode and Okoh, 2009). It is therefore important that frequent water quality monitoring be carried out and that strict water quality guidelines be set and enforced by the South African government. Problems may also be alleviated by upgrading these treatment plants and ensuring that proper design, planning and construction is being carried out.

CHAPTER THREE

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *Escherichia coli* ISOLATES RECOVERED FROM TREATED WASTEWATER EFFLUENT AND RECEIVING AQUATIC *MILIEU* IN DURBAN, SOUTH AFRICA

Abstract

The extensive and uncontrolled use of antimicrobial agents in veterinary medicine, agriculture and by humans has led to the rise in resistance to new and commonly used antimicrobial agents. This phenomenon is proof that bacteria have the ability to exchange and rearrange genomic sequences to gain resistance traits through the horizontal gene transfer (HGT) of mobile genetic elements such as integrons. In this study, 200 *Escherichia coli* isolates recovered from treated effluent of two wastewater treatment plants and their respective receiving rivers were screened for their antimicrobial resistance as well as for the prevalence of classes 1, 2 and 3 integrons. The presence and identity of gene cassettes was determined via PCR, Restriction Fragment Length Polymorphism (RFLP) and sequence analysis, while conjugation experiments were conducted to determine if plasmid encoded integrons are transferrable to recipient strains. The results revealed high resistance rates towards tetracycline (56%), ampicillin (52.5%) and trimethoprim (50.5%). Class 1 integrons were detectd in 50.5% of isolates while class 2 was found in only 1%. Gene cassettes were detected in all integron-positive isolates with the most abundant gene being *dfrA17*, which encodes resistance to trimethoprim. All detected integrons were located on both the plasmid and chromosome found to be transferrable to recipient strains. Majority (86%) of integron-positive isolates were multi-drug resistant and exhibited higher resistance rates to antimicrobial agents when compared to integron negative strains. Random Amplified Polymorphism DNA (RAPD) analysis of integron positive strains shows clonal relationships among isolates with similar resistance phenotypes. These results confirm integrons as possibly important contributors to the widespread occurrence of antibiotic resistance in *E. coli*.

3.1 Introduction

To date, bacterial evolution has been shaped by the high plasticity of bacterial genomes, and this has led to their adaptations to most ecosystems (Stalder *et al*., 2012). Antimicrobial agents are considered as one of the most significant contributors to modern medicine, having the ability to kill or inhibit the growth of a plethora of microorganisms (Gungchao *et al*., 2013). The extensive and uncontrolled use of antimicrobial agents in veterinary medicine, agriculture and for human use has led to the emerging rise of resistance to new and commonly used antimicrobial agents. This phenomenon is proof that bacteria have the ability to exchange and rearrange genomic sequences to gain new traits such as resistance to these agents (Stalder *et al*., 2012). Therefore, microbial mediated infections still remain as one of the greatest public health concerns in the 21st century, due to complications experienced in the treatment of infections (Yang *et al*., 2009;Gungchao *et al*., 2013). Resistance to antimicrobial agents may arise due to mutations in the bacterial chromosome, or through the horizontal transfer of mobile genetic elements (MGEs) such as plasmids, transposons and integrons (Koczura *et al*., 2012). However, horizontal gene transfer (HGT) events, which allow for the loss and acquisition of functional modules is the preferred method for the transfer of antibiotic resistance genes (ARGs) as opposed to random mutational events (Stalder *et al*., 2012). Integrons are one of the main types of MGEs currently known to be involved in the acquisition and spread of ARGs amongst gram-negative bacteria (Koczura *et al*., 2012).

A functional integron comprises of three key elements namely: an integrase gene (*intl*), which encodes an integrase; a recombination site (*attl*); and a promoter gene (*Pc*). Characterized by the presence of invariant RHRY amino acids, integrase, belongs to the tyrosine-recombinase family, and is responsible for mediating the recombination between the *attl* site and a secondary target

called the *attC* site (Gungchao *et al*., 2013). These recombination sites are generally associated with a single open reading frame, which can be found in a structure called gene cassette (Stalder *et al*., 2012). Gene cassettes (GCs) are one of the smallest MGEs and can exist in either a circular or linear form. They are not always found in integrons, but once integrated, they become part of the integron (Fluit and Schmitz, 1999). GCs encode ARGs, and to date, more than one hundred different antibiotic resistance gene cassettes having unique *attC* sites have been characterized (Gungchao *et al*., 2013). According to Mazel (2006), an integron itself is not movable, but GCs within integrons can be mobilized to another integron or another site within the genome. There are two major groups of integrons: chromosomal and mobile integrons, with the latter being found on MGEs that promote their dissemination amongst bacteria (Stalder *et al*., 2012). Studies by Skurnik *et al*. (2005), demonstrated that mobile integrons are most prevalent in bacterial communities who are subjected to direct or indirect antibiotic pressure in clinical, agricultural and environmental settings. In addition, several classes of integrons have been characterized based on the divergence in their *intl* sequences. Classes 1, 2 and 3 integrons are most commonly screened for, while class 4 is rare and is termed a 'super integron' (Gungchao *et al*., 2013). Class 1 integrons have been reported in a number of gram-negative organisms, including *E. coli*, *Aeromonas*, *Shigella* and *Serratia spp*., and have therefore been studied extensively. They have been detected in 22 to 77% of clinical isolates obtained from human and animal sources (Gungchao *et al*., 2013;Koczura *et al*., 2012;Stalder *et al*., 2012). Class 2 integrons contain a defective integrase gene and is considered to be less prevalent when compared to the widely distributed class 1 integron. It is most commonly found in *E. coli*, *Salmonella enterica* and *Acinetobacter baumanii* (Ahmed *et al*., 2005). Class 3 intergrons are the least common and was previously detected in *Vibrio and Delftia* spp. (Koczura *et al*., 2012; Rizzo *et al*., 2013).

Wastewater treatment plants (WWTPs) are the interface between human waste and the aquatic and soil environments. The convergence of gut-associated bacteria such as *E. coli*, antibiotic residues and a rich diversity of microbial consortium suggest that WWTPs provide a favorable environment for the transfer of MGEs in microbial communities (Rizzo *et al*., 2013). Reports by Rizzo *et al*. (2013) show that on average, WWTPs discharge approximately $10^9 – 10^{12}$ colony forming units per day and of this, around 10^9 - 10^{10} are resistant. However, WWTPs are ineffective in completely removing ARB in final effluent. Studies by Ferreria da Silva *et al*. (2006) showed that the total number of resistant *E. coli* isolates recovered from wastewater increased by 10.30% after tertiary wastewater treatment, while Plaza *et al*. (2013) reported that 90% of *E.coli* isolates recovered from WWTPs were resistant to at least one antibiotic. In addition, approximately 30% - 60% of *E. coli* isolates have been reported to contain class 1 integrons (Moura *et al*., 2007; Zhang *et al*., 2009a). The current study therefore evaluated the antimicrobial resistance patterns of integron bearing strains of *E. coli* isolated from treated wastewater effluent and receiving surface waters and identified the gene cassettes harbored by these isolates. In addition, the ability of integrons to be transferred via conjugation was evaluated while the genotypic relationship among multi-drug resistant phenotypes was determined.

3.2 Materials and Methods

3.2.1 *E. coli* isolates and antibiotic susceptibility testing

A total of 200 *E. coli* isolates previously recovered from the treated effluent of two WWTPs and the receiving surface water were characterized in this study. Presumptive isolates obtained following membrane filtration and plating on selective chromogenic media were biochemically identified via iMViC tests, and confirmed via PCR amplification of the *mdh* gene. Confirmed isolates were grown for 24 h in nutrient broth, standardized to a 0.5 McFarland standard according to CLSI guidelines (CLSI, 2013), swabbed onto Mueller Hinton agar and thereafter incubated at 37 °C for 24 h. Determination of the antibiotic resistance profile of each isolate was done using the Kirby-Bauer disc diffusion assay using the following antibiotics (µg per disc): Penicillins: ampicillin (10), amoxicillin (10); Cephems: cefazolin (30), cephalothin (30), ceftazidime (30); Tetracyclines: tetracycline (30), doxycycline (10); Phenicols: chloramphenicol (30); Aminoglycosides: amikacin (30), gentamicin (10); Quinolones: nalidixic acid (30); Fluoroquinolones: norfloxacin (10), ciprofloxacin (5); Fosfomycin: fosfomycin (200) and Trimethoprim (10).

3.2.2 Identification of integrase genes

A multiplex PCR procedure was used to detect the presence of classes 1, 2 and 3 integrase genes in all confirmed isolates (Dillon *et al*., 2005). DNA was extracted from the isolates using the boiling method and used as template in the PCR assay (Bai *et al*., 2010). The 50 µl PCR assay mixture contained 2 µl of DNA, 1 µl of each primer (Table 3.1), 0.2 µl of *Taq* DNA Polymerase, 10 µl of 1 mM dNTP, 3 µl $MgCl_2$, 5 µl of 10 × reaction buffer and 23.8 µl of RNAse free water.

Amplification of target genes was performed in a T100 Thermal Cycler (Biorad, USA) under the following conditions: Initial denaturation at 94 ºC for 5 min, 30 cycles of denaturation at 94 ºC for 1 min, annealing at 59 ºC for 1 min, elongation at 72 ºC for 1 min and a final elongation step at 72 ºC for 8 min. Amplified DNA was resolved by electrophoresis on a 1.5 % agarose gel (Seakem), and products visualized after staining of gel with ethidium bromide (0.5 µg/ml) using the Chemigenius Bioimaging System (Syngiene, England). Representative bands of positive amplicons were excised, sequenced (Inqaba Biotech Pty.) and compared against GenBank database by using Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3.2.3 Identification of gene cassette arrays

Primers 3CS and 5CS were used for the detection of gene cassette arrays within integron positive isolates (Table 3.1). DNA was extracted using the boiling method and used as a template in the PCR reaction (Bai *et al*., 2010). The PCR assay consisted of the following components: 0.75 µl Long PCR Enzyme Mix with $MgCl_2$ (ThermoFisher Scientific), 10 µl of 1mM dNTP, 5 µl of 10 × reaction buffer, 5 µl of each primer, 3 µl of DNA and 21.25 µl of RNAse free water. Amplification of target genes was performed in a T100 Thermal Cycler (Biorad,USA) under the following conditions: Initial denaturation at 94 ºC for 2 min, followed by 30 cycles of denaturation at 94 ºC for 20 s, annealing at 57 ºC for 30 s and elongation at 68 ºC for 90 s. A final elongation step was done at 68 ºC for 10 min. Amplified DNA was resolved on a 1.5% agarose gel (Seakem) and stained with ethidium bromide (0.5 µg/ml). The various base pair sizes were noted.

3.2.4 Restriction Fragment Length Polymorphism (RFLP)

Isolates showing positive amplification for gene cassettes were further characterized by RFLP analysis using the restriction enzymes *HinfI* and *RsaI* (ThermoFisher Scientific). Each reaction mixture contained 15 µl amplification product and 0.5 µl (5 U) restriction enzyme. Both mixtures were incubated for 24 h at 37 ºC. The restricted fragments were resolved on a 1.5 % agarose gel, stained with ethidium bromide (0.5 µg/ml) and visualized. The different RFLP types were designated with letters (A-D), and PCR products of amplicons representing different restriction patterns were sequenced (Inqaba Biotech Pty.) and compared against the Genbank database using the BLAST tool.

3.2.5 Determination of integrons location

To determine if identified integrons are plasmid or chromosomally encoded, plasmid and genomic DNA was extracted from integron positive isolates using the GeneJet Plasmid MiniPrep Kit (Fermentas) and the GeneJet Genomic DNA Purification Kit (Fermentas), respectively. Extracted DNA was used as a template in a PCR assay to determine the presence of class 1, 2 and 3 integrase genes as previously described. The location of the identified integron was noted.

3.2.6 Conjugation

Ten isolates with plasmid encoded integrons were chosen at random and selected as donor strains in conjugation experiments. Donor (n=10) and recipient cells were grown seperately in Luria Bertani (LB) broth (Merck, USA) for 18 h. Each culture was then serially diluted and plated onto LB agar and incubated at 37 °C for 18 h. After incubation, the number of colonies were counted and expressed as CFU/ml. Donor and recipient cells were mixed (2:3) and incubated for 18 h.

Threafter, cultures were spread onto LB agar containing nalidixic acid (25 µg/ml) and trimethoprim (15 µg/ml) to select for transconjugants. The frequency of transfer was calculated as shown in equation 1, and all transconjugants were isolated and screened for the presence of integrons as previously described. *E. coli* CSH56 which has a natural resistance to nalidixic acid was used as the recipient strain.

$$\text{Frequency of transfer} = \frac{no\ of\ transconjugants/ml}{no\ of\ donor\ cells/ml}$$

3.2.7 Random Amplified Polymorphism DNA (RAPD) analysis

All integron positive isolates were subjected to RAPD analysis. Each PCR mixture (50 µl) contained 2.5 µl 10 × reaction buffer, 6 µl $MgCl_2$, 10 µl dNTP, 2 µl of the arbitrary primer 1247 (Table 3.1), 0.5 µl *Taq* polymerase, 2 µl DNA and 23 µl RNAse free water. Amplification was performed under the following conditions: 2 cycles of 94°C for 30 s, 42°C for 7 s and 72°C for 70 s, 38 cycles of: 94°C for 1 s, 42°C for 7 s and 72°C for 70 s. A final elongation step was done at 72°C for 5 min (Salehi *et al*., 2008). The PCR products were analysed by electrophoresis on a 1.5% agarose gel (Seakem), stained with ethidium bromide (0.5 µg/ml), and visualized. Photographs of RAPD-PCR patterns were taken using the Chemigenius Bioimaging System (Syngiene). Conversion, normalization and further analysis of RAPD patterns were done using the Bionumerics 6 software (Applied Maths). The dendogram was drawn by using the unweighted pair group procedure (UPGMA) clustering and tree building program in the same software.

Table 3.1: Primer sequences used in this study

Type of Gene	Sequence(5′→3′)	Expected product size (bp)	Reference
Int1-F	CAGTGGACATAAGCCTGTTC	160	Dillon *et al*., 2005
Int1-R	CCCGAGGCATAGACTGTA		
Int2-F	CACGGATATGCGACAAAAAGGT	788	Dillon *et al*., 2005
Int2-R	GTAGCAAACGAGTGACGAAATG		
Int3-F	GCCTCCGGCAGCGACTTTCAG	979	Dillon *et al*., 2005
Int3-R	ACGGATCTGCCAAACCTGACT		
5CS	GGCATCCAAGCAGCAAG	Variable	Meervenne *et al*., 2013
3CS	AAGCAGACTTGACCTGA		
1274	AAGAGCCCG	Variable	Salehi *et al*., 2008

3.3 Results

3.3.1 Antibiotic resistance profiling of *E. coli* isolates

The antibiotic resistance pattern(s) of the isolates against the 15 antibiotics are illustrated in Figures 3.1 and 3.2. All the 200 *E. coli* isolates tested were susceptible to amikacin and cefazolin. The highest resistance was observed towards trimethoprim (69%), followed by tetracycline (56%), ampicillin (52.5%), amoxycillin (40%) and doxycycline (31%). Furthermore, 27%, 21.5%, 20.5%, 18% and 16.5% of the isolates were resistant towards nalidixic acid, ciprofloxacin, norfloxacin, chloramphenicol and cephalothin, respectively, while only 3.5% of the isolates exhibited resistance towards gentamicin and fosfomycin, and 7.5% of the isolates were resistant to ceftazidime. Five percent of isolates were resistant to 8 antibiotics (Figure 3.1 A), while 9% showed resistance to 7 antibiotics (Figure 3.1 B). A large portion (14.5%, 15%, and 39%) of isolates were resistant to 6, 5, and 4 antibiotics as seen in Figure 3.1 C and D, and Figure 3.2 E respectively. Six percent of *E. coli* isolates exhibited resistance towards 3 antibiotics (Figure 3.2

F) while 11% (Figure 3.2 G) and 10.5% (Figure 2 H) of isolates were resistant to 2 and 1 antimicrobial agent respectively. None of the isolates were susceptible to all antibiotics tested.

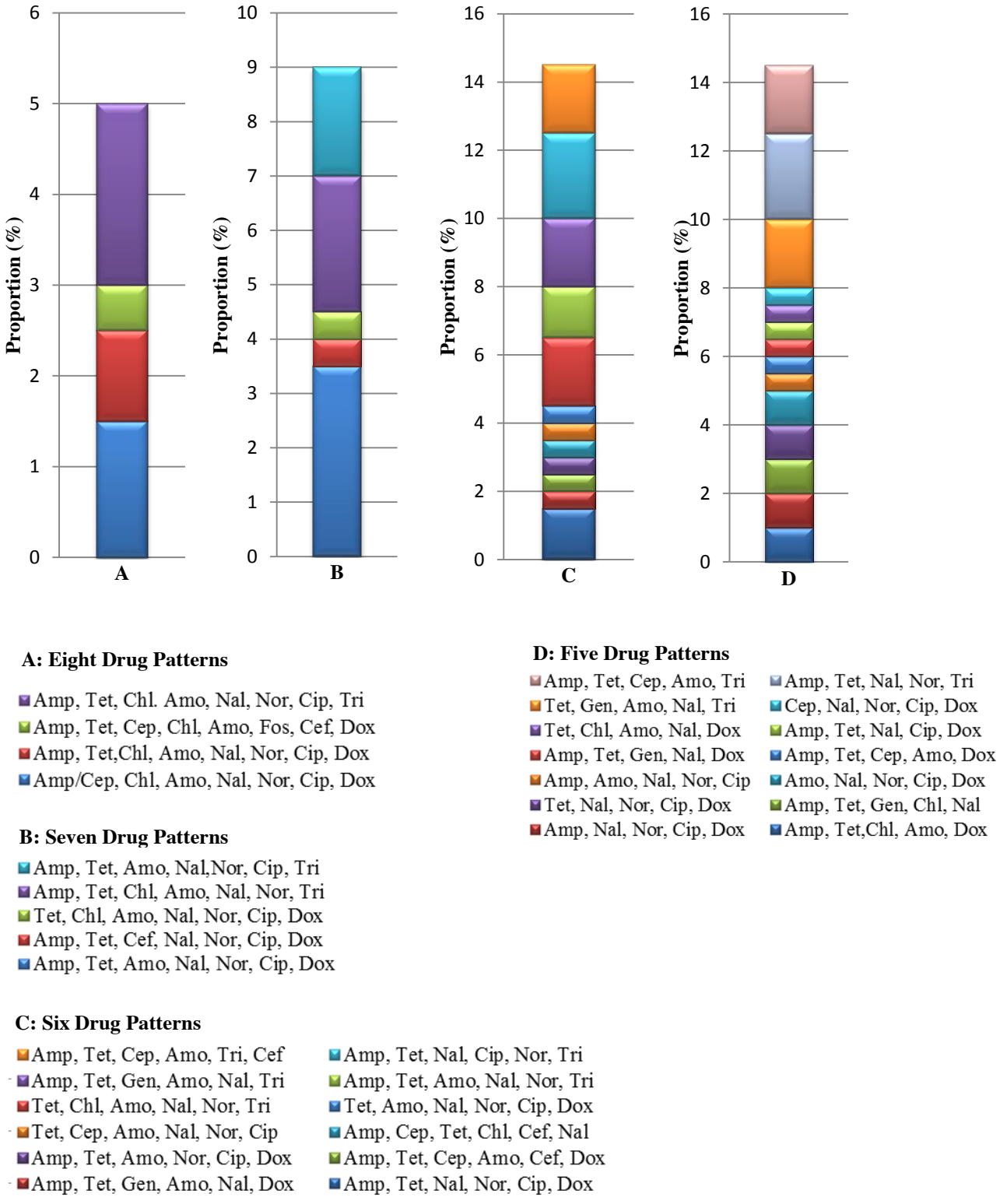


Figure 3.1: Resistance spectra of *E. coli* isolates having eight (A), seven (B), six (C) and five (D) drug patterns. Amp: Ampicillin; Tet: Tetracycline; Chl: Chloramphenicol; Amo: Amoxycillin; Nal: Nalidixic Acid; Nor: Norfloxacin; Cip: Ciprofloxacin; Tri: Trimethoprim; Cep: Cephalothin; Dox: Doxycycline; Gen: Gentamicin; Cef: Ceftazidime; Fos: Fosfomycin

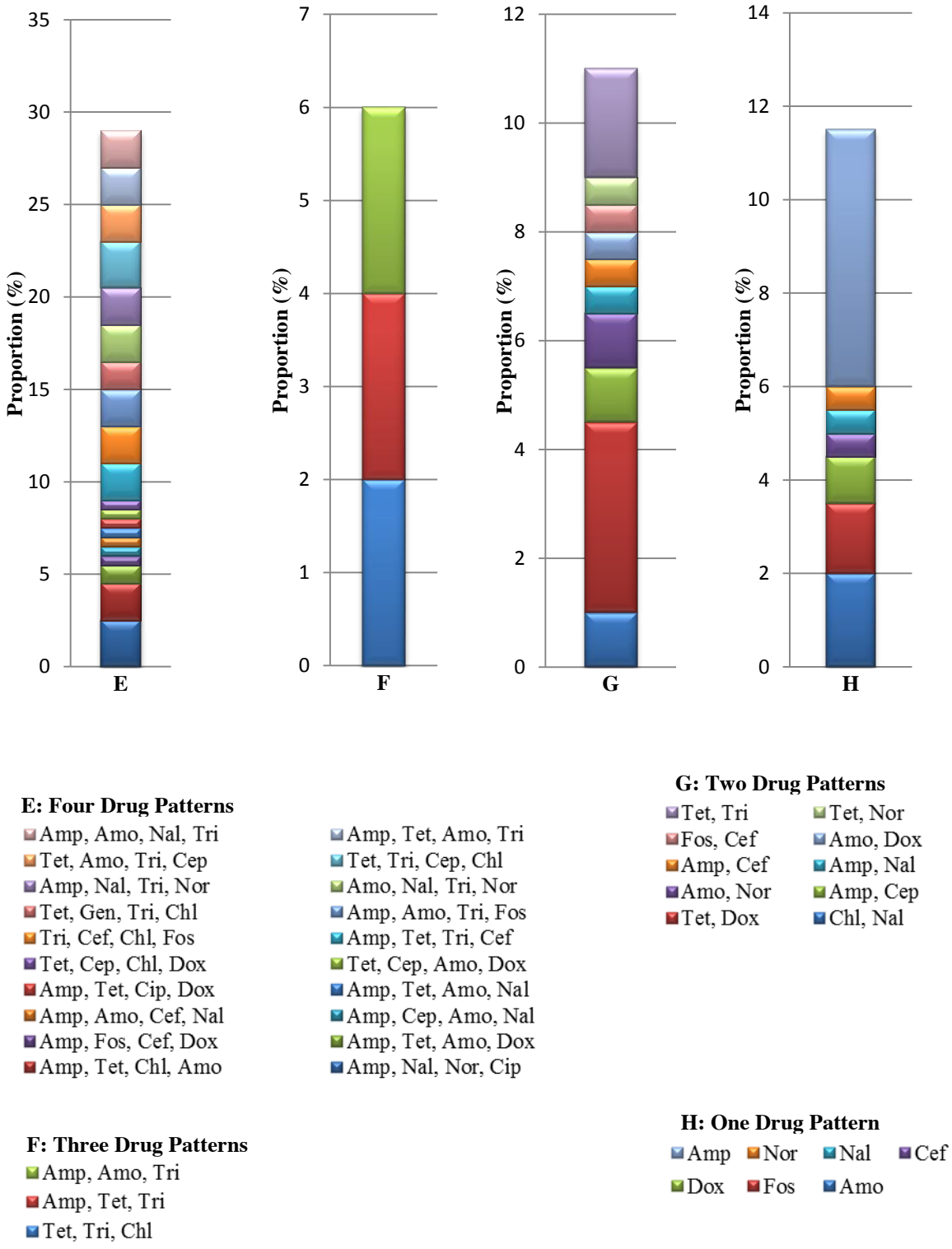


Figure 3.2: Resistance spectra of *E. coli* isolates having four (E), three (F), two (G) and one (H) drug pattern/s. Amp: Ampicillin; Tet: Tetracycline; Chl: Chloramphenicol; Amo: Amoxycillin; Nal: Nalidixic acid; Nor: Norfloxacin; Cip: Ciprofloxacin; Tri: Trimethoprim; Cep: Cephalothin; Dox: Doxycycline; Gen: Gentamicin; Cef: Ceftazidime; Fos: Fosfomycin

Of the 200 *E. coli* isolates analysed for the presence of integrons by PCR, 101 isolates were found to contain integrons. Class 1 integrons were detected in 50.5% of the isolates while class 2 was found in only 1%. One isolate carried both class 1 and class 2. PCR amplification using the 3CS and 5CS primer sets to detect gene cassette arrays revealed amplicon sizes ranging between 100 and 1900 bp. RFLP analysis of these fragments with the restriction enzymes *Hin*fI and *Rsa*I revealed four different RFLP types (Table 3.2). The identity of these cassettes determined via sequence analysis showed the presence of 3 gene cassettes, encoding genes *dfrA17*, *dfrA12-aadA2* and *dfrA12-aadA1*. Majority (76/101) of the isolates (75.24%) harbored *dfrA17* which is associated with trimethoprim resistance, whilst 21.78% and 1.98% harbored *dfrA12-aadA2* and *dfrA12-aadA1* respectively, which is associated with trimethoprim and streptomycin resistance. Whilst streptomycin resistance/susceptibility was not evaluated in this study, all isolates harboring these cassettes were resistant to trimethoprim. PCR analysis showed that all detected integrons were located on both the plasmid and chromosome.

Conjugation experiments yielded a frequency of transfer of 9.4×10^{6}. Amongst the integron positive *E. coli* isolates, 3.96% were resistant to 8 antibiotics, 9.90% to 7 antibiotics and 18.81% were resistant to 6 antibiotics. Resistance to 5, 4, 3 and 2 antibiotics was observed in 11.88%, 39.60%, 11.88% and 3.96% of the isolates. Trimethoprim resistance was not observed in integron negative strains while doxycycline resistance was not observed in integron positive strains. Eighty six percent of integron positive isolates were resistant to 3 or more classes of antibiotics and were therefore considered to be multi-drug resistant. Majority (30.69% and 20.70%) of integron bearing, drug-resistant strains were isolated from the discharge and before chlorination points of the wastewater treatment process while 19.80% of strains were isolated from both the upstream and downstream points of the receiving river sources. Figure 3.3 shows the gel image depicting the

presence of *intI* in all transconjugants (n=10), indicating that tested isolates are capable of transferring integrons via conjugation.

Table 3.2: Antibiotic resistance phenotypes, gene cassette arrays and integron locations of integron-positive *E. coli* isolates recovered from treated wastewater effluent and receiving aquatic *milieu*.

Isolate no:	Resistant Phenotype	*intI* gene	Gene cassettes: amplicon size (bp)	Gene cassette array	Integron location
1	Amp,Tet,Amo, Tri	1	1650 – 1900	*dfrA17*	C, P
2	Tet, Amo, Nal, Nor, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
3	Amp, Tet, Cep, Amo, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
4	Tet, Gen, Tri, Chl	1	1650 – 1900	*dfrA12-aadA2*	C, P
5	Amp, Tet, Amo, Nal, Tri, Nor, Cip	1	1650 – 1750	*dfrA17*	C, P
6	Amp, Tet, Amo Nal, Tri, Nor, Chl, Cip	1	1650 – 1900	*dfrA17*	C, P
7	Amp, Nal, Tri, Nor	1	1650 – 1900	*dfrA12-aadA1*	C, P
8	Amp, Amo, Tri	1	2000	*dfrA12-aadA1*	C, P
9	Tet, Amo, Nal, Nor, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
10	Tet, Amo, Tri, Cep	1	100	*dfrA17*	C, P
11	Amp, Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
12	Amp, Nal, Tri, Nor	1	800	*dfrA17*	C, P
13	Amp, Tet, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA12-aadA2*	C, P
14	Amp, Tet, Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
15	Amp, Tet, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
16	Amp, Amo, Tri, Fos	1	1650 – 1900	*dfrA17*	C, P
17	Amp, Tet, Amo, Nal, Tri, Nor, Chl	1	2000	*dfrA12-aadA2*	C, P
18	Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
19	Amp, Tet, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
20	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P
21	Amp, Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
22	Tri, Cef, Chl, Fos	1	2000	*dfrA17*	C, P
23	Amp, Tet, Amo, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
24	Amp, Amo, Nal, Tri	1	1650 – 1750	*dfrA17*	C, P
25	Tet, Amo, Tri, Cep	1	1650 – 1900	*dfrA17*	C, P
26	Tet, Tri, Chl	1	800	*dfrA17*	C, P
27	Amp, Amo, Tri	1,2	1650 – 1900	*dfrA17*	C, P
28	Amp, Tet, Amo, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA17*	C, P
29	Amp, Tet, Amo Nal, Tri, Nor, Chl, Cip	1	1650 – 1900	*dfrA17*	C, P
30	Tet, Amo, Nal, Nor, Tri, Chl	1	2000	*dfrA17*	C, P
31	Amp, Tet, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA17*	C, P
32	Amp, Tet, Amo, Tri, Cep	1	1650 – 1900	*dfrA12-aadA2*	C, P
33	Amp, Tet, Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA12-aadA2*	C, P
34	Amp, Tet, Cep, Amo, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
35	Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
36	Tet, Tri	1,2	2000	*dfrA12-aadA2*	C, P
37	Amp, Tet, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
38	Amp, Amo, Tri, Fos	1	1650 – 1900	*dfrA17*	C, P
39	Amp, Tet, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P

Table 3.2 continued…

Isolate no:	Resistant Phenotype	*intI* gene	Gene cassettes: amplicon size (bp)	Gene cassette array	Integron location
40	Amp, Tet, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
41	Tet, Gen, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
42	Amp, Tet, Amo, Nal, Tri, Nor, Chl	1	1650 – 1900	*dfrA17*	C, P
43	Amp, Amo, Tri	1	1650 – 1900	*dfrA17*	C, P
44	Tet, Tri, Cep, Chl	1	1650 – 1900	*dfrA17*	C, P
45	Amp, Amo, Tri	1	1650 – 1900	*dfrA17*	C, P
46	Amp, Tet, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA17*	C, P
47	Tet, Tri, Chl	1	2000	*dfrA17*	C, P
48	Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
49	Tet, Amo, Tri, Cep	1	1650 – 1900	*dfrA17*	C, P
50	Amp, Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
51	Tet, Tri, Cep, Chl	1	1650 – 1900	*dfrA12-aadA2*	C, P
52	Amp, Tet, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA12-aadA2*	C, P
53	Amp, Nal, Tri, Nor	1	100	*dfrA17*	C, P
54	Amp, Tet, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
55	Amp, Tet, Amo, Nal, Tri, Nor, Chl	1	1650 – 1900	*dfrA17*	C, P
56	Amp, Tet, Cep, Amo, Tri, Cef	1	1650 – 1900	*dfrA12-aadA2*	C, P
57	Tet, Gen, Tri, Chl	1	2000	*dfrA17*	C, P
58	Amp, Tet, Amo, Tri	1	1650 – 1900	*dfrA17*	C, P
59	Tet, Tri, Cep, Chl	1	1650 – 1900	*dfrA12-aadA2*	C, P
60	Amp, Tet, Tri	1	1650 – 1900	*dfrA17*	C, P
61	Amp, Tet, Amo, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
62	Tet, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
63	Amp, Tet, Amo Nal, Tri, Nor, Chl, Cip	1	1650 – 1900	*dfrA17*	C, P
64	Amp, Nal, Tri, Nor	1	2000	*dfrA17*	C, P
65	Amp, Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
66	Amp, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
67	Tet, Amo, Nal, Nor, Tri, Chl	1	1650 – 1900	*dfrA12-aadA2*	C, P
68	Amp, Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
69	Tet, Tri, Cep, Chl	1	1650 – 1900	*dfrA17*	C, P
70	Amp, Tet, Amo, Nal, Tri, Nor, Chl	1	1650 – 1900	*dfrA12-aadA2*	C, P
71	Amp, Tet, Amo, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA17*	C, P
72	Amp, Tet, Amo, Tri, Cep	1	1650 – 1900	*dfrA17*	C, P
73	Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
74	Amo, Nal, Tri, Nor	1	800	*dfrA17*	C, P
75	Amp, Tet, Nal, Tri, Nor, Cip	1	2000	*dfrA12-aadA2*	C, P
76	Tet, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
77	Amp, Amo, Tri, Fos	1	1650 – 1900	*dfrA17*	C, P
78	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P

Table 3.2 continued…

Isolate no:	Resistant Phenotype	*intI* gene	Gene cassettes: amplicon size (bp)	Gene cassette array	Integron location
74	Amo, Nal, Tri, Nor	1	800	*dfrA17*	C, P
75	Amp, Tet, Nal, Tri, Nor, Cip	1	2000	*dfrA12-aadA2*	C, P
76	Tet, Tri, Chl	1	1650 – 1900	*dfrA17*	C, P
77	Amp, Amo, Tri, Fos	1	1650 – 1900	*dfrA17*	C, P
78	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P
79	Tet, Tri, Cep, Chl	1	1650 – 1900	*dfrA17*	C, P
80	Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA12-aadA2*	C, P
81	Amp, Tet, Amo, Nal, Tri, Nor	1	1650 – 1900	*dfrA12-aadA2*	C, P
82	Amp, Tet, Nal, Tri, Nor	1	1650 – 1900	*dfrA17*	C, P
83	Amp, Tet, Amo, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA17*	C, P
84	Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA12-aadA2*	C, P
85	Amp, Tet, Nal, Tri, Nor	1	1650 – 1750	*dfrA17*	C, P
86	Amp, Tet, Amo Nal, Tri, Nor, Chl, Cip	1	1650 – 1900	*dfrA17*	C, P
87	Amp, Tet, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
88	Amp, Tet, Cep, Amo, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
89	Tet, Tri	1	1650 – 1900	*dfrA17*	C, P
90	Tet, Tri	1	1650 – 1900	*dfrA17*	C, P
91	Amp, Tet, Amo, Nal, Tri, Nor, Cip	1	1650 – 1900	*dfrA12-aadA2*	C, P
92	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P
93	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P
94	Tet, Gen, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
95	Amp, Tet, Tri	1	1650 – 1900	*dfrA17*	C, P
96	Amp, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
97	Tri, Cef, Chl, Fos	1	1650 – 1900	*dfrA17*	C, P
98	Amp, Amo, Nal, Tri	1	1650 – 1900	*dfrA17*	C, P
99	Amp, Tet, Amo, Tri, Cep	1	2000	*dfrA12-aadA2*	C, P
100	Amp, Tet, Tri, Cef	1	1650 – 1900	*dfrA17*	C, P
101	Tet, Amo, Tri, Cep	1	2000	*dfrA17*	C, P

Amp: Ampicilin; Tet: Tetracycline; Chl: Chloramphenicol; Amo: Amoxycillin; Nal: Nalidixic acid; Nor: Norfloxacin; Cip: Ciprofloxacin; Tri: Trimethoprim; Tet: Tetracycline; Gen: Gentamicin; Fos: Fosfomycin; Cef: Ceftadizime; Cep: Cephalothin; C: Chromosome; P: Plasmid

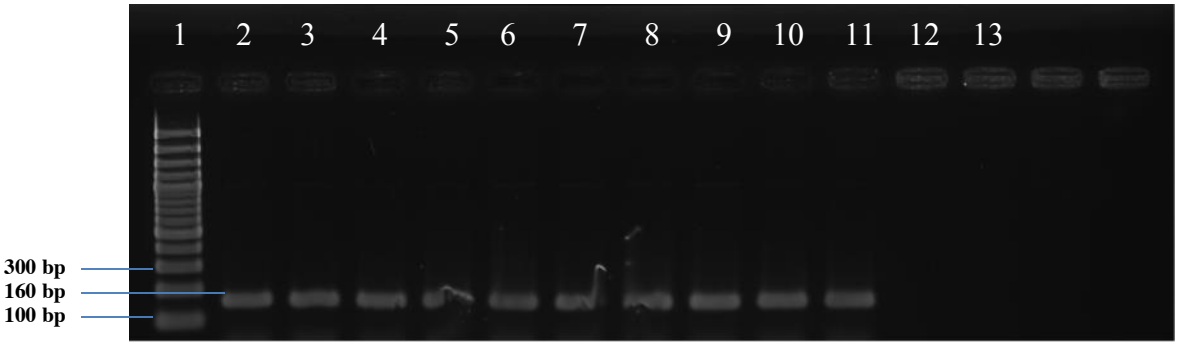


Figure 3.3: Agarose gel showing the presence of class 1 integrons in positive transconjugants (n=10)

Where: Lane 1: Molecular weight marker; Lanes 2-10: Amplification of class 1 integron (160bp) in positive transconjugants

3.3.3 RAPD analysis of integron positive *E. coli* isolates

Analysis of RAPD-PCR patterns obtained with primer 1247 for all integron bearing isolates resulted in the UPGMA dendogram depicted in Figure 4. The dendogram indicates the presence of 7 clusters. Cluster I comprised of 7 isolates, with majority of isolates having similar resistance phenotypes. Majority of the isolates in cluster I (85.71%) were resistant to the penicillin and tetracycline class of antibiotics whilst no isolates showed resistance to fosfomycin. Cluster II comprised a large portion of the total number of isolates tested with 31.68% (31/101) of tested isolates being grouped into this cluster. Similarities to cluster I was observed in which a majority of isolates were resistant to the penicillin (ampicillin and amoxicillin) and tetracycline (tetracycline) class of antibiotics. However, the frequency of tetracycline resistance was lower in isolates belonging to cluster II when compared to those in cluster I. In addition, 4 isolates (12.50%) showed resistance to fosfomycin, which is a phenotypic trait not observed in isolates belonging to cluster I. Cluster III is the largest cluster within the group which contains 36.63% of the tested

isolates. Once again, resistance to tetracycline was prevalent among the isolates in this cluster followed by resistance to ampicillin (59.46%) and amoxicillin (54.05%). However, isolates in cluster III showed an increased resistance to cephalothin (18.92%) and ciprofloxacin (18.92%), and a decrease in resistance to the penicillin class of antibiotics when compared to clusters I and II. Resistance to fosfomycin was not observed in this cluster. Cluster IV contained 6 isolates, all of which are susceptible to ciprofloxacin and gentamicin. Fifty percent of isolates in this cluster exhibited resistance towards ampicillin, amoxicillin, tetracycline, norfloxacin and nalidixic acid. All *E. coli* isolates in cluster V (n=7) showed resistance to tetracycline and susceptibility towards fosfomycin. Cluster VI comprises of 9 isolates, all of which show susceptibility to cephalothin and ciprofloxacin. The highest resistance was observed towards tetracycline (55.56%) and the penicillin class of antibiotics (ampicillin: 66.67%; amoxicillin: 55.56%). In addition a decrease in resistance towards norfloxacin (11.11%), which belongs to the class quinolones was noted in this cluster when compared to previously defined clusters. All isolates belonging to cluster VII (n=3) showed susceptibility towards gentamicin (aminoglycosides), chloramphenicol (phenicols), fosfomycin (fosfomycins) and all tested antibiotics belonging to the quinolone class (norfloxacin, ciprofloxacin and nalidixic acid). Tetracycline resistance (100%) was observed in all isolates within this cluster. All isolates tested (n=101) displayed phenotypic resistance towards trimethoprim.

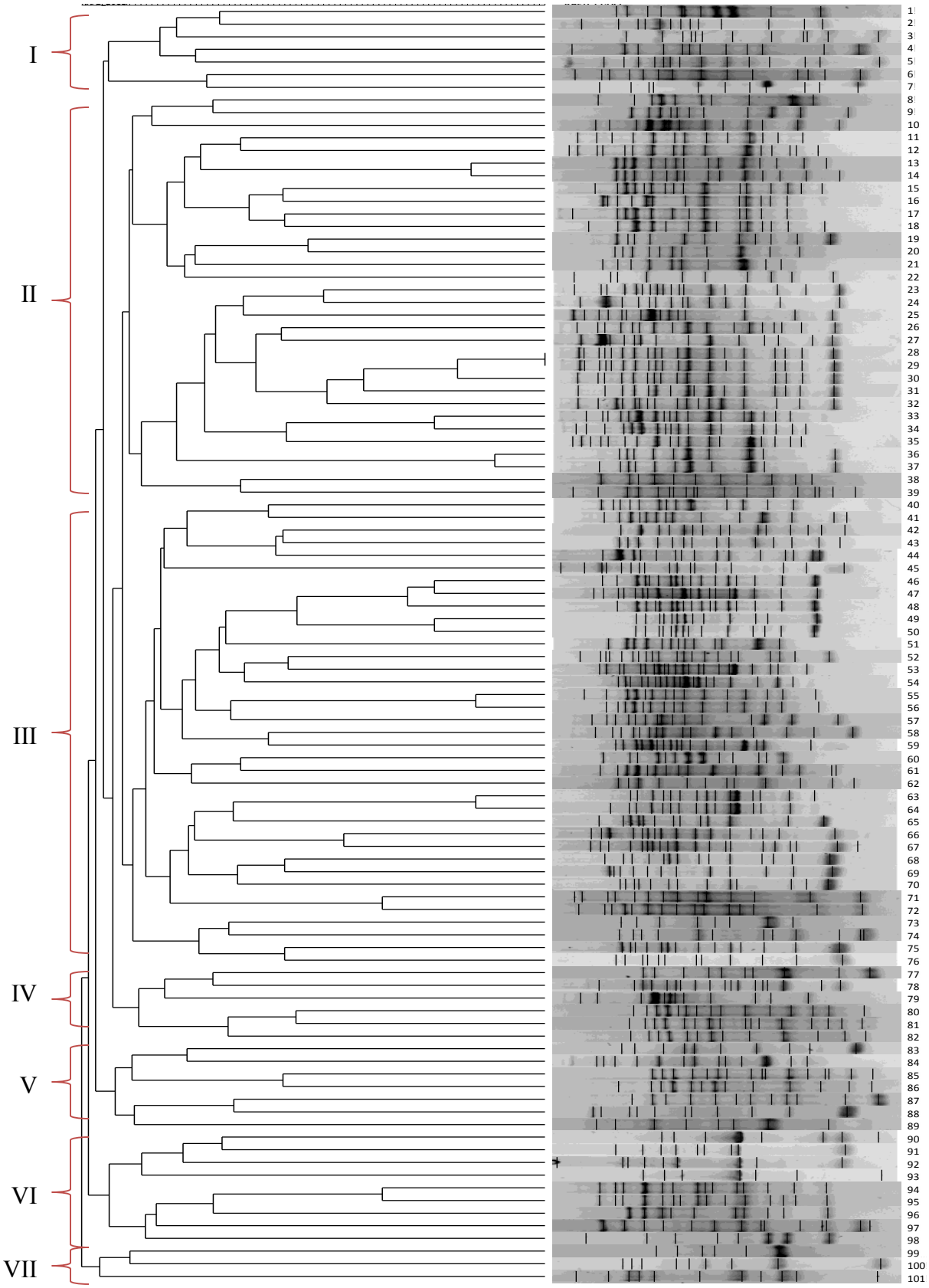


Figure 3.4: UPGMA dendogram derived from a comparison of RAPD patterns of *E. coli* isolates recovered from treated effluent of wastewater treatment plants and the receiving rivers

3.4 Discussion

Integrons play a major role in the dissemination of antibiotic resistance genes in the environment. The detection frequency of integrons (50.5%) in the present study (Table 3.2) is much higher than reported values for *E. coli* isolates from surface water studies in France (11%) (Laroche *et al*., 2009), and the Czech Republic (29.6%) (Dolejska *et al*., 2009), and higher than values obtained using clinical isolates (Vinue *et al*., 2010). This may suggest that a stronger selective pressure exists in the WWTPs and respective receiving surface waters chosen for this study. The results obtained in this study is consistent with previous studies by Chen *et al*. (2010) in which only classes 1 and 2 integrons were detected. The high prevalence of class 1 integrons over class 2 integrons obtained in this study is also consistent with studies by Han *et al*. (2012). The results demonstrate that class 1 integrons are more prevalent as antibiotic resistant *E. coli* strains in the Durban area. Class 3 integrons are considered rare even in clinical isolates and were not detected in this study. So far, only a few class 3 integrons have been studied in detail (Correia *et al*., 2003; Lu *et al*., 2010).

The prevalence of gene cassettes observed in this study is higher than that obtained in similar studies (Lu *et al*., 2010). However, the identity of the gene cassettes in the present study was similar to that reported by Lu *et al*. (2010) and Sanchez *et al*. (2002) in which resistance to trimethoprim and aminoglycosides were widespread among isolates harboring class 1 integrons. Results from this study also corroborate previous reports by Povilonis *et al*. (2010) and Lu *et al*. (2010) implicating dihydrofolate reductase genes (*dfrA12*, *dfrA1* and *dfrA17*) as the most prevalent genes found in integron positive isolates. This was often accompanied by *aadA1* or *aadA2* which confers resistance to aminoglycosides (Lu *et al*., 2010). These two types belong to the most common integron types found in *E. coli* (van Meervenne *et al*., 2013). However, not all isolates

which harbored the *aadA1* cassettes in this study exhibited phenotypic resistance towards aminoglycosides. There were no novel gene cassette arrangements or gene cassettes found in the *E. coli* isolates in this study when compared to previous studies.

In this study, high resistance rates of *E. coli* to tetracycline, ampicillin and amoxicillin was observed. This most likely arose from the long term and widespread use of these antimicrobials for the treatment of various diseases in clinical and agricultural settings in South Africa. According to Mellon *et al*. (2011), the most extensively sold antibiotics in South Africa include: tylosin, tetracycline, sulfonamides and penicillins. In addition, the application of these drugs in food and animals in particular may lead to a selection of resistant strains of bacteria, which could lead to treatment problems in infected humans and animals. The highest resistance amongst integron positive isolates was observed towards sulfonamides, tetracyclines, penicillins and phenicols. Integron positive strains were more resistant to these classes of antibiotics when compared to integron negative strains. The resistance to trimethoprim observed in this study can be attributed to the presence of integrons while resistance to tetracycline and ampicillin could be due to the association of mobile integrons with other MGEs such as plasmids and transposons (White *et al*., 2001; van Meervenne *et al*., 2013).

According to Vo *et al*. (2006), the location of antimicrobial resistance genes in the bacterial chromosome contributes to clonal spread of resistance genes on the conjugative plasmid, which promotes horizontal transfer. Hsu *et al*. (2006) demonstrated that class 1 integrons were located on conjugative plasmids, while others reported integrons to be located mainly on the chromosome (Vo *et al*., 2006). In the current study, integrons were found on both the chromosome and plasmid in all isolates, indicating that there were both vertical and horizontal transfers of the resistance gene among these isolates. Previous studies on the location of integrons have also revealed the

presence of 70.7% of class 1 integrons on both the plasmid and chromosome (Lu *et al*., 2010). The current study also demonstrated that plasmid encoded class 1 integrons could be transferred via conjugation to recipient strains, similar to the observations in previous reports (Zhang *et al*., 2009). The transfer of integrons via conjugation enables resistance determinants on gene cassettes to be transferred to strains devoid of such traits, thus contributing to the dissemination of antibiotic resistance genes (ARGs) in bacteria. It can be said that the environment in a WWTP could facilitate HGT as they combine several favourable factors namely: a high cell density sustained by a nutrient rich environment, and a recurrent contamination with both antibiotics and antibiotic resistant bacteria (ARB) (Rizzo *et al*., 2013).

The RAPD technique accurately differentiates strains of *E. coli* by means of the number and position of the amplified DNA fragments. Polymorphism is then observed and rated on the presence or absence of a DNA fragment and relates sequence variation due to nucleotide insertion, deletion or substitution (McGregor *et al*., 2000). The greater the differences in the individual patterns, the more unrelated the strains are from one another (Fei *et al*., 2003). This technique was used to confirm the phenotypic and genotypic identities of integron bearing, *E. coli* strains and to determine if these strains shared any clonal relationships. All isolates with similar banding patterns were grouped into a single cluster, resulting in the presence of 7 distinct clusters as shown by the UPGMA dendogram (Figure 3.4). All clusters contained many sub-clusters, indicating that there is considerable polymorphism amongst the strains in each group. However, all strains containing similar resistant phenotypes appear to be genetically related. This is confirmed by the presence of many roots within the tree, which not only indicate a relationship between strains in the same cluster but show a common link between *E. coli* strains in all clusters. It can be assumed that this close relationship may be due to the presence of integrons, which have been shown to

have the ability to move between strains and confer resistance to antibiotics.

3.5 Conclusion

In summary, the present study characterized and compared antibiotic resistant *E. coli* isolates obtained from treated wastewater effluent and receiving aquatic milieu. The presence of integrons was also determined and the impact of their presence within antibiotic resistant isolates was evaluated. In addition, the ability to transfer plasmids encoding resistance determinants via conjugation was evaluated. The evidence suggests that WWTPs are potential hotspots for ARB and could possibly promote the dissemination of ARGs amongst bacteria due to the unique selective pressures that exist within this environment. The clonal relationships seen between antimicrobial resistant, integron bearing strains highlights the fact that isolates exhibiting resistance to certain classes of antibiotics are most often related. This may be due to a common ancestor or may be attributed to the presence of MGEs, which transfer resistance genes found in gene cassettes. The RAPD technique is therefore useful in characterizing isolates, which may have experienced genomic changes before or after resistance has developed. The current study is in agreement with previous reports by Gallert *et al*. (2005), Ferreira daSilva *et al*. (2006), Goñi-Urriza *et al*. (2000), Baquero *et al*. (2008), Martínez (2009), and Servais and Passerat (2009) on antimicrobial resistance in WWTPs, which have all indicated that WWTPs are among the leading reservoirs of ARB and ARGs in the environment. This calls for more stringent monitoring of antimicrobial use or the search for alternate treatment options in an attempt to prevent the spread of multi-drug resistant bacteria worldwide.

CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSION

4.1 Research in perspective

Water remains as an essential resource needed for everyday activities. Usafe and insufficient drinking water, and inadequate sanitation are frequently identified as one of the principal causes of water-related diseases (DWAF, 2013). Unrelenting urbanization as a result of increased population also places strain on already stressed water supplies (Ashbolt, 2004). Inadequately treated effluent from wastewater treatment plants (WWTPs) are largely responsible for the high microbial loads found in receiving surface waters. These microbial populations are resistant to commonly used antibiotics and have the potential to cause disease (Stalder *et al*., 2012). In addition, they have the ability to transfer resistant determinants, which pose challenges to the treatment of bacterial infections in clinical settings.

Bacterial analysis of the treated effluent and receiving surface water samples from the Northern wastewater treatment plant (NWWTP) and the New Germany treatment works (NGTWs) as presented in chapter two, indicated variability in *E*. *coli* populations obtained from all sampling points. It can be deduced that the larger of the plants, NWWTP was inefficient in reducing *E*. *coli* populations for 3 out of the 6 months of sampling. Treatment efficiency rates of 62.82%, 25.00% and 19.25%, were recorded for the months of May, June and July respectively. The higher microbial loads obtained at the DP during March, April and August at NWWTP may be attributed to the upgrade of plant infrastructure going on at the time. However, poorly maintained outfall pipes seen at the plant were not being upgraded and this may be one of the major contributing

factors to the increased bacterial load. The discharge of waste by informal settlement dwellers into the surface waters did not seem to impact the microbial load obtained at the US and DS points of the uMgeni River as *E. coli* populations at these points were relatively low when compared to the DP which is directly under the influence of the wastewater treatment plant. The low populations obtained downstream of the uMgeni River receiving effluent from the NWWTP may be due to the dilution effect of "clean" water flowing from the US point. In contrast, the NGTWs appeared to be efficient, having the ability to reduce *E. coli* populations by over 93% for 4 out of the 6 months of sampling. This efficiency may be attributed to the smaller operating capacity of the plant as well as the influx of chemicals and dyes from nearby industries, which were suspected to have inhibitory effects on the *E. coli* populations. However, although both plants displayed the ability to treat wastewater, this was not to acceptable standards as stipulated by the Department of Water Affairs, which requires 0 CFU/ml for *E. coli* in water used for domestic purposes. Results from this study therefore highlight the ineffectiveness of WWTPs in question. In addition, it demonstrates the ability of certain strains of *E. coli* to survive tertiary treatment (chlorination) and pollute receiving surface waters.

The physico-chemical profile of any water sample is easily affected by the wastewater treatment process. The amount of dissolved oxygen is easily affected by the temperature of the water during the treatment process, while the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) is affected by the influx of organic waste, as well as failing septic systems. This study showed that although the NWWTP was not as efficient in reducing the *E. coli* loads compared to NGTWs, it was efficient in reducing majority of the physico-chemical parameters tested. BOD and COD concentrations for the NWWTP showed that this plant is not the only source of pollution

of receiving surface waters, but that the NGTWs definitely is a major contributor to the bacterial pollution of the Aller River. This is evident by an increase in COD, TDS, nitrate and phosphate levels at the DP of the NGTWs after the treatment process. Elevated concentrations of any of these parameters has been shown to alter the normal biological functioning of ecosystems, which not only affects freshwater life, but human populations too (DWAF, 1996). The physico-chemical profile of the water samples studied, discourages the use of this water for domestic purposes based on the South African water quality guidelines for domestic use (DWAF, 1996).

The virulence gene and antimicrobial resistance profile of *E. coli* isolates were also investigated and reported in chapter two and provide further insight into the potentially harmful nature of *E. coli* isolates being discharged into surface waters as a result of improperly treated wastewater effluent. Determination of the presence of *stx1*, *stx2*, *hly*, *eaeA* and *fliC* genes revealed that the frequency of these genes in *E. coli* isolates detected in the current study were lower than that of previous studies. No definite conclusions could be drawn without further research. However, it should be noted that 14% of tested isolates contained the *eaeA* gene in conjunction with either *stx1* or *stx2*, which is characteristic of *E. coli* having the ability to cause hemorrhagic colitis. In addition, this study showed that certain isolates (18%) are now resistant to drugs (chloramphenicol), which is worrisome as these drugs are only reserved for life threatening situations. In 2014, the World Health Organization (WHO) named *E. coli* as an organism of interest due to the rise of resistance towards fluoroquinolones (WHO, 2014). As presented in chapter two, the *E. coli* isolates exhibited 20.5% and 21.5% resistance towards norfloxacin and ciprofloxacin respectively, which are antibiotics belonging to fluroquinolone class of antibiotics. Trends of resistance observed towards gentamicin and the β-lactam group of antimicrobial agents were similar to that of Ram *et al*. (2008)

and Luczkiewicz *et al*. (2010), while an increase in resistance towards the β-lactam group was observed when compared to previous studies conducted in the Durban area in 2009 (Olaniran *et al*., 2009). Rizzo *et al*. (2013) has indicated that WWTPs are one of the main sources of antibiotics, antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) into the environment.

Resistance to antimicrobial agents has been attributed to the spread of mobile genetic elements (MGEs) which confer resistance to other organisms (Olaniran *et al*., 2009;WHO, 2014). MGEs play an important role in the dissemination of ARGs in the environment through the mobilization of gene cassettes by horizontal gene transfer (HGT). The frequency of integrons in the current study (50.5%) is much higher than reported values from previous surface water studies in France (11%) and the Czech Republic (29.6%) (Dolejska *et al*., 2009; Laroche *et al*., 2009). To the best of our knowledge, no study has been undertaken to determine the presence of integrons within *E. coli* isolates obtained from effluents of WWTPs and receiving surface waters in Durban, South Africa. The detection of class 1 and 2 integrons were consistent with previously reported studies as the presence of class 3 integrons in *E. coli* is rare. The presence of the *dfrA17*/*dfrA12* and *aadA2* gene cassettes through RFLP sequence analysis were also consistent with previous reports (Sanchez *et al*., 2002;Lu *et al*., 2010). The presence of these specific cassettes corresponded to the phenotypic characteristics displayed by isolates harboring class 1 integrons, in which both resistance to trimethoprim and aminoglygosides were noted.

As the mobilization of integrons via conjugation allows for resistance determinants on gene cassettes to be transferred, conjugation experiments involving integron positive strains were carried out as mentioned in chapter three. It was observed that all integron-bearing isolates

contained integrons on both the plasmid and chromosome, indicating that horizontal and vertical transfers of resistance genes between strains did exist. Additionally, these plasmids could be transferred to isolates devoid of integrons, emphasizing the fact that the rise in antimicrobial resistance may be due to the transfer of resistance genes to non-resistant strains. A review by Rizzo *et al*. (2013) suggests that WWTPs provide a favourable environment for the facilitation of HGT due to the nutrient rich environment and a recurrent contamination of antibiotics. Further analysis of integron bearing isolates using the RAPD-PCR technique indicated that isolates having similar resistance patterns could be grouped together, indicating a common linkage. Linkages (roots), which can be seen in the UPGMA dendogram presented in chapter three indicates a relationship between all strains and possibly a common ancestor. It can also be speculated that this relationship involves the transfer of integrons.

The overall findings of this study emphasizes the need for constant evaluation and monitoring of WWTPs by the relevant authorities, to ensure that plants are efficient and comply with the set guidelines. This will in-turn ensure better public health and safety. This study indicates that WWTPs are indeed hotspots for antibiotic resistance, and that the inefficiency of WWTPs resulted in the discharge of potentially pathogenic and multi-drug resistant bacteria into surface waters used for domestic purposes. In addition, these bacteria had the ability to transfer resistance determinants through HGT, which further compounds the problem of an ever-increasing rise in global antibiotic resistance.

4.2 Potential for future development of the study

The epidemiological importance of pathogenic *E. coli* continues to be of public health concern (EFSA, 2011; Dadie *et al*., 2014). Although all *E. coli* strains are potentially virulent, only some have acquired virulence factors and are responsible for the recent outbreaks of digestive and extra –intestinal infections as observed in Germany in 2011 and the USA in 2014 (Dadie *et al*., 2014). According to Dadie *et al*. (2014), one of the alternatives for the control of such infections involves epidemiological surveillance, which itself would be based on spatio-temporal documentation of phenotypic and molecular determinants of pathotypes. The data generated would be useful in implementing outbreak prevention models, to reveal the diversity of *E. coli* strains, risk factors, and also provide traceability and evolutionary history of pathotypes (Dadie *et al*., 2014). Therefore, future work on these *E. coli* isolates would involve the differentiation of isolates into pathotypes based on the PCR detection of virulence genes, which are specific to each pathotype. It is also proposed that the sample size be increased so as to maximize the chances of detecting the desired pathotypes. Pathotypes to be investigated would include: enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and uropathogenic *E. coli* (UPEC). Uropathogenic *E. coli* (UPEC) is responsible for over 90% of urinary tract infections (UTIs). Cytotoxic necrotozing factor type 1 (CNF-1) is a bacterial toxin, which is found in certain UPEC and diarrheagenic strains of *E. coli* and which is known to target the Rho family of small GTP-binding proteins (Mills *et al*., 2000). Reports by Mills *et al*. (2000) show that this toxin has a number of effects on mammalian tissue, some of which include: necrosis of skin with intradermal inoculation of *E. coli* lysates containing the toxin; enlargement and multinucleation of tissue culture cells after treatment with crude toxin preparations; actin stress fiber formation after

exposure to CNF-1; phagocytic activity in human Hep-2 epithelial cells and impairment of cell repair in human bladder cells. Although the effects of CNF-1 have been investigated, the exact role of CNF-1 in the pathogenesis of UTIs remain unknown. It is therefore important that the contribution of CNF-1 to uropathogenesis be investigated so as to add to the pool of existing knowledge. Thus far, no work has been done in the Durban area involving UPEC strains obtained from wastewater and the effect of resulting toxins on human cell lines from relevant physiological sites. It would be of interest that future work involve determining the effect of CNF-1 on human bladder, ureter and kidney cell lines. All cell lines would be treated with CNF-1 and the intoxication phenotype would be determined using specific assays and immunofluorescence microscopy. Intoxication phenotypes to be evaluated include cytotoxicity, actin fiber formation, Rho modification and multi-nucleation (Mills *et al*., 2000). The relationship between virulent clones of *E. coli* and their phylogenetic groups can also be established, therefore, molecular serotyping (by PCR-RFLP detection of the operon O rfb gene) and differentiation into distinct phylogenetic groups could also provide valuable insight into the study, as data on the molecular characteristics of pathogenic *E. coli* isolated from wastewater in South Africa is rare.

Virulent strains of *E. coli* may also possess other traits, which make infections mediated by them difficult to treat. Pathogens carrying Extended-Spectrum-β-Lactamases (ESBLs) are one of the main challenges to antibiotic therapy (Zarfel *et al*., 2013). ESBLs are defined as enzymes, which have the ability to hydrolyze penicillins, aztreonam and 1st, 2nd, and 3rd generation cephalosporins. They are mainly found in the family Enterobacteriaceae, in particular *E. coli* and *Klebsiella* (Zarfel *et al*., 2013). Till now, over 200 different ESBL genes encoding β-lactamases have been identified. According to Zarfel *et al*. (2013), these genes are genetically diverse and highly mobile, due to

their presence on MGEs. In addition, HGT is said to play an important role in spreading resistance to different strains. Not only has ESBL-producing bacteria been found in wastewater, but, the distribution of ESBL genes in non-human reservoirs is said to be significantly different (Zarfel *et al*., 2013). Since the concepts of antibiotic resistance, virulence genes, MGEs and HGT has already been explored, it would be interesting to establish if any relationships exist between ESBL producing *E. coli* within this group. Analysis of environmental samples of *E. coli* in this regard is important to understand how antibiotic resistance is transferred to humans (Zarfel *et al*., 2013).

Antibiotic resistant bacteria and associated resistance determinants have become an important environmental contamination issue. Information on the prevalence of ARB which are potentially pathogenic, could serve as a basis for proper selection of optimal wastewater treatment processes as it has been established that WWTPs and receiving surface waters are reservoirs for ARGs and ARB (Titilawo *et al*., 2015). Genes encoding resistance towards aminoglycosides, sulfonamides, tetracyclines and β-lactams have been frequently detected in bacterial isolates from wastewater (Lee *et al*., 1998; Lin and Biyela, 2005; Park *et al*., 2003; Schwartz *et al*., 2003; Mukherjee and Chakraborty, 2006; Poppe *et al*., 2006). Numerous waterborne outbreaks have been attributed to multi-drug resistant *E. coli* worldwide, and the high prevalence of multi-drug resistance warrants a need for antibiotic surveillance and the planning of active interventions to reduce drug resistance worldwide (Olayinka *et al*., 2004; Titilawo *et al*., 2015). It is then proposed that future work involve the testing of a broader range of antibiotics using the Kirby-Bauer disc diffusion assay. Isolates will be exposed to a panel of antibiotics not previously tested, which include: streptomycin, kanamycin, neomycin, cefepime, meropenem, imipenem, gatilofloxacin,

sulfamethoxazole and nitrofurantoin. In addition, isolates will be screened for the presence of 19 ARGs belonging to 5 classes of antibiotics.

According to Yu *et al*. (2010), resistance to the aminoglycosides class of antibiotics is becoming a serious clinical problem. Gram-negative rods such as *E. coli* produce many aminoglycoside-modifying enzymes such as phosphotransferases, nucleotidyltransferases and acetyltransferases (Bercot *et al*., 2011). In addition, plasmid-mediated 16S rRNA methylases identified in members of the family *Enterobacteriaceae* have been shown to confer high levels of resistance to aminoglycosides (Bercot *et al*., 2011). These methylases have now emerged as unique mechanisms for high-level resistance to arbekacin, amikacin, tobramycin and gentamicin, which has become quite problematic in clinical settings (Yu *et al*., 2010). To date, six plasmid-encoded methylases (ArmA, RmtA, RmtB, RmtC, RmtD and NpmA) have been identified in gram-negative bacilli, and many of these have been identified specifically in *E. coli* (Yu *et al*., 2010; Bercot *et al*., 2011). In addition to this, recent studies have shown that 16S rRNA methylase genes are most commonly detected in ESBL producing isolates which harbor a gene encoding a carbapenemase NDM-1, which further exacerbates the issue of multi-drug resistance. There is clearly an emerging spread of 16S rRNA methylase producers and epidemiological data regarding 16S rRNA methylase production in *E. coli* isolates in South Africa is lacking. Future work on these *E. coli* isolates from wastewater would involve the identification of carbapenemase NDM-1, screening of the isolates for methylase genes via PCR, as well as conjugation experiments to determine if aminoglycoside resistance is transferable in 16S rRNA methylase bearing plasmids. The relationship between the ESBL producing isolates, isolates harboring carpapenemase NDM-1 and methylase producers will also be established.

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APPENDIX I: MICROBIAL ANALYSIS

A: Northern Wastewater Treatment Plant

Table 1: CFU/ml obtained across all sampling points in March

NWWTP: March												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	212	4.24	30	0.60	6	0.12	2	0.04
BC 2	-	-	Tmtc	-	272	5.44	23	0.46	4	0.08	0	0
DP 1	Tmtc	-	528	10.56	312	6.24	-	-	-	-	-	-
DP 2	Tmtc	-	1040	20.80	720	14.40	-	-	-	-	-	-
US 1	-	-	-	-	19	0.38	8	0.16	4	0.08	1	0.02
US 2	-	-	-	-	4	0.08	10	0.20	1	0.02	2	0.04
DS 1	-	-	-	-	40	0.80	1	0.02	1	0.02	0	0
DS 2	-	-	-	-	30	0.60	4	0.08	0	0	2	0.04

Table 2: CFU/ml obtained across all sampling points in April

NWWTP: April												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	320	6.4	108	2.16	40	0.80	3	0.06
BC 2	-	-	Tmtc	-	340	6.8	115	2.30	44	0.88	6	0.12
DP 1	Tmtc	-	Tmtc	-	Tmtc	-	348	6.96	168	3.36	79	1.58
DP 2	Tmtc	-	Tmtc	-	Tmtc	-	232	4.64	153	3.06	80	1.60
US 1	-	-	-	-	22	0.44	8	0.16	1	0.02	0	0
US 2	-	-	-	-	35	0.70	4	0.08	0	0	10	0.20
DS 1	-	-	-	-	580	11.60	103	2.06	12	0.24	0	0
DS 2	-	-	-	-	512	10.24	96	1.92	15	0.3	3	0.06

Table 3: CFU/ml obtained across all sampling points in May

NWWTP: May												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	Tmtc	Tmtc	-	Tmtc	-	132	2.64	35	0.7
BC 2	-	-	Tmtc	Tmtc	Tmtc	-	Tmtc	-	180	3.6	29	0.58
DP 1	-	-	Tmtc	Tmtc	Tmtc	Tmtc	Tmtc	-	48	0.96	38	0.76
DP 2	Tmtc	-	Tmtc	Tmtc	Tmtc	Tmtc	Tmtc	-	68	1.36	40	0.8
US 1	Tmtc	-	-	-	39	0.78	11.00	0.22	4.0	0.08	2	0.04
US 2	-	-	-	-	37	0.74	8.00	0.16	5.0	0.10	1.08.08	0.02
DS 1	-	-	-	-	30	0.60	5.00	0.10	0	0	0	0
DS 2	-	-	-	-	34	0.68	5.00	0.10	0	0	0	0

Table 4: CFU/ml obtained across all sampling points in June

NWWTP: June												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	Tmtc	Tmtc	-	80	1.6	13	0.26	12	0.24
BC 2	-	-	Tmtc	Tmtc	Tmtc	-	100	2	17	0.34	9	0.18
DP 1	-	-	Tmtc	Tmtc	Tmtc	Tmtc	67	1.34	10	0.2	5	0.1
DP 2	-	-	Tmtc	Tmtc	Tmtc	Tmtc	68	1.36	16	0.32	4	0.08
US 1	-	-	-	-	Tmtc	-	13	0.26	2	0.04	0	0
US 2	-	-	-	-	Tmtc	-	10	0.2	6	0.12	1	0.02
DS 1	-	-	-	-	Tmtc	-	17	0.34	12	0.24	5	0.1
DS 2	-	-	-	-	Tmtc	-	10	0.2	8	0.16	11	0.22

Table 5: CFU/ml obtained across all sampling points in July

NWWTP: July												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	Tmtc	-	75	1.5	7	0.14	0	0
BC 2	-	-	Tmtc	-	Tmtc	-	69	1.38	5	0.1	0	0
DP 1	6	0.12	3	0.06	0	0	0	0	0	0	0	0
DP 2	8	0.16	1	0.02	0	0	0	0	0	0	0	0
US 1	-	-	-	-	22	0.44	5	0.1	1	0.02	0	0
US 2	-	-	-	-	19	0.38	3	0.06	0	0	0	0
DS 1	3	0.06	1	0.02	0	0	0	0	0	0	0	0
DS 2	4	0.08	0	0	0	0	0	0	0	0	0	0

Table 6: CFU/ml obtained across all sampling points in August

NWWTP: August												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	Tmtc	Tmtc	-	248	4.96	190	3.80	79	1.58
BC 2	-	-	Tmtc	Tmtc	Tmtc	-	245	4.9	185	3.70	86	1.72
DP 1	-	-	Tmtc	Tmtc	Tmtc	-	264	5.28	189	3.78	112	2.24
DP 2	-	-	Tmtc	Tmtc	Tmtc	-	265	5.3	193	3.86	99	1.98
US 1	-	-	-	-	36	-	9	0.18	6	0.12	1	0.02
US 2	-	-	-	-	41	-	12	0.24	3	0.06	2	0.04
DS 1	-	-	-	-	10	-	2	0.04	0	0	0	0
DS 2	-	-	-	-	9	-	1	0.02	0	0	0	0

Table 7: Average CFU/ml for March

Average cfu/ml x 10^3 : March				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	0.424	0.544	0.484	0.85
DP	0.624	1.44	1.032	5.77
US	0.038	0.038	0.038	0.21
DS	0.08	0.06	0.07	0.14

Table 8: Average CFU/ml for April

Average cfu/ml x 10^3 : April				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	2.16	2.3	2.23	2.27
DP	6.96	4.64	5.8	3.41
US	0.16	0.08	0.12	1.08
DS	2.06	1.92	1.99	0.10

Table 9: Average CFU/ml for May

Average cfu/ml x 10^3 : May				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	26.4	36	31.2	5.54
DP	9.6	13.6	11.6	2.31
US	0.8	1	0.9	0.12
DS	1	1	1	0.00

Table 10: Average CFU/ml for June

Average cfu/ml x 10^3 : June				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	1.6	2	1.8	0.23
DP	1.34	1.36	1.35	0.01
US	0.26	0.2	0.23	0.03
DS	0.34	0.2	0.27	0.08

Table 11: Average CFU/ml for July

Average cfu/ml x 10^3 : July				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	3.28	3.16	3.22	0.07
DP	2.64	2.56	2.6	0.05
US	0.7	0.6	0.65	0.06
DS	0.52	0.6	0.56	0.05

Table 12: Average CFU/ml for August

Average cfu/ml x 10^3 : August				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	4.96	245	4.93	0.08
DP	5.28	265	5.27	0.40
US	0.08	12	0.21	0.08
DS	0.04	1	0.03	0.06

B: New Germany Treatment Works

Table 1: CFU/ml obtained across all sampling points in March

NGTW: March												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	260	5.2	64	1.28	7	0.14	1	0.02
BC 2	-	-	Tmtc	-	-	-	56	1.12	4	0.08	1	0.02
DP 1	6	0.12	3	0.06	6	0.12	0	0	0	0	-	-
DP 2	11	0.22	3	0.06	0	0	0	0	0	0	-	-
US 1	-	-	-	-	184	3.68	8	34	1	0.02	0	0
US 2	-	-	-	-	232	4.6	10	17	3	0.06	1	0.02
DS 1	-	-	-	-	0	0	0	0	0	0	0	0
DS 2	-	-	-	-	0	0	0	0	0	0	0	0

Table 2: CFU/ml obtained across all sampling points in April

NGTW: April												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	48	0.96	11	0.22	1	0.02	0	0	0	0
BC 2	-	-	50	1	7	0.14	0	0	0	0	0	0
DP 1	-	-	0	0	0	0	0	0	-	-	-	-
DP 2	-	-	1	0.02	0	0	3	0.06	-	-	-	-
US 1	-	-	-	-	360	7.2	33	0.66	3	0.06	0	0
US 2	-	-	-	-	400	8	31	0.62	4	0.08	0	0
DS 1	-	-	-	-	0	0	0	0	0	0	2	0.04
DS 2	-	-	-	-	0	0	0	0	0	0	0	0

Table 3: CFU/ml obtained across all sampling points in May

NGTW: May												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	Tmtc	-	350	7	270	5.4	106	2.12
BC 2	-	-	Tmtc	-	Tmtc	-	325	6.5	258	5.16	123	2.46
DP 1	-	-	Tmtc	-	Tmtc	-	Tmtc	-	156	3.12	96	1.92
DP 2	-	-	Tmtc	-	Tmtc	-	Tmtc	-	140	2.8	80	1.60
US 1	-	-	-	-	19	0.38	7	0.14	2	0.04	0	0
US 2	-	-	-	-	15	0.30	7	0.14	0	0	0	0
DS 1	-	-	-	-	Tmtc	-	Tmtc	-	330	6.6	279	5.58
DS 2	-	-	-	-	Tmtc	-	Tmtc	-	300	6	280	5.60

Table 4: CFU/ml obtained across all sampling points in June

NGTW: June												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	Tmtc	-	140	2.8	13	0.26	3	0.06
BC 2	-	-	Tmtc	-	Tmtc	-	100	2	18	0.36	5	0.1
DP 1	-	-	Tmtc	-	Tmtc	-	100	2	16	0.32	6	0.12
DP 2	-	-	Tmtc	-	Tmtc	-	120	2.4	11	0.22	8	0.16
US 1	-	-	-	-	Tmtc	-	10	0.2	8	0.16	2	0.04
US 2	-	-	-	-	Tmtc	-	16	0.32	9	0.18	3	0.06
DS 1	-	-	-	-	Tmtc	-	60	1.2	18	0.36	2	0.04
DS 2	-	-	-	-	tmtc	-	54	1.08	16	0.32	3	0.06

Table 5: CFU/ml obtained across all sampling points in July

NGTW: July												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	Tmtc	-	75	1.5	7	0.14	0	0
BC 2	-	-	Tmtc	-	Tmtc	-	69	1.38	5	0.10	0	0
DP 1	6	0.12	3	0.06	0	0	0	0	0	0	0	0
DP 2	8	0.16	1	0.02	0	0	0	0	0	0	0	0
US 1	-	-	-	-	22	0.44	5	0.1	1	0.02	0	0
US 2	-	-	-	-	19	0.38	3	0.06	0	0	0	0
DS 1	3	0.06	1	0.02	0	0	0	0	0	0	0	0
DS 2	4	0.08	0	0	0	0	0	0	0	0	0	0

Table 6: CFU/ml obtained across all sampling points in August

NWWTP: August												
	1×10^0		1×10^1		1×10^2		1×10^3		1×10^4		1×10^5	
`	1		10		100		1000		10000		100000	
	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml	Count	CFU/ml
BC 1	-	-	Tmtc	-	51	1.02	9	0.18	4	0.08	0	0
BC 2	-	-	Tmtc	-	59	1.18	7	0.14	3	0.06	1	0.02
DP 1	-	-	2	0.04	0	0	0	0	0	0	0	0
DP 2	-	-	1	0.02	0	0	0	0	0	0	0	0
US 1	-	-	-	-	216	4.32	133	2.66	126	2.52	50	1
US 2	-	-	-	-	220	4.4	131	2.62	118	2.36	46	0.92
DS 1	-	-	-	-	29	0.58	15	0.3	4	0.08	1	0.02
DS 2	-	-	-	-	32	0.64	12	0.24	3	0.06	0	0

Table 7: Average CFU/ml for March

Average cfu/ml x 10^3 : March				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	0.52	1.12	0.82	0.53
DP	0.012	0	0.006	0.24
US	0.36	0.46	0.41	0.24
DS	0	0	0	0.00

Table 8: Average CFU/ml for April

Average cfu/ml x 10^3 : March				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	0.02	0	0.01	1.50
DP	0	3	1.5	1.32
US	0.66	0.62	0.64	0.37
DS	0	0	0	0.00

Table 9: Average CFU/ml for May

Average cfu/ml x 10^3 : May				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	7	6.5	6.75	0.29
DP	0.312	0.28	0.296	0.02
US	0.14	0.14	0.14	0.00
DS	0.66	0.6	0.63	0.03

Table 10: Average CFU/ml for June

Average cfu/ml x 10^3 : June				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	2	2.8	2.4	0.46
DP	2.4	2	2.2	0.23
US	0.2	0.32	0.26	0.07
DS	1.2	1.08	1.14	0.07

Table 11: Average CFU/ml for July

Average cfu/ml x 10^3 : July				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	1.5	1.38	1.44	0.07
DP	0.0006	0.0002	0.0004	0.00
US	0.1	0.06	0.08	0.02
DS	0.0002	0	0.0001	0.00

Table 12: Average CFU/ml for August

Average cfu/ml x 10^3 : August				
Sample Point	**Value 1**	**Value 2**	**Avg**	**Std dev**
BC	0.18	0.14	0.16	0.04
DP	0	0	0	0.00
US	2.66	2.62	2.64	0.10
DS	0.3	0.24	0.27	0.05

APPENDIX II: PHYSICOCHEMICAL ANALYSIS

A: Northern Wastewater Treatment Plant

Table 1: Temperature values obtained over the six month period

Temperature °C						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	26.00	26.00	26.00	26.00	0.00
	DP	25.00	25.00	25.00	25.00	0.00
	US	26.00	26.00	26.00	26.00	0.00
	DS	25.50	25.50	25.50	25.50	0.00
April	**BC**	22.00	22.00	22.00	22.00	0.00
	DP	22.00	22.00	22.00	22.00	0.00
	US	21.00	21.00	21.00	21.00	0.00
	DS	21.00	21.00	21.00	21.00	0.00
May	**BC**	22.00	22.00	21.80	21.93	0.12
	DP	21.00	21.00	21.00	21.00	0.00
	US	21.00	21.00	21.00	21.00	0.00
	DS	22.00	22.00	22.00	22.00	0.00
June	**BC**	13.50	13.50	13.50	13.50	0.00
	DP	13.00	12.50	12.50	12.67	0.29
	US	13.00	13.00	13.00	13.00	0.00
	DS	12.00	12.00	12.00	12.00	0.00
July	**BC**	14.80	14.80	14.80	14.80	0.00
	DP	15.50	15.20	15.20	15.30	0.17
	US	15.00	15.00	14.80	14.93	0.12
	DS	16.00	16.00	15.50	15.83	0.29
August	**BC**	21.00	21.00	21.00	21.00	0.00
	DP	19.00	19.00	19.00	19.00	0.00
	US	20.00	20.00	20.00	20.00	0.00
	DS	19.00	19.00	19.00	19.00	0.00

Table 2: Salinity of water samples obtained over the six month period

Salinity %						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	0.41	0.41	0.41	0.41	0
	DP	0.44	0.44	0.44	0.44	0
	US	0.37	0.37	0.37	0.37	0
	DS	0.35	0.35	0.35	0.35	0
April	**BC**	0.38	0.38	0.38	0.38	0
	DP	0.44	0.44	0.45	0.44	0.01
	US	0.49	0.49	0.49	0.49	0
	DS	0.33	0.33	0.33	0.33	0
May	**BC**	0.42	0.42	0.42	0.42	0
	DP	0.48	0.48	0.48	0.48	0
	US	1.07	1.08	1.08	1.08	0.01
	DS	0.30	0.30	0.30	0.30	0
June	**BC**	0.34	0.34	0.34	0.34	0
	DP	0.37	0.37	0.37	0.37	0
	US	0.53	0.54	0.54	0.54	0
	DS	0.31	0.31	0.31	0.31	0
July	**BC**	0.34	0.34	0.35	0.34	0.01
	DP	0.42	0.42	0.42	0.42	0
	US	0.71	0.72	0.72	0.72	0
	DS	0.29	0.29	0.29	0.29	0
August	**BC**	0.37	0.37	0.37	0.37	0
	DP	0.41	0.41	0.41	0.41	0
	US	0.44	0.44	0.44	0.44	0
	DS	0.33	0.33	0.33	0.33	0

Table 3: Conductivity of water samples over the six month period

Conductivity(µS/cm)						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	839	840	840	839.67	0.58
	DP	889	888	887	888	1
	US	755	758	760	757.67	2.52
	DS	714	713	717	714.67	2.08
April	**BC**	785	781	784	783.33	2.08
	DP	902	903	906	903.67	2.08
	US	1001	996	996	997.67	2.89
	DS	676	677	676	676.33	0.58
May	**BC**	853	853	853	853	0
	DP	966	966	966	966	0
	US	2116	2115	2116	2115.67	0.58
	DS	619	624	620	621	2.65
June	**BC**	702	703	704	703	1
	DP	759	755	758	757.33	2.08
	US	1080	1083	1084	1082.33	2.08
	DS	634	633	633	633.33	0.58
July	**BC**	718	725	731	724.67	6.51
	DP	851	849	849	849.67	1.15
	US	1424	1430	1432	1428.67	4.16
	DS	604	607	607	606	1.73
August	**BC**	753	752	759	754.67	3.79
	DP	841	840	839	840	1
	US	903	898	896	899	3.61
	DS	673	676	679	676	3

Table 4: Resistivity of the water samples over the six month period

Resistivity (Ω.cm)						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	1192.00	1191.00	1190.00	1191.00	1.00
	DP	1125.00	1126.00	1127.00	1126.00	1.00
	US	1320.00	1316.00	1325.00	1320.33	4.51
	DS	1402.00	1403.00	1395.00	1400.00	4.36
April	**BC**	1275.00	1280.00	1275.00	1276.67	2.89
	DP	1108.00	1108.00	1104.00	1106.67	2.31
	US	1004.00	1004.00	1004.00	1004.00	0.00
	DS	1480.00	1476.00	1480.00	1478.67	2.31
May	**BC**	1173.00	1173.00	1173.00	1173.00	0.00
	DP	1037.00	1035.00	1036.00	1036.00	1.00
	US	473.00	473.00	473.00	473.00	0.00
	DS	1616.00	1612.00	1609.00	1612.33	3.51
June	**BC**	1426.00	1423.00	1421.00	1423.33	2.52
	DP	1318.00	1324.00	1319.00	1320.33	3.21
	US	926.00	923.00	923.00	924.00	1.73
	DS	1578.00	1581.00	1581.00	1580.00	1.73
July	**BC**	1368.00	1365.00	1379.00	1370.67	7.37
	DP	1176.00	1178.00	1177.00	1177.00	1.00
	US	702.00	699.00	699.00	700.00	1.73
	DS	1655.00	1648.00	1648.00	1650.33	4.04
August	**BC**	1327.00	1314.00	1317.00	1319.33	6.81
	DP	1189.00	1190.00	1192.00	1190.33	1.53
	US	1107.00	1114.00	1116.00	1112.33	4.73
	DS	1487.00	1478.00	1472.00	1479.00	7.55

Table 5: TDS concentrations over the six month period

Total Dissolved Solids mg/L						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	411	411	412	411.33	0.58
	DP	436	436	436	436	0
	US	370	371	369	370	1
	DS	348	348	350	348.67	1.15
April	**BC**	384	382	383	383	1
	DP	443	444	445	444	1
	US	493	490	490	491	1.73
	DS	329	330	329	329.33	0.58
May	**BC**	418	418	418	418	0
	DP	475	475	475	475	0
	US	1063	1069	1069	1067	3.46
	DS	301	303	302	302	1
June	**BC**	342	343	343	342.67	0.58
	DP	366	369	370	368.33	2.08
	US	535	533	535	534.33	1.15
	DS	308	308	308	308	0
July	**BC**	340	351	354	348.33	7.37
	DP	417	416	416	416.33	0.58
	US	705	712	713	710	4.36
	DS	294	295	295	294.67	0.58
August	**BC**	368	372	371	370.33	2.08
	DP	412	412	411	411.67	0.58
	US	443	441	440	441.33	1.53
	DS	328	330	331	329.67	1.53

Table 6: Turbidity of the water samples over the six month period

Turbidity (NTU)												
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Ave**	**Std Dev**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Ave**	**Std Dev**	**Final Ave**	**Std Dev**
MARCH	**BC**	8.08	8.12	7.53	7.91	0.33	8.08	8.12	7.53	7.91	0.33	7.91	0.33
	DP	37.40	16.60	16.20	23.40	12.13	37.40	16.60	16.20	23.40	12.13	23.40	12.13
	US	17.10	16.40	16.50	16.67	0.38	17.10	16.40	16.50	16.67	0.38	16.67	0.38
	DS	15.20	15.40	15.20	15.27	0.12	15.20	15.40	15.20	15.27	0.12	15.27	0.12
APRIL	**BC**	56.60	56.60	56.40	56.53	0.12	51.50	51.50	51.30	51.43	0.12	53.98	2.94
	DP	76.60	76.60	76.10	76.43	0.29	65.40	65.40	65.90	65.60	0.26	71.02	6.25
	US	19.70	19.70	19.70	19.70	0.00	17.00	17.00	17.00	17.00	0.00	18.35	1.56
	DS	14.80	14.80	14.80	14.80	0.00	14.30	14.40	14.50	14.40	0.10	14.60	0.23
MAY	**BC**	19.60	19.60	19.60	19.60	0.00	25.90	20.10	19.10	21.70	3.67	20.65	1.21
	DP	14.10	14.10	14.00	14.07	0.06	14.40	14.30	14.40	14.37	0.06	14.22	0.17
	US	12.80	12.80	12.80	12.80	0.00	14.70	14.30	14.10	14.37	0.31	13.58	0.90
	DS	12.90	12.90	12.90	12.90	0.00	14.30	14.50	14.00	14.27	0.25	13.58	0.79
JUNE	**BC**	7.37	7.37	7.37	7.37	0.00	7.23	7.24	7.24	7.24	0.01	7.30	0.08
	DP	7.34	7.35	7.35	7.35	0.00	7.23	7.24	7.25	7.24	0.01	7.29	0.06
	US	7.64	7.65	7.65	7.65	0.01	7.54	7.50	7.53	7.54	0.01	7.59	0.06
	DS	7.84	7.85	7.84	7.84	0.01	7.74	7.75	7.76	7.75	0.02	7.80	0.05
JULY	**BC**	9.28	9.22	9.20	9.23	0.04	4.04	3.95	3.96	3.98	0.05	6.61	3.03
	DP	8.29	8.29	8.29	8.29	0.00	4.85	5.01	4.93	4.93	0.08	6.61	1.94
	US	9.91	9.91	9.91	9.91	0.00	4.69	4.61	4.68	4.66	0.04	7.29	3.03
	DS	16.90	16.90	16.90	16.90	0.00	4.72	4.97	4.97	4.89	0.14	10.89	6.94
AUGUST	**BC**	56.70	56.00	56.40	56.37	0.35	56.70	56.00	56.40	56.37	0.35	56.37	0.35
	DP	68.70	67.90	69.00	68.53	0.57	68.70	67.90	69.00	68.53	0.57	68.53	0.57
	US	28.70	28.70	28.80	28.73	0.06	28.70	28.70	28.80	28.73	0.06	28.73	0.06
	DS	20.70	20.80	20.80	20.77	0.06	20.70	20.80	20.80	20.77	0.06	20.77	0.06

Table 7: pH of the water samples over the six month period

pH													
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Final Ave**	**Std dev**
March	**BC**	7.15	7.05	7.12	7.11	0.05	7.15	7.05	7.12	7.11	0.05	7.11	0.05
	DP	7.31	7.44	7.34	7.36	0.07	7.31	7.44	7.34	7.36	0.07	7.36	0.07
	US	7.29	7.15	7.31	7.25	0.09	7.29	7.15	7.31	7.25	0.09	7.25	0.09
	DS	7.23	7.30	7.19	7.24	0.06	7.23	7.30	7.19	7.24	0.06	7.24	0.06
April	**BC**	7.70	7.60	7.70	7.67	0.05	7.12	7.11	7.03	7.09	0.04	7.38	0.33
	DP	7.50	7.30	7.40	7.40	0.10	7.13	7.05	7.02	7.04	0.02	7.22	0.21
	US	7.50	7.30	7.50	7.43	0.12	7.16	7.13	7.14	7.14	0.01	7.29	0.17
	DS	7.70	7.60	7.60	7.63	0.06	7.16	7.16	7.15	7.16	0.01	7.40	0.28
May	**BC**	7.05	7.07	7.11	7.08	0.03	7.18	6.93	6.76	6.96	0.21	20.65	1.21
	DP	7.26	7.29	7.28	7.28	0.02	6.97	6.80	6.75	6.84	0.12	14.22	0.17
	US	6.88	6.95	6.91	6.91	0.04	6.79	6.77	6.92	6.83	0.08	13.58	0.90
	DS	7.15	7.10	7.13	7.13	0.03	6.69	6.51	6.50	6.57	0.11	13.58	0.79
June	**BC**	7.37	7.37	7.37	7.37	0.00	7.23	7.24	7.24	7.24	0.01	7.30	0.08
	DP	7.34	7.35	7.35	7.35	0.01	7.23	7.24	7.25	7.24	0.01	7.29	0.06
	US	7.64	7.65	7.65	7.65	0.01	7.54	7.54	7.53	7.54	0.01	7.59	0.06
	DS	7.84	7.85	7.84	7.84	0.01	7.74	7.75	7.76	7.75	0.02	7.80	0.05
July	**BC**	7.47	7.49	7.48	7.48	0.01	6.92	7.00	6.85	6.92	0.08	7.20	0.32
	DP	7.70	7.70	7.70	7.70	0.00	6.26	6.37	6.28	6.30	0.06	7.00	0.81
	US	7.54	7.55	7.54	7.54	0.01	6.40	6.37	6.38	6.38	0.02	6.96	0.67
	DS	7.85	7.88	7.87	7.87	0.02	6.05	5.90	6.03	5.99	0.08	6.93	1.08
August	**BC**	6.73	6.86	6.95	6.85	0.11	6.73	6.86	6.95	6.85	0.11	6.85	0.11
	DP	7.05	7.09	7.13	7.09	0.04	7.05	7.09	7.13	7.09	0.04	7.09	0.04
	US	7.10	7.11	7.15	7.12	0.03	7.10	7.11	7.15	7.12	0.03	7.12	0.03
	DS	7.24	7.26	7.28	7.26	0.02	7.24	7.26	7.28	7.26	0.02	7.26	0.02

Table 8: COD concentrations obtained over the six month period

COD mg/L															
		Replicate	Replicate 2	Replicate 3	Average	Average 1	Std Dev	Replicate 1	Replicate 2	Replicate 3	Average	Std Dev	Average 2	Final Ave	Std Dev
March	**BC 1**	90.00	80.00	97.00	89.00		8.54	90.00	80.00	97.00	89.00	8.54			
	BC2	115.00	113.00	114.00	114.00		1.00	115.00	113.00	114.00	114.00	1.00			
	BC3	112.00	111.00	111.00	111.33	104.78	0.58	112.00	111.00	111.00	111.33	0.58	104.78	104.77	0.00
	DP 1	<10	<10	<10	< 10		0.00	<10	<10	<10	<10	0.00			
	DP 2	<10	<10	<10	< 10		0.00	<10	<10	<10	<10	0.00			
	DP 3	<10	<10	<10	< 10	< 10	0.00	<10	<10	<10	<10	0.00	< 10	< 10	0.00
	US 1	163.00	169.00	164.00	165.33		3.21	163.00	169.00	164.00	165.33	3.21			
	US 2	157.00	157.00	156.00	156.67		0.58	157.00	157.00	156.00	156.67	0.58			
	US 3	161.00	164.00	161.00	162.00	161.33	1.73	164.00	161.00	161.00	162.00	1.73	161.33	161.33	0.00
	DS 1	112.00	113.00	114.00	113.00		1.00	121.00	113.00	114.00	116.00	4.36			
	DS 2	309.00	310.00	309.00	309.33		0.58	309.00	310.00	309.00	309.33	0.58			
	DS 3	152.00	152.00	152.00	152.00	191.44	0.00	152.00	152.00	152.00	152.00	0.00	192.44	191.94	0.58
April	**BC 1**	126.00	126.00	127.00	126.33		0.58	126.00	126.00	127.00	126.33	0.58			
	BC2	129.00	129.00	128.00	128.67		0.58	129.00	129.00	128.00	128.67	0.58			
	BC3	127.00	128.00	131.00	128.67	127.89	2.08	127.00	128.00	131.00	128.67	2.08	127.89	127.88	0.01
	DP 1	151.00	150.00	150.00	150.33		0.58	151.00	150.00	150.00	150.33	0.58			
	DP 2	149.00	149.00	145.00	147.67		2.31	149.00	149.00	145.00	147.67	2.31			
	DP 3	162.00	160.00	160.00	160.67	152.89	1.15	162.00	160.00	160.00	160.67	1.15	152.89	152.88	0.01
	US 1	309.00	308.00	310.00	309.00		1.00	309.00	313.00	311.00	311.00	2.00			
	US 2	311.00	310.00	309.00	310.00		1.00	309.00	313.00	311.00	311.00	0.00			
	US 3	309.00	309.00	308.00	308.67	309.22	0.58	309.00	313.00	311.00	311.00	0.00	311.00	310.11	1.02
	DS 1	304.00	304.00	304.00	304.00		0.00	304.00	304.00	304.00	304.00	0.00			
	DS 2	302.00	302.00	302.00	302.00		0.00	302.00	302.00	302.00	302.00	0.00			
	DS 3	303.00	303.00	303.00	303.00	303.00	0.00	303.00	303.00	303.00	303.00	0.00	303.00	303.00	0.00
May	**BC 1**	52.00	49.00	50.00	50.33		1.53	92.00	92.00	92.00	92.00	0.00			
	BC2	37.00	36.00	38.00	37.00		1.00	86.00	85.00	86.00	85.67	0.52			
	BC3	28.00	27.00	27.00	27.33	38.22	0.58	37.00	37.00	37.00	37.00	0.00	71.56	54.89	0.00
	DP 1	<10	<10	<10	<10		0.00	< 10	< 10	< 10	<10	0.00			
	DP 2	<10	<10	<10	<10		0.00	< 10	< 10	< 10	<10	0.00			
	DP 3	<10	<10	<10	<10	<10	0.00	< 10	< 10	< 10	<10	0.00	<10	<10	0.00
	US 1	18.00	19.00	18.00	18.33		0.58	79.00	79.00	79.00	79.00	0.00			
	US 2	20.00	21.00	21.00	20.67		0.58	35.00	35.00	35.00	35.00	0.00			
	US 3	19.00	20.00	22.00	20.33	19.78	1.53	37.00	40.00	40.00	39.00	1.55	51	35.39	18.02
	DS 1	308.00	310.00	308.00	308.67		1.15	308.00	308.00	308.00	308.00	0.00			
	DS 2	307.00	309.00	307.00	307.67		1.15	300.00	300.00	300.00	300.00	0.00			
	DS 3	311.00	311.00	311.00	311.00	309.11	0.00	304.00	306.00	305.00	305.00	0.89	304.33	306.72	2.75

Table 8 continued…

		COD mg/L													
		Replicate	Replicate	Replicate	Average	Average 1	Std Dev	Replicate	Replicate	Replicate	Average	Std Dev	Average 2	Final Ave	Std Dev
June	**BC 1**	110.00	109.00	110.00	109.67		0.58	110.00	109.00	110.00	109.67	0.58			
	BC2	117.00	117.00	117.00	117.00		0.00	117.00	117.00	117.00	117.00	0.00			
	BC3	114.00	113.00	113.00	113.33	113.33	0.58	114.00	113.00	113.00	113.33	0.58	113.33	113.33	0.001
	DP 1	286.00	293.00	292.00	290.33		3.79	286.00	293.00	292.00	290.33	3.79			
	DP 2	303.00	302.00	303.00	302.67		0.58	303.00	302.00	303.00	302.67	0.58			
	DP 3	307.00	307.00	307.00	307.00	300.00	0.00	307.00	307.00	307.00	307.00	0.00	300	300.00	0
	US 1	107.00	107.00	106.00	106.67		0.58	127.00	114.00	112.00	117.67	8.14			
	US 2	113.00	113.00	112.00	112.67		0.58	127.00	114.00	112.00	117.67	8.14			
	US 3	110.00	111.00	111.00	110.67	110.00	0.58	127.00	114.00	112.00	117.67	8.14	117.67	113.83	4.42
	DS 1	86.00	86.00	86.00	86.00		0.00	88.00	94.00	88.00	90.00	3.46			
	DS 2	90.00	90.00	90.00	90.00		0.00	88.00	94.00	88.00	90.00	3.46			
	DS 3	90.00	91.00	90.00	90.33	88.78	0.58	88.00	94.00	88.00	90.00	3.46	90	89.38	0.705
July	**BC 1**	123.00	122.00	123.00	122.67		0.58	123.00	121.00	122.00	122.00	1.00			
	BC2	117.00	116.00	118.00	117.00		1.00	97.00	108.00	99.00	101.33	5.86			
	BC3	113.00	114.00	119.00	115.33	118.33	3.21	121.00	121.00	121.00	121.00	0.00	114.78	116.55	2.05
	DP 1	295.00	295.00	294.00	294.67		0.58	292.00	291.00	291.00	291.33	0.58			
	DP 2	293.00	293.00	293.00	293.00		0.00	291.00	291.00	291.00	291.00	0.00			
	DP 3	287.00	288.00	288.00	287.67	291.78	0.58	290.00	290.00	289.00	289.67	0.58	290.67	291.22	0.6
	US 1	312.00	311.00	312.00	311.67		0.58	312.00	312.00	312.00	312.00	0.00			
	US 2	312.00	312.00	311.00	311.67		0.58	311.00	311.00	311.00	311.00	0.00			
	US 3	315.00	314.00	313.00	314.00	312.44	1.00	310.00	310.00	310.00	310.00	0.00	311	311.72	0.8
	DS 1	312.00	313.00	313.00	312.67		0.58	314.00	313.00	312.00	313.00	1.00			
	DS 2	311.00	312.00	313.00	312.00		1.00	311.00	311.00	310.00	310.67	0.58			
	DS 3	312.00	313.00	313.00	312.67	314.44	0.58	309.00	309.00	310.00	309.33	0.58	311	311.72	0.8
August	**BC 1**	311.00	310.00	310.00	310.33		0.58	311.00	310.00	310.00	310.33	0.58			
	BC2	309.00	309.00	310.00	309.33		0.58	309.00	309.00	310.00	309.33	0.58			
	BC3	311.00	310.00	311.00	310.67	310.11	0.58	311.00	310.00	311.00	310.67	0.58	310.11	310.11	0
	DP 1	183.00	188.00	185.00	185.33		2.52	183.00	188.00	185.00	185.33	2.52			
	DP 2	181.00	181.00	181.00	181.00		0.00	181.00	181.00	181.00	181.00	0.00			
	DP 3	184.00	180.00	182.00	182.00	182.78	2.00	184.00	180.00	182.00	182.00	2.00	182.78	182.78	0
	US 1	111.00	110.00	109.00	110.00		1.00	111.00	110.00	109.00	110.00	1.00			
	US 2	105.00	105.00	106.00	105.33		0.58	105.00	105.00	106.00	105.33	0.58			
	US 3	99.00	100.00	108.00	102.33	105.89	4.93	99.00	100.00	108.00	102.33	4.93	105.89	105.89	0
	DS 1	307.00	308.00	309.00	308.00		1.00	307.00	308.00	309.00	308.00	1.00			
	DS 2	313.00	312.00	311.00	312.00		1.00	313.00	312.00	311.00	312.00	1.00			
	DS 3	308.00	309.00	309.00	308.67	309.56	0.58	308.00	309.00	309.00	308.67	0.58	309.56	309.56	0

Table 9: BOD concentrations obtained in March

March																		
	BOD DAY 0					BOD DAY 7												
Point	1	2	3	Average	Std Dev	1	2	3	Average	Std Dev	P Value	BOD 1	BOD 2	BOD 3	Average	Std Dev	FINAL	Std Dev
NW BC 2	7.63	7.63	7.70	7.65	0.04	6.89	6.41	6.03	6.44	0.43	0.67	1.10	1.82	2.49	1.81	0.69	2.23	0.36
NW BC 2	7.36	7.43	7.43	7.41	0.04	6.79	6.45	6.5	6.58	0.18	0.72	0.79	1.36	1.29	1.15	0.31		
NW BC 2	7.34	7.50	7.46	7.43	0.08	5.68	5.63	5.42	5.58	0.14	0.92	1.80	2.03	2.22	2.02	0.21		
NW BC 3	7.69	7.56	7.63	7.63	0.07	3.51	3.66	3.86	3.68	0.18	1.00	4.18	3.90	3.77	3.95	0.21		
NW AC 2	7.80	7.98	7.99	7.92	0.11	4.04	3.96	4	4.00	0.04	0.67	5.61	6.00	5.96	5.86	0.21	5.13	0.18
NW AC 2	8.14	8.20	8.26	8.20	0.06	3.14	3.37	3.58	3.36	0.22	0.72	6.94	6.71	6.50	6.72	0.22		
NW AC 2	8.36	8.35	8.37	8.36	0.01	4.96	5.23	4.94	5.04	0.16	0.92	3.70	3.39	3.73	3.61	0.19		
NW AC 3	8.09	8.04	8.11	8.08	0.04	3.71	3.62	3.88	3.74	0.13	1.00	4.38	4.42	4.23	4.34	0.10		
NW US 6	8.09	8.16	8.15	8.13	0.04	6.2	5.02	6	5.74	0.63	0.20	9.45	15.70	10.75	11.97	3.30	5.62	1.01
NW US 1	8.38	8.36	8.34	8.36	0.02	6.61	6.67	6.44	6.57	0.12	0.50	3.54	3.38	3.80	3.57	0.21		
NW US 2	7.97	7.91	7.94	7.94	0.03	5.98	5.8	5.61	5.80	0.19	0.67	2.97	3.15	3.48	3.20	0.26		
NW US 3	8.14	8.13	8.11	8.13	0.02	4.66	4.07	4.4	4.38	0.30	1.00	3.48	4.06	3.71	3.75	0.29		
NW DS 6	8.16	8.10	8.15	8.14	0.03	5.58	5.57	5.69	5.61	0.07	0.20	12.90	12.65	12.30	12.62	0.30	5.62	0.24
NW DS15	7.90	7.92	7.91	7.91	0.01	5.42	5.52	5.63	5.52	0.11	0.50	4.96	4.80	4.56	4.77	0.20		
NW DS 2	7.72	8.02	8.03	7.92	0.18	5.75	5.81	5.8	5.79	0.03	0.67	2.94	3.30	3.33	3.19	0.22		
NW DS 3	7.81	7.71	7.80	7.77	0.06	5.99	5.96	5.61	5.85	0.21	1.00	1.82	1.75	2.19	1.92	0.24		
CONTRO	8.37	8.32	8.36	**8.35**	**0.03**	6.68	6.69	6.79	**6.72**	**0.06**	**1.00**	**1.69**	**1.63**	**1.57**	**1.63**	**0.06**		

Table 10: BOD concentrations obtained in April

April																		
BOD DAY 0						BOD DAY 7												
Point	1	2	3	Average	Std Dev	1	2	3	Average	Std Dev	P Value	BOD 1	BOD 2	BOD 3	Average	Std Dev	FINAL	Std Dev
NW BC 200	7.68	7.82	7.71	7.74	0.07	5.86	4.54	4.84	5.08	0.69	0.67	2.72	4.90	4.28	3.97	1.12	3.30	0.97
NW BC 225	8.17	7.31	7.7	7.73	0.43	3.95	4.99	4.3	4.41	0.53	0.72	5.86	3.22	4.72	4.60	1.32		
NW BC 275	7.34	7.08	7.29	7.24	0.14	5.5	3.43	4.79	4.57	1.05	0.92	2.00	3.97	2.72	2.89	1.00		
NW BC 300	7.87	7.58	7.59	7.68	0.16	6.6	5.58	5.6	5.93	0.58	1.00	1.27	2.00	1.99	1.75	0.42		
NW AC 200	7.69	7.45	7.62	7.59	0.12	5.19	5.62	5.01	5.27	0.31	0.67	3.73	2.73	3.90	3.45	0.63	3.44	0.67
NW AC 225	7.36	7.78	7.9	7.68	0.28	4.77	4.21	4.92	4.63	0.37	0.72	3.60	4.96	4.14	4.23	0.69		
NW AC 275	7.14	7.61	7.63	7.46	0.28	4.28	4.13	4.78	4.40	0.34	0.92	3.11	3.78	3.10	3.33	0.39		
NW AC 300	7.95	7.92	7.95	7.94	0.02	6.28	4.83	4.45	5.19	0.97	1.00	1.67	3.09	3.50	2.75	0.96		
NW US 60	8.52	8.53	8.52	8.52	0.01	4.17	4.37	4.49	4.34	0.16	0.20	21.75	20.80	20.15	20.90	0.80	8.49	0.47
NW US 150	8.48	8.5	8.5	8.49	0.01	5.63	5.55	5.83	5.67	0.14	0.50	5.70	5.90	5.34	5.65	0.28		
NW US 200	8.51	8.53	8.51	8.52	0.01	5.45	5.96	5.29	5.57	0.35	0.67	4.57	3.84	4.81	4.40	0.51		
NW US 300	8.49	8.47	8.48	8.48	0.01	5.54	5.19	5.73	5.49	0.27	1.00	2.95	3.28	2.75	2.99	0.27		
NW DS 60	8.31	8.31	8.28	8.30	0.02	5.15	5.14	5.05	5.11	0.06	0.20	15.80	15.85	16.15	15.93	0.19	6.33	0.21
NW DS150	8.05	8.05	7.98	8.03	0.04	5.89	5.97	5.92	5.93	0.04	0.50	4.32	4.16	4.12	4.20	0.11		
NW DS 200	7.76	7.74	7.89	7.80	0.08	5.78	5.8	5.43	5.67	0.21	0.67	2.96	2.90	3.67	3.17	0.43		
NW DS 300	7.23	7.27	7.45	7.32	0.12	5.24	5.13	5.49	5.29	0.18	1.00	1.99	2.14	1.96	2.03	0.10		
CONTROL	**8.37**	**8.32**	**8.36**	**8.35**	**0.03**	**6.68**	**6.69**	**6.79**	**6.72**	**0.06**	**1.00**	**1.69**	**1.63**	**1.57**	**1.63**	**0.06**		

Table 11: BOD concentrations obtained in May

May																		
BOD DAY 0						BOD DAY 5												
Point	1	2	3	Average	Std Dev	1	2	3	Average	Std Dev	P Value	BOD 1	BOD 2	BOD 3	Average	Std Dev	FINAL	Std Dev
NW BC 20	6.78	6.75	6.72	6.75	0.03	6.69	6.21	6.22	6.37	0.27	0.67	0.13	0.81	0.75	0.56	0.37	1.03	0.19
NW BC 22	6.78	6.9	6.81	6.83	0.06	5.25	5.18	5.27	5.23	0.05	0.72	2.13	2.39	2.14	2.22	0.15		
NW BC 27	6.23	6.28	6.11	6.21	0.09	6.21	6.49	6.36	6.35	0.14	0.92	0.02	-0.23	-0.27	-0.16	0.16		
NW BC 30	6.86	6.93	6.98	6.92	0.06	5.35	5.53	5.42	5.43	0.09	1.00	1.51	1.40	1.56	1.49	0.08		
NW AC 20	8	8.02	7.99	8.00	0.02	5.45	5.09	5.1	5.21	0.21	0.67	3.81	4.37	4.31	4.16	0.31	3.25	0.17
NW AC 22	7.95	7.94	7.95	7.95	0.01	5.12	5.05	5.02	5.06	0.05	0.72	3.93	4.01	4.07	4.00	0.07		
NW AC 27	8.09	8.05	8.07	8.07	0.02	5.42	5.81	5.49	5.57	0.21	0.92	2.90	2.43	2.80	2.71	0.25		
NW AC 30	7.94	7.95	7.97	7.95	0.02	5.8	5.78	5.9	5.83	0.06	1.00	2.14	2.17	2.07	2.13	0.05		
NW US 60	8.12	8.11	8.09	8.11	0.02	6.25	6.45	6.9	6.53	0.33	0.20	9.35	8.30	5.95	7.87	1.74	4.29	0.79
NW US 15	8.13	8.11	8.14	8.13	0.02	6.14	6.3	6.61	6.35	0.24	0.50	3.98	3.62	3.06	3.55	0.46		
NW US 200																		
NW US 30	8.01	8.02	7.98	8.00	0.02	6.49	6.75	6.4	6.55	0.18	1.00	1.52	1.27	1.58	1.46	0.16		
NW DS 60	8.17	8.16	8.15	8.16	0.01	5.61	5.53	5.39	5.51	0.11	0.20	12.80	13.15	13.80	13.25	0.51	5.68	0.30
NW DS150	8.09	8.1	8.15	8.11	0.03	5.99	6.1	6.09	6.06	0.06	0.50	4.20	4.00	4.12	4.11	0.10		
NW DS 20	8.14	8.17	8.19	8.17	0.03	5.76	5.4	5.95	5.70	0.28	0.67	3.55	4.13	3.34	3.68	0.41		
NW DS 30	8.11	8.14	8.1	8.12	0.02	6.54	6.22	6.49	6.42	0.17	1.00	1.57	1.92	1.61	1.70	0.19		
CONTRO	**8.13**	**8.16**	**8.14**	**8.14**	**0.02**	**7.92**	**8.03**	**8.07**	**8.01**	**0.08**	**1.00**	**0.21**	**0.13**	**0.07**	**0.14**	**0.07**		

Table 11 continued…

BOD DAY 0						BOD DAY 5								
Point	**1**	**2**	**3**	**Average**	**Std Dev**	**1**	**2**	**3**	**Average**	**Std Dev**	**P Value**	**BOD 1**	**BOD 2**	**BOD 3**
NW BC 200	7.65	7.6	7.57	7.61	0.04	5.74	5.89	5.62	5.75	0.12	0.67	2.79	1.79	0.62
NW BC 225	7.3	7.28	7.3	7.29	0.01	6.18	6.2	6.27	6.22	0.04	0.72	1.44		
NW BC 275	7.39	7.4	7.39	7.39	0.01	6.05	6.08	6.1	6.08	0.02	0.92	1.44		
NW BC 300	7.59	7.6	7.61	7.6	0.01	6.07	6.15	6.11	6.11	0.04	1.00	1.49		
NW AC 200	7.99	5.92	2.07	0.67	3.11	5.93	5.91	5.93	5.92	0.01	0.67	3.11	2.82	0.48
NW AC 225	7.97	6.36	1.61	0.75	2.15	6.45	6.3	6.32	6.36	0.07	0.72	2.15		
NW AC 275	8.07	5.59	2.48	0.92	2.71	5.57	5.65	5.55	5.59	0.05	0.92	2.71		
NW AC 300	8.08	5.86	2.22	1.00	3.33	5.83	5.96	5.78	5.86	0.08	1.00	3.33		
NW US 200	8.15	8.15	8.16	8.15	0.01	5.99	6.02	5.93	5.98	0.04	0.20	10.87	4.75	3.80
NW US 225	8.12	8.1	8.09	8.10	0.01	6.37	6.53	6.31	6.40	0.10	0.50	3.40		
NW US 275	8.14	8.14	8.14	8.14	0.00	6.42	6.4	6.4	6.41	0.01		2.60		
NW US 300	8.17	8.1	8.15	8.14	0.03	5.94	6.08	5.95	5.99	0.07	1.00	2.15		
NW DS 60	8.24	8.22	8.22	8.23	0.01	6.07	5.98	6.01	6.02	0.04	0.20	11.03	4.45	5.17
NW DS 200	8.16	8.17	8.17	8.17	0.01	8.09	8	7.98	8.02	0.05	0.67	0.21		
NW DS 300	8	8.09	8.11	8.07	0.05	6.04	5.95	5.92	5.97	0.06	1.00	2.10		
CONTROL	8.29	8.29	8.22	8.27	0.04	6.81	6.85	6.96	6.87	0.07	**1.00**		**1.39**	

Average BOD of water samples in May

Final BOD Average: May				
	AVG 1	**AVG 2**	**AVG**	**STD DEV**
NW BC	**1.03**	**1.79**	**1.41**	**0.44**
NW DP	**3.25**	**2.82**	**3.04**	**0.25**
NW US	**4.29**	**4.75**	**4.52**	**0.27**
NW DS	**5.68**	**4.45**	**5.07**	**0.71**

Table 12: BOD concentrations obtained in June

June																		
BOD DAY 0						BOD DAY 5												
Point	1	2	3	Average	Std Dev	1	2	3	Average	Std Dev	P Value	BOD 1	BOD 2	BOD 3	Average	Std Dev	FINAL	Std Dev
NW BC 20	8.26	8.18	8.24	8.23	0.04	4.4	4.75	4.23	4.46	0.27	0.67	5.76	5.12	5.99	5.62	0.45	3.66	0.30
NW BC 22	8.04	8.09	8.12	8.08	0.04	5.52	5.36	5.22	5.37	0.15	0.72	3.50	3.79	4.03	3.77	0.26		
NW BC 27	7.74	7.83	7.73	7.77	0.06	5.05	5.47	5.15	5.22	0.22	0.92	2.92	2.57	2.80	2.76	0.18		
NW BC 30	7.79	7.96	7.89	7.88	0.09	5.65	5.22	5.36	5.41	0.22	1.00	2.14	2.74	2.53	2.47	0.30		
NW AC 20	8.67	8.64	8.68	8.66	0.02	5.43	5.71	5.43	5.52	0.16	0.67	4.84	4.37	4.85	4.69	0.27	4.08	0.21
NW AC 22	8.56	8.59	8.57	8.57	0.02	5.09	5	5.17	5.09	0.09	0.72	4.82	4.99	4.72	4.84	0.13		
NW AC 27	8.63	8.69	8.7	8.67	0.04	5.2	5.36	5.44	5.33	0.12	0.92	3.73	3.62	3.54	3.63	0.09		
NW AC 30	8.68	8.66	8.67	8.67	0.01	5.55	5.82	5.17	5.51	0.33	1.00	3.13	2.84	3.50	3.16	0.33		
NW US 60	8.6	8.56	8.54	8.57	0.03	4.62	4.62	4.45	4.56	0.10	0.20	19.90	19.70	20.45	20.02	0.39	9.80	0.44
NW US 15	8.48	8.42	8.43	8.44	0.03	5.48	5.92	5.38	5.59	0.29	0.50	6.00	5.00	6.10	5.70	0.61		
NW US 200																		
NW US 30	8.37	8.33	8.26	8.32	0.06	4.93	4.26	4.69	4.63	0.34	1.00	3.44	4.07	3.57	3.69	0.33		
NW DS 60	8.47	8.48	8.51	8.49	0.02	5.77	5.66	5.68	5.70	0.06	0.20	13.50	14.10	14.15	13.92	0.36	6.93	0.35
NW DS150	8.5	8.51	8.48	8.50	0.02	5.78	5.8	5.53	5.70	0.15	0.50	5.44	5.42	5.90	5.59	0.27		
NW DS 20	8.36	8.37	8.41	8.38	0.03	5.33	5.68	5.17	5.39	0.26	0.67	4.52	4.01	4.84	4.46	0.41		
NW DS 30	8.37	8.33	8.36	8.35	0.02	4.22	4.69	4.87	4.59	0.34	1.00	4.15	3.64	3.49	3.76	0.35		
CONTRO	**8.67**	**8.58**	**8.51**	**8.59**	**0.08**	**7.66**	**7.67**	**7.59**	**7.64**	**0.04**	**1.00**	**1.01**	**0.91**	**0.92**	**0.95**	**0.06**		

Table 12 continued…

BOD DAY 0						BOD DAY 5												
Point	**1**	**2**	**3**	**Average**	**Std Dev**	**1**	**2**	**3**	**Average**	**Std Dev**	**P Value**	**BOD 1**	**BOD 2**	**BOD 3**	**Average**	**Std Dev**	**FINAL**	**Std Dev**
NW BC 20	8	7.96	7.96	7.97	0.02	4.76	4.77	4.5	4.68	0.15	0.67	4.84	4.76	5.16	4.92	0.21	4.36	0.16
NW BC 22	8.09	8.1	7.9	8.03	0.11	4.33	4.29	4.39	4.34	0.05	0.72	5.22	5.29	4.88	5.13	0.22		
NW BC 27	7.78	7.77	7.79	7.78	0.01	3.88	3.85	3.63	3.79	0.14	0.92	4.24	4.26	4.52	4.34	0.16		
NW BC 30	7.59	7.59	7.6	7.59	0.01	4.46	4.53	4.59	4.53	0.07	1.00	3.13	3.06	3.01	3.07	0.06		
NW AC 20	8.45	8.42	8.44	8.44	0.02	4.45	4.42	4.32	4.40	0.07	0.67	5.97	5.97	6.15	6.03	0.10	4.54	0.12
NW AC 22	8.43	8.41	8.42	8.42	0.01	5.11	5.27	5.35	5.24	0.12	0.72	4.61	4.36	4.26	4.41	0.18		
NW AC 27	8.56	8.57	8.57	8.57	0.01	4.69	4.78	4.65	4.71	0.07	0.92	4.21	4.12	4.26	4.20	0.07		
NW AC 30	8.69	8.66	8.68	8.68	0.02	5.07	5.32	5.13	5.17	0.13	1.00	3.62	3.34	3.55	3.50	0.15		
NW US 20	8.1	8.12	8.14	8.12	0.02	4.72	4.79	4.79	4.77	0.04	0.20	16.90	16.65	16.75	16.77	0.13	9.42	0.15
NW US 22	8.31	8.3	8.32	8.31	0.01	4.45	4.24	4.31	4.33	0.11	0.50	7.72	8.12	8.02	7.95	0.21		
NW US 27	8.34	8.34	8.35			4.4	4.33	4.26										
NW US 30	8.21	8.16	8.21	8.19	0.03	4.69	4.75	4.56	4.67	0.10	1.00	3.52	3.41	3.65	3.53	0.12		
NW DS 20	8.41	8.48	8.48	8.46	0.04	4.6	4.46	4.49	4.52	0.07	0.20	19.05	20.10	19.95	19.70	0.57	10.32	0.42
NW DS 22	8.56	8.57	8.52	8.55	0.03	4.87	4.5	4.79	4.72	0.19	0.50	7.38	8.14	7.46	7.66	0.42		
NW DS 275																		
NW DS 30	8.44	8.48	8.5	8.47	0.03	5.04	4.97	4.6	4.87	0.24	1.00	3.40	3.51	3.90	3.60	0.26		
CONTRO	8.42	8.43	8.43	**8.43**	**0.01**	8.14	8.13	8.15	**8.14**	**0.01**	**1.00**	**0.28**	**0.30**	**0.28**	**0.29**	**0.01**		

Average BOD of water samples in June

Final BOD Average: June				
	AVG 1	**AVG 2**	**AVG**	**STD DEV**
NW BC	**3.66**	**4.36**	**4.01**	**0.41**
NW DP	**4.08**	**4.54**	**4.31**	**0.26**
NW US	**9.80**	**9.42**	**9.61**	**0.22**
NW DS	**6.93**	**7.74**	**7.34**	**0.47**

Table 13: BOD concentrations obtained in July

July																		
BOD DAY 0						**BOD DAY 5**												
Point	**1**	**2**	**3**	**Average**	**Std Dev**	**1**	**2**	**3**	**Average**	**Std Dev**	**P Value**	**BOD 1**	**BOD 2**	**BOD 3**	**Average**	**Std Dev**	**FINAL**	**Std Dev**
NW BC 200	6.61	6.92	6.8	6.78	0.16	4.07	4.91	4.27	4.42	0.44	0.67	3.79	3.00	3.78	3.52	0.45	1.70	0.38
NW BC 225	6.17	6.68	6.67	6.51	0.29	5.46	5.1	5.2	5.25	0.19	0.72	0.99	2.19	2.04	1.74	0.66		
NW BC 275											0.92	0.00	0.00	0.00	0.00	0.00		
NW BC 300	6.48	5.74	6.18	6.13	0.37	4.84	4.64	4.31	4.60	0.27	1.00	1.64	1.10	1.87	1.54	0.40		
NW AC 200	7.73	7.71	7.66	7.70	0.04	4.76	4.93	4.72	4.80	0.11	0.67	4.43	4.15	4.39	4.32	0.15	3.07	0.19
NW AC 225	7.71	7.13	7.4	7.41	0.29	5.44	5.23	5.1	5.26	0.17	0.72	3.15	2.64	3.19	3.00	0.31		
NW AC 275	7.7	7.21	7.35	7.42	0.25	5.46	5.05	5	5.17	0.25	0.92	2.43	2.35	2.55	2.45	0.10		
NW AC 300	7.27	7.36	7.42	7.35	0.08	4.92	4.85	4.72	4.83	0.10	1.00	2.35	2.51	2.70	2.52	0.18		
NW US 60	8.35	8.45	8.32	8.37	0.07	4.85	4.05	4.96	4.62	0.50	0.20	17.50	22.00	16.80	18.77	2.82	9.84	1.22
NW US 150	8.22	8.25	8.24	8.24	0.02	4.51	4.34	4.93	4.59	0.30	0.50	7.42	7.82	6.62	7.29	0.61		
NW US 200	8.21	8.18	8.35	8.25	0.09	4.96	4.27	4.16										
NW US 300	8.23	8.17	8.16	8.19	0.04	4.9	4.83	4.41	4.71	0.27	1.00	3.33	3.34	3.75	3.47	0.24		
NW DS 60	8.51	8.37	8.33	8.40	0.09	4.64	4.52	4.84	4.67	0.16	0.20	19.35	19.25	17.45	18.68	1.07	8.80	0.64
NW DS150	8.31	8.26	8.21	8.26	0.05	4.81	4.81	4.93	4.85	0.07	0.50	7.00	6.90	6.56	6.82	0.23		
NW DS 200	8.3	8.21	8.51	8.34	0.15	4.8	4.77	3.94	4.50	0.49	0.67	5.22	5.13	6.82	5.73	0.95		
NW DS 300	8.34	8.28	8.31	8.31	0.03	4.7	4.2	4.07	4.32	0.33	1.00	3.64	4.08	4.24	3.99	0.31		
CONTROL	**8.51**	**8.45**	**8.39**	**8.45**	**0.06**	**7.35**	**7.18**	**7.29**	**7.27**	**0.09**	**1.00**	**1.16**	**1.27**	**1.10**	**1.18**	**0.09**		

Table 13 continued…

BOD DAY 0						BOD DAY 7												
Point	**1**	**2**	**3**	**Average**	**Std Dev**	**1**	**2**	**3**	**Average**	**Std Dev**	**P Value**	**BOD 1**	**BOD 2**	**BOD 3**	**Average**	**Std Dev**	**FINAL**	**Std Dev**
NW BC 200	6.27	6.30	6.37	6.31	0.05	3.52	3.4	3.47	3.46	0.06	0.67	4.10	4.33	4.33	4.25	0.13	2.56	0.58
NW BC 225	6.52	6.45	6.42	6.46	0.05	4.46	4.99	4.3	4.58	0.36	0.72	2.86	2.03	2.94	2.61	0.51		
NW BC 275	6.31	6.32	6.24	6.29	0.04	4.44	3.43	4.79	4.22	0.71	0.92	2.03	3.14	1.58	2.25	0.80		
NW BC 300	6.42	6.24	6.17	6.28	0.13	4.27	5.58	5.6	5.15	0.76	1.00	2.15	0.66	0.57	1.13	0.89		
NW AC 200	7.43	7.25	7.53	7.40	0.14	5.15	5.62	5.01	5.26	0.32	0.67	3.40	2.43	3.76	3.20	0.69	3.12	0.62
NW AC 225	7.49	7.36	7.34	7.40	0.08	5.03	4.21	4.92	4.72	0.45	0.72	3.42	4.38	3.36	3.72	0.57		
NW AC 275	7.58	7.44	7.63	7.55	0.10	5.64	4.13	4.78	4.85	0.76	0.92	2.11	3.60	3.10	2.93	0.76		
NW AC 300	7.54	7.49	7.54	7.52	0.03	5.4	4.83	4.45	4.89	0.48	1.00	2.14	2.66	3.09	2.63	0.48		
NW US 60	7.93	7.89	7.88	7.90	0.03	5.51	4.37	4.49	4.79	0.63	0.20	12.10	17.60	16.95	15.55	3.01	7.68	1.37
NW US 150	7.81	7.78	7.75	7.78	0.03	5.97	5.55	5.83	5.78	0.21	0.50	3.68	4.46	3.84	3.99	0.41		
NW US 200	7.97	7.75	7.88	7.87	0.11	5.34	5.96	5.29	5.53	0.37	0.67	3.93	2.67	3.87	3.49	0.71		
NW US 300																		
NW DS 60	7.89	7.88	7.84	7.87	0.03	6.01	5.92	5.82	5.92	0.10	0.20	9.40	9.80	10.10	9.77	0.35	4.69	0.23
NW DS150	7.95	7.78	7.77	7.83	0.10	5.91	5.78	5.97	5.89	0.10	0.50	4.08	4.00	3.60	3.89	0.26		
NW DS 200	7.89	7.78	7.71	7.79	0.09	5.68	5.71	5.8	5.73	0.06	0.67	3.30	3.09	2.85	3.08	0.22		
NW DS 300	7.96	7.88	7.91	7.92	0.04	5.9	5.95	5.81	5.89	0.07	1.00	2.06	1.93	2.10	2.03	0.09		
CONTROL	8.19	8.21	8.17	**8.19**	**0.02**	8.14	8.21	8.09	**8.15**	**0.06**	**1.00**	**0.05**	**0.00**	**0.08**	**0.04**	**0.04**		

Average of water samples in July

Final BOD Average: July				
	AVG 1	**AVG 2**	**AVG**	**STD DEV**
NW BC	**1.70**	**2.56**	**2.13**	**0.50**
NW DP	**3.07**	**3.12**	**3.10**	**0.03**
NW US	**9.84**	**5.76**	**7.80**	**2.36**
NW DS	**8.80**	**4.69**	**6.75**	**2.37**

Table 14: BOD concentrations obtained in August

August																		
BOD DAY 0						BOD DAY 5												
Point	**1**	**2**	**3**	**Average**	**Std Dev**	**1**	**2**	**3**	**Average**	**Std Dev**	**P Value**	**BOD 1**	**BOD 2**	**BOD 3**	**Average**	**Std Dev**	**FINAL**	**Std Dev**
NW BC 200	6.89	6.96	6.8	6.88	0.08	5.89	5.5	5.75	5.71	0.20	0.67	1.49	2.18	1.57	1.75	0.38	1.54	0.30
NW BC 225	6.63	6.78	6.64	6.68	0.08	5.29	5.84	5.8	5.64	0.31	0.72	1.86	1.31	1.17	1.44	0.37		
NW BC 275																		
NW BC 300	6.17	6.17	6.03	6.12	0.08	4.85	4.76	4.43	4.68	0.22	1.00	1.32	1.41	1.60	1.44	0.14		
NW AC 200	6.47	6.47	6.04	6.33	0.25	5.25	5.33	5.12	5.23	0.11	0.67	1.82	1.70	1.37	1.63	0.23	2.23	0.28
NW AC 225	6.07	6.02	6.18	6.09	0.08	4.44	4.73	4.63	4.60	0.15	0.72	2.26	1.79	2.15	2.07	0.25		
NW AC 275	5.66	5.3	5.47	5.48	0.18	4.6	4.66	4.79	4.68	0.10								
NW AC 300	5.85	5.77	5.9	5.84	0.07	5.15	5.8	5.7	5.55	0.35	1.00	0.70	-0.03	0.20	3.00	0.37		
NW US 60	7.91	7.91	7.82	7.88	0.05	4.72	4.79	4.77	4.76	0.04	0.20	15.95	15.60	15.25	15.60	0.35	7.80	0.21
NW US 150	7.6	7.52	7.58	7.57	0.04	4.67	4.76	4.65	4.69	0.06	0.50	5.86	5.52	5.86	5.75	0.20		
NW US 200	7.82	7.79	7.72	7.78	0.05	5.14	5.37	5.6										
NW US 300	7.54	7.54	7.55	7.54	0.01	5.42	5.48	5.6	5.50	0.09	1.00	2.12	2.06	1.95	2.04	0.09		
NW DS 60	8.11	8	8.02	8.04	0.06	5.35	5.67	5.72	5.58	0.20	0.20	13.80	11.65	11.50	12.32	1.29	5.74	0.50
NW DS150	8	8	8.01	8.00	0.01	5.05	5.18	5.27	5.17	0.11	0.50	5.90	5.64	5.48	5.67	0.21		
NW DS 200	8.01	8.04	8	8.02	0.02	6.22	6.3	6.04	6.19	0.13	0.67	2.67	2.60	2.93	2.73	0.17		
NW DS 300	8.02	8.04	8.05	8.04	0.02	5.8	5.46	6.1	5.79	0.32	1.00	2.22	2.58	1.95	2.25	0.32		
CONTROL	**8.04**	**8.02**	**8.01**	**8.02**	**0.02**	**7.85**	**7.43**	**7.63**	**7.64**	**0.21**	**1.00**	**0.19**	**0.59**	**0.38**	**0.39**	**0.20**		

Table 15: TSS values obtained in March

March								
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG	SD
NW BC 1	12.5988	12.6899	0.0911	12.6943	0.0955	**0.0176**	**0.01**	**0.003355**
NW BC 2	12.7003	12.7931	0.0928	12.796	0.0957	**0.0116**		
NW BC 3	13.0776	13.1697	0.0921	13.1727	0.0951	**0.012**		
NW AC 1	12.7741	12.8652	0.0911	12.865	0.0909	**-0.0008**	**0.03**	**0.023829**
NW AC 2	12.4964	12.5895	0.0931	12.598	0.1016	**0.034**		
NW AC 3	13.469	13.5594	0.0904	13.5706	0.1016	**0.0448**		
NW US 1	12.7538	12.8458	0.092	14.3782	1.6244	**6.1296**	**1.24**	**4.299678**
NW US 2	12.7145	12.8056	0.0911	12.6976	-0.0169	**-0.432**		
NW US 3	12.8333	12.9256	0.0923	12.4344	-0.3989	**-1.9648**		
NW DS 1	12.2821	12.3749	0.0928	12.849	0.5669	**1.8964**	**1.13**	**1.023156**
NW DS 2	12.3402	12.431	0.0908	12.8102	0.47	**1.5168**		
NW DS 3	12.602	12.938	0.336	12.9293	0.3273	**-0.0348**		

Table 16: TSS values obtained in April

April								
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG	SD
NW BC 1	13.0773	13.1687	0.0914	13.1877	0.1104	**0.095**	**0.096**	**0.018021**
NW BC 2	12.7	12.7927	0.0927	12.8156	0.1156	**0.1145**		
NW BC 3	12.5994	12.6925	0.0931	12.7082	0.1088	**0.0785**		
NW AC 1	12.7741	12.8661	0.092	12.8815	0.1074	**0.0616**	**0.066**	**0.022666**
NW AC 2	14.2819	14.3749	0.093	14.3862	0.1043	**0.0452**		
NW AC 3	13.4686	13.5615	0.0929	13.584	0.1154	**0.09**		
NW US 1	12.7533	12.8447	0.0914	12.8488	0.0955	**0.0164**	**0.015**	**0.001222**
NW US 2	12.4966	12.5895	0.0929	12.5934	0.0968	**0.0156**		
NW US 3	12.8333	12.9268	0.0935	12.9303	0.097	**0.014**		
NW DS 1	12.6015	12.6936	0.0921	12.6969	0.0954	**0.0132**	**0.012**	**0.006503**
NW DS 2	12.3404	12.4311	0.0907	12.4355	0.0951	**0.0176**		
NW DS 3	12.7148	12.808	0.0932	12.8092	0.0944	**0.0048**		
C0NTROL	12.4698	12.5611	0.0913	12.5612	0.0914	**0.0004**		

Table 17: TSS values obtained in May

May								
SAMPLE	**WEIGHT OF PETRI-DISH**	**WEIGHT OF PD + FILTER**	**WEIGHT OF FILTER**	**WEIGHT OF PD + FILTER AFTER FILTRATION**	**WEIGHT OF FILTER AFTER FILTRATION**	**TSS**	**AVG**	**SD**
NW BC 1	24.9914	25.3208	0.3294	25.3274	0.336	**0.033**	**0.035**	**0.004444**
NW BC 2	24.424	24.756	0.332	24.7639	0.3399	**0.0395**		
NW BC 3	37.4848	37.8149	0.3301	37.8211	0.3363	**0.031**		
NW AC 1	21.9558	22.2827	0.3269	22.2858	0.33	**0.0124**	**0.016**	**0.005787**
NW AC 2	22.2848	22.6155	0.3307	22.6188	0.334	**0.0132**		
NW AC 3	24.431	24.7605	0.3295	24.7662	0.3352	**0.0228**		
NW US 1	24.3661	24.6937	0.3276	24.6982	0.3321	**0.018**	**0.013**	**0.004277**
NW US 2	22.4333	22.7659	0.3326	22.7683	0.335	**0.0096**		
NW US 3	24.37	24.7013	0.3313	24.7044	0.3344	**0.0124**		
NW DS 1	59.4128	59.739	0.3262	59.746	0.3332	**0.028**	**5.925**	**9.129095**
NW DS 2	24.3979	24.9689	0.571	25.2957	0.8978	**1.3072**		
NW DS 3	19.9517	20.2856	0.3339	24.3958	4.4441	**16.4408**		
C0NTROL	21.9423	22.2762	0.3339	22.2768	0.3345	**0.0024**		

Table 18: TSS values obtained in June

June								
SAMPLE	**WEIGHT OF PETRI-DISH**	**WEIGHT OF PD + FILTER**	**WEIGHT OF FILTER**	**WEIGHT OF PD + FILTER AFTER FILTRATION**	**WEIGHT OF FILTER AFTER FILTRATION**	**TSS**	**FINAL AVG**	**SD**
NW BC 1	13.1784	13.273	0.0946	13.276	0.0976	**0.015**	**0.017667**	**0.002754**
NW BC 2	12.5997	12.6929	0.0932	12.697	0.0973	**0.0205**		
NW BC 3	12.7012	12.7954	0.0942	12.7989	0.0977	**0.0175**		
NW AC 1	14.5535	14.6457	0.0922	14.651	0.0975	**0.0212**	**0.0204**	**0.002498**
NW AC 2	14.2797	14.372	0.0923	14.3776	0.0979	**0.0224**		
NW AC 3	13.4671	13.5608	0.0937	13.5652	0.0981	**0.0176**		
NW US 1	12.7521	12.8455	0.0934	12.8471	0.095	**0.0064**	**0.0068**	**0.000693**
NW US 2	12.4958	12.5899	0.0941	12.5918	0.096	**0.0076**		
NW US 3	12.8323	12.9258	0.0935	12.9274	0.0951	**0.0064**		
NW DS 1	12.5412	12.6336	0.0924	12.6358	0.0946	**0.0088**	**0.0112**	**0.002227**
NW DS 2	12.34	12.4326	0.0926	12.4359	0.0959	**0.0132**		
NW DS 3	12.7144	12.8064	0.092	12.8093	0.0949	**0.0116**		
C0NTROL	12.4691	12.5602	0.0911	12.5611	0.092	**0.0036**		

Table 19: TSS values obtained in July

July								
SAMPLE	**WEIGHT OF PETRI-DISH**	**WEIGHT OF PD + FILTER**	**WEIGHT OF FILTER**	**WEIGHT OF PD + FILTER AFTER FILTRATION**	**WEIGHT OF FILTER AFTER FILTRATION**	**TSS**	**FINAL AVG**	**SD**
NW BC 1	13.0768	13.1695	0.0927	13.1765	0.0997	**0.035**	**0.03**	**0.005568**
NW BC 2	12.5997	12.6924	0.0927	12.6972	0.0975	**0.024**		
NW BC 3	12.6995	12.7923	0.0928	12.7985	0.099	**0.031**		
NW AC 1	14.5553	14.6482	0.0929	14.6554	0.1001	**0.0288**	**0.028**	**0.00485**
NW AC 2	14.2811	14.375	0.0939	14.3807	0.0996	**0.0228**		
NW AC 3	13.4686	13.5619	0.0933	13.57	0.1014	**0.0324**		
NW US 1	12.7533	12.8455	0.0922	12.8473	0.094	**0.0072**	**0.007067**	**0.001007**
NW US 2	12.4961	12.5889	0.0928	12.5909	0.0948	**0.008**		
NW US 3	12.774	12.866	0.092	12.8675	0.0935	**0.006**		
NW DS 1	12.5399	12.6328	0.0929	12.6377	0.0978	**0.0196**	**0.0164**	**0.002884**
NW DS 2	12.6013	12.6946	0.0933	12.6985	0.0972	**0.0156**		
NW DS 3	13.1786	13.2718	0.0932	13.2753	0.0967	**0.014**		
C0NTROL	12.4683	12.5601	0.0918	12.5602	0.0919	**0.0004**		

Table 20: TSS values obtained in August

August								
SAMPLE	**WEIGHT OF PETRI-DISH**	**WEIGHT OF PD + FILTER**	**WEIGHT OF FILTER**	**WEIGHT OF PD + FILTER AFTER FILTRATION**	**WEIGHT OF FILTER AFTER FILTRATION**	**TSS**	**FINAL AVG**	**SD**
NW BC 1	13.0776	13.1695	0.0919	13.1789	0.1013	**0.047**	**0.037333**	**0.01193**
NW BC 2	12.5983	12.6913	0.093	12.6995	0.1012	**0.041**		
NW BC 3	12.6991	12.793	0.0939	12.7978	0.0987	**0.024**		
NW AC 1	14.5545	14.6474	0.0929	14.6587	0.1042	**0.0452**	**0.048533**	**0.003885**
NW AC 2	12.7131	12.8069	0.0938	12.8188	0.1057	**0.0476**		
NW AC 3	13.4671	13.56	0.0929	13.5732	0.1061	**0.0528**		
NW US 1	12.7516	12.8449	0.0933	12.8519	0.1003	**0.028**	**0.023867**	**0.003585**
NW US 2	12.4952	12.5876	0.0924	12.5931	0.0979	**0.022**		
NW US 3	12.7725	12.8649	0.0924	12.8703	0.0978	**0.0216**		
NW DS 1	12.541	12.6328	0.0918	12.6368	0.0958	**0.016**	**0.015467**	**0.002053**
NW DS 2	12.6	12.6929	0.0929	12.6962	0.0962	**0.0132**		
NW DS 3	13.1756	13.2688	0.0932	13.2731	0.0975	**0.0172**		
C0NTROL	12.4684	12.5609	0.0925	12.561	0.0926	**0.0004**		

B: New Germany Wastewater Works

Table 1: Temperature values obtained over the six month period

Temperature °C						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	26.00	25.50	25.50	25.67	0.29
	DP	26.00	26.00	26.00	26.00	0.00
	US	26.00	26.00	26.00	26.00	0.00
	DS	25.50	25.50	25.50	25.50	0.00
April	**BC**	20.00	20.50	20.00	20.17	0.29
	DP	20.50	20.00	20.00	20.17	0.29
	US	18.00	18.00	17.00	17.67	0.58
	DS	19.00	19.00	19.00	19.00	0.00
May	**BC**	19.00	19.00	19.00	19.00	0.00
	DP	18.50	18.50	18.50	18.50	0.00
	US	13.00	14.00	14.00	13.67	0.58
	DS	16.00	16.20	16.00	16.07	0.12
June	**BC**	18.00	18.00	18.00	18.00	0.00
	DP	17.50	17.50	17.50	17.50	0.00
	US	16.00	16.00	16.00	16.00	0.00
	DS	14.00	13.80	13.80	13.87	0.12
July	**BC**	16.50	16.50	16.50	16.50	0.00
	DP	17.00	17.00	17.00	17.00	0.00
	US	13.50	13.50	13.50	13.50	0.00
	DS	14.50	14.50	14.50	14.50	0.00
August	**BC**	16.80	16.80	16.80	16.80	0.00
	DP	16.50	16.50	16.50	16.50	0.00
	US	14.50	14.50	14.50	14.50	0.00
	DS	12.00	12.00	12.00	12.00	0.00

Table 2: Salinity of the water samples over the six month period

Salinity %						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	0.44	0.43	0.44	0.44	0.01
	DP	0.47	0.48	0.48	0.48	0.01
	US	0.21	0.21	0.21	0.21	0.00
	DS	0.30	0.30	0.30	0.30	0.00
April	**BC**	0.39	0.39	0.38	0.39	0.01
	DP	0.43	0.43	0.44	0.43	0.01
	US	0.16	0.16	0.16	0.16	0.00
	DS	0.30	0.31	0.30	0.30	0.00
May	**BC**	0.57	0.57	0.57	0.57	0.00
	DP	0.63	0.62	0.62	0.62	0.01
	US	0.17	0.17	0.17	0.17	0.00
	DS	0.46	0.46	0.46	0.46	0.00
June	**BC**	0.53	0.53	0.54	0.53	0.01
	DP	0.58	0.58	0.58	0.58	0.00
	US	0.18	0.18	0.18	0.18	0.00
	DS	0.40	0.40	0.40	0.40	0.00
July	**BC**	0.46	0.47	0.47	0.47	0.01
	DP	0.52	0.52	0.53	0.52	0.01
	US	0.15	0.15	0.15	0.15	0.00
	DS	0.34	0.34	0.34	0.34	0.00
August	**BC**	0.41	0.42	0.42	0.42	0.01
	DP	0.47	0.47	0.47	0.47	0.00
	US	0.35	0.34	0.34	0.34	0.00
	DS	0.37	0.37	0.37	0.37	0.00

Table 3: Conductivity of the water samples over the six month period

Conductivity (µS/cm)						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	893.00	854.00	888.00	878.33	21.22
	DP	957.00	976.00	978.00	970.33	11.59
	US	428.00	433.00	433.00	431.33	2.89
	DS	616.00	613.00	616.00	615.00	1.73
April	**BC**	791.00	793.00	787.00	790.33	3.06
	DP	883.00	880.00	889.00	884.00	4.58
	US	330.00	329.00	327.00	328.67	1.53
	DS	627.00	628.00	627.00	627.33	0.58
May	**BC**	1143.00	1148.00	1154.00	1148.33	5.51
	DP	1257.00	1251.00	1253.00	1253.67	3.06
	US	365.00	363.00	363.00	363.67	1.15
	DS	939.00	941.00	939.00	939.67	1.15
June	**BC**	1086.00	1082.00	1085.00	1084.33	2.08
	DP	1164.00	1167.00	1168.00	1166.33	2.08
	US	384.00	383.00	384.00	383.67	0.58
	DS	828.00	827.00	825.00	826.67	1.53
July	**BC**	974.00	975.00	975.00	974.67	0.58
	DP	1069.00	1068.00	1070.00	1069.00	1.00
	US	322.00	322.00	321.00	321.67	0.58
	DS	708.00	707.00	705.00	706.67	1.53
August	**BC**	849.00	851.00	848.00	849.33	1.53
	DP	960.00	958.00	956.00	958.00	2.00
	US	699.00	707.00	714.00	706.67	7.51
	DS	756.00	756.00	760.00	757.33	2.31

Table 4: Resistivity of the water samples over the six month period

Resistivity (Ω.cm)						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	1120.00	1131.00	1126.00	1125.67	5.51
	DP	1025.00	1022.00	1024.00	1023.67	1.53
	US	2340.00	2310.00	2310.00	2320.00	17.32
	DS	1623.00	1630.00	1615.00	1622.67	7.51
April	**BC**	1265.00	1261.00	1270.00	1265.33	4.51
	DP	1132.00	1137.00	1125.00	1131.33	6.03
	US	303.00	304.00	306.00	304.33	1.53
	DS	1596.00	1592.00	1595.00	1594.33	2.08
May	**BC**	875.00	871.00	867.00	871.00	4.00
	DP	795.00	800.00	798.00	797.67	2.52
	US	2.74	2.76	2.76	2.75	0.01
	DS	1065.00	1063.00	1065.00	1064.33	1.15
June	**BC**	926.00	924.00	926.00	925.33	1.15
	DP	859.00	857.00	856.00	857.33	1.53
	US	2610.00	2610.00	2610.00	2610.00	0.00
	DS	1208.00	1210.00	1210.00	1209.33	1.15
July	**BC**	1026.00	1027.00	1027.00	1026.67	0.58
	DP	936.00	935.00	935.00	935.33	0.58
	US	3110.00	3110.00	3110.00	3110.00	0.00
	DS	1413.00	1414.00	1418.00	1415.00	2.65
August	**BC**	1175.00	1206.00	1180.00	1187.00	16.64
	DP	1042.00	1044.00	1046.00	1044.00	2.00
	US	1432.00	1414.00	1411.00	1419.00	11.36
	DS	1323.00	1323.00	1316.00	1320.67	4.04

Table 5: TDS concentrations obtained over the six month period

Total Dissolved Solids mg/L						
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std dev**
March	**BC**	438.00	434.00	436.00	436.00	2.00
	DP	471.00	480.00	481.00	477.33	5.51
	US	206.00	209.00	208.90	207.97	1.70
	DS	300.00	298.00	299.00	299.00	1.00
April	**BC**	387.00	388.00	385.00	386.67	1.53
	DP	433.00	432.00	436.00	433.67	2.08
	US	158.30	157.80	156.90	157.67	0.71
	DS	305.00	306.00	305.00	305.33	0.58
May	**BC**	565.00	567.00	570.00	567.33	2.52
	DP	623.00	620.00	621.00	621.33	1.53
	US	175.40	174.40	174.20	174.67	0.64
	DS	461.00	463.00	462.00	462.00	1.00
June	**BC**	536.00	534.00	533.00	534.33	1.53
	DP	576.00	578.00	578.00	577.33	1.15
	US	184.20	184.60	184.80	184.53	0.31
	DS	405.00	404.00	405.00	404.67	0.58
July	**BC**	464.00	467.00	470.00	467.00	3.00
	DP	523.00	527.00	527.00	525.67	2.31
	US	154.30	154.30	153.90	154.17	0.23
	DS	345.00	345.00	344.00	344.67	0.58
August	**BC**	416.00	417.00	417.00	416.67	0.58
	DP	472.00	471.00	470.00	471.00	1.00
	US	341.00	345.00	348.00	344.67	3.51
	DS	369.00	369.00	371.00	369.67	1.15

Table 6: pH values obtained over the six month period

pH												
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Final Ave**	**Std dev**
March	**BC**	7.08	6.93	7.35	7.12	0.21	7.08	6.93	7.35	7.12	0.21	7.12	0.00
	DP	7.29	7.05	7.21	7.18	0.12	7.29	7.05	7.21	7.18	0.12	7.18	0.00
	US	7.61	7.51	7.44	7.52	0.09	7.61	7.51	7.44	7.52	0.09	7.52	0.00
	DS	7.43	7.60	7.49	7.51	0.09	7.43	7.60	7.49	7.51	0.09	7.51	0.00
April	**BC**	6.61	6.63	6.58	6.61	0.03	7.01	7.02	7.08	7.04	0.03	6.82	0.25
	DP	6.54	6.56	6.60	6.57	0.03	6.83	6.82	6.82	6.82	0.01	6.70	0.15
	US	6.80	6.68	6.81	6.76	0.07	7.06	7.09	7.10	7.08	0.02	6.92	0.18
	DS	7.16	7.09	7.00	7.08	0.08	6.93	6.93	6.93	6.93	0.00	7.01	0.09
May	**BC**	6.97	6.94	6.81	6.91	0.09	7.18	6.93	6.76	6.96	0.19	6.93	0.03
	DP	7.02	7.02	7.03	7.02	0.01	6.97	6.80	6.75	6.84	0.10	6.93	0.11
	US	6.39	6.40	6.48	6.42	0.05	6.79	6.77	6.92	6.83	0.07	6.63	0.23
	DS	7.10	7.10	7.10	7.10	0.00	6.69	6.51	6.50	6.57	0.10	6.83	0.31
June	**BC**	7.57	7.58	7.59	7.58	0.01	7.61	7.63	7.63	7.62	0.01	7.60	0.03
	DP	7.66	7.67	7.68	7.67	0.01	7.54	7.55	7.55	7.55	0.01	7.61	0.07
	US	7.93	7.91	7.93	7.92	0.01	7.94	7.92	7.93	7.93	0.01	7.93	0.00
	DS	7.79	7.78	7.78	7.78	0.01	7.83	7.83	7.83	7.83	0.00	7.81	0.03
July	**BC**	6.92	7.00	6.85	6.92	0.07	6.63	6.43	6.53	6.53	0.09	6.73	0.23
	DP	6.26	6.37	6.28	6.30	0.05	6.88	6.89	6.90	6.89	0.01	6.60	0.34
	US	6.40	6.37	6.38	6.38	0.01	6.29	6.30	6.31	6.30	0.01	6.34	0.05
	DS	6.05	5.90	6.03	5.99	0.07	6.82	6.99	7.00	6.94	0.09	6.47	0.54
August	**BC**	6.75	6.26	6.95	6.65	0.36	6.75	6.26	6.95	6.65	0.36	6.65	0.00
	DP	7.05	7.09	7.18	7.11	0.07	7.05	7.09	7.18	7.11	0.07	7.11	0.00
	US	7.10	7.11	7.15	7.12	0.03	7.10	7.11	7.15	7.12	0.03	7.12	0.00
	DS	7.24	7.26	7.28	7.26	0.02	7.24	7.26	7.28	7.26	0.02	7.26	0.00

Table 7: Turbidity of the water samples over the six month period

Turbidity (Ntu)													
	Point	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Average**	**Std Dev**	**Final Ave**	**Std dev**
March	**BC**	6.41	6.86	6.67	6.65	0.23	6.41	6.86	6.67	6.65	0.23	6.65	0.00
	DP	5.45	5.29	6.38	5.71	0.59	5.45	5.29	6.38	5.71	0.59	5.71	0.00
	US	5.22	5.15	5.12	5.16	0.05	5.22	5.15	5.12	5.16	0.05	5.16	0.00
	DS	7.70	7.13	7.14	7.32	0.33	7.70	7.13	7.14	7.32	0.33	7.32	0.00
April	**BC**	1.52	1.52	1.52	1.52	0.00	1.15	1.15	1.15	1.15	0.00	1.34	0.21
	DP	1.43	1.43	1.40	1.42	0.02	1.44	1.46	1.45	1.45	0.01	1.44	0.02
	US	8.23	8.23	8.23	8.23	0.00	9.27	9.27	9.28	9.27	0.01	8.75	0.60
	DS	17.00	17.00	17.00	17.00	0.00	17.20	17.20	17.20	17.20	0.00	17.10	0.12
May	**BC**	28.70	28.70	28.70	28.70	0.00	25.90	20.10	19.10	21.70	3.28	25.20	4.04
	DP	30.30	30.30	30.30	30.30	0.00	14.40	14.30	14.40	14.37	0.05	22.33	9.20
	US	3.18	3.18	3.18	3.18	0.00	14.70	14.30	14.10	14.37	0.27	8.77	6.46
	DS	17.80	17.80	17.80	17.80	0.00	14.30	14.50	14.00	14.27	0.23	16.03	2.04
June	**BC**	4.68	4.73	4.78	4.73	0.04	10.50	10.20	11.20	10.63	0.46	7.68	3.41
	DP	5.94	5.96	5.88	5.93	0.04	8.91	9.15	8.99	9.02	0.11	7.47	1.78
	US	4.11	4.11	4.11	4.11	0.00	9.65	9.65	9.60	9.63	0.03	6.87	3.19
	DS	4.19	4.19	4.19	4.19	0.00	14.00	14.20	14.00	14.07	0.10	9.13	5.70
July	**BC**	23.40	20.70	19.70	21.27	1.91	20.20	20.00	20.00	20.07	0.15	20.67	0.69
	DP	27.70	28.70	28.40	28.27	0.51	20.70	20.60	20.90	20.73	0.00	24.50	4.35
	US	12.30	12.60	12.20	12.37	0.21	2.43	2.43	2.43	2.43	0.00	7.40	5.74
	DS	23.80	23.30	23.40	23.50	0.26	16.10	16.10	16.10	16.10	0.00	19.80	4.27
August	**BC**	19.60	19.70	19.90	19.73	0.15	19.60	19.70	19.90	19.73	0.15	19.73	0.00
	DP	16.90	16.60	16.90	16.80	0.17	16.90	16.60	16.90	16.80	0.17	16.80	0.00
	US	40.10	40.30	40.80	40.40	0.36	40.10	40.30	40.80	40.40	0.36	40.40	0.00
	DS	14.00	14.10	14.20	14.10	0.10	14.00	14.10	14.20	14.10	0.10	14.10	0.00

Table 8: COD concentrations obtained over the six month period

COD mg/L															
		Replicate	Replicate 2	Replicate	Average	Average 1	Std Dev	Replicate 1	Replicate 2	Replicate 3	Average	Std Dev	Average 2	Final Ave	Std Dev
March	BC 1	149.00	150.00	150.00	149.67		0.58	151.00	150.00	149.00	150.00	1.00			
	BC2	174.00	169.00	163.00	168.67		5.51	166.00	167.00	167.00	166.67	0.58			
	BC3	147.00	145.00	145.00	145.67	154.67	1.15	145.00	146.00	145.00	145.33	0.58	154.00	154.33	0.38
	DP 1	245.00	251.00	251.00	249.00		3.46	247.00	249.00	249.00	248.33	1.15			
	DP 2	239.00	239.00	239.00	239.00		0.00	235.00	237.00	237.00	236.33	1.15			
	DP 3	229.00	234.00	234.00	232.33	240.11	2.89	229.00	230.00	229.00	229.33	0.58	238.00	239.05	1.21
	US 1	153.00	153.00	153.00	153.00		0.00	314.00	314.00	314.00	314.00	0.00			
	US 2	142.00	141.00	141.00	141.33		0.58	314.00	314.00	314.00	314.00	0.00			
	US 3	131.00	131.00	132.00	131.33	141.89	0.58	310.00	311.00	312.00	311.00	1.00	141.40	141.67	0.25
	DS 1	311.00	314.00	314.00	313.00		1.73	153.00	153.00	153.00	153.00	0.00			
	DS 2	315.00	314.00	316.00	315.00		1.00	140.00	140.00	140.00	140.00	0.00			
	DS 3	313.00	315.00	316.00	314.67	314.22	1.53	132.00	131.00	131.00	131.33	0.58	313.00	313.61	0.70
April	BC 1	212.00	210.00	211.00	211.00		1.00	201.00	201.00	201.00	201.00	0.00			
	BC2	193.00	193.00	193.00	193.00		0.00	195.00	195.00	195.00	195.00	0.00			
	BC3	204.00	205.00	204.00	204.33	202.78	0.58	191.00	191.00	192.00	191.33	0.58	195.78	199.28	4.04
	DP 1	190.00	190.00	190.00	190.00		0.00	180.00	181.00	181.00	180.67	0.58			
	DP 2	173.00	173.00	175.00	173.67		1.15	180.00	180.00	180.00	180.00	0.00			
	DP 3	179.00	179.00	180.00	179.33	181.00	0.58	179.00	178.00	178.00	178.33	0.58	179.67	180.34	0.76
	US 1	101.00	101.00	101.00	101.00		0.00	106.00	106.00	106.00	106.00	0.00			
	US 2	106.00	105.00	105.00	105.33		0.58	100.00	100.00	100.00	100.00	0.00			
	US 3	105.00	107.00	107.00	106.33	104.22	1.15	95.00	95.00	95.00	95.00	0.00	100.00	102.27	2.24
	DS 1	112.00	112.00	112.00	112.00		0.00	113.00	113.00	113.00	113.00	0.00			
	DS 2	114.00	114.00	114.00	114.00		0.00	116.00	116.00	116.00	116.00	0.00			
	DS 3	125.00	125.00	123.00	124.33	116.78	1.15	113.00	113.00	113.00	113.00	0.00	114.00	115.39	1.60
May	BC 1	312.00	312.00	312.00	312.00		0.00	315.00	314.00	314.00	314.33	0.52			
	BC2	310.00	313.00	312.00	311.67		1.53	312.00	312.00	311.00	311.67	0.52			
	BC3	313.00	313.00	313.00	313.00	312.22	0.00	312.00	313.00	317.00	314.00	1.30	313.33	312.76	0.64
	DP 1	247.00	247.00	247.00	247.00		0.00	238.00	240.00	245.00	241.00	3.22			
	DP 2	249.00	249.00	249.00	249.00		0.00	246.00	245.00	246.00	245.67	0.52			
	DP 3	241.00	243.00	245.00	243.00	246.33	2.00	247.00	245.00	247.00	246.33	1.03	244.33	245.33	1.15
	US 1	298.00	299.00	298.00	298.33		0.58	311.00	311.00	310.00	310.67	0.52			
	US 2	299.00	299.00	298.00	298.67		0.58	313.00	314.00	313.00	313.33	0.52			
	US 3	299.00	299.00	299.00	299.00	298.67	0.00	313.00	315.00	313.00	313.67	1.03	312.56	305.61	8.01
	DS 1	314.00	314.00	312.00	313.33		1.15	292.00	292.00	292.00	292.00	0.00			
	DS 2	313.00	312.00	314.00	313.00		1.00	290.00	293.00	296.00	293.00	2.68			
	DS 3	308.00	310.00	310.00	309.33	311.89	1.15	294.00	296.00	296.00	295.33	1.03	293.44	302.67	10.64

Table 8 continued…

COD mg/L															
		Replicate	Replicate	Replicate	Average	Average 1	Std Dev	Replicate	Replicate	Replicate	Average	Std Dev	Average 2	Final Ave	Std Dev
June	**BC 1**	303.00	303.00	302.00	302.67		0.58	312.00	309.00	309.00	310.00	1.55			
	BC2	306.00	305.00	304.00	305.00		1.00	312.00	309.00	309.00	310.00	1.55			
	BC3	306.00	305.00	305.00	305.33	304.33	0.58	312.00	309.00	309.00	310.00	1.55	310	307.17	3.27
	DP 1	110.00	111.00	111.00	110.67		0.58	110.00	111.00	111.00	110.67	0.52			
	DP 2	113.00	113.00	113.00	113.00		0.00	113.00	113.00	113.00	113.00	0.00			
	DP 3	109.00	108.00	108.00	108.33	110.67	0.58	109.00	108.00	108.00	108.33	0.52	110	110.33	0.38
	US 1	34.00	32.00	33.00	33.00		1.00	25.00	18.00	24.00	22.33	3.39			
	US 2	29.00	29.00	29.00	29.00		0.00	25.00	18.00	24.00	22.33	3.39			
	US 3	29.00	29.00	29.00	29.00	30.33	0.00	25.00	18.00	24.00	22.33	3.39	22.33	26.33	4.61
	DS 1	75.00	75.00	75.00	75.00		0.00	78.00	72.00	70.00	73.33	3.72			
	DS 2	76.00	75.00	75.00	75.33		0.58	78.00	72.00	70.00	73.33	3.72			
	DS 3	63.00	63.00	64.00	63.33	71.22	0.58	78.00	72.00	70.00	73.33	3.72	73.33	72.28	1.21
July	**BC 1**	194.00	193.00	194.00	193.67		0.58	194.00	193.00	194.00	193.67	0.58			
	BC2	190.00	190.00	190.00	190.00		0.00	190.00	190.00	190.00	190.00	0.00			
	BC3	197.00	198.00	197.00	197.33	193.67	0.58	197.00	198.00	197.00	197.33	0.58	193.67	193.67	0
	DP 1	309.00	309.00	309.00	309.00		0.00	309.00	309.00	309.00	309.00	0.00			
	DP 2	309.00	308.00	308.00	308.33		0.58	309.00	308.00	308.00	308.33	0.58			
	DP 3	310.00	307.00	309.00	308.67	308.67	1.53	310.00	309.00	307.00	308.67	1.53	308.67	308.67	0
	US 1	314.00	310.00	311.00	311.67		2.08	314.00	310.00	311.00	311.67	2.08			
	US 2	310.00	310.00	310.00	310.00		0.00	310.00	310.00	310.00	310.00	0.00			
	US 3	304.00	309.00	304.00	305.67	309.00	2.89	309.00	309.00	304.00	307.33	2.89	309.67	309.39	0.32
	DS 1	292.00	292.00	291.00	291.67		0.58	298.00	299.00	298.00	298.33	0.58			
	DS 2	295.00	295.00	295.00	295.00		0.00	295.00	296.00	296.00	295.67	0.58			
	DS 3	305.00	302.00	302.00	303.00	296.56	1.73	304.00	305.00	305.00	304.67	0.58	299.56	298.06	1.73
August	**BC 1**	138.00	139.00	139.00	138.67		0.58	138.00	139.00	139.00	138.67	0.58			
	BC2	142.00	140.00	140.00	140.67		1.15	142.00	140.00	140.00	140.67	1.15			
	BC3	139.00	139.00	140.00	139.33	139.56	0.58	139.00	139.00	140.00	139.33	0.58	139.56	139.56	0
	DP 1	308.00	307.00	308.00	307.67		0.58	308.00	307.00	308.00	307.67	0.58			
	DP 2	309.00	310.00	308.00	309.00		1.00	309.00	310.00	308.00	309.00	1.00			
	DP 3	310.00	310.00	311.00	310.33	309.00	0.58	310.00	310.00	311.00	310.33	0.58	309	309.00	0
	US 1	205.00	207.00	207.00	206.33		1.15	205.00	207.00	207.00	206.33	1.15			
	US 2	208.00	208.00	208.00	208.00		0.00	208.00	208.00	208.00	208.00	0.00			
	US 3	209.00	208.00	208.00	208.33	207.55	0.58	209.00	208.00	208.00	208.33	0.58	207.55	207.55	0
	DS 1	313.00	313.00	312.00	312.67		0.58	313.00	313.00	312.00	312.67	0.58			
	DS 2	312.00	312.00	311.00	311.67		0.58	312.00	312.00	311.00	311.67	0.58			
	DS 3	311.00	312.00	310.00	311.00	311.78	1.00	311.00	312.00	310.00	311.00	1.00	311.78	311.78	

Table 9: BOD concentrations obtained in March

March																		
	BOD DAY 0					BOD DAY 5												
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NW BC 200	8.37	8.17	8.25	8.26	0.10	7.15	7.05	7.23	7.14	0.09	0.67	1.82	1.67	1.52	1.67	0.15	2.20	0.13
NW BC 225	8.06	8.08	8.03	8.06	0.03	6.18	6.36	6.15	6.23	0.11	0.72	2.61	2.39	2.61	2.54	0.13		
NW BC 275	8.24	8.15	8.04	8.14	0.10	5.89	5.73	5.76	5.79	0.09	0.92	2.55	2.63	2.48	2.55	0.08		
NW BC 300	8.27	8.12	8.15	8.18	0.08	6.12	6.04	6.31	6.16	0.14	1.00	2.15	2.08	1.84	2.02	0.16		
NW AC 200	8.37	8.27	8.15	8.26	0.11	5.64	5.86	5.43	5.64	0.22	0.67	4.07	3.60	4.06	3.91	0.27	3.12	0.27
NW AC 225	8.23	8.25	8.26	8.25	0.02	6.69	6.41	6.33	6.48	0.19	0.72	2.14	2.56	2.68	2.46	0.28		
NW AC 275	8.15	8	8	8.05	0.09	6.06	5.83	5.93	5.94	0.12								
NW AC 300	7.97	8.18	8.08	8.08	0.11	5.99	5.73	5.96	5.89	0.14	1.00	1.98	2.45	2.12	3.00	0.24		
NW US 60	8.47	8.56	8.56	8.53	0.05	5.57	5.21	5.05	5.28	0.27	0.20	14.50	16.75	17.55	16.27	1.58	7.79	0.83
NW US 150	8.64	8.59	8.59	8.61	0.03	6.59	6.38	6.93	6.63	0.28	0.50	4.10	4.42	3.32	3.95	0.57		
NW US 200	8.61	8.58	8.58			4.24	4.12	4.72										
NW US 300	8.63	8.62	8.6	8.62	0.02	5.83	5.15	5.36	5.45	0.35	1.00	2.80	3.47	3.24	3.17	0.34		
NW DS 60	8.69	8.63	8.62	8.65	0.04	6.68	6.62	6.18	6.49	0.27	0.20	10.05	10.05	12.20	10.77	1.24	4.97	0.59
NW DS150	8.59	8.58	8.57	8.58	0.01	6.76	6.25	6.86	6.62	0.33	0.50	3.66	4.66	3.42	3.91	0.66		
NW DS 200	8.65	8.66	8.64	8.65	0.01	6.41	6.1	6.62	6.38	0.26	0.67	3.34	3.82	3.01	3.39	0.41		
NW DS 300	8.66	8.61	8.59	8.62	0.04	6.86	6.72	6.82	6.80	0.07	1.00	1.80	1.89	1.77	1.82	0.06		
CONTROL	8.71	8.7	8.69	**8.70**	**0.01**	6.84	6.19	6.19	**6.41**	**0.38**	**1.00**	**1.87**	**2.51**	**2.50**	**2.29**	**0.37**		

Table 10: BOD concentrations obtained in April

April																		
	BOD DAY 0					BOD DAY 5												
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NG BC 200	8.49	8.45	8.45	8.46	0.02	5.73	5.33	5.49	5.52	0.20	0.67	4.12	4.66	4.42	4.40	0.27	3.75	0.29
NG BC 225	8.37	8.36	8.35	8.36	0.01	5.31	5.52	5.86	5.56	0.28	0.72	4.25	3.94	3.46	3.88	0.40		
NG BC 275																		
NG BC 300	8.35	8.38	8.38	8.37	0.02	5.17	5.59	5.46	5.41	0.22	1.00	3.18	2.79	2.92	2.96	0.20		
NG AC 200	8.49	8.49	8.46	8.48	0.02	6.33	6.5	6.3	6.38	0.11	0.67	3.22	2.97	3.22	3.14	0.15	3.72	0.14
NG AC 225	8.5	8.49	8.5	8.50	0.01	5.31	5.37	5.41	5.36	0.05	0.72	4.43	4.33	4.29	4.35	0.07		
NG AC 275	8.52	8.52	8.54	8.53	0.01	5.38	5.39	5.09	5.29	0.17	0.92	3.41	3.40	3.75	3.52	0.20		
NG AC 300	8.56	8.52	8.5	8.53	0.03	4.74	4.47	4.71	4.64	0.15	1.00	3.82	4.05	3.79	3.89	0.14		
NG US 60	8.51	8.47	8.46	8.48	0.03	4.93	4.98	4.83	4.91	0.08	0.20	17.90	17.45	18.15	17.83	0.35	9.26	0.24
NG US 150	8.64	8.68	8.68	8.67	0.02	4.22	4.22	4.35	4.26	0.08	0.50	8.84	8.92	8.66	8.81	0.13		
NG US 200	8.73	8.78	8.78	8.76	0.03	4.72	4.25	4.34	4.44	0.25	0.67	5.99	6.76	6.63	6.46	0.41		
NG US 300	8.83	8.86	8.89	8.86	0.03	4.97	4.95	4.88	4.93	0.05	1.00	3.86	3.91	4.01	3.93	0.08		
NG DS 60	8.54	8.52	8.56	8.54	0.02	4.25	4.95	4.2	4.47	0.42	0.20	21.45	17.85	21.80	20.37	2.19	9.34	0.83
NG DS150	8.64	8.64	8.63	8.64	0.01	4.63	4.35	4.01	4.33	0.31	0.50	8.02	8.58	9.24	8.61	0.61		
NG DS 200	8.71	8.72	8.75	8.73	0.02	5.69	5.86	5.32	5.62	0.28	0.67	4.51	4.27	5.12	4.63	0.44		
NG DS 300	8.85	8.85	8.81	8.84	0.02	5.07	5.05	5.13	5.08	0.04	1.00	3.78	3.80	3.68	3.75	0.06		
CONTROL	**8.38**	**8.38**	**8.35**	**8.37**	**0.02**	5.13	5.8	5.64	**5.52**	**0.35**	**1.00**	**3.25**	**2.58**	**2.71**	**2.85**	**0.36**		

Table 11: BOD concentrations obtained in May

May																		
	BOD DAY 0						BOD DAY 5											
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NW BC 200	8.34	8.36	8.47	8.39	0.07	5.99	5	5.1	5.36	0.55	0.67	3.51	5.01	5.03	4.52	0.87	3.15	0.25
NW BC 225	8.38	8.34	8.35	8.36	0.02	4.96	4.88	4.81	4.88	0.08	0.72	4.75	4.81	4.92	4.82	0.08		
NW BC 275											0.92	0.00	0.00	0.00	0.00	0.00		
NW BC 300	8.17	8.16	8.08	8.14	0.05	4.95	4.88	4.81	4.88	0.07	1.00	3.22	3.28	3.27	3.26	0.03		
NW AC 200	8.08	8.16	8.1	8.11	0.04	4.44	4.35	4.27	4.35	0.09	0.67	5.43	5.69	5.72	5.61	0.16	4.72	0.16
NW AC 225	8.41	8.45	8.35	8.40	0.05	4.39	4.61	4.24	4.41	0.19	0.72	5.58	5.33	5.71	5.54	0.19		
NW AC 275	8.09	8	8.1	8.06	0.06	4.41	4.67	4.4	4.49	0.15	0.92	4.00	3.62	4.02	3.88	0.23		
NW AC 300	8.25	8.13	8.14	8.17	0.07	4.43	4.24	4.36	4.34	0.10	1.00	3.82	3.89	3.78	3.83	0.06		
NW US 60	8.79	8.73	8.7	8.74	0.05	4.88	4.87	4.24	4.66	0.37	0.20	19.55	19.30	22.30	20.38	1.66	11.04	0.97
NW US 150	8.61	8.65	8.68	8.65	0.04	4.84	4.09	4.44	4.46	0.38	0.50	7.54	9.12	8.48	8.38	0.79		
NW US 200	8.79	8.87	8.89	8.85	0.05	4.92	4.38	4.64										
NW US 300	8.83	8.87	8.89	8.86	0.03	4.94	4.07	4.52	4.51	0.44	1.00	3.89	4.80	4.37	4.35	0.46		
NW DS 60	8.49	8.56	8.59	8.55	0.05	4.35	4.07	4.24	4.22	0.14	0.20	20.70	22.45	21.75	21.63	0.88	9.67	0.55
NW DS150	8.64	8.64	8.61	8.63	0.02	4.95	4.21	4.56	4.57	0.37	0.50	7.38	8.86	8.10	8.11	0.74		
NW DS 200	8.68	8.65	8.65	8.66	0.02	4.3	4.87	4.62	4.60	0.29	0.67	6.54	5.64	6.01	6.06	0.45		
NW DS 300	8.57	8.49	8.51	8.52	0.04	5.84	5.53	5.55	5.64	0.17	1.00	2.73	2.96	2.96	2.88	0.13		
CONTROL	**8.65**	**8.63**	**8.60**	**8.63**	**0.03**	**7.66**	**7.67**	**7.69**	**7.67**	**0.02**	**1.00**	**0.99**	**0.96**	**0.91**	**0.95**	**0.04**		

Table 12: BOD concentrations obtained in June

June																		
	BOD DAY 0						BOD DAY 5											
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NW BC 200	7.86	7.81	7.84	7.84	0.03	4.65	4.15	4.31	4.37	0.26	0.67	4.79	5.46	5.27	5.17	0.35	4.17	0.39
NW BC 225	7.82	7.85	7.88	7.85	0.03	4.81	4.13	4.47	4.47	0.34	0.72	4.18	5.17	4.74	4.69	0.49		
NW BC 275	7.91	7.93	7.91	7.92	0.01	4.7	4.99	4.33	4.67	0.33	0.92	3.49	3.20	3.89	3.53	0.35		
NW BC 300	7.93	7.96	7.99	7.96	0.03	4.95	4.79	4.33	4.69	0.32	1.00	2.98	3.17	3.66	3.27	0.35		
NW AC 200	8.24	8.11	8.11	8.15	0.08	5.57	5.57	5.07	5.40	0.29	0.67	3.99	3.79	4.54	4.10	0.39	3.68	0.18
NW AC 225	8.04	8.06	8.03	8.04	0.02	5.23	5.18	5.21	5.21	0.03	0.72	3.90	4.00	3.92	3.94	0.05		
NW AC 275																		
NW AC 300	8.1	8.08	8.1	8.09	0.01	5.02	5.05	5.22	5.10	0.11	1.00	3.08	3.03	2.88	3.00	0.10		
NW US 60	7.96	7.98	7.97	7.97	0.01	4.89	4.88	4.46	4.74	0.25	0.20	15.35	15.50	17.55	16.13	1.23	8.90	0.63
NW US 150	8.1	8.13	8.16	8.13	0.03	4.69	4.98	4.54	4.74	0.22	0.50	6.82	6.30	7.24	6.79	0.47		
NW US 200	8.22	8.23	8.25			4.82	4.48	4.38										
NW US 300	8.58	8.56	8.53	8.56	0.03	4.98	4.57	4.76	4.77	0.21	1.00	3.60	3.99	3.77	3.79	0.20		
NW DS 60	7.82	7.82	7.79	7.81	0.02	5.78	5.82	5.5	5.70	0.17	0.20	10.20	10.00	11.45	10.55	0.79	6.73	0.46
NW DS150	8.04	8.03	8.02	8.03	0.01	4.18	4.39	4.3	4.29	0.11	0.50	7.72	7.28	7.44	7.48	0.22		
NW DS 200	7.74	7.74	7.62	7.70	0.07	4.26	4.7	4.62	4.53	0.23	0.67	5.19	4.54	4.48	4.74	0.40		
NW DS 300	8.64	8.67	8.73	8.68	0.05	4.5	4.9	4.13	4.51	0.39	1.00	4.14	3.77	4.60	4.17	0.42		
CONTROL	**8.23**	**8.13**	**8.15**	**8.17**	**0.05**	**7.40**	**7.56**	**7.60**	**7.52**	**0.11**	**1.00**	**0.83**	**0.57**	**0.55**	**0.65**	**0.16**		

Table 12 continued…

	BOD DAY 0					BOD DAY 5												
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NW BC 200	7.31	7.43	7.37	7.37	0.06	4.21	4.18	4.11	4.17	0.05	0.67	4.63	4.85	4.87	4.78	0.13	4.19	0.11
NW BC 225	7.53	7.55	7.51	7.53	0.02	3.97	3.91	3.85	3.91	0.06	0.72	4.94	5.06	5.08	5.03	0.07		
NW BC 275	7.48	7.49	7.46	7.48	0.02	4.06	4.24	4.19	4.16	0.09	0.92	3.72	3.53	3.55	3.60	0.10		
NW BC 300	7.47	7.43	7.43	7.44	0.02	4.19	3.9	4.16	4.08	0.16	1.00	3.28	3.53	3.27	3.36	0.15		
NW AC 200	8.04	8.01	8.03	8.03	0.02	3.55	3.71	3.68	3.65	0.09	0.67	6.70	6.42	6.49	6.54	0.15	5.03	0.07
NW AC 225	8.25	8.23	8.23	8.24	0.01	4.27	4.24	4.22	4.24	0.03	0.72	5.53	5.54	5.57	5.55	0.02		
NW AC 275	8.18	8.17	8.15			3.72	3.67	3.84										
NW AC 300	8.23	8.23	8.21	8.22	0.01	4	4.04	3.92	3.99	0.06	1.00	4.23	4.19	4.29	3.00	0.05		
NW US 200	8.31	8.32	8.32	8.32	0.01	4.34	4.32	4.04	4.23	0.17	0.20	19.85	20.00	21.40	20.42	0.85	10.80	0.41
NW US 225	8.37	8.37	8.36	8.37	0.01	4.41	4.54	4.71	4.55	0.15	0.50	7.92	7.66	7.30	7.63	0.31		
NW US 275	8.5	8.51	8.52			4.45	4.44	4.37										
NW US 300	8.68	8.71	8.73	8.71	0.03	4.4	4.31	4.31	4.34	0.05	1.00	4.28	4.40	4.42	4.37	0.08		
NW DS 200	8.23	8.25	8.23	8.24	0.01	4.59	4.71	4.51	4.60	0.10	0.20	18.20	17.70	18.60	18.17	0.45	9.55	2.09
NW DS 225	8.46	8.45	8.45	8.45	0.01	4.1	4.04	8.85	5.66	2.76	0.50	8.72	8.82	-0.80	5.58	5.53		
NW DS 275	8.65	8.67	8.68	8.67	0.02	5.29	5.62	5.24	5.38	0.21	0.67	5.01	4.55	5.13	4.90	0.31		

Average BOD obtained in June

Final BOD: June				
	AVG 1	AVG 2	AVG	STD DEV
NW BC	4.165984	4.19258	4.179282	0.015355
NW DP	3.681431	5.02787	4.35465	0.777367
NW US	8.902222	10.80333	9.852778	1.097607
NW DS	6.73408	7.161791	6.947935	0.246939

Table 13: BOD concentrations obtained in July

July																		
	BOD DAY 0						**BOD DAY 5**											
SAMPLE	**1**	**2**	**3**	**AVG**	**SD**	**1**	**2**	**3**	**AVG**	**SD**	**p value**	**bod 1**	**bod 2**	**bod 3**	**avg**	**sd**	**FINAL**	**sd**
NW BC 200	7.75	7.67	7.41	7.61	0.18	4.82	4.8	5.09	4.90	0.16	0.67	4.37	4.28	3.46	4.04	0.50	2.64	0.32
NW BC 225	7.54	7.34	7.33	7.40	0.12	4.15	4.6	4.35	4.37	0.23	0.72	4.71	3.81	4.14	4.22	0.46		
NW BC 275				0.00	0.00				0.00	0.00	0.92	0.00	0.00	0.00	0.00	0.00		
NW BC 300	6.52	6.8	6.39	6.57	0.21	4.59	4.21	4.06	4.29	0.27	1.00	1.93	2.59	2.33	2.28	0.33		
NW AC 200	8.62	8.59	8.65	8.62	0.03	4.94	4.49	4.59	4.67	0.24	0.67	5.49	6.12	6.06	5.89	0.35	4.94	0.26
NW AC 225	8.66	8.66	8.66	8.66	0.00	4.22	4.38	4.6	4.40	0.19	0.72	6.17	5.94	5.64	5.92	0.26		
NW AC 275	8.68	8.69	8.72	8.70	0.02	4.26	4.52	4.97	4.58	0.36								
NW AC 300	8.8	8.89	8.88	8.86	0.05	4.46	4.22	4.35	4.34	0.12	1.00	4.34	4.67	4.53	3.00	0.17		
NW US 60	8.47	8.36	8.31	8.38	0.08	4.12	4.31	4.59	4.34	0.24	0.20	21.75	20.25	18.60	20.20	1.58	11.01	0.71
NW US 150	8.61	8.58	8.58	8.59	0.02	4.15	4.19	4.39	4.24	0.13	0.50	8.92	8.78	8.38	8.69	0.28		
NW US 200	8.37	8.42	8.53			4.98	4.27	4.82										
NW US 300	8.69	8.74	8.84	8.76	0.08	4.39	4.91	4.57	4.62	0.26	1.00	4.30	3.83	4.27	4.13	0.26		
NW DS 60	8.16	8.25	8.28	8.23	0.06	4.92	4.56	4.85	4.78	0.19	0.20	16.20	18.45	17.15	17.27	1.13	9.42	0.55
NW DS150	8.32	8.38	8.41	8.37	0.05	3.97	3.61	3.62	3.73	0.21	0.50	8.70	9.54	9.58	9.27	0.50		
NW DS 200	8.4	8.59	8.55	8.51	0.10	4.59	4.08	4.29	4.32	0.26	0.67	5.69	6.73	6.36	6.26	0.53		
NW DS 300	8.57	8.73	8.82	8.71	0.13	3.73	3.89	3.87	3.83	0.09	1.00	4.84	4.84	4.95	4.88	0.06		
CONTROL	**8.15**	**8.17**	**8.17**	**8.16**	**0.01**	**7.21**	**7.26**	**7.37**	**7.28**	**0.08**	**1.00**	**0.94**	**0.91**	**0.80**	**0.88**	**0.07**		
	BOD DAY 0						**BOD DAY 5**											
SAMPLE	**1**	**2**	**3**	**AVG**	**SD**	**1**	**2**	**3**	**AVG**	**SD**	**p value**	**bod 1**	**bod 2**	**bod 3**	**avg**	**sd**	**FINAL**	**sd**
NW BC 200	6.86	6.97	7.04	6.96	0.09	5.2	5.3	5.32	5.27	0.06	0.67	2.48	2.49	2.57	2.51	0.05	2.12	0.17
NW BC 225	6.83	7.08	7.11	7.01	0.15	5.52	5.36	5.61	5.50	0.13	0.72	1.82	2.39	2.08	2.10	0.28		
NW BC 275	7.06	7.15	7.18	7.13	0.06	5.21	5.15	5.09	5.15	0.06	0.92	2.01	2.17	2.27	2.15	0.13		
NW BC 300	7.14	7.12	7.21	7.16	0.05	5.66	5.37	5.34	5.46	0.18	1.00	1.48	1.75	1.87	1.70	0.20		
NW AC 200	7.93	7.95	7.90	7.93	0.03	4.75	4.46	5.1	4.77	0.32	0.67	4.75	5.21	4.18	4.71	0.52	3.95	0.34
NW AC 225	7.83	7.85	7.87	7.85	0.02	4.79	5.15	4.65	4.86	0.26	0.72	4.22	3.75	4.47	4.15	0.37		
NW AC 275	7.79	7.84	7.78	7.80	0.03	5.09	4.85	4.86	4.93	0.14								
NW AC 300	7.85	7.87	7.90	7.87	0.03	5.14	5.04	4.92	5.03	0.11	1.00	2.71	2.83	2.98	3.00	0.14		
NW US 60	8.16	8.13	8.11	8.13	0.03	5.66	5.99	6.01	5.89	0.20	0.20	12.50	10.70	10.50	11.23	1.10	5.27	0.41
NW US 150	8.00	7.98	7.95	7.98	0.03	5.76	5.62	5.67	5.68	0.07	0.50	4.48	4.72	4.56	4.59	0.12		
NW US 200	7.97	7.99	7.97			5.52	5.67	5.59										
NW US 300				0.00	0.00				0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00		
NW DS 60	1.95	7.89	7.90	5.91	3.43	5.27	5	5.07	5.11	0.14	0.20	-16.60	14.45	14.15	4.00	17.84	4.32	4.59
NW DS150	8.00	7.90	7.99	7.96	0.06	5.16	4.98	4.93	5.02	0.12	0.50	5.68	5.84	6.12	5.88	0.22		
NW DS 200	7.95	7.93	7.98	7.95	0.03	5.05	4.94	4.97	4.99	0.06	0.67	4.33	4.46	4.49	4.43	0.09		
NW DS 300	8.02	8.03	8.03	8.03	0.01	5.12	5.18	4.82	5.04	0.19	1.00	2.90	2.85	3.21	2.99	0.20		
CONTROL	8.08	8.08	8.09	**8.08**	**0.01**	7.34	7.41	7.4	**7.38**	**0.04**	**1.00**	**0.74**	**0.67**	**0.69**	**0.70**	**0.04**		

Average BOD for July

Final BOD: July				
	AVG 1	AVG 2	AVG	STD DEV
NW BC	2.635182	2.115458	2.37532	0.300062
NW DP	4.935738	3.953197	4.444467	0.56727
NW US	11.00889	5.273333	8.141111	3.311425
NW DS	9.418843	4.323632	6.871238	2.941722

Table 14: BOD concentrations obtained in August

August																		
BOD DAY 0						BOD DAY 5												
SAMPLE	1	2	3	AVG	SD	1	2	3	AVG	SD	p value	bod 1	bod 2	bod 3	avg	sd	FINAL	sd
NW BC 200	8.08	7.88	7.92	7.96	0.11	4.83	4.36	4.76	4.65	0.25	0.67	4.85	5.25	4.72	4.94	0.28	4.10	0.31
NW BC 225	8.08	7.94	7.99	8.00	0.07	4.27	4.55	4.94	4.59	0.34	0.72	5.29	4.71	4.24	4.75	0.53		
NW BC 275																		
NW BC 300	7.86	7.83	7.83	7.84	0.02	5.2	5.35	5.14	5.23	0.11	1.00	2.66	2.48	2.69	2.61	0.11		
NW AC 200	8.28	8.13	8.06	8.16	0.11	4.12	4.43	4.42	4.32	0.18	0.67	6.21	5.52	5.43	5.72	0.42	4.49	0.38
NW AC 225	8.19	8.02	8.11	8.11	0.09	4.5	4.92	4.66	4.69	0.21	0.72	5.13	4.31	4.79	4.74	0.41		
NW AC 275	8.06	8.11	7.99	8.05	0.06	4.82	4.8	4.77	4.80	0.03								
NW AC 300	7.96	7.93	8.01	7.97	0.04	4.79	4.77	4.34	4.63	0.25	1.00	3.17	3.16	3.67	3.00	0.29		
NW US 60	8	7.99	7.98	7.99	0.01	3.39	3.85	3.64	3.63	0.23	0.20	23.05	20.70	21.70	21.82	1.18	10.61	0.63
NW US 150	7.66	7.61	7.65	7.64	0.03	4.13	4.47	4.59	4.40	0.24	0.50	7.06	6.28	6.12	6.49	0.50		
NW US 200	7.34	7.22	7.28			4.91	4.92	4.21										
NW US 300	7.12	7.2	6.8	7.04	0.21	3.64	3.47	3.47	3.53	0.10	1.00	3.48	3.73	3.33	3.51	0.20		
NW DS 60	8.14	8.18	8.21	8.18	0.04	4.74	4.34	4.66	4.58	0.21	0.20	17.00	19.20	17.75	17.98	1.12	8.84	0.60
NW DS150	8.59	8.53	8.5	8.54	0.05	4.47	4.51	4.98	4.65	0.28	0.50	8.24	8.04	7.04	7.77	0.64		
NW DS 200	8.46	8.53	8.57	8.52	0.06	4.76	4.66	4.97	4.80	0.16	0.67	5.52	5.78	5.37	5.56	0.20		
NW DS 300	8.43	8.55	8.54	8.51	0.07	4.83	4.05	4.53	4.47	0.39	1.00	3.60	4.50	4.01	4.04	0.45		
CONTROL	**8.44**	**8.27**	**8.19**	**8.30**	**0.13**	**7.20**	**7.26**	**7.42**	**7.29**	**0.11**	**1.00**	**1.24**	**1.01**	**0.77**	**1.01**	**0.24**		

Table 15: TSS values obtained in March

March							
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG
NW BC 1	13.0782	13.1705	0.0923	13.1727	0.0945	0.0088	**0.01027**
NW BC 2	12.7017	12.7932	0.0915	12.7958	0.0941	0.0104	
NW BC 3	12.5996	12.692	0.0924	12.6949	0.0953	0.0116	
NW AC 1	12.7748	12.8674	0.0926	12.87	0.0952	0.0104	**0.0164**
NW AC 2	14.2826	14.3755	0.0929	14.3811	0.0985	0.0224	
NW AC 3	13.4693	13.5615	0.0922	13.5656	0.0963	0.0164	
NW US 1	12.7538	12.8464	0.0926	12.8475	0.0937	0.0044	**0.00427**
NW US 2	12.497	12.5886	0.0916	12.5898	0.0928	0.0048	
NW US 3	12.8334	12.9275	0.0941	12.9284	0.095	0.0036	
NW DS 1	12.6001	12.6932	0.0931	12.6966	0.0965	0.0136	**0.0116**
NW DS 2	12.714	12.8055	0.0915	12.4348	-0.2792	-1.4828	
NW DS 3	12.3403	12.4327	0.0924	12.8087	0.4684	1.504	
control	12.4763	12.5673	0.091	12.5647	0.0884	-0.0104	

Table 16: TSS values obtained in April

April							
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG
NW BC 1	24.3989	24.7295	0.3306	24.7563	0.3574	**0.134**	**0.04633**
NW BC 2	12.7016	12.7953	0.0937	12.7954	0.0938	**0.0005**	
NW BC 3	12.5997	12.6928	0.0931	12.6937	0.094	**0.0045**	
NW AC 1	24.9109	25.2493	0.3384	25.2712	0.3603	**0.0876**	**0.03107**
NW AC 2	14.282	14.3742	0.0922	14.3751	0.0931	**0.0036**	
NW AC 3	13.4684	13.5619	0.0935	13.5624	0.094	**0.002**	
NW US 1	12.7534	12.8454	0.092	12.8479	0.0945	**0.01**	**0.01093**
NW US 2	12.4959	12.5862	0.0903	12.5894	0.0935	**0.0128**	
NW US 3	12.8327	12.9247	0.092	12.9272	0.0945	**0.01**	
NW DS 1	24.1408	24.472	0.3312	24.5059	0.3651	**0.1356**	**0.05707**
NW DS 2	12.3394	12.4304	0.091	12.4331	0.0937	**0.0108**	
NW DS 3	12.7131	12.8057	0.0926	12.8119	0.0988	**0.0248**	
CONTROL	12.4675	12.5624	0.0949	12.5635	0.096	**0.0044**	

Table 17: TSS values obtained in May

May							
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG
NW BC 1	14.559	14.6491	0.0901	14.657	0.098	**0.0395**	**0.0375**
NW BC 2	14.2817	14.3742	0.0925	14.3822	0.1005	**0.04**	
NW BC 3	13.4486	13.5613	0.1127	13.5679	0.1193	**0.033**	
NW AC 1	13.1787	13.2719	0.0932	13.2782	0.0995	**0.0252**	**0.02667**
NW AC 2	12.7012	12.7936	0.0924	12.6991	-0.0021	**-0.378**	
NW AC 3	12.5997	12.6921	0.0924	12.8003	0.2006	**0.4328**	
NW US 1	12.7533	12.8461	0.0928	12.8461	0.0928	**0**	**0.00027**
NW US 2	12.4966	12.5893	0.0927	12.5893	0.0927	**0**	
NW US 3	12.8332	12.9264	0.0932	12.9266	0.0934	**0.0008**	
NW DS 1	12.5415	12.6353	0.0938	12.6392	0.0977	**0.0156**	**0.01573**
NW DS 2	12.3402	12.434	0.0938	12.438	0.0978	**0.016**	
NW DS 3	12.7148	12.8076	0.0928	12.8115	0.0967	**0.0156**	
C0NTROI	12.4695	12.5635	0.094	12.5638	0.0943	**0.0012**	

Table 18: TSS values obtained in June

June							
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG
NW BC 1	13.178	13.2715	0.0935	13.2745	0.0965	**0.015**	**0.01383**
NW BC 2	12.599	12.6914	0.0924	12.694	0.095	**0.013**	
NW BC 3	12.7005	12.7936	0.0931	12.7963	0.0958	**0.0135**	
NW AC 1	14.5552	14.6475	0.0923	14.65	0.0948	**0.01**	**0.0092**
NW AC 2	14.281	14.3736	0.0926	14.3755	0.0945	**0.0076**	
NW AC 3	13.4683	13.5611	0.0928	13.5636	0.0953	**0.01**	
NW US 1	12.7527	12.8451	0.0924	12.8455	0.0928	**0.0016**	**0.00227**
NW US 2	12.496	12.5886	0.0926	12.5892	0.0932	**0.0024**	
NW US 3	12.8321	12.9249	0.0928	12.9256	0.0935	**0.0028**	
NW DS 1	12.3397	12.4325	0.0928	12.4342	0.0945	**0.0068**	**0.0068**
NW DS 2	12.5411	12.6338	0.0927	12.6354	0.0943	**0.0064**	
NW DS 3	12.7143	12.8074	0.0931	12.8092	0.0949	**0.0072**	
C0NTROI	12.4683	12.56	0.0917	12.5602	0.0919	**0.0008**	

Table 19: TSS values obtained in July

July							
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG
NW BC 1	13.0776	13.1706	0.093	13.1747	0.0971	**0.0205**	**0.02117**
NW BC 2	12.5998	12.6937	0.0939	12.6978	0.098	**0.0205**	
NW BC 3	12.7007	12.7935	0.0928	12.798	0.0973	**0.0225**	
NW AC 1	14.5559	14.6504	0.0945	14.6533	0.0974	**0.0116**	**0.02093**
NW AC 2	12.7139	12.8074	0.0935	12.8146	0.1007	**0.0288**	
NW AC 3	13.4684	13.5618	0.0934	13.5674	0.099	**0.0224**	
NW US 1	12.7532	12.847	0.0938	12.847	0.0938	**0**	**0**
NW US 2	12.4959	12.5908	0.0949	12.5908	0.0949	**0**	
NW US 3	12.7739	12.8679	0.094	12.8679	0.094	**0**	
NW DS 1	12.5408	12.6349	0.0941	12.6398	0.099	**0.0196**	**0.01547**
NW DS 2	12.6008	12.6936	0.0928	12.6978	0.097	**0.0168**	
NW DS 3	13.1783	13.2707	0.0924	13.2732	0.0949	**0.01**	
C0NTROI	12.4685	12.5631	0.0946	12.5631	0.0946	**0**	

Table 20: TSS values obtained in August

August								
SAMPLE	WEIGHT OF PETRI-DISH	WEIGHT OF PD + FILTER	WEIGHT OF FILTER	WEIGHT OF PD + FILTER AFTER FILTRATION	WEIGHT OF FILTER AFTER FILTRATION	TSS	FINAL AVG	SD
NW BC 1	13.0777	13.1702	0.0925	13.1733	0.0956	**0.0155**	**0.01517**	**0.00058**
NW BC 2	12.5992	12.6925	0.0933	12.6956	0.0964	**0.0155**		
NW BC 3	12.701	12.794	0.093	12.7969	0.0959	**0.0145**		
NW AC 1	14.5561	14.6489	0.0928	14.6529	0.0968	**0.016**	**0.01373**	**0.00205**
NW AC 2	12.7142	12.807	0.0928	12.81	0.0958	**0.012**		
NW AC 3	13.4688	13.5614	0.0926	13.5647	0.0959	**0.0132**		
NW US 1	12.7527	12.8471	0.0944	12.8528	0.1001	**0.0228**	**0.032**	**0.01172**
NW US 2	12.4962	12.5879	0.0917	12.5949	0.0987	**0.028**		
NW US 3	12.774	12.8661	0.0921	12.8774	0.1034	**0.0452**		
NW DS 1	12.5414	12.634	0.0926	12.6373	0.0959	**0.0132**	**0.01653**	**0.00577**
NW DS 2	12.6012	12.6945	0.0933	12.6978	0.0966	**0.0132**		
NW DS 3	13.1788	13.2718	0.093	13.2776	0.0988	**0.0232**		
C0NTROI	12.4688	12.5621	0.0933	12.561	0.0922	**-0.004**		

APPENDIX III - ANTIMICROBIAL RESISTANCE PROFILING

Table 1: Zones of inhibition (mm) of *E. coli* isolates tested against ampicillin

AMPICILLIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	7	7	7	0.00	R	29	11.5	12	11.75	0.35	R
2	14	14	14	0.00	S	30	14	14	14	0.00	S
3	8	7	7.5	0.71	R	31	12	12	12	0.00	R
4	15	15.5	15.25	0.35	S	32	12	11	11.5	0.71	R
5	9	8	8.5	0.71	R	33	10	11	10.5	0.71	R
6	4	4.5	4.25	0.35	R	34	11	11.5	11.25	0.35	R
7	6	5	5.5	0.71	R	35	15	15	15	0.00	S
8	8	8	8	0.00	R	36	15	15	15	0.00	S
9	14.5	14.5	14.5	0.00	S	37	12	13	12.5	0.71	R
10	13.5	13.5	13.5	0.00	S	38	11	11	11	0.00	R
11	8	8	8	0.00	R	39	12	13	12.5	0.71	R
12	11	10	10.5	0.71	R	40	10	10	10	0.00	R
13	9.5	8	8.75	1.06	R	41	15	15	15	0.00	S
14	8	8.5	8.25	0.35	R	42	9	8.5	8.75	0.35	R
15	8	9	8.5	0.71	R	43	11	11	11	0.00	R
16	10	11	10.5	0.71	R	44	14	14	14	0.00	S
17	12	11.5	11.75	0.35	R	45	12	12.5	12.25	0.35	R
18	14	15	14.5	0.71	S	46	13	11	12	1.41	R
19	9	9.5	9.25	0.35	R	47	16	15	15.5	0.71	S
20	16	16.5	16.25	0.35	S	48	15	15	15	0.00	S
21	8	8	8	0.00	R	49	17	17	17	0.00	S
22	14	14.5	14.25	0.35	S	50	11	12	11.5	0.71	R
23	10	10	10	0.00	R	51	18	17.5	17.75	0.35	S
24	11	12	11.5	0.71	R	52	10	12	11	1.41	R
25	16	15	15.5	0.71	S	53	12	12	12	0.00	R
26	15	15.5	15.25	0.35	S	54	12	13	12.5	0.71	R
27	10	11	10.5	0.71	R	55	11	11.5	11.25	0.35	R
28	11	12	11.5	0.71	R	56	10	10	10	0.00	R

Table 1 continued…

AMPICILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	11	11.5	11.25	0.35	R	81	11	12	11.5	0.71	R
56	10	10	10	0.00	R	82	13	9	11	2.83	R
57	18	18.5	18.25	0.35	S	83	12	11	11.5	0.71	R
58	11	11	11	0.00	R	84	15	16	15.5	0.71	S
59	15	15	15	0.00	S	85	13	9	11	2.83	R
60	12	12	12	0.00	R	86	9	8.5	8.75	0.35	R
61	11	11.5	11.25	0.35	R	87	8.5	9	8.75	0.35	R
62	16	15	15.5	0.71	S	88	10	9	9.5	0.71	R
63	13	13	13	0.00	R	89	16	15	15.5	0.71	S
64	12	12.5	12.25	0.35	R	90	16	15	15.5	0.71	S
65	12	13	12.5	0.71	R	91	12	12	12	0.00	R
66	13	12.5	12.75	0.35	R	92	17.5	17.5	17.5	0.00	S
67	15	15	15	0.00	S	93	16	15.5	15.75	0.35	S
68	11	10	10.5	0.71	R	94	18	17	17.5	0.71	S
69	18	17.5	17.75	0.35	S	95	10	10	10	0.00	R
70	9	8.5	8.75	0.35	R	96	11	11	11	0.00	R
71	8.5	8.5	8.5	0.00	R	97	15	14.5	14.75	0.35	S
72	11	12	11.5	0.71	R	98	11	11	11	0.00	R
73	16	15.5	15.75	0.35	S	99	12.5	12.5	12.5	0.00	R
74	15	15	15	0.00	S	100	12	12	12	0.00	R
75	12	13	12.5	0.71	R	101	19	18	18.5	0.71	S
76	16.5	16.5	16.5	0.00	S	102	12	12	12	0.00	R
77	11	11.5	11.25	0.35	R	103	11	12	11.5	0.71	R
78	17	16	16.5	0.71	S	104	10	10	10	0.00	R
79	16	16	16	0.00	S	105	10	11	10.5	0.71	R
80	16	16	16	0.00	S	106	11	10.5	10.75	0.35	R

Table 1 continued…

AMPICILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	11	12	11.5	0.71	R	133	15	16	15.5	0.71	S
108	12	13	12.5	0.71	R	134	15	16	15.5	0.71	S
109	13	12.5	12.75	0.35	R	135	17	17.5	17.25	0.35	S
110	12.5	12	12.25	0.35	R	136	12	12	12	0.00	R
111	10	11	10.5	0.71	R	137	11	11.5	11.25	0.35	R
112	10.5	10.5	10.5	0.00	R	138	11	10.5	10.75	0.35	R
113	9	9	9	0.00	R	139	10.5	10	10.25	0.35	R
114	12	11	11.5	0.71	R	140	12.5	12	12.25	0.35	R
115	11	10	10.5	0.71	R	141	18	18.5	18.25	0.35	S
116	15	16	15.5	0.71	S	142	16	16	16	0.00	S
117	12	12	12	0.00	R	143	12	12	12	0.00	R
118	12	12	12	0.00	R	144	12.5	11.5	12	0.71	R
119	12	12	12	0.00	R	145	11	12.5	11.75	1.06	R
120	11	11	11	0.00	R	146	11	11	11	0.00	R
121	10.5	10.5	10.5	0.00	R	147	10.5	11	10.75	0.35	R
122	13	12.5	12.75	0.35	R	148	12.5	12	12.25	0.35	R
123	12	11	11.5	0.71	R	149	11	11	11	0.00	R
124	17	17.5	17.25	0.35	S	150	12.5	12	12.25	0.35	R
125	18	18	18	0.00	S	151	13	13	13	0.00	R
126	12	11.5	11.75	0.35	R	152	10.5	10.5	10.5	0.00	R
127	9	9	9	0.00	R	153	9.5	9	9.25	0.35	R
128	9	10	9.5	0.71	R	154	10.5	10	10.25	0.35	R
129	10.5	10.5	10.5	0.00	R	155	11.5	11	11.25	0.35	R
130	11	10	10.5	0.71	R	156	12.5	12.5	12.5	0.00	R
131	12.5	12	12.25	0.35	R	157	9.5	9	9.25	0.35	R
132	16	16	16	0.00	S	158	9	10	9.5	0.71	R

Table 1 continued…

AMPICILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	15	16.5	15.75	1.06	S	185	15.5	16	15.75	0.35	S
160	14.5	16	15.25	1.06	S	186	15	15	15	0.00	S
161	12.5	12	12.25	0.35	R	187	16	16	16	0.00	S
162	11	10	10.5	0.71	R	188	16	16.5	16.25	0.35	S
163	10.5	10.5	10.5	0.00	R	189	16	16.5	16.25	0.35	S
164	11	11	11	0.00	R	190	15.5	16	15.75	0.35	S
165	15	15	15	0.00	S	191	15	15	15	0.00	S
166	16	16	16	0.00	S	192	15.5	16	15.75	0.35	S
167	15	15.5	15.25	0.35	S	193	15	15	15	0.00	S
168	17	17	17	0.00	S	194	15	15	15	0.00	S
169	17.5	17.5	17.5	0.00	S	195	15.5	16	15.75	0.35	S
170	18	18	18	0.00	S	196	12	12	12	0.00	R
171	19	19	19	0.00	S	197	12.5	11	11.75	1.06	R
172	15	15.5	15.25	0.35	S	198	11	11.5	11.25	0.35	R
173	19.5	19.5	19.5	0.00	S	199	10.5	10.5	10.5	0.00	R
174	12.5	12.5	12.5	0.00	R	200	9	8.5	8.75	0.35	R
175	11	10	10.5	0.71	R						
176	18.5	18.5	18.5	0.00	S						
177	18	18	18	0.00	S						
178	11	11	11	0.00	R						
179	10	10	10	0.00	R						
180	12.5	12.5	12.5	0.00	R						
181	16	16	16	0.00	S						
182	16	16.5	16.25	0.35	S						
183	15.5	16	15.75	0.35	S						
184	15	15	15	0.00	S						

Table 2: Zones of inhibition (mm) of *E. coli* isolates tested against tetracycline

TETRACYCLINE											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	12	12	12	0.00	S	29	10	10	10	0.00	R
2	8	8	8	0.00	R	30	11	11	11	0.00	R
3	7	7	7	0.00	R	31	10.1	9	9.55	0.78	R
4	9	9.5	9.25	0.35	R	32	10	9.5	9.75	0.35	R
5	9	9	9	0.00	R	33	9.5	10	9.75	0.35	R
6	10	9	9.5	0.71	R	34	10	11	10.5	0.71	R
7	14	14	14	0.00	S	35	11	9.5	10.25	1.06	R
8	14.5	14.5	14.5	0.00	S	36	12	10	11	1.41	R
9	9	9.5	9.25	0.35	R	37	10.5	11	10.75	0.35	R
10	10	11	10.5	0.71	R	38	11	9.5	10.25	1.06	R
11	9	9	9	0.00	R	39	8	10	9	1.41	R
12	12.5	13	12.75	0.35	S	40	9	10	9.5	0.71	R
13	10	10	10	0.00	R	41	8.5	10	9.25	1.06	R
14	10	10	10	0.00	R	42	9	9	9	0.00	R
15	9	8.5	8.75	0.35	R	43	12	12	12	0.00	S
16	10	10	10	0.00	R	44	8.5	9	8.75	0.35	R
17	14	14	14	0.00	S	45	13	13	13	0.00	S
18	15	15.5	15.25	0.35	S	46	9	10	9.5	0.71	R
19	9	9.5	9.25	0.35	R	47	10	11	10.5	0.71	R
20	14	14	14	0.00	S	48	13.5	13.5	13.5	0.00	S
21	9	10	9.5	0.71	R	49	14	14	14	0.00	S
22	15.5	15	15.25	0.35	S	50	11	9.5	10.25	1.06	R
23	10	11	10.5	0.71	R	51	11	10	10.5	0.71	R
24	16	16	16	0.00	S	52	10	10	10	0.00	R
25	10	9	9.5	0.71	R	53	15	15.5	15.25	0.35	S
26	10	10.5	10.25	0.35	R	54	9.5	10	9.75	0.35	R
27	15	15	15	0.00	S						
28	9	10	9.5	0.71	R						

Table 2 continued…

TETRACYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	9	9	9	0.00	R	81	9	10	9.5	0.71	R
56	10	10	10	0.00	R	82	9	10	9.5	0.71	R
57	11	11	11	0.00	R	83	9	10	9.5	0.71	R
58	11.5	10	10.75	1.06	R	84	10	9	9.5	0.71	R
59	8	9	8.5	0.71	R	85	10	9	9.5	0.71	R
60	8.5	10	9.25	1.06	R	86	11	10	10.5	0.71	R
61	10	11	10.5	0.71	R	87	10.5	11	10.75	0.35	R
62	11	10	10.5	0.71	R	88	11	11	11	0.00	R
63	10.5	10	10.25	0.35	R	89	9	10	9.5	0.71	R
64	15	15	15	0.00	S	90	8.5	9	8.75	0.35	R
65	10	10	10	0.00	R	91	9	10	9.5	0.71	R
66	14	14	14	0.00	S	92	14	14.5	14.25	0.35	S
67	11	11	11	0.00	R	93	12	12	12	0.00	S
68	10	9	9.5	0.71	R	94	10	10	10	0.00	R
69	10	9	9.5	0.71	R	95	11	10.5	10.75	0.35	R
70	9	11	10	1.41	R	96	12.5	13	12.75	0.35	S
71	11	9	10	1.41	R	97	14.5	14.5	14.5	0.00	S
72	10	10	10	0.00	R	98	13	13	13	0.00	S
73	10.5	10.5	10.5	0.00	R	99	11	11	11	0.00	R
74	14	14	14	0.00	S	100	10.5	11	10.75	0.35	R
75	9	9.5	9.25	0.35	R	101	9	10	9.5	0.71	R
76	10	10	10	0.00	R	102	14.5	14	14.25	0.35	S
77	15	15	15	0.00	S	103	15	16	15.5	0.71	S
78	12	12	12	0.00	S	104	16	16.5	16.25	0.35	S
79	9	9	9	0.00	R	105	10	10	10	0.00	R
80	10	10	10	0.00	R	106	10	10	10	0.00	R

Table 2 continued…

TETRACYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	10	10	10	0.00	R	133	14.5	14.5	14.5	0.00	S
108	9	9	9	0.00	R	134	14	14	14	0.00	S
109	9.5	9.5	9.5	0.00	R	135	16	15.5	15.75	0.35	S
110	10	10	10	0.00	R	136	16	17	16.5	0.71	S
111	9	9.5	9.25	0.35	R	137	9	9	9	0.00	R
112	8.5	9	8.75	0.35	R	138	8.5	8.5	8.5	0.00	R
113	9.5	9	9.25	0.35	R	139	9	9	9	0.00	R
114	8	9	8.5	0.71	R	140	10	10	10	0.00	R
115	9.5	9	9.25	0.35	R	141	9.5	9	9.25	0.35	R
116	10	10	10	0.00	R	142	12	12	12	0.00	S
117	10	10	10	0.00	R	143	13	12	12.5	0.71	S
118	9.5	9.5	9.5	0.00	R	144	13.5	13.5	13.5	0.00	S
119	8.5	9	8.75	0.35	R	145	14.5	14.5	14.5	0.00	S
120	9	9	9	0.00	R	146	15	15	15	0.00	S
121	10	10	10	0.00	R	147	16	16	16	0.00	S
122	9	8.5	8.75	0.35	R	148	10	10	10	0.00	R
123	8.5	9	8.75	0.35	R	149	10	10	10	0.00	R
124	9	8.5	8.75	0.35	R	150	9.5	9	9.25	0.35	R
125	9.5	9.5	9.5	0.00	R	151	8.5	8.5	8.5	0.00	R
126	10	10	10	0.00	R	152	9	9.5	9.25	0.35	R
127	9.5	9.5	9.5	0.00	R	153	10	10	10	0.00	R
128	12.5	12.5	12.5	0.00	S	154	14.5	14.5	14.5	0.00	S
129	13	13	13	0.00	S	155	14.5	15	14.75	0.35	S
130	9.5	9.5	9.5	0.00	R	156	15	15	15	0.00	S
131	10.5	10.5	10.5	0.00	R	157	9.5	9.5	9.5	0.00	R
132	10	10	10	0.00	R	158	10	10	10	0.00	R

Table 2 continued…

TETRACYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	9.5	10	9.75	0.35	R	185	13.5	13.5	13.5	0.00	S
160	10	9.5	9.75	0.35	R	186	15	15	15	0.00	S
161	17	17	17	0.00	S	187	15	15	15	0.00	S
162	14	14.5	14.25	0.35	S	188	14.5	14.5	14.5	0.00	S
163	14.5	14.5	14.5	0.00	S	189	16	16	16	0.00	S
164	15.5	15	15.25	0.35	S	190	15	15	15	0.00	S
165	15	15	15	0.00	S	191	14.5	14.5	14.5	0.00	S
166	15	15	15	0.00	S	192	16	16	16	0.00	S
167	8.5	9	8.75	0.35	R	193	15	15	15	0.00	S
168	9	9	9	0.00	R	194	13.5	13.5	13.5	0.00	S
169	10	10	10	0.00	R	195	15	15	15	0.00	S
170	10	10	10	0.00	R	196	15	15	15	0.00	S
171	9.5	9.5	9.5	0.00	R	197	14.5	14.5	14.5	0.00	S
172	8.5	9	8.75	0.35	R	198	16	16	16	0.00	S
173	9.5	9.5	9.5	0.00	R	199	15	15	15	0.00	S
174	12.5	12.5	12.5	0.00	S	200	14.5	14.5	14.5	0.00	S
175	13.5	13.5	13.5	0.00	S						
176	15	15	15	0.00	S						
177	15	15	15	0.00	S						
178	14.5	14.5	14.5	0.00	S						
179	16	16	16	0.00	S						
180	16.5	16.5	16.5	0.00	S						
181	15	15	15	0.00	S						
182	15	15.5	15.25	0.35	S						
183	9.5	10	9.75	0.35	R						
184	17	17	17	0.00	S						

Table 3: Zones of inhibition (mm) of *E. coli* isolates tested against cephalothin

CEPHALOTHIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	16	16	16	0.00	S	29	15.5	15.5	15.5	0.00	S
2	15	15	15	0.00	S	30	15.5	15.5	15.5	0.00	S
3	15.5	15.5	15.5	0.00	S	31	15	15	15	0.00	S
4	16.5	16.5	16.5	0.00	S	32	17	17	17	0.00	S
5	16	17	16.5	0.71	S	33	18	18	18	0.00	S
6	17	17	17	0.00	S	34	11	12	11.5	0.71	R
7	18	18	18	0.00	S	35	18.5	18.5	18.5	0.00	S
8	18.5	18.5	18.5	0.00	S	36	18	18	18	0.00	S
9	19	19	19	0.00	S	37	19.5	19.5	19.5	0.00	S
10	12	13	12.5	0.71	R	38	20	20	20	0.00	S
11	20	20.5	20.25	0.35	S	39	15.5	15.5	15.5	0.00	S
12	22.5	22.5	22.5	0.00	S	40	20	20	20	0.00	S
13	21	21	21	0.00	S	41	21.5	21.5	21.5	0.00	S
14	22	22	22	0.00	S	42	20	20	20	0.00	S
15	18.5	18.5	18.5	0.00	S	43	21	21	21	0.00	S
16	18	18	18	0.00	S	44	13	12.5	12.75	0.35	R
17	18	18	18	0.00	S	45	21	21.5	21.25	0.35	S
18	17	17.5	17.25	0.35	S	46	22	22.5	22.25	0.35	S
19	17.5	17.5	17.5	0.00	S	47	22.5	22.5	22.5	0.00	S
20	19	19	19	0.00	S	48	22	22	22	0.00	S
21	20	20	20	0.00	S	49	12	12	12	0.00	R
22	21.5	21.5	21.5	0.00	S	50	23	23	23	0.00	S
23	20	20	20	0.00	S	51	13	13	13	0.00	R
24	22	22	22	0.00	S	52	25	24.5	24.75	0.35	S
25	12	13	12.5	0.71	R	53	22	23	22.5	0.71	S
26	21.5	22	21.75	0.35	S	54	25	25	25	0.00	S
27											
28											

Table 3 continued…

CEPHALOTHIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	16.5	16.5	16.5	0.00	S	81	18.5	18.5	18.5	0.00	S
56	13	13	13	0.00	R	82	18	18	18	0.00	S
57	16	16	16	0.00	S	83	19.5	19.5	19.5	0.00	S
58	15	15	15	0.00	S	84	20	20	20	0.00	S
59	10	11.5	10.75	1.06	R	85	15.5	15.5	15.5	0.00	S
60	16	16	16	0.00	S	86	19.5	19.5	19.5	0.00	S
61	15	15	15	0.00	S	87	20	20	20	0.00	S
62	15.5	15.5	15.5	0.00	S	88	11	11	11	0.00	R
63	16.5	16.5	16.5	0.00	S	89	18.5	18.5	18.5	0.00	S
64	16	17	16.5	0.71	S	90	18	18	18	0.00	S
65	17	17	17	0.00	S	91	18	18	18	0.00	S
66	18	18	18	0.00	S	92	19.5	19.5	19.5	0.00	S
67	18.5	18.5	18.5	0.00	S	93	15.5	15.5	15.5	0.00	S
68	19	19	19	0.00	S	94	16.5	16.5	16.5	0.00	S
69	11	12	11.5	0.71	R	95	16	17	16.5	0.71	S
70	15.5	17	16.25	1.06	S	96	17	17	17	0.00	S
71	17	17	17	0.00	S	97	18	18	18	0.00	S
72	12	11	11.5	0.71	R	98	18.5	18.5	18.5	0.00	S
73	16	16	16	0.00	S	99	10	11.5	10.75	1.06	R
74	15	15	15	0.00	S	100	17.5	17.5	17.5	0.00	S
75	15.5	15.5	15.5	0.00	S	101	12	12	12	0.00	R
76	16.5	16.5	16.5	0.00	S	102	11	11	11	0.00	R
77	15	15	15	0.00	S	103	10.5	10.5	10.5	0.00	R
78	15.5	15.5	15.5	0.00	S	104	13	13	13	0.00	R
79	10	11	10.5	0.71	R	105	21	21	21	0.00	S
80	19	19.5	19.25	0.35	S	106	20	20	20	0.00	S

Table 3 continued…

CEPHALOTHIN										
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	12	12.5	12.25	0.35	R	133	19.5	19.5	19.5	0.00	S
108	16	16	16	0.00	S	134	20	20	20	0.00	S
109	15	15	15	0.00	S	135	15.5	15.5	15.5	0.00	S
110	15.5	15.5	15.5	0.00	S	136	20	20	20	0.00	S
111	16.5	16.5	16.5	0.00	S	137	13.5	12.5	13	0.71	R
112	15	15	15	0.00	S	138	16.5	16.5	16.5	0.00	S
113	15.5	15.5	15.5	0.00	S	139	14	14	14	0.00	R
114	16.5	16.5	16.5	0.00	S	140	16.5	16.5	16.5	0.00	S
115	15	15	15	0.00	S	141	17	17	17	0.00	S
116	15.5	15.5	15.5	0.00	S	142	11	10.5	10.75	0.35	R
117	18	18.5	18.25	0.35	S	143	18	18	18	0.00	S
118	18	18	18	0.00	S	144	19.5	19.5	19.5	0.00	S
119	17.5	18	17.75	0.35	S	145	20	20	20	0.00	S
120	19	19	19	0.00	S	146	15.5	15.5	15.5	0.00	S
121	13.5	13.5	13.5	0.00	R	147	20	20	20	0.00	S
122	15	15	15	0.00	S	148	15.5	15.5	15.5	0.00	S
123	14	14	14	0.00	R	149	16	16	16	0.00	S
124	12.5	13	12.75	0.35	R	150	16.5	16.5	16.5	0.00	S
125	18	18	18	0.00	S	151	17	17	17	0.00	S
126	19.5	19.5	19.5	0.00	S	152	17	17	17	0.00	S
127	20	20	20	0.00	S	153	19.5	20	19.75	0.35	S
128	20	20	20	0.00	S	154	20	20	20	0.00	S
129	15.5	15.5	15.5	0.00	S	155	9.5	10	9.75	0.35	R
130	18	18	18	0.00	S	156	16.5	17	16.75	0.35	S
131	18	18	18	0.00	S	157	16	16	16	0.00	S
132	19	18.5	18.75	0.35	S	158	15.5	15.5	15.5	0.00	S

Table 3 continued…

CEPHALOTHIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	11	10.5	10.75	0.35	R	185	16	16	16	0.00	S
160	10	10.5	10.25	0.35	R	186	15	15	15	0.00	S
161	15.5	15.5	15.5	0.00	S	187	17	17.5	17.25	0.35	S
162	16	16	16	0.00	S	188	19	19	19	0.00	S
163	15.5	15	15.25	0.35	S	189	15	15	15	0.00	S
164	15.5	15	15.25	0.35	S	190	17	17.5	17.25	0.35	S
165	15	15	15	0.00	S	191	19	19	19	0.00	S
166	15	15	15	0.00	S	192	15	15	15	0.00	S
167	17	17.5	17.25	0.35	S	193	15	15	15	0.00	S
168	19	19	19	0.00	S	194	17	17.5	17.25	0.35	S
169	23	22.5	22.75	0.35	S	195	19	19	19	0.00	S
170	18.5	18	18.25	0.35	S	196	15	15	15	0.00	S
171	16	16.5	16.25	0.35	S	197	17	17.5	17.25	0.35	S
172	17	17	17	0.00	S	198	19	19	19	0.00	S
173	17	17.5	17.25	0.35	S	199	15	15	15	0.00	S
174	12.5	13	12.75	0.35	R	200	15	15	15	0.00	S
175	10	10	10	0.00	R						
176	18.5	18.5	18.5	0.00	S						
177	16	16	16	0.00	S						
178	15.5	15.5	15.5	0.00	S						
179	15.5	15.5	15.5	0.00	S						
180	15	15	15	0.00	S						
181	16	16	16	0.00	S						
182	16	15.5	15.75	0.35	S						
183	16	15	15.5	0.71	S						
184	18.5	17	17.75	1.06	S						

Table 4: Zones of inhibition (mm) of *E. coli* isolates tested against gentamicin

GENTAMICIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	15	15	15	0.00	S	29	17	17.5	17.25	0.35	S
2	15	15.5	15.25	0.35	S	30	18	18	18	0.00	S
3	16	16	16	0.00	S	31	15.5	15.5	15.5	0.00	S
4	11	11	11	0.00	R	32	15	15	15	0.00	S
5	17.5	17	17.25	0.35	S	33	16.5	17	16.75	0.35	S
6	17	17.5	17.25	0.35	S	34	16	16	16	0.00	S
7	18	18	18	0.00	S	35	10	10	10	0.00	R
8	15.5	15.5	15.5	0.00	S	36	20	20	20	0.00	S
9	15	15	15	0.00	S	37	16.5	17	16.75	0.35	S
10	16	16.5	16.25	0.35	S	38	16	16	16	0.00	S
11	11	10	10.5	0.71	R	39	16	16	16	0.00	S
12	17	17	17	0.00	S	40	19	20	19.5	0.71	S
13	17	17.5	17.25	0.35	S	41	10	10.5	10.25	0.35	R
14	18.5	18	18.25	0.35	S	42	16.5	17	16.75	0.35	S
15	15.5	16	15.75	0.35	S	43	16	16	16	0.00	S
16	20	20	20	0.00	S	44	15	15	15	0.00	S
17	16.5	17	16.75	0.35	S	45	15	15	15	0.00	S
18	16	16	16	0.00	S	46	16.5	16.5	16.5	0.00	S
19	16	16	16	0.00	S	47	16	16	16	0.00	S
20	17	17	17	0.00	S	48	21.5	21	21.25	0.35	S
21	10	10.5	10.25	0.35	R	49	22	22	22	0.00	S
22	17.5	17.5	17.5	0.00	S	50	12	12	12	0.00	R
23	17.5	17	17.25	0.35	S	51	15	15.5	15.25	0.35	S
24	15	15	15	0.00	S	52	16	16	16	0.00	S
25	15	15	15	0.00	S	53	15	15.5	15.25	0.35	S
26	16.5	16.5	16.5	0.00	S	54	17	18	17.5	0.71	S
27	16	16	16	0.00	S						
28	15.5	16	15.75	0.35	S						

Table 4 continued…

GENTAMICIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	13	13	13	0.00	S	81	15	15.5	15.25	0.35	S
56	13	13	13	0.00	S	82	16	16	16	0.00	S
57	11	11.5	11.25	0.35	R	83	16	17	16.5	0.71	S
58	15	15.5	15.25	0.35	S	84	12	12	12	0.00	R
59	16	16	16	0.00	S	85	17	17.5	17.25	0.35	S
60	16	17	16.5	0.71	S	86	18.5	18	18.25	0.35	S
61	18.5	18	18.25	0.35	S	87	15.5	16	15.75	0.35	S
62	19	19	19	0.00	S	88	20	20	20	0.00	S
63	15.5	16	15.75	0.35	S	89	16.5	17	16.75	0.35	S
64	15	16	15.5	0.71	S	90	16	16	16	0.00	S
65	12	12	12	0.00	R	91	16	16	16	0.00	S
66	11	11.5	11.25	0.35	R	92	17	17	17	0.00	S
67	15	15.5	15.25	0.35	S	93	19	20	19.5	0.71	S
68	11	11	11	0.00	R	94	15	15.5	15.25	0.35	S
69	16	17	16.5	0.71	S	95	16	16	16	0.00	S
70	12	12	12	0.00	R	96	16	17	16.5	0.71	S
71	17	17.5	17.25	0.35	S	97	16.5	17	16.75	0.35	S
72	18.5	18	18.25	0.35	S	98	16	16	16	0.00	S
73	11	11.5	11.25	0.35	R	99	16	16	16	0.00	S
74	16	17	16.5	0.71	S	100	17	17.5	17.25	0.35	S
75	15	15.5	15.25	0.35	S	101	18.5	18	18.25	0.35	S
76	16	16	16	0.00	S	102	15	15.5	15.25	0.35	S
77	15	15.5	15.25	0.35	S	103	16	16	16	0.00	S
78	15.5	16	15.75	0.35	S	104	15	15.5	15.25	0.35	S
79	15	16	15.5	0.71	S	105	17	18	17.5	0.71	S
80	15	15.5	15.25	0.35	S	106	16	17	16.5	0.71	S

Table 4 continued…

GENTAMICIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	17	17.5	17.25	0.35	S	133	17	17	17	0.00	S
108	18.5	18	18.25	0.35	S	134	19	20	19.5	0.71	S
109	15.5	16	15.75	0.35	S	135	15	15.5	15.25	0.35	S
110	20	20	20	0.00	S	136	16	16	16	0.00	S
111	16.5	17	16.75	0.35	S	137	19	20	19.5	0.71	S
112	16	16	16	0.00	S	138	15	15.5	15.25	0.35	S
113	16	16	16	0.00	S	139	16	16	16	0.00	S
114	17	17	17	0.00	S	140	19	20	19.5	0.71	S
115	19	20	19.5	0.71	S	141	15	15.5	15.25	0.35	S
116	15	15.5	15.25	0.35	S	142	16	16	16	0.00	S
117	16	16	16	0.00	S	143	19	20	19.5	0.71	S
118	16	17	16.5	0.71	S	144	17	17	17	0.00	S
119	21	21.5	21.25	0.35	S	145	19	20	19.5	0.71	S
120	10	10.5	10.25	0.35	R	146	15	15.5	15.25	0.35	S
121	16	16	16	0.00	S	147	16	16	16	0.00	S
122	19	20	19.5	0.71	S	148	17	17	17	0.00	S
123	15	15.5	15.25	0.35	S	149	19	20	19.5	0.71	S
124	16	16	16	0.00	S	150	15	15.5	15.25	0.35	S
125	19	20	19.5	0.71	S	151	16	16	16	0.00	S
126	17	17	17	0.00	S	152	16	16	16	0.00	S
127	19	20	19.5	0.71	S	153	16	16	16	0.00	S
128	22.5	21.5	22	0.71	S	154	17	17	17	0.00	S
129	20	22	21	1.41	S	155	19	20	19.5	0.71	S
130	11	11	11	0.00	R	156	15	15.5	15.25	0.35	S
131	10	10	10	0.00	R	157	18.5	18.5	18.5	0.00	S
132	15	16	15.5	0.71	S	158	20	20	20	0.00	S

Table 4 continued…

GENTAMICIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	20	20	20	0.00	S	185	15.5	16	15.75	0.35	S
160	16.5	17	16.75	0.35	S	186	20	20	20	0.00	S
161	16	16	16	0.00	S	187	20	20	20	0.00	S
162	19	20	19.5	0.71	S	188	16	16	16	0.00	S
163	15.5	16	15.75	0.35	S	189	19	20	19.5	0.71	S
164	20	20	20	0.00	S	190	15	15.5	15.25	0.35	S
165	16	16	16	0.00	S	191	15.5	16	15.75	0.35	S
166	16	16	16	0.00	S	192	20	20	20	0.00	S
167	19	20	19.5	0.71	S	193	16	16	16	0.00	S
168	15.5	16	15.75	0.35	S	194	18	18	18	0.00	S
169	20	20	20	0.00	S	195	18.5	19	18.75	0.35	S
170	20	20	20	0.00	S	196	17	17.5	17.25	0.35	S
171	16.5	17	16.75	0.35	S	197	17	17	17	0.00	S
172	16	16	16	0.00	S	198	19	19.5	19.25	0.35	S
173	16	16	16	0.00	S	199	23	22	22.5	0.71	S
174	17	17	17	0.00	S	200	22	20	21	1.41	S
175	16	16	16	0.00	S						R
176	19	20	19.5	0.71	S						R
177	15	15.5	15.25	0.35	S						R
178	16	16	16	0.00	S						R
179	16	16	16	0.00	S						R
180	19	20	19.5	0.71	S						R
181	15	15.5	15.25	0.35	S						R
182	15.5	16	15.75	0.35	S						R
183	19	19.5	19.25	0.35	S						R
184	21	21	21	0.00	S						R

Table 5: Zones of inhibition (mm) of *E. coli* isolates tested against chloramphenicol

CHLORAMPHENICOL											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	13	13	13	0.00	S	29	10	10	10	0.00	R
2	11	11	11	0.00	R	30	11	11	11	0.00	R
3	14.5	15	14.75	0.35	S	31	12.5	12.3	12.4	0.14	S
4	10	10	10	0.00	R	32	14	14	14	0.00	S
5	14	14	14	0.00	S	33	16	16	16	0.00	S
6	11	11.5	11.25	0.35	R	34	15.5	16	15.75	0.35	S
7	15	15	15	0.00	S	35	14	13	13.5	0.71	S
8	14.5	15	14.75	0.35	S	36	15.5	16	15.75	0.35	S
9	11	12	11.5	0.71	R	37	14	14.5	14.25	0.35	S
10	15	14	14.5	0.71	S	38	17.5	17.5	17.5	0.00	S
11	14	15	14.5	0.71	S	39	17	17	17	0.00	S
12	12.5	12.5	12.5	0.00	S	40	17	17	17	0.00	S
13	16	16.5	16.25	0.35	S	41	12	12	12	0.00	R
14	13	13	13	0.00	S	42	11	11	11	0.00	R
15	13	13.5	13.25	0.35	S	43	13	13	13	0.00	S
16	14	14	14	0.00	S	44	11	11	11	0.00	R
17	11	12	11.5	0.71	R	45	13	13	13	0.00	S
18	17	16	16.5	0.71	S	46	14	15	14.5	0.71	S
19	12.5	13	12.75	0.35	S	47	12	12	12	0.00	R
20	12	10	11	1.41	R	48	16	16	16	0.00	S
21	15	15	15	0.00	S	49	15	16	15.5	0.71	S
22	9.5	10	9.75	0.35	R	50	15	15	15	0.00	S
23	12.5	12.5	12.5	0.00	S	51	12	12	12	0.00	R
24	14	14	14	0.00	S	52	16	16	16	0.00	S
25	14	14	14	0.00	S	53	13	13	13	0.00	S
26	11	12	11.5	0.71	R	54	19	19	19	0.00	S

Table 5 continued…

CHLORAMPHENICOL											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	12	12	12	0.00	R	81	16	16	16	0.00	S
56	14	14	14	0.00	S	82	18.5	18.5	18.5	0.00	S
57	11	11	11	0.00	R	83	19	19	19	0.00	S
58	14	14	14	0.00	S	84	13.5	14	13.75	0.35	S
59	10	10	10	0.00	R	85	14	14	14	0.00	S
60	15	15	15	0.00	S	86	19	19	19	0.00	S
61	15	15.5	15.25	0.35	S	87	13.5	14	13.75	0.35	S
62	12	12	12	0.00	R	88	19	19	19	0.00	S
63	10	11	10.5	0.71	R	89	19	19	19	0.00	S
64	15	16	15.5	0.71	S	90	14	14	14	0.00	S
65	16	17	16.5	0.71	S	91	19	17	18	1.41	S
66	15	17	16	1.41	S	92	12	12	12	0.00	R
67	10	10	10	0.00	R	93	10	10.5	10.25	0.35	R
68	18	18.5	18.25	0.35	S	94	16.5	16	16.25	0.35	S
69	9	10	9.5	0.71	R	95	16	18	17	1.41	S
70	11	11	11	0.00	R	96	18.5	17.5	18	0.71	S
71	18	18	18	0.00	S	97	12	11.5	11.75	0.35	R
72	16.5	15	15.75	1.06	S	98	18	18	18	0.00	S
73	15	15	15	0.00	S	99	14	14	14	0.00	S
74	15	14.5	14.75	0.35	S	100	12.5	13	12.75	0.35	S
75	16	16	16	0.00	S	101	12	12	12	0.00	R
76	11	11.5	11.25	0.35	R	102	11	11	11	0.00	R
77	19	19	19	0.00	S	103	10	10	10	0.00	R
78	12	11.5	11.75	0.35	R	104	9.5	9	9.25	0.35	R
79	10	11	10.5	0.71	R	105	11	10	10.5	0.71	R
80	19	19	19	0.00	S	106	11.5	11.5	11.5	0.00	R

Table 5 continued…

CHLORAMPHENICOL											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	10.5	10.5	10.5	0.00	R	133	14	14.5	14.25	0.35	S
108	16	16	16	0.00	S	134	14	15	14.5	0.71	S
109	14.5	15	14.75	0.35	S	135	19	19	19	0.00	S
110	15	15	15	0.00	S	136	12.5	13	12.75	0.35	S
111	15	16	15.5	0.71	S	137	13	13	13	0.00	S
112	16	17	16.5	0.71	S	138	15	17	16	1.41	S
113	15	17	16	1.41	S	139	13	13	13	0.00	S
114	14	15	14.5	0.71	S	140	12	14	13	1.41	S
115	14.5	15	14.75	0.35	S	141	12	12	12	0.00	R
116	11	12	11.5	0.71	R	142	14	14.5	14.25	0.35	S
117	16	16	16	0.00	S	143	14	15	14.5	0.71	S
118	17	17	17	0.00	S	144	19	19	19	0.00	S
119	16	16	16	0.00	S	145	12.5	13	12.75	0.35	S
120	16	16.5	16.25	0.35	S	146	14	15	14.5	0.71	S
121	17	17	17	0.00	S	147	19	19	19	0.00	S
122	12.5	13	12.75	0.35	S	148	10	10	10	0.00	R
123	10.5	10.5	10.5	0.00	R	149	11	11	11	0.00	R
124	13	13	13	0.00	S	150	10	10	10	0.00	R
125	13	13	13	0.00	S	151	11.5	12	11.75	0.35	R
126	11.5	11.5	11.5	0.00	R	152	16.5	17	16.75	0.35	S
127	10	10	10	0.00	R	153	14	14	14	0.00	S
128	19	19	19	0.00	S	154	18.5	17	17.75	1.06	S
129	12.5	13	12.75	0.35	S	155	19	19	19	0.00	S
130	13	13	13	0.00	S	156	12.5	13	12.75	0.35	S
131	12	12	12	0.00	R	157	13	13	13	0.00	S
132	13.5	14	13.75	0.35	S	158	15	15	15	0.00	S

Table 5 continued…

CHLORAMPHENICOL											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	18	18	18	0.00	S	185	12.5	12.3	12.4	0.14	S
160	12	12	12	0.00	R	186	14	14	14	0.00	S
161	16	16	16	0.00	S	187	16	16	16	0.00	S
162	14.5	15	14.75	0.35	S	188	15.5	16	15.75	0.35	S
163	15	15	15	0.00	S	189	14	13	13.5	0.71	S
164	15	16	15.5	0.71	S	190	15.5	16	15.75	0.35	S
165	16	16	16	0.00	S	191	16	16	16	0.00	S
166	14.5	15	14.75	0.35	S	192	14.5	15	14.75	0.35	S
167	15	15	15	0.00	S	193	15	15	15	0.00	S
168	15	16	15.5	0.71	S	194	15	16	15.5	0.71	S
169	15	16	15.5	0.71	S	195	16	16	16	0.00	S
170	19	19	19	0.00	S	196	14.5	15	14.75	0.35	S
171	12.5	13	12.75	0.35	S	197	15	15	15	0.00	S
172	13	13	13	0.00	S	198	15	16	15.5	0.71	S
173	15	16	15.5	0.71	S	199	15	15	15	0.00	S
174	16	17	16.5	0.71	S	200	15	15.5	15.25	0.35	S
175	14	14.5	14.25	0.35	S						
176	14.5	15	14.75	0.35	S						
177	15	15	15	0.00	S						
178	15	16	15.5	0.71	S						
179	15	15	15	0.00	S						
180	14	15	14.5	0.71	S						
181	12.5	13	12.75	0.35	S						
182	12.5	13	12.75	0.35	S						
183	13	13	13	0.00	S						
184	16	17	16.5	0.71	S						

Table 6: Zones of inhibition (mm) of *E. coli* isolates tested against amoxicillin

AMOXYCILLIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	12	12	12	0.00	R	29	10	9	9.5	0.71	R
2	11	11	11	0.00	R	30	9.5	10	9.75	0.35	R
3	12	12	12	0.00	R	31	14	14	14	0.00	S
4	15	15	15	0.00	S	32	12	11	11.5	0.71	R
5	12	12	12	0.00	R	33	11	12	11.5	0.71	R
6	11	11	11	0.00	R	34	10	11	10.5	0.71	R
7	14	15	14.5	0.71	S	35	10.5	10	10.25	0.35	R
8	10	10.5	10.25	0.35	R	36	14.5	15	14.75	0.35	S
9	10	11	10.5	0.71	R	37	16	16	16	0.00	S
10	11	12	11.5	0.71	R	38	12	11	11.5	0.71	R
11	12	11	11.5	0.71	R	39	13.5	13.5	13.5	0.00	S
12	14	14	14	0.00	S	40	16	17	16.5	0.71	S
13	14	14	14	0.00	S	41	17	17	17	0.00	S
14	12	12	12	0.00	R	42	10	10	10	0.00	R
15	15	15	15	0.00	S	43	12	12	12	0.00	R
16	11	11	11	0.00	R	44	18	17	17.5	0.71	S
17	13	13	13	0.00	R	45	10	10.5	10.25	0.35	R
18	10	10.5	10.25	0.35	R	46	16	16	16	0.00	S
19	16	16.5	16.25	0.35	S	47	16	16	16	0.00	S
20	17	18	17.5	0.71	S	48	16.5	15	15.75	1.06	S
21	12	12	12	0.00	R	49	12	12	12	0.00	R
22	13.5	13.5	13.5	0.00	S	50	12	12	12	0.00	R
23	12	12	12	0.00	R	51	14	15	14.5	0.71	S
24	10	10	10	0.00	R	52	15	15	15	0.00	S
25	11	11	11	0.00	R	53	15	16.5	15.75	1.06	S
26	14	14	14	0.00	S	54	17	18	17.5	0.71	S
27	15	15	15	0.00	S	55	12	12	12	0.00	R
28	12	12	12	0.00	R	56	11	11.5	11.25	0.35	R

Table 6 continued…

AMOXYCILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	16	15	15.5	0.71	S	81	12	12	12	0.00	R
56	16	15	15.5	0.71	S	82	14	13	13.5	0.71	S
57	16	16	16	0.00	S	83	10	10	10	0.00	R
58	12	12	12	0.00	R	84	10	10	10	0.00	R
59	14	14	14	0.00	S	85	15.5	15	15.25	0.35	S
60	14	14	14	0.00	S	86	10.5	10.5	10.5	0.00	R
61	11	11.5	11.25	0.35	R	87	16	16	16	0.00	S
62	15	15	15	0.00	S	88	10	10	10	0.00	R
63	15	15	15	0.00	S	89	16	16	16	0.00	S
64	15	15	15	0.00	S	90	17	17	17	0.00	S
65	12	12	12	0.00	R	91	12	12.5	12.25	0.35	R
66	10	9.5	9.75	0.35	R	92	16	16	16	0.00	S
67	10	11	10.5	0.71	R	93	18	18	18	0.00	S
68	12	10	11	1.41	R	94	13	13	13	0.00	R
69	14.5	15	14.75	0.35	S	95	14	14	14	0.00	S
70	10	11	10.5	0.71	R	96	13	12.5	12.75	0.35	R
71	11	12	11.5	0.71	R	97	14	14	14	0.00	S
72	9	10.5	9.75	1.06	R	98	12.5	12.5	12.5	0.00	R
73	11	11	11	0.00	R	99	12	11	11.5	0.71	R
74	12	12	12	0.00	R	100	19	19	19	0.00	S
75	15	15	15	0.00	S	101	12.5	11.5	12	0.71	R
76	16.5	17	16.75	0.35	S	102	11	11	11	0.00	R
77	10	8.5	9.25	1.06	R	103	12	12	12	0.00	R
78	13.5	13.5	13.5	0.00	S	104	10.5	10.5	10.5	0.00	R
79	14.5	15	14.75	0.35	S	105	11	12	11.5	0.71	R
80	12	12	12	0.00	R	106	13	13	13	0.00	R

Table 6 continued…

AMOXYCILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	12	12	12	0.00	R	133	18	18	18	0.00	S
108	11.5	11	11.25	0.35	R	134	12	12.5	12.25	0.35	R
109	10.5	10	10.25	0.35	R	135	11	11	11	0.00	R
110	12.5	12.5	12.5	0.00	R	136	11	11	11	0.00	R
111	10.5	10.5	10.5	0.00	R	137	11	11	11	0.00	R
112	12	12	12	0.00	R	138	16	16	16	0.00	S
113	10.5	10.5	10.5	0.00	R	139	16.5	17	16.75	0.35	S
114	9	10.5	9.75	1.06	R	140	15	15	15	0.00	S
115	15	15	15	0.00	S	141	12.5	12.5	12.5	0.00	R
116	13	13	13	0.00	R	142	15.5	16	15.75	0.35	S
117	16	16	16	0.00	S	143	16	16	16	0.00	S
118	16.5	17	16.75	0.35	S	144	14	15	14.5	0.71	S
119	18	18	18	0.00	S	145	15	15.5	15.25	0.35	S
120	12	11.5	11.75	0.35	R	146	16	14.5	15.25	1.06	S
121	12	12	12	0.00	R	147	15	15	15	0.00	S
122	12.5	12.5	12.5	0.00	R	148	11	12	11.5	0.71	R
123	13.5	14	13.75	0.35	S	149	10.5	10.5	10.5	0.00	R
124	12	12	12	0.00	R	150	10.5	10.5	10.5	0.00	R
125	10.5	10.5	10.5	0.00	R	151	11	10.5	10.75	0.35	R
126	10.5	10.5	10.5	0.00	R	152	12.5	11.5	12	0.71	R
127	9.5	10	9.75	0.35	R	153	12.5	13	12.75	0.35	R
128	16	16	16	0.00	S	154	14	14	14	0.00	S
129	16.5	17	16.75	0.35	S	155	12	12	12	0.00	R
130	18	18	18	0.00	S	156	13	13	13	0.00	R
131	16	16	16	0.00	S	157	11	10.5	10.75	0.35	R
132	18	17	17.5	0.71	S	158	16.5	16.5	16.5	0.00	S

Table 6 continued…

AMOXYCILLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	10.5	10.5	10.5	0.00	R	185	12.5	12.5	12.5	0.00	R
160	14	14	14	0.00	S	186	11	12	11.5	0.71	R
161	14	15	14.5	0.71	S	187	15.5	16	15.75	0.35	S
162	15	15	15	0.00	S	188	16	16	16	0.00	S
163	15	16.5	15.75	1.06	S	189	14	15	14.5	0.71	S
164	17	18	17.5	0.71	S	190	15	15.5	15.25	0.35	S
165	16	16	16	0.00	S	191	15	15	15	0.00	S
166	15.5	16	15.75	0.35	S	192	15	16.5	15.75	1.06	S
167	15	16	15.5	0.71	S	193	17	18	17.5	0.71	S
168	19	19	19	0.00	S	194	13.5	14	13.75	0.35	S
169	16	15	15.5	0.71	S	195	16	16	16	0.00	S
170	15	15	15	0.00	S	196	15	15	15	0.00	S
171	15	16.5	15.75	1.06	S	197	15	16	15.5	0.71	S
172	17	18	17.5	0.71	S	198	18	18	18	0.00	S
173	17.5	18	17.75	0.35	S	199	16.5	17	16.75	0.35	S
174	18	18	18	0.00	S	200	18	18	18	0.00	S
175	17.5	18	17.75	0.35	S						
176	12.5	12.5	12.5	0.00	R						
177	13	13	13	0.00	R						
178	14.5	15	14.75	0.35	S						
179	15	16	15.5	0.71	S						
180	16	16	16	0.00	S						
181	10.5	11	10.75	0.35	R						
182	15.5	16	15.75	0.35	S						
183	16.5	17	16.75	0.35	S						
184	10.5	10.5	10.5	0.00	R						

Table 7: Zones of inhibition (mm) of *E. coli* isolates tested against nalidixic acid

NALIDIXIC ACID											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	15	15	15	0.00	S	29	11	12	11.5	0.71	R
2	11	11	11	0.00	R	30	12	12	12	0.00	R
3	16	16	16	0.00	S	31	11	13	12	1.41	R
4	16	15	15.5	0.71	S	32	15	15	15	0.00	S
5	11	11	11	0.00	R	33	10	10	10	0.00	R
6	10	10	10	0.00	R	34	16	16	16	0.00	S
7	11	11	11	0.00	R	35	11	11.5	11.25	0.35	R
8	14	15	14.5	0.71	S	36	15	15	15	0.00	S
9	12	12	12	0.00	R	37	14.5	15	14.75	0.35	S
10	15	15	15	0.00	S	38	15	15	15	0.00	S
11	11	11	11	0.00	R	39	12	12	12	0.00	R
12	12	12	12	0.00	R	40	16	16	16	0.00	S
13	10	12	11	1.41	R	41	18	17.5	17.75	0.35	S
14	10.5	10.5	10.5	0.00	R	42	12	12	12	0.00	R
15	11	11	11	0.00	R	43	19.5	20	19.75	0.35	S
16	19	19	19	0.00	S	44	19	19	19	0.00	S
17	12	12	12	0.00	R	45	14.5	15	14.75	0.35	S
18	10	10	10	0.00	R	46	12	12	12	0.00	R
19	12	11	11.5	0.71	R	47	13.5	15	14.25	1.06	S
20	16	16	16	0.00	S	48	10	10.5	10.25	0.35	R
21	12	12	12	0.00	R	49	14	14	14	0.00	S
22	14	14	14	0.00	S	50	12	12.5	12.25	0.35	R
23	15	15.5	15.25	0.35	S	51	15	15	15	0.00	S
24	11	11	11	0.00	R	52	12	12	12	0.00	R
25	14	14	14	0.00	S	53	11	11.5	11.25	0.35	R
26	15	15	15	0.00	S	54	14	14	14	0.00	S
27	13.5	14	13.75	0.35	S						
28	13	13	13	0.00	R						

Table 7 continued…

NALIDIXIC ACID											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	12.5	11	11.75	1.06	R	81	12	12	12	0.00	R
56	15	15	15	0.00	S	82	12	11	11.5	0.71	R
57	15	15	15	0.00	S	83	13	13	13	0.00	R
58	14.5	15	14.75	0.35	S	84	11	12	11.5	0.71	R
59	15	15	15	0.00	S	85	10	10.5	10.25	0.35	R
60	16	15	15.5	0.71	S	86	9	10	9.5	0.71	R
61	17.5	18	17.75	0.35	S	87	14.5	15	14.75	0.35	S
62	17	17	17	0.00	S	88	18	18	18	0.00	S
63	12	12	12	0.00	R	89	13.5	14	13.75	0.35	S
64	12	12	0	0.00	R	90	15	15	15	0.00	S
65	11	11	11	0.00	R	91	12	12	12	0.00	R
66	12	10	11	1.41	R	92	15.5	16	15.75	0.35	S
67	12.5	12	12.25	0.35	R	93	15	15	15	0.00	S
68	12	12.5	12.25	0.35	R	94	10	10	10	0.00	R
69	15	15	15	0.00	S	95	16	16	16	0.00	S
70	9	9	9	0.00	R	96	11	10	10.5	0.71	R
71	10	10	10	0.00	R	97	14.5	15	14.75	0.35	S
72	13.5	14	13.75	0.35	S	98	10	11	10.5	0.71	R
73	12	12	12	0.00	R	99	15.5	15.5	15.5	0.00	S
74	13	13	13	0.00	R	100	16	16	16	0.00	S
75	10.5	10.5	10.5	0.00	R	101	14	14	14	0.00	S
76	14	14	14	0.00	S	102	11	11	11	0.00	R
77	15	15	15	0.00	S	103	12	12	12	0.00	R
78	14.5	15	14.75	0.35	S	104	12	12.5	12.25	0.35	R
79	13.5	14.5	14	0.71	S	105	13	13	13	0.00	R
80	11	11	11	0.00	R	106	10.5	10.5	10.5	0.00	R

Table 7 continued…

NALIDIXIC ACID											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	15	15	15	0.00	S	133	12	13	12.5	0.71	R
108	12	12	12	0.00	R	134	11	11	11	0.00	R
109	11.5	12	11.75	0.35	R	135	10.5	10.5	10.5	0.00	R
110	12	12	12	0.00	R	136	13	13	13	0.00	R
111	12	12	12	0.00	R	137	15	15	15	0.00	S
112	12	12	12	0.00	R	138	18	17	17.5	0.71	S
113	10	10	10	0.00	R	139	16	16	16	0.00	S
114	10	10	10	0.00	R	140	12	12.5	12.25	0.35	R
115	10.5	10.5	10.5	0.00	R	141	12	13	12.5	0.71	R
116	9.5	9.5	9.5	0.00	R	142	12	12	12	0.00	R
117	12	12	12	0.00	R	143	12.5	12.5	12.5	0.00	R
118	11	11.5	11.25	0.35	R	144	10	10.5	10.25	0.35	R
119	11.5	11	11.25	0.35	R	145	11.5	11.5	11.5	0.00	R
120	11.5	12	11.75	0.35	R	146	12	12	12	0.00	R
121	15	15	15	0.00	S	147	13	13	13	0.00	R
122	16	16.5	16.25	0.35	S	148	18	18.5	18.25	0.35	S
123	12	12	12	0.00	R	149	16	15.5	15.75	0.35	S
124	12.5	12.5	12.5	0.00	R	150	16	16	16	0.00	S
125	12.5	13	12.75	0.35	R	151	16.5	16.5	16.5	0.00	S
126	15	15	15	0.00	S	152	17	17	17	0.00	S
127	14.5	14.5	14.5	0.00	S	153	14.5	15	14.75	0.35	S
128	11	11	11	0.00	R	154	14	14	14	0.00	S
129	10	10.5	10.25	0.35	R	155	10.5	11	10.75	0.35	R
130	10	10.5	10.25	0.35	R	156	11	11.5	11.25	0.35	R
131	10	10	10	0.00	R	157	11	11	11	0.00	R
132	10.5	9.5	10	0.71	R	158	16	16	16	0.00	S

Table 7 continued…

NALIDIXIC ACID											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	13.5	15	14.25	1.06	S	185	15.5	16	15.75	0.35	S
160	15	15	15	0.00	S	186	15	15	15	0.00	S
161	16	16.5	16.25	0.35	S	187	16.5	17	16.75	0.35	S
162	15.5	16	15.75	0.35	S	188	18	17	17.5	0.71	S
163	15	15.5	15.25	0.35	S	189	17.5	18	17.75	0.35	S
164	13.5	14	13.75	0.35	S	190	15	15	15	0.00	S
165	12	12	12	0.00	R	191	14.5	14.5	14.5	0.00	S
166	13	13	13	0.00	R	192	13.5	14	13.75	0.35	S
167	14	14	14	0.00	S	193	20	20	20	0.00	S
168	14	14	14	0.00	S	194	13	13	13	0.00	R
169	15.5	16	15.75	0.35	S	195	18	18	18	0.00	S
170	19	19	19	0.00	S	196	19.5	20	19.75	0.35	S
171	18	18	18	0.00	S	197	13.5	13.5	13.5	0.00	S
172	15	15	15	0.00	S	198	14	14	14	0.00	S
173	14.5	15	14.75	0.35	S	199	15	15	15	0.00	S
174	15	15.5	15.25	0.35	S	200	15	15	15	0.00	S
175	16.5	17	16.75	0.35	S						
176	16	16	16	0.00	S						
177	17	17.5	17.25	0.35	S						
178	10.5	11	10.75	0.35	R						
179	12.5	12.5	12.5	0.00	R						
180	20	20	20	0.00	S						
181	15	16	15.5	0.71	S						
182	14.5	15	14.75	0.35	S						
183	16	16	16	0.00	S						
184	13.5	14	13.75	0.35	S						

Table 8: Zones of inhibition (mm) of *E. coli* isolates tested against trimethoprim

TRIMETHOPRIM											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	9	10	9.5	0.71	R	29	9	10.5	9.75	1.06	R
2	9	10	9.5	0.71	R	30	8	9	8.5	0.71	R
3	9	10	9.5	0.71	R	31	9	8	8.5	0.71	R
4	8	9	8.5	0.71	R	32	8	10	9	1.41	R
5	8	9	8.5	0.71	R	33	9	9	9	0.00	R
6	9	8	8.5	0.71	R	34	10	8	9	1.41	R
7	8	9	8.5	0.71	R	35	8	9	8.5	0.71	R
8	9	9	9	0.00	R	36	10	9	9.5	0.71	R
9	8	8	8	0.00	R	37	9	9	9	0.00	R
10	9	10	9.5	0.71	R	38	8	8	8	0.00	R
11	8	9	8.5	0.71	R	39	9.5	8	8.75	1.06	R
12	9	8	8.5	0.71	R	40	9.5	10	9.75	0.35	R
13	8	10	9	1.41	R	41	8	10	9	1.41	R
14	9	10	9.5	0.71	R	42	10	10	10	0.00	R
15	10	10	10	0.00	R	43	9	8	8.5	0.71	R
16	9	8	8.5	0.71	R	44	8	9	8.5	0.71	R
17	10	9	9.5	0.71	R	45	8.5	10	9.25	1.06	R
18	8	9	8.5	0.71	R	46	10	9	9.5	0.71	R
19	9	9	9	0.00	R	47	10	8	9	1.41	R
20	10	8	9	1.41	R	48	10	10	10	0.00	R
21	9	8	8.5	0.71	R	49	9	9	9	0.00	R
22	8	9.5	8.75	1.06	R	50	8	8	8	0.00	R
23	10	8.5	9.25	1.06	R	51	10	10	10	0.00	R
24	9	10	9.5	0.71	R	52	9	9	9	0.00	R
25	10	9.5	9.75	0.35	R	53	8.5	9	8.75	0.35	R
26	8	10	9	1.41	R	54	9	8.5	8.75	0.35	R
27											
28											

Table 8 continued…

TRIMETHOPRIM											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	10	10	10	0.00	R	81	9	8	8.5	0.71	R
56	9	9	9	0.00	R	82	8	9	8.5	0.71	R
57	8	8	8	0.00	R	83	10	10	10	0.00	R
58	8	10	9	1.41	R	84	10	9	9.5	0.71	R
59	9	9	9	0.00	R	85	10	10	10	0.00	R
60	8	8	8	0.00	R	86	9.5	10	9.75	0.35	R
61	8	10	9	1.41	R	87	8.5	9	8.75	0.35	R
62	9	9.5	9.25	0.35	R	88	9	8	8.5	0.71	R
63	9	8	8.5	0.71	R	89	8	9	8.5	0.71	R
64	9	9	9	0.00	R	90	10	9	9.5	0.71	R
65	10	10	10	0.00	R	91	9	8	8.5	0.71	R
66	10	10	10	0.00	R	92	8	8	8	0.00	R
67	10	10	10	0.00	R	93	8	8	8	0.00	R
68	10	9	9.5	0.71	R	94	8	8	8	0.00	R
69	10	9	9.5	0.71	R	95	9.5	9	9.25	0.35	R
70	8	7	7.5	0.71	R	96	9	9	9	0.00	R
71	9	9	9	0.00	R	97	8.5	9	8.75	0.35	R
72	8	8	8	0.00	R	98	9	9	9	0.00	R
73	9	9	9	0.00	R	99	10	10	10	0.00	R
74	9	9	9	0.00	R	100	10	9	9.5	0.71	R
75	9	9	9	0.00	R	101	9	9	9	0.00	R
76	8	8	8	0.00	R	102	11	11	11	0.00	S
77	8	9	8.5	0.71	R	103	14	14	14	0.00	S
78	8.5	9	8.75	0.35	R	104	15	15	15	0.00	S
79	8	9	8.5	0.71	R	105	12.5	13	12.75	0.35	S
80	10	9	9.5	0.71	R	106	13	13	13	0.00	S

Table 8 continued…

TRIMETHOPRIM											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	12	12	12	0.00	S	133	11	11.5	11.25	0.35	S
108	13	13	13	0.00	S	134	14.5	15	14.75	0.35	S
109	11	11	11	0.00	S	135	12	12	12	0.00	S
110	12	12.5	12.25	0.35	S	136	13	13	13	0.00	S
111	15	15	15	0.00	S	137	16	16	16	0.00	S
112	16	16	16	0.00	S	138	16	16	16	0.00	S
113	13	13	13	0.00	S	139	16.5	17	16.75	0.35	S
114	17	17.5	17.25	0.35	S	140	16	16	16	0.00	S
115	18	18	18	0.00	S	141	17.5	17.5	17.5	0.00	S
116	14	14.5	14.25	0.35	S	142	11	11	11	0.00	S
117	13.5	13.5	13.5	0.00	S	143	10.5	11	10.75	0.35	S
118	14.5	15	14.75	0.35	S	144	14	14.5	14.25	0.35	S
119	15	15	15	0.00	S	145	12.5	13	12.75	0.35	S
120	16.5	16.5	16.5	0.00	S	146	12	12	12	0.00	S
121	14	15	14.5	0.71	S	147	14	14	14	0.00	S
122	13.5	14	13.75	0.35	S	148	11.5	11.5	11.5	0.00	S
123	12.5	13	12.75	0.35	S	149	11	11	11	0.00	S
124	14.5	14	14.25	0.35	S	150	10.5	11	10.75	0.35	S
125	15	15.5	15.25	0.35	S	151	16	16	16	0.00	S
126	16	16	16	0.00	S	152	15.5	15.5	15.5	0.00	S
127	14	14.5	14.25	0.35	S	153	17	17	17	0.00	S
128	14	15	14.5	0.71	S	154	12.5	13	12.75	0.35	S
129	15	16	15.5	0.71	S	155	14	14	14	0.00	S
130	11	12.5	11.75	1.06	S	156	15	15	15	0.00	S
131	12.5	13	12.75	0.35	S	157	11	11	11	0.00	S
132	19	20	19.5	0.71	S	158	11	11	11	0.00	S

Table 8 continued…

TRIMETHOPRIM											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	14	14	14	0.00	S	185	19	19	19	0.00	S
160	19	19	19	0.00	S	186	16	16	16	0.00	S
161	15.5	15.5	15.5	0.00	S	187	15.5	16	15.75	0.35	S
162	13	13	13	0.00	S	188	16.5	17	16.75	0.35	S
163	12.5	13	12.75	0.35	S	189	16.5	17	16.75	0.35	S
164	14.5	15	14.75	0.35	S	190	14.5	15.5	15	0.71	S
165	16	16	16	0.00	S	191	18	17.5	17.75	0.35	S
166	17	17	17	0.00	S	192	13	12.5	12.75	0.35	S
167	14.5	15	14.75	0.35	S	193	10.5	11	10.75	0.35	S
168	15	15.5	15.25	0.35	S	194	11	11	11	0.00	S
169	11	10.5	10.75	0.35	S	195	12.5	12.5	12.5	0.00	S
170	10.5	10.5	10.5	0.00	S	196	15.5	16	15.75	0.35	S
171	10.5	11	10.75	0.35	S	197	14.5	15	14.75	0.35	S
172	10	10.5	10.25	0.35	S	198	13.5	15	14.25	1.06	S
173	13	14	13.5	0.71	S	199	12	12.5	12.25	0.35	S
174	15.5	16	15.75	0.35	S	200	12	12	12	0.00	S
175	13.5	12.5	13	0.71	S						
176	13	12.5	12.75	0.35	S						
177	14.5	13	13.75	1.06	S						
178	16	16	16	0.00	S						
179	19	19	19	0.00	S						
180	21	21	21	0.00	S						
181	11.5	12	11.75	0.35	S						
182	10	12	11	1.41	S						
183	10	10.5	10.25	0.35	S						
184	17.5	18	17.75	0.35	S						

Table 9: Zones of inhibition (mm) of *E. coli* isolates tested against norfloxacin

NORFLOXACIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	18	18	18	0.00	S	29	12	12	12	0.00	R
2	10	10	10	0.00	R	30	11	11	11	0.00	R
3	15	15.5	15.25	0.35	S	31	11	11.5	11.25	0.35	R
4	16	16	16	0.00	S	32	13	13	13	0.00	S
5	11	11	11	0.00	R	33	9.5	10	9.75	0.35	R
6	11	11	11	0.00	R	34	18.5	17	17.75	1.06	S
7	10	10	10	0.00	R	35	14	14	14	0.00	S
8	12.5	12.5	12.5	0.00	S	36	15	15	15	0.00	S
9	8	8.5	8.25	0.35	R	37	15	15.5	15.25	0.35	S
10	16	16	16	0.00	S	38	15	15	15	0.00	S
11	14	14	14	0.00	S	39	9.5	9.5	9.5	0.00	R
12	11	11	11	0.00	R	40	13	13.5	13.25	0.35	S
13	12	12	12	0.00	R	41	14.5	14.5	14.5	0.00	S
14	10	10.5	10.25	0.35	R	42	10.5	10.5	10.5	0.00	R
15	10.5	11	10.75	0.35	R	43	16	16	16	0.00	S
16	15	15	15	0.00	S	44	15	15.5	15.25	0.35	S
17	11	10	10.5	0.71	R	45	16	16.5	16.25	0.35	S
18	10	9	9.5	0.71	R	46	11	11	11	0.00	R
19	10.5	9.5	10	0.71	R	47	14.5	15	14.75	0.35	S
20	36	36	36	0.00	S	48	12	11.5	11.75	0.35	R
21	33	32.5	32.75	0.35	S	49	13.5	14	13.75	0.35	S
22	37	36	36.5	0.71	S	50	16	17	16.5	0.71	S
23	12.5	13	12.75	0.35	S	51	16	16	16	0.00	S
24	13.5	14	13.75	0.35	S	52	12	11	11.5	0.71	R
25	16	16	16	0.00	S	53	10.5	11	10.75	0.35	R
26	17	17	17	0.00	S	54	32	33	32.5	0.71	S
27	12.5	13	12.75	0.35	S						
28	12	12	12	0.00	R						

Table 9 continued…

NORFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	11	11	11	0.00	R	81	25	26.5	25.75	1.06	S
56	30.5	31	30.75	0.35	S	82	12	10.5	11.25	1.06	R
57	31	32	31.5	0.71	S	83	11	11	11	0.00	R
58	30	30.5	30.25	0.35	S	84	30.5	31	30.75	0.35	S
59	32	32.5	32.25	0.35	S	85	12	12	12	0.00	R
60	31	32	31.5	0.71	S	86	9	9	9	0.00	R
61	39	40	39.5	0.71	S	87	38	38	38	0.00	S
62	30.5	31	30.75	0.35	S	88	35	35	35	0.00	S
63	10	10	10	0.00	R	89	35	35	35	0.00	S
64	9	9.5	9.25	0.35	R	90	36	35	35.5	0.71	S
65	36	36	36	0.00	S	91	9	9	9	0.00	R
66	33	32.5	32.75	0.35	S	92	38	38	38	0.00	S
67	12	12	12	0.00	R	93	35	35	35	0.00	S
68	33	33	33	0.00	S	94	35	35	35	0.00	S
69	32	32	32	0.00	S	95	35	35	35	0.00	S
70	11	11	11	0.00	R	96	36	37	36.5	0.71	S
71	11	11	11	0.00	R	97	38	39	38.5	0.71	S
72	27	28.5	27.75	1.06	S	98	37	36	36.5	0.71	S
73	28	29	28.5	0.71	S	99	25	26	25.5	0.71	S
74	12	11	11.5	0.71	R	100	38	38	38	0.00	S
75	10.5	10	10.25	0.35	R	101	35	35	35	0.00	S
76	38	38	38	0.00	S	102	10	10	10	0.00	R
77	35	35	35	0.00	S	103	10	10	10	0.00	R
78	30	32	31	1.41	S	104	9.5	10	9.75	0.35	R
79	33	33	33	0.00	S	105	12	12	12	0.00	R
80	9.5	9.5	9.5	0.00	R	106	11.5	11.5	11.5	0.00	R

Table 9 continued…

NORFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	26	27	26.5	0.71	S	133	10	10	10	0.00	R
108	11	11	11	0.00	R	134	11	11.5	11.25	0.35	R
109	10	10	10	0.00	R	135	12	12	12	0.00	R
110	11	11	11	0.00	R	136	10.5	10	10.25	0.35	R
111	10.5	10.5	10.5	0.00	R	137	35	36	35.5	0.71	S
112	10	10	10	0.00	R	138	35	35.5	35.25	0.35	S
113	10.5	9.5	10	0.71	R	139	37	36	36.5	0.71	S
114	11	11	11	0.00	R	140	30.5	31	30.75	0.35	S
115	9.5	9.5	9.5	0.00	R	141	36	35.5	35.75	0.35	S
116	11	12	11.5	0.71	R	142	9.5	9.5	9.5	0.00	R
117	12	12	12	0.00	R	143	10	10	10	0.00	R
118	10.5	10.5	10.5	0.00	R	144	11	10	10.5	0.71	R
119	35	36	35.5	0.71	S	145	12	11	11.5	0.71	R
120	35	35.5	35.25	0.35	S	146	10.5	10.5	10.5	0.00	R
121	37	36	36.5	0.71	S	147	10.5	10.5	10.5	0.00	R
122	11	11	11	0.00	R	148	35	36	35.5	0.71	S
123	18	18	18	0.00	S	149	35	35.5	35.25	0.35	S
124	12	12	12	0.00	R	150	37	36	36.5	0.71	S
125	11	11.5	11.25	0.35	R	151	36	35.5	35.75	0.35	S
126	36	35.5	35.75	0.35	S	152	26	27	26.5	0.71	S
127	26	27	26.5	0.71	S	153	35	35	35	0.00	S
128	10.5	10.5	10.5	0.00	R	154	33	33	33	0.00	S
129	11	11	11	0.00	R	155	34	32.5	33.25	1.06	S
130	33	33	33	0.00	S	156	36	36	36	0.00	S
131	32	32	32	0.00	S	157	31	30	30.5	0.71	S
132	10.5	11	10.75	0.35	R	158	36	35	35.5	0.71	S

Table 9 continued…

NORFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	35	35	35	0.00	S	185	30.5	31	30.75	0.35	S
160	33	33	33	0.00	S	186	31	32	31.5	0.71	S
161	28	28	28	0.00	S	187	30	30.5	30.25	0.35	S
162	30	30	30	0.00	S	188	36	35.5	35.75	0.35	S
163	32	31	31.5	0.71	S	189	28	28	28	0.00	S
164	38	39	38.5	0.71	S	190	30	30	30	0.00	S
165	35	36	35.5	0.71	S	191	32	31	31.5	0.71	S
166	35	35.5	35.25	0.35	S	192	35	36.5	35.75	1.06	S
167	37	36	36.5	0.71	S	193	36	36	36	0.00	S
168	36	35.5	35.75	0.35	S	194	25	25	25	0.00	S
169	32	33	32.5	0.71	S	195	9.5	10	9.75	0.35	R
170	35	35	35	0.00	S	196	33	33	33	0.00	S
171	37	36	36.5	0.71	S	197	28	28	28	0.00	S
172	25.5	26	25.75	0.35	S	198	30	30	30	0.00	S
173	26.5	27	26.75	0.35	S	199	32	31	31.5	0.71	S
174	27	28	27.5	0.71	S	200	38	39	38.5	0.71	S
175	32	33	32.5	0.71	S						R
176	11.5	11.5	11.5	0.00	R						R
177	12	12	12	0.00	R						R
178	36	36	36	0.00	S						R
179	33	32.5	32.75	0.35	S						R
180	35	36	35.5	0.71	S						R
181	36.5	37	36.75	0.35	S						R
182	37	37.5	37.25	0.35	S						R
183	10.5	10	10.25	0.35	R						R
184	30	30	30	0.00	S						R

Table 10: Zones of inhibition (mm) of *E. coli* isolates tested against ciprofloxacin

CIPROFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	39	38	38.5	0.71	S	29	12	12	12	0.00	R
2	37	37.5	37.25	0.35	S	30	27	27	27	0.00	S
3	40	40	40	0.00	S	31	34	34	34	0.00	S
4	41	41	41	0.00	S	32	11	10	10.5	0.71	R
5	12	13	12.5	0.71	R	33	37	37.5	37.25	0.35	S
6	33	33	33	0.00	S	34	20	21	20.5	0.71	S
7	39	38	38.5	0.71	S	35	38	38.5	38.25	0.35	S
8	40	41	40.5	0.71	S	36	37	36	36.5	0.71	S
9	41	41	41	0.00	S	37	29	28.5	28.75	0.35	S
10	41	45	43	2.83	S	38	38	38.5	38.25	0.35	S
11	45	35	40	7.07	S	39	39	39	39	0.00	S
12	35	32	33.5	2.12	S	40	35	35.5	35.25	0.35	S
13	14	13.5	13.75	0.35	R	41	35	34	34.5	0.71	S
14	39	39.5	39.25	0.35	S	42	35	34	34.5	0.71	S
15	37	38	37.5	0.71	S	43	36	36	36	0.00	S
16	36	37	36.5	0.71	S	44	21	21	21	0.00	S
17	37	38	37.5	0.71	S	45	32	32	32	0.00	S
18	36	36	36	0.00	S	46	12	10.5	11.25	1.06	R
19	35	35	35	0.00	S	47	36	36	36	0.00	S
20	35.6	36	35.8	0.28	S	48	34	34	34	0.00	S
21	39	39	39	0.00	S	49	33	33	33	0.00	S
22	34	34	34	0.00	S	50	33	34	33.5	0.71	S
23	29	28	28.5	0.71	S	51	40	40	40	0.00	S
24	28	28	28	0.00	S	52	10	10	10	0.00	R
25	35	34.5	34.75	0.35	S	53	32	33	32.5	0.71	S
26	27	26	26.5	0.71	S	54	36	35	35.5	0.71	S
27	23	22	22.5	0.71	S						
28	10.5	11	10.75	0.35	R						

Table 10 continued…

CIPROFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	33	33	33	0.00	S	81	35	36.5	35.75	1.06	S
56	32	32	32	0.00	S	82	32.5	33	32.75	0.35	S
57	33	33	33	0.00	S	83	14	14	14	0.00	R
58	39	38	38.5	0.71	S	84	29.5	29	29.25	0.35	S
59	40	41	40.5	0.71	S	85	21	22	21.5	0.71	S
60	37	39	38	1.41	S	86	14	13	13.5	0.71	R
61	31	32	31.5	0.71	S	87	27	28	27.5	0.71	S
62	30	30	30	0.00	S	88	25	25	25	0.00	S
63	12	10	11	1.41	R	89	26	26	26	0.00	S
64	28	28	28	0.00	S	90	30	30	30	0.00	S
65	33	32	32.5	0.71	S	91	10	9	9.5	0.71	R
66	32	31	31.5	0.71	S	92	27	27	27	0.00	S
67	21	20	20.5	0.71	S	93	30	29.5	29.75	0.35	S
68	39	38.5	38.75	0.35	S	94	33	32	32.5	0.71	S
69	33	32	32.5	0.71	S	95	37	38	37.5	0.71	S
70	32	31	31.5	0.71	S	96	32	31	31.5	0.71	S
71	10	10	10	0.00	R	97	21	20	20.5	0.71	S
72	32	33.5	32.75	1.06	S	98	39	38.5	38.75	0.35	S
73	35	36	35.5	0.71	S	99	32	32	32	0.00	S
74	36	36	36	0.00	S	100	33	33	33	0.00	S
75	11.5	12	11.75	0.35	R	101	25	25.5	25.25	0.35	S
76	34	35	34.5	0.71	S	102	12	11	11.5	0.71	R
77	27	27	27	0.00	S	103	13	12	12.5	0.71	R
78	30	29.5	29.75	0.35	S	104	12	12.5	12.25	0.35	R
79	27	27	27	0.00	S	105	15	15	15	0.00	R
80	30	30.5	30.25	0.35	S	106	14	14	14	0.00	R

Table 10 continued…

CIPROFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	35	35	35	0.00	S	133	14	14	14	0.00	R
108	13	13	13	0.00	R	134	12.5	12.5	12.5	0.00	R
109	13	13	13	0.00	R	135	13	12	12.5	0.71	R
110	14	13.5	13.75	0.35	R	136	13	13	13	0.00	R
111	14.5	14.5	14.5	0.00	R	137	33	32	32.5	0.71	S
112	14	14	14	0.00	R	138	32	32	32	0.00	S
113	11	11	11	0.00	R	139	9.5	10	9.75	0.35	R
114	11	11	11	0.00	R	140	11	11	11	0.00	R
115	11.5	11.5	11.5	0.00	R	141	36	36	36	0.00	S
116	12	12	12	0.00	R	142	14	14	14	0.00	R
117	13.5	13	13.25	0.35	R	143	14	14.5	14.25	0.35	R
118	14	14	14	0.00	R	144	13.5	13	13.25	0.35	R
119	14.5	14	14.25	0.35	R	145	12.5	12.5	12.5	0.00	R
120	33	32	32.5	0.71	S	146	12.5	12.5	12.5	0.00	R
121	32	32	32	0.00	S	147	12	12	12	0.00	R
122	10	10.5	10.25	0.35	R	148	33	32	32.5	0.71	S
123	33	32	32.5	0.71	S	149	32	33	32.5	0.71	S
124	12	11	11.5	0.71	R	150	29	30	29.5	0.71	S
125	12	12.5	12.25	0.35	R	151	34	35	34.5	0.71	S
126	34	34.5	34.25	0.35	S	152	27	27	27	0.00	S
127	37	39	38	1.41	S	153	30	29.5	29.75	0.35	S
128	12.5	12.5	12.5	0.00	R	154	33	32	32.5	0.71	S
129	13	13	13	0.00	R	155	37	38	37.5	0.71	S
130	33	35	34	1.41	S	156	37	37	37	0.00	S
131	37	37	37	0.00	S	157	41	40	40.5	0.71	S
132	14	14	14	0.00	R	158	15	15	15	0.00	R

Table 10 continued…

CIPROFLOXACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	38	38	38	0.00	S	185	33	34.5	33.75	1.06	S
160	26	26	26	0.00	S	186	37	37	37	0.00	S
161	28	28	28	0.00	S	187	37	38	37.5	0.71	S
162	31	31	31	0.00	S	188	37	37	37	0.00	S
163	25	25.5	25.25	0.35	S	189	41	40	40.5	0.71	S
164	29	29	29	0.00	S	190	12	13	12.5	0.71	R
165	33	34	33.5	0.71	S	191	36	35	35.5	0.71	S
166	34	34.5	34.25	0.35	S	192	30	30	30	0.00	S
167	28	28	28	0.00	S	193	32	31	31.5	0.71	S
168	33	32	32.5	0.71	S	194	27	28	27.5	0.71	S
169	32	31	31.5	0.71	S	195	37	38	37.5	0.71	S
170	21	20	20.5	0.71	S	196	32	33	32.5	0.71	S
171	39	38.5	38.75	0.35	S	197	34	35	34.5	0.71	S
172	25	25	25	0.00	S	198	38	39	38.5	0.71	S
173	32	32	32	0.00	S	199	36	37	36.5	0.71	S
174	31	30	30.5	0.71	S	200	35	36	35.5	0.71	S
175	33	32	32.5	0.71	S						
176	37.5	36	36.75	1.06	S						
177	32	32.5	32.25	0.35	S						
178	36	36	36	0.00	S						
179	32	32	32	0.00	S						
180	30	30	30	0.00	S						
181	28	28	28	0.00	S						
182	33	32	32.5	0.71	S						
183	32	31	31.5	0.71	S						
184	37	35	36	1.41	S						

Table 11: Zones of inhibition (mm) of *E. coli* isolates tested against ceftazidime

CEFTAZIDIME											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	20	20	20	0.00	S	29	32	33	32.5	0.71	S
2	21	21	21	0.00	S	30	34	34	34	0.00	S
3	11	11	11	0.00	R	31	20.5	20.5	20.5	0.00	S
4	23	23	23	0.00	S	32	25	25	25	0.00	S
5	21	21	21	0.00	S	33	28	28	28	0.00	S
6	24	24	24	0.00	S	34	12	12	12	0.00	R
7	22	22	22	0.00	S	35	25	25	25	0.00	S
8	18	18	18	0.00	S	36	29	29	29	0.00	S
9	21	21	21	0.00	S	37	24	24	24	0.00	S
10	21	21	21	0.00	S	38	25	26	25.5	0.71	S
11	24	23	23.5	0.71	S	39	25	24.5	24.75	0.35	S
12	19	20	19.5	0.71	S	40	11.5	12	11.75	0.35	R
13	20	20	20	0.00	S	41	21	21	21	0.00	S
14	26	25	25.5	0.71	S	42	18	18	18	0.00	S
15	24	24	24	0.00	S	43	31	30	30.5	0.71	S
16	22	22	22	0.00	S	44	34	34	34	0.00	S
17	25	25	25	0.00	S	45	33	32	32.5	0.71	S
18	26	26	26	0.00	S	46	28	28	28	0.00	S
19	20	21	20.5	0.71	S	47	24.5	23	23.75	1.06	S
20	13	12.5	12.75	0.35	R	48	26	21	23.5	3.54	S
21	25	25	25	0.00	S	49	26	24	25	1.41	S
22	10	10.5	10.25	0.35	R	50	32	32	32	0.00	S
23	26	26	26	0.00	S	51	33	33	33	0.00	S
24	34	33	33.5	0.71	S	52	21	21.5	21.25	0.35	S
25	32	32	32	0.00	S	53	22	22	22	0.00	S
26	28	28	28	0.00	S	54	13	12.5	12.75	0.35	R
27	35	34	34.5	0.71	S	55	29	29	29	0.00	S
28	33	32	32.5	0.71	S	56	32	33	32.5	0.71	S

Table 11 continued…

CEFTAZIDIME											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	25	25	25	0.00	S	81	24	24	24	0.00	S
56	12	13	12.5	0.71	R	82	25	25	25	0.00	S
57	24	24	24	0.00	S	83	22	22	22	0.00	S
58	24	24	24	0.00	S	84	21	21	21	0.00	S
59	21	21	21	0.00	S	85	20.5	21	20.75	0.35	S
60	21	21	21	0.00	S	86	26	26	26	0.00	S
61	21	21	21	0.00	S	87	10	10	10	0.00	R
62	24	24	24	0.00	S	88	10	10	10	0.00	R
63	24	24	24	0.00	S	89	24	24	24	0.00	S
64	22	23	22.5	0.71	S	90	25	26	25.5	0.71	S
65	21	20.5	20.75	0.35	S	91	22	23	22.5	0.71	S
66	23	22	22.5	0.71	S	92	12	11	11.5	0.71	R
67	22	22	22	0.00	S	93	10	10.5	10.25	0.35	R
68	22	22	22	0.00	S	94	25	25	25	0.00	S
69	22	21	21.5	0.71	S	95	25	24	24.5	0.71	S
70	21	20	20.5	0.71	S	96	24	23.5	23.75	0.35	S
71	20	21	20.5	0.71	S	97	13	12.5	12.75	0.35	R
72	23	23	23	0.00	S	98	20	20	20	0.00	S
73	25	25	25	0.00	S	99	21	21	21	0.00	S
74	26	26	26	0.00	S	100	10	11	10.5	0.71	R
75	22	23	22.5	0.71	S	101	31	30	30.5	0.71	S
76	22	22	22	0.00	S	102	34	34	34	0.00	S
77	23	23	23	0.00	S	103	33	32	32.5	0.71	S
78	12	12	12	0.00	R	104	28	28	28	0.00	S
79	20.5	21	20.75	0.35	S	105	27	28	27.5	0.71	S
80	22	22	22	0.00	S	106	33	30	31.5	2.12	S

Table 11 continued…

CEFTAZIDIME											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	12	12	12	0.00	R	133	30	30	30	0.00	S
108	31	30	30.5	0.71	S	134	33	33	33	0.00	S
109	34	34	34	0.00	S	135	30	28	29	1.41	S
110	33	32	32.5	0.71	S	136	27	27	27	0.00	S
111	28	28	28	0.00	S	137	31	30	30.5	0.71	S
112	24.5	23	23.75	1.06	S	138	34	34	34	0.00	S
113	26	26	26	0.00	S	139	33	32	32.5	0.71	S
114	26	24	25	1.41	S	140	28	28	28	0.00	S
115	13	13.5	13.25	0.35	R	141	24.5	23	23.75	1.06	S
116	24	25	24.5	0.71	S	142	26	26	26	0.00	S
117	26	26	26	0.00	S	143	26	24	25	1.41	S
118	20.5	21	20.75	0.35	S	144	24.5	22	23.25	1.77	S
119	21	22	21.5	0.71	S	145	27	27	27	0.00	S
120	23	23	23	0.00	S	146	29.5	29	29.25	0.35	S
121	14	14	14	0.00	R	147	23.5	21	22.25	1.77	S
122	25	26	25.5	0.71	S	148	29.5	30	29.75	0.35	S
123	13	13	13	0.00	R	149	25.5	26	25.75	0.35	S
124	36	36	36	0.00	S	150	31.5	32	31.75	0.35	S
125	32	32	32	0.00	S	151	22.5	25	23.75	1.77	S
126	25	24	24.5	0.71	S	152	32.5	33	32.75	0.35	S
127	20.5	23	21.75	1.77	S	153	25	37	31	8.49	S
128	21	22	21.5	0.71	S	154	11	11.5	11.25	0.35	R
129	28	28	28	0.00	S	155	33	35	34	1.41	S
130	34	34	34	0.00	S	156	12	11.5	11.75	0.35	R
131	39	39	39	0.00	S	157	29	29	29	0.00	S
132	32	33	32.5	0.71	S	158	32	30.5	31.25	1.06	S

Table 11 continued…

CEFTAZIDIME											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	31	31	31	0.00	S	185	31	32	31.5	0.71	S
160	33	33	33	0.00	S	186	32	32	32	0.00	S
161	31	31	31	0.00	S	187	32.5	33	32.75	0.35	S
162	31	31	31	0.00	S	188	25	25	25	0.00	S
163	28	28	28	0.00	S	189	22	22	22	0.00	S
164	31	30	30.5	0.71	S	190	27	27	27	0.00	S
165	30	30	30	0.00	S	191	30.5	30	30.25	0.35	S
166	31	32	31.5	0.71	S	192	38	39	38.5	0.71	S
167	30.5	30	30.25	0.35	S	193	10	10	10	0.00	R
168	27	27	27	0.00	S	194	32	33	32.5	0.71	S
169	29	28	28.5	0.71	S	195	35	35	35	0.00	S
170	31	31	31	0.00	S	196	20.5	21	20.75	0.35	S
171	24	24	24	0.00	S	197	21	21	21	0.00	S
172	28	27.5	27.75	0.35	S	198	30.5	30.5	30.5	0.00	S
173	30	29.5	29.75	0.35	S	199	36	36	36	0.00	S
174	30.5	30	30.25	0.35	S	200	34	35	34.5	0.71	S
175	27	27	27	0.00	S						
176	23.5	24	23.75	0.35	S						
177	24.5	25	24.75	0.35	S						
178	26.5	27	26.75	0.35	S						
179	27	28	27.5	0.71	S						
180	13	11.5	12.25	1.06	R						
181	23	23	23	0.00	S						
182	12	12	12	0.00	R						
183	31	30	30.5	0.71	S						
184	28	29	28.5	0.71	S						

Table 12: Zones of inhibition (mm) of *E. coli* isolates tested against fosfomycin

FOSFOMYCIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	25	25	25	0.00	S	29	23	23	23	0.00	S
2	18	18	18	0.00	S	30	24	25	24.5	0.71	S
3	26	26	26	0.00	S	31	26	26	26	0.00	S
4	26	26	26	0.00	S	32	23	24	23.5	0.71	S
5	23	24	23.5	0.71	S	33	20.5	21	20.75	0.35	S
6	25	26	25.5	0.71	S	34	26	26	26	0.00	S
7	25	25	25	0.00	S	35	23	24	23.5	0.71	S
8	22	23	22.5	0.71	S	36	25	25	25	0.00	S
9	23	22	22.5	0.71	S	37	29	29	29	0.00	S
10	23	23	23	0.00	S	38	12	12	12	0.00	R
11	24	25	24.5	0.71	S	39	23	23	23	0.00	S
12	26	26	26	0.00	S	40	23	23	23	0.00	S
13	23	24	23.5	0.71	S	41	25	24	24.5	0.71	S
14	25	26	25.5	0.71	S	42	25	25	25	0.00	S
15	27	29	28	1.41	S	43	23	23	23	0.00	S
16	10	10	10	0.00	R	44	24	25	24.5	0.71	S
17	26	25	25.5	0.71	S	45	26	26	26	0.00	S
18	27	26	26.5	0.71	S	46	23	24	23.5	0.71	S
19	28	29	28.5	0.71	S	47	25	26	25.5	0.71	S
20	11	11	11	0.00	R	48	27	27	27	0.00	S
21	25	25	25	0.00	S	49	25	25	25	0.00	S
22	11	11	11	0.00	R	50	30	30	30	0.00	S
23	25	25	25	0.00	S	51	30.5	31	30.75	0.35	S
24	23	23	23	0.00	S	52	32	33	32.5	0.71	S
25	24	25	24.5	0.71	S	53	24	24	24	0.00	S
26	26	26	26	0.00	S	54	26	26	26	0.00	S
27	23	24	23.5	0.71	S						
28	25	26	25.5	0.71	S						

Table 12 continued…

FOSFOMYCIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	23	23	23	0.00	S	81	23	23	23	0.00	S
56	25	25	25	0.00	S	82	24	25	24.5	0.71	S
57	23	23	23	0.00	S	83	26	26	26	0.00	S
58	24	24	24	0.00	S	84	23	24	23.5	0.71	S
59	23	23	23	0.00	S	85	25	26	25.5	0.71	S
60	24	25	24.5	0.71	S	86	24.5	25	24.75	0.35	S
61	26	26	26	0.00	S	87	26	26	26	0.00	S
62	23	24	23.5	0.71	S	88	27	27	27	0.00	S
63	25	26	25.5	0.71	S	89	30	30	30	0.00	S
64	25	25	25	0.00	S	90	32	32	32	0.00	S
65	22	23	22.5	0.71	S	91	30.5	31	30.75	0.35	S
66	23	22	22.5	0.71	S	92	10	10.5	10.25	0.35	R
67	20	20	20	0.00	S	93	11	11	11	0.00	R
68	24	24	24	0.00	S	94	35	35	35	0.00	S
69	25	25	25	0.00	S	95	24	24	24	0.00	S
70	25	26	25.5	0.71	S	96	26	26	26	0.00	S
71	23.5	24	23.75	0.35	S	97	12	12	12	0.00	R
72	24	25	24.5	0.71	S	98	28	29	28.5	0.71	S
73	30	30	30	0.00	S	99	22	22	22	0.00	S
74	35	32	33.5	2.12	S	100	21	22	21.5	0.71	S
75	20	20	20	0.00	S	101	19	20	19.5	0.71	S
76	20	20	20	0.00	S	102	23.5	24	23.75	0.35	S
77	10	10	10	0.00	R	103	26	26	26	0.00	S
78	11	11	11	0.00	R	104	22	23	22.5	0.71	S
79	25	25	25	0.00	S	105	24	25	24.5	0.71	S
80	28	28	28	0.00	S	106	24	24	24	0.00	S

Table 12 continued…

FOSFOMYCIN											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	12	12	12	0.00	R	133	25	24	24.5	0.71	S
108	23	23	23	0.00	S	134	23.5	25.5	24.5	1.41	S
109	25	25	25	0.00	S	135	21	25	23	2.83	S
110	23	23	23	0.00	S	136	22	20	21	1.41	S
111	24	24	24	0.00	S	137	26	24	25	1.41	S
112	23	23	23	0.00	S	138	22	24	23	1.41	S
113	24	25	24.5	0.71	S	139	21	25	23	2.83	S
114	26	26	26	0.00	S	140	29	29	29	0.00	S
115	23	22	22.5	0.71	S	141	22	26	24	2.83	S
116	23	22	22.5	0.71	S	142	19	20	19.5	0.71	S
117	19	20	19.5	0.71	S	143	29	28	28.5	0.71	S
118	25	25.5	25.25	0.35	S	144	25	25	25	0.00	S
119	25	25	25	0.00	S	145	23	24	23.5	0.71	S
120	21	20	20.5	0.71	S	146	27	26	26.5	0.71	S
121	25	24	24.5	0.71	S	147	24	23	23.5	0.71	S
122	24	24	24	0.00	S	148	22	23	22.5	0.71	S
123	25	25	25	0.00	S	149	26	24	25	1.41	S
124	26	25.5	25.75	0.35	S	150	24	25	24.5	0.71	S
125	23	26	24.5	2.12	S	151	25	26	25.5	0.71	S
126	23	26	24.5	2.12	S	152	25	25	25	0.00	S
127	23	24	23.5	0.71	S	153	27	27	27	0.00	S
128	22	26	24	2.83	S	154	10	10	10	0.00	R
129	25	25	25	0.00	S	155	30	32	31	1.41	S
130	26	23	24.5	2.12	S	156	32	32	32	0.00	S
131	26	22	24	2.83	S	157	26	26	26	0.00	S
132	22	20	21	1.41	S	158	28	28	28	0.00	S

Table 12 continued…

FOSFOMYCIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	19	18	18.5	0.71	S	185	19	20	19.5	0.71	S
160	31	32	31.5	0.71	S	186	31	33	32	1.41	S
161	24	23	23.5	0.71	S	187	24	23	23.5	0.71	S
162	22	23	22.5	0.71	S	188	10	10	10	0.00	R
163	26	24	25	1.41	S	189	12	11.5	11.75	0.35	R
164	24	25	24.5	0.71	S	190	10.5	10.5	10.5	0.00	R
165	29	27	28	1.41	S	191	23.5	24	23.75	0.35	S
166	25	25	25	0.00	S	192	23	23	23	0.00	S
167	23	25	24	1.41	S	193	23	26	24.5	2.12	S
168	27	26	26.5	0.71	S	194	23	24	23.5	0.71	S
169	24.5	25	24.75	0.35	S	195	25	25	25	0.00	S
170	23	23	23	0.00	S	196	24	24	24	0.00	S
171	21	25	23	2.83	S	197	26	26	26	0.00	S
172	29	28.5	28.75	0.35	S	198	27	27	27	0.00	S
173	23.5	24	23.75	0.35	S	199	20	20	20	0.00	S
174	23	23	23	0.00	S	200	23	23	23	0.00	S
175	23	26	24.5	2.12	S						
176	23	24	23.5	0.71	S						
177	22	20	21	1.41	S						
178	25	25	25	0.00	S						
179	26	25	25.5	0.71	S						
180	26	25	25.5	0.71	S						
181	22	20	21	1.41	S						
182	11	11.5	11.25	0.35	R						
183	25	25	25	0.00	S						
184	27	26.5	26.75	0.35	S						

Table 13: Zones of inhibition (mm) of *E. coli* isolates tested against amikacin

AMIKACIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	15	16	15.5	0.71	S	29	16	15	15.5	0.71	S
2	16	18	17	1.41	S	30	17	16	16.5	0.71	S
3	15	17	16	1.41	S	31	17	15	16	1.41	S
4	17	16	16.5	0.71	S	32	15	17	16	1.41	S
5	18	17	17.5	0.71	S	33	16	18	17	1.41	S
6	15	15	15	0.00	S	34	18	17	17.5	0.71	S
7	17	16	16.5	0.71	S	35	15	16	15.5	0.71	S
8	17	16	16.5	0.71	S	36	16	17	16.5	0.71	S
9	15	17	16	1.41	S	37	17	15	16	1.41	S
10	16	15	15.5	0.71	S	38	17	16	16.5	0.71	S
11	18	16	17	1.41	S	39	16	16	16	0.00	S
12	17	18	17.5	0.71	S	40	17	18	17.5	0.71	S
13	16	15	15.5	0.71	S	41	15	17	16	1.41	S
14	17	16	16.5	0.71	S	42	16	16	16	0.00	S
15	15	17	16	1.41	S	43	15	17	16	1.41	S
16	16	16	16	0.00	S	44	16	15	15.5	0.71	S
17	18	17	17.5	0.71	S	45	17	17	17	0.00	S
18	15	15	15	0.00	S	46	18	18.5	18.25	0.35	S
19	16	15.5	15.75	0.35	S	47	20	19.5	19.75	0.35	S
20	17	18	17.5	0.71	S	48	16	16	16	0.00	S
21	18	18	18	0.00	S	49	17	17	17	0.00	S
22	20	20	20	0.00	S	50	17	16	16.5	0.71	S
23	15	16	15.5	0.71	S	51	16	17	16.5	0.71	S
24	16	17	16.5	0.71	S	52	17	17	17	0.00	S
25	15.5	16	15.75	0.35	S	53	15	16	15.5	0.71	S
26	18	17	17.5	0.71	S	54	16	16	16	0.00	S
27	15	15	15	0.00	S						
28	16	15	15.5	0.71	S						

Table 13 continued…

AMIKACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	16	17	16.5	0.71	S	81	16	17	16.5	0.71	S
56	17	16	16.5	0.71	S	82	18	16	17	1.41	S
57	17	17	17	0.00	S	83	16	17	16.5	0.71	S
58	15	15	15	0.00	S	84	18	15	16.5	2.12	S
59	16	16	16	0.00	S	85	17	16	16.5	0.71	S
60	18	17	17.5	0.71	S	86	18	17	17.5	0.71	S
61	15	15	15	0.00	S	87	15	15	15	0.00	S
62	16	15	15.5	0.71	S	88	16	15	15.5	0.71	S
63	17	16	16.5	0.71	S	89	17	16	16.5	0.71	S
64	17	18	17.5	0.71	S	90	17	18	17.5	0.71	S
65	18	18	18	0.00	S	91	18	18	18	0.00	S
66	20	21	20.5	0.71	S	92	20	21	20.5	0.71	S
67	21	21	21	0.00	S	93	21	21	21	0.00	S
68	16	17	16.5	0.71	S	94	16	17	16.5	0.71	S
69	17	16	16.5	0.71	S	95	17	16	16.5	0.71	S
70	15	17	16	1.41	S	96	15	17	16	1.41	S
71	15.5	15	15.25	0.35	S	97	15.5	15	15.25	0.35	S
72	18.5	17	17.75	1.06	S	98	18.5	15	16.75	2.47	S
73	15	16	15.5	0.71	S	99	15	16	15.5	0.71	S
74	16	17	16.5	0.71	S	100	16	17	16.5	0.71	S
75	19	17	18	1.41	S	101	18	16	17	1.41	S
76	20	21	20.5	0.71	S	102	17	18	17.5	0.71	S
17	20	21	20.5	0.71	S	103	16	15	15.5	0.71	S
18	18	18	18	0.00	S	104	17	16	16.5	0.71	S
79	16	16	16	0.00	S	105	15	17	16	1.41	S
80	16	16	16	0.00	S	106	16	17	16.5	0.71	S

Table 13 continued…

AMIKACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	16	17	16.5	0.71	S	133	18	17	17.5	0.71	S
108	18	17	17.5	0.71	S	134	15	15	15	0.00	S
109	17	16	16.5	0.71	S	135	20	21	20.5	0.71	S
110	18	17	17.5	0.71	S	136	21	21	21	0.00	S
111	17	16	16.5	0.71	S	137	16	15	15.5	0.71	S
112	15	17	16	1.41	S	138	18	16	17	1.41	S
113	16	15	15.5	0.71	S	139	17	18	17.5	0.71	S
114	18	16	17	1.41	S	140	16	15	15.5	0.71	S
115	17	18	17.5	0.71	S	141	18	16	17	1.41	S
116	16	15	15.5	0.71	S	142	16	15	15.5	0.71	S
117	17	16	16.5	0.71	S	143	18	16	17	1.41	S
118	15	17	16	1.41	S	144	17	18	17.5	0.71	S
119	16	16	16	0.00	S	145	16	15	15.5	0.71	S
120	18	17	17.5	0.71	S	146	25	25	25	0.00	S
121	15	15	15	0.00	S	147	23	22	22.5	0.71	S
122	16	15.5	15.75	0.35	S	148	24	23	23.5	0.71	S
123	15	16	15.5	0.71	S	149	24	25	24.5	0.71	S
124	16	16	16	0.00	S	150	25	23	24	1.41	S
125	19	19	19	0.00	S	151	24	23	23.5	0.71	S
126	19	20	19.5	0.71	S	152	17	18	17.5	0.71	S
127	20	20.5	20.25	0.35	S	153	16	15	15.5	0.71	S
128	20	20.5	20.25	0.35	S	154	17	16	16.5	0.71	S
129	15	15	15	0.00	S	155	18	19	18.5	0.71	S
130	15.5	17	16.25	1.06	S	156	19	19	19	0.00	S
131	17	17	17	0.00	S	157	25	25	25	0.00	S
132	18	18	18	0.00	S	158	22	23	22.5	0.71	S

Table 13 continued…

AMIKACIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	18	16	17	1.41	S	185	18	16	17	1.41	S
160	17	18	17.5	0.71	S	186	17	18	17.5	0.71	S
161	16	15	15.5	0.71	S	187	18	17	17.5	0.71	S
162	17	16	16.5	0.71	S	188	15	15	15	0.00	S
163	15	17	16	1.41	S	189	16	15.5	15.75	0.35	S
164	16	16	16	0.00	S	190	16	16	16	0.00	S
165	18	17	17.5	0.71	S	191	18	17	17.5	0.71	S
166	22	21	21.5	0.71	S	192	15	15	15	0.00	S
167	24	24	24	0.00	S	193	16	15.5	15.75	0.35	S
168	23	23	23	0.00	S	194	23	22	22.5	0.71	S
169	23	23	23	0.00	S	195	25	25	25	0.00	S
170	24	23	23.5	0.71	S	196	25	25	25	0.00	S
171	24	25	24.5	0.71	S	197	24	24	24	0.00	S
172	23	24	23.5	0.71	S	198	23	22	22.5	0.71	S
173	22	22	22	0.00	S	199	23	23	23	0.00	S
174	22	22	22	0.00	S	200	25	24	24.5	0.71	S
175	25	25	25	0.00	S						
176	18	17	17.5	0.71	S						
177	15	15	15	0.00	S						
178	16	15.5	15.75	0.35	S						
179	15	16	15.5	0.71	S						
180	16	16	16	0.00	S						
181	19	19	19	0.00	S						
182	19	20	19.5	0.71	S						
183	20	20.5	20.25	0.35	S						
184	20	20.5	20.25	0.35	S						

Table 14: Zones of inhibition (mm) of *E. coli* isolates tested against cefazolin

CEFAZOLIN											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	30	30	30	0.00	S	29	28	29	28.5	0.71	S
2	32	31	31.5	0.71	S	30	17	16	16.5	0.71	S
3	30	30	30	0.00	S	31	16	16	16	0.00	S
4	28	28	28	0.00	S	32	15	17	16	1.41	S
5	30	29	29.5	0.71	S	33	16	15	15.5	0.71	S
6	27	27	27	0.00	S	34	18	16	17	1.41	S
7	28	28	28	0.00	S	35	17	18	17.5	0.71	S
8	29	29	29	0.00	S	36	16	15	15.5	0.71	S
9	30	30	30	0.00	S	37	17	16	16.5	0.71	S
10	25	25	25	0.00	S	38	15	17	16	1.41	S
11	27	26	26.5	0.71	S	39	16	16	16	0.00	S
12	27	26	26.5	0.71	S	40	18	17	17.5	0.71	S
13	30	29	29.5	0.71	S	41	15	15	15	0.00	S
14	26	27	26.5	0.71	S	42	16	15	15.5	0.71	S
15	27	28	27.5	0.71	S	43	17	16	16.5	0.71	S
16	23	24	23.5	0.71	S	44	17	18	17.5	0.71	S
17	27	27	27	0.00	S	45	18	18	18	0.00	S
18	29	29	29	0.00	S	46	20	21	20.5	0.71	S
19	30	31	30.5	0.71	S	47	28.5	29	28.75	0.35	S
20	30	31	30.5	0.71	S	48	21	22	21.5	0.71	S
21	27	26	26.5	0.71	S	49	25	25	25	0.00	S
22	26	26.5	26.25	0.35	S	50	22	25	23.5	2.12	S
23	30	30	30	0.00	S	51	24	26	25	1.41	S
24	29	29.5	29.25	0.35	S	52	25	26	25.5	0.71	S
25	30	30	30	0.00	S	53	24	24	24	0.00	S
26	29	29	29	0.00	S	54	16	16	16	0.00	S
27	27	27	27	0.00	S						
28	27	27	27	0.00	S						

Table 14 continued…

CEFAZOLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	18	16	17	1.41	S	81	18	15	16.5	2.12	S
56	17	18	17.5	0.71	S	82	17	16	16.5	0.71	S
57	18	17	17.5	0.71	S	83	18	17	17.5	0.71	S
58	15	15	15	0.00	S	84	15	15	15	0.00	S
59	16	15	15.5	0.71	S	85	16	15	15.5	0.71	S
60	17	16	16.5	0.71	S	86	17	16	16.5	0.71	S
61	17	15	16	1.41	S	87	17	18	17.5	0.71	S
62	17	16	16.5	0.71	S	88	18	18	18	0.00	S
63	16	16	16	0.00	S	89	20	21	20.5	0.71	S
64	15	17	16	1.41	S	90	21	21	21	0.00	S
65	16	15	15.5	0.71	S	91	16	17	16.5	0.71	S
66	18	16	17	1.41	S	92	17	16	16.5	0.71	S
67	17	18	17.5	0.71	S	93	15	17	16	1.41	S
68	16	15	15.5	0.71	S	94	26.5	27	26.75	0.35	S
69	17	16	16.5	0.71	S	95	26	26	26	0.00	S
70	15	17	16	1.41	S	96	24	24	24	0.00	S
71	16	16	16	0.00	S	97	26.5	26.5	26.5	0.00	S
72	26	26	26	0.00	S	98	24.5	25	24.75	0.35	S
73	25	25	25	0.00	S	99	25	26	25.5	0.71	S
74	30	30	30	0.00	S	100	25	26	25.5	0.71	S
75	16	16	16	0.00	S	101	20	21	20.5	0.71	S
76	15.5	16	15.75	0.35	S	102	20	20	20	0.00	S
77	21.5	22	21.75	0.35	S	103	26	26	26	0.00	S
78	32	33	32.5	0.71	S	104	31	32	31.5	0.71	S
79	18.5	18.5	18.5	0.00	S	105	16	17	16.5	0.71	S
80	21	22	21.5	0.71	S	106	17.5	18	17.75	0.35	S

Table 14 continued…

CEFAZOLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	27	26	26.5	0.71	S	133	24.5	25	24.75	0.35	S
108	29	28	28.5	0.71	S	134	19.5	20	19.75	0.35	S
109	29	29	29	0.00	S	135	24	25	24.5	0.71	S
110	28	28	28	0.00	S	136	26	26	26	0.00	S
111	27	27	27	0.00	S	137	16	15	15.5	0.71	S
112	30	30	30	0.00	S	138	18	16	17	1.41	S
113	30	31	30.5	0.71	S	139	17	18	17.5	0.71	S
114	25	24.5	24.75	0.35	S	140	16	15	15.5	0.71	S
115	24	25	24.5	0.71	S	141	17	16	16.5	0.71	S
116	25	25	25	0.00	S	142	15	17	16	1.41	S
117	23	23	23	0.00	S	143	16	16	16	0.00	S
118	23	24	23.5	0.71	S	144	18	17	17.5	0.71	S
119	25	26	25.5	0.71	S	145	15	15	15	0.00	S
120	21	22	21.5	0.71	S	146	16	15.5	15.75	0.35	S
121	23	23	23	0.00	S	147	15	16	15.5	0.71	S
122	17	18	17.5	0.71	S	148	16	16	16	0.00	S
123	19.5	20	19.75	0.35	S	149	19	19	19	0.00	S
124	24	25	24.5	0.71	S	150	19	20	19.5	0.71	S
125	24	26	25	1.41	S	151	20	20.5	20.25	0.35	S
126	23	24	23.5	0.71	S	152	20	20.5	20.25	0.35	S
127	24	25	24.5	0.71	S	153	23	24	23.5	0.71	S
128	22	23	22.5	0.71	S	154	25	26	25.5	0.71	S
129	26	28	27	1.41	S	155	28.5	29	28.75	0.35	S
130	26	24	25	1.41	S	156	25	26	25.5	0.71	S
131	21.5	22	21.75	0.35	S	157	25.5	25	25.25	0.35	S
132	24	25	24.5	0.71	S	158	16	15	15.5	0.71	S

Table 14 continued…

CEFAZOLIN											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	18	17	17.5	0.71	S	185	21.5	22	21.75	0.35	S
160	15	15	15	0.00	S	186	22.5	23	22.75	0.35	S
161	20	21	20.5	0.71	S	187	17.5	18	17.75	0.35	S
162	21	21	21	0.00	S	188	20	20	20	0.00	S
163	16	15	15.5	0.71	S	189	28.5	29	28.75	0.35	S
164	18	16	17	1.41	S	190	25	26	25.5	0.71	S
165	17	18	17.5	0.71	S	191	25.5	25	25.25	0.35	S
166	16	15	15.5	0.71	S	192	25	25	25	0.00	S
167	17	16	16.5	0.71	S	193	19.5	20	19.75	0.35	S
168	15	17	16	1.41	S	194	25	25	25	0.00	S
169	16	16	16	0.00	S	195	21.5	22	21.75	0.35	S
170	18	17	17.5	0.71	S	196	15.5	16	15.75	0.35	S
171	17	18	17.5	0.71	S	197	17	18	17.5	0.71	S
172	16	15	15.5	0.71	S	198	23.5	24	23.75	0.35	S
173	17	16	16.5	0.71	S	199	22.5	22.5	22.5	0.00	S
174	15	17	16	1.41	S	200	26	26	26	0.00	S
175	16	16	16	0.00	S						
176	18	17	17.5	0.71	S						
177	15	15	15	0.00	S						
178	16	15.5	15.75	0.35	S						
179	20	20	20	0.00	S						
180	27	27	27	0.00	S						
181	22	23	22.5	0.71	S						
182	16	16	16	0.00	S						
183	15	15	15	0.00	S						
184	16	17	16.5	0.71	S						

Table 15: Zones of inhibition (mm) of *E. coli* isolates tested against doxycycline

DOXYCYCLINE											
					R	S					
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
1	16	16	16	0.00	S	29	16	16	16	0.00	S
2	18	17	17.5	0.71	S	30	17	17.5	17.25	0.35	S
3	15	15	15	0.00	S	31	16	16	16	0.00	S
4	10	11	10.5	0.71	S	32	25	24.5	24.75	0.35	S
5	10	11	10.5	0.71	S	33	17	17.5	17.25	0.35	S
6	18	17.5	17.75	0.35	S	34	11.5	12	11.75	0.35	S
7	10	11	10.5	0.71	S	35	17	17	17	0.00	S
8	16	15.5	15.75	0.35	S	36	10	11.5	10.75	1.06	S
9	17	17	17	0.00	S	37	22	23	22.5	0.71	S
10	17	17	17	0.00	S	38	10.5	11	10.75	0.35	S
11	17	18	17.5	0.71	S	39	16	16.5	16.25	0.35	S
12	19	19.5	19.25	0.35	S	40	15	16.5	15.75	1.06	S
13	10	11.5	10.75	1.06	S	41	17	17	17	0.00	S
14	19	19	19	0.00	S	42	11	12	11.5	0.71	S
15	16	17.5	16.75	1.06	S	43	27.5	27.5	27.5	0.00	S
16	19	19.5	19.25	0.35	S	44	17	18	17.5	0.71	S
17	16	16.5	16.25	0.35	S	45	16	17.5	16.75	1.06	S
18	15	17	16	1.41	S	46	15	16	15.5	0.71	S
19	13	14.5	13.75	1.06	S	47	14	14	14	0.00	S
20	16	15.5	15.75	0.35	S	48	24	23.5	23.75	0.35	S
21	11	12	11.5	0.71	S	49	19	19	19	0.00	S
22	15	15.5	15.25	0.35	S	50	17	17.5	17.25	0.35	S
23	15.5	15.5	15.5	0.00	S	51	14.5	15	14.75	0.35	S
24	10	10	10	0.00	R	52	15	15	15	0.00	S
25	14	14.5	14.25	0.35	S	53	16.5	17	16.75	0.35	S
26	15.5	16	15.75	0.35	S	54	16	17.5	16.75	1.06	S
27	18	17.5	17.75	0.35	S						
28	18	19	18.5	0.71	S						

Table 15 continued…

DOXYCYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
55	14	15.5	14.75	1.06	S	81	11	12	11.5	0.71	S
56	12	13	12.5	0.71	S	82	14	14	14	0.00	S
57	15.5	16	15.75	0.35	S	83	11.5	12	11.75	0.35	S
58	10	11	10.5	0.71	S	84	10.5	11	10.75	0.35	S
59	15	14.5	14.75	0.35	S	85	17	17	17	0.00	S
60	11	12	11.5	0.71	S	86	17	18.5	17.75	1.06	S
61	10	12	11	1.41	S	87	10	11	10.5	0.71	S
62	11	12	11.5	0.71	S	88	10	12	11	1.41	S
63	16	15	15.5	0.71	S	89	16	17	16.5	0.71	S
64	16	15.5	15.75	0.35	S	90	11	12.5	11.75	1.06	S
65	16	15.5	15.75	0.35	S	91	16	17	16.5	0.71	S
66	16	16	16	0.00	S	92	10	11.5	10.75	1.06	S
67	14	14	14	0.00	S	93	12	12	12	0.00	S
68	16	16	16	0.00	S	94	14	14	14	0.00	S
69	13	13	13	0.00	S	95	11	11	11	0.00	S
70	16	16	16	0.00	S	96	11	11	11	0.00	S
71	13	13	13	0.00	S	97	13	13	13	0.00	S
72	13	14	13.5	0.71	S	98	19	19.5	19.25	0.35	S
73	12	13	12.5	0.71	S	99	15	16	15.5	0.71	S
74	13	13	13	0.00	S	100	17	18	17.5	0.71	S
75	15	15	15	0.00	S	101	12.5	14	13.25	1.06	S
76	16	16	16	0.00	S	102	9	9	9	0.00	R
77	18	18.5	18.25	0.35	S	103	8.5	8.5	8.5	0.00	R
78	12.5	13	12.75	0.35	S	104	9	9	9	0.00	R
79	16	16	16	0.00	S	105	10	10	10	0.00	R
80	15	14.5	14.75	0.35	S	106	10	10	10	0.00	R

Table 15 continued…

DOXYCYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
107	9	9	9	0.00	R	133	9.5	9.5	9.5	0.00	R
108	10	10	10	0.00	R	134	9	9	9	0.00	R
109	8.5	9	8.75	0.35	R	135	8.5	8	8.25	0.35	R
110	9.5	9.5	9.5	0.00	R	136	10	10	10	0.00	R
111	10	10	10	0.00	R	137	9.5	9.5	9.5	0.00	R
112	10	10	10	0.00	R	138	9.5	10	9.75	0.35	R
113	10	9.5	9.75	0.35	R	139	9	8.5	8.75	0.35	R
114	9	9.5	9.25	0.35	R	140	9	9	9	0.00	R
115	9.5	9	9.25	0.35	R	141	10	10	10	0.00	R
116	9.5	9.5	9.5	0.00	R	142	9.5	10	9.75	0.35	R
117	9	9	9	0.00	R	143	12	12	12	0.00	S
118	8.5	8.5	8.5	0.00	R	144	18	18	18	0.00	S
119	9.5	9.5	9.5	0.00	R	145	16	16	16	0.00	S
120	10	10	10	0.00	R	146	17	17.5	17.25	0.35	S
121	10	10	10	0.00	R	147	13.5	14	13.75	0.35	S
122	10	10	10	0.00	R	148	16.5	16	16.25	0.35	S
123	11	11	11	0.00	S	149	14	14	14	0.00	S
124	15	12	13.5	2.12	S	150	16	16	16	0.00	S
125	9.5	9.5	9.5	0.00	R	151	13	14	13.5	0.71	S
126	10	10	10	0.00	R	152	9.5	10	9.75	0.35	R
127	10	10	10	0.00	R	153	10	10	10	0.00	R
128	9.5	9.5	9.5	0.00	R	154	10	10	10	0.00	R
129	9	9	9	0.00	R	155	12	13	12.5	0.71	S
130	11	13	12	1.41	S	156	11.5	12	11.75	0.35	S
131	12.5	13	12.75	0.35	S	157	10.5	11	10.75	0.35	S
132	9.5	9.5	9.5	0.00	R	158	9.5	8.5	9	0.71	R

Table 15 continued…

DOXYCYCLINE											
NO:	REP 1	REP 2	AVE	STD DEV	R/S	NO:	REP 1	REP 2	AVE	STD DEV	R/S
159	9	9	9	0.00	R	185	16	16	16	0.00	S
160	9.5	9.5	9.5	0.00	R	186	12	12	12	0.00	S
161	11.5	11	11.25	0.35	S	187	12.5	11	11.75	1.06	S
162	14	14	14	0.00	S	188	13	13.5	13.25	0.35	S
163	13	13.5	13.25	0.35	S	189	14	14	14	0.00	S
164	16	16	16	0.00	S	190	11	12	11.5	0.71	S
165	16	16	16	0.00	S	191	10	10	10	0.00	R
166	15	15.5	15.25	0.35	S	192	10	9.5	9.75	0.35	R
167	8.5	9	8.75	0.35	R	193	17	17	17	0.00	S
168	9.5	9.5	9.5	0.00	R	194	17	18.5	17.75	1.06	S
169	9.5	10	9.75	0.35	R	195	10	11	10.5	0.71	S
170	10	10	10	0.00	R	196	10	12	11	1.41	S
171	9.5	9.5	9.5	0.00	R	197	16	17	16.5	0.71	S
172	9.5	9.5	9.5	0.00	R	198	11	12.5	11.75	1.06	S
173	8.5	8.5	8.5	0.00	R	199	16	17	16.5	0.71	S
174	19	19.5	19.25	0.35	S	200	10	11.5	10.75	1.06	S
175	17.5	18.5	18	0.71	S						
176	13	13	13	0.00	S						
177	16	16	16	0.00	S						
178	11	11	11	0.00	S						
179	11	12	11.5	0.71	S						
180	12	13	12.5	0.71	S						
181	9.5	10	9.75	0.35	R						
182	17	17	17	0.00	S						
183	18	18	18	0.00	S						
184	15	16	15.5	0.71	S						