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Survival of free-living and plankton-associated *Escherichia coli* in the final effluents of a waste water treatment facility in a peri-urban community of the Eastern Cape Province of South Africa

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Escherichia coli **remains a major threat in many places around the globe as a major causative agent of diarrhea and its reservoir in the estuarine environment may play an important role in the survival and transport of pathogenic strains. The final effluents of a peri-urban waste water treatment facility were assessed for surviving *E. coli* community as free-living or plankton-associated cells in relation to some physicochemical parameter for a year period. The free-living *E. coli* population densities varied from 0 to 3.13 × 101 cfu/ml, while the plankton- associated *E. coli* densities vary with plankton sizes as follows: 180 µm (0 - 4.30 × 10^1 cfu/ml), 60 µm (0 - 4.20 × 10^1 cfu/ml), 20 µm (0 - 5.00 × 101 cfu/ml). The seasonal variations in the *E. coli* densities among the plankton size categories were significant (P < 0.05). Correlation analysis suggested that the counts of *E. coli* correlated negatively with salinity (P < 0.001) and positively with temperature, pH, turbidity and dissolved oxygen (P < 0.001) in the final effluent. Target genes that encode pathogenicity for *E. coli* were successfully amplified by PCR. The study suggested that final effluents are a significant sources of pathogenic *E. coli* in the receiving watershed.**

Key words: Escherichia coli, free-living, plankton-associated, wastewater final effluent.

INTRODUCTION

Escherichia coli are member of the Enterobactericeae (the intestinal bacteria) and belong to the order Eubacteria (Berg, 1978; Berg, 2000; Chapelle, 2001). These bacteria are facultatively anaerobic, gram negative rods that can grow under both aerobic and anaerobic conditions (Chapelle, 2001). If molecular oxygen is available, the bacteria rely on respiratory metabolism to survive. In the absence of molecular oxygen, the organisms use fermentation as an alternate means of survival (Berg, 2000; Chapelle, 2001). Bacteria belonging to the genus Escherichia are motile by means of peritrichous/multiple flagellum and it is unable to form spores to survive unfavourable environmental conditions (Todar, 2002). *E. coli* are important cause of disease in animals and humans worldwide. Strains of *E. coli* can be classified as (i) commensal, (ii) intestinal pathogenic (enteric/diarrheagenic), or (iii) extraintestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson, 2000). An increasing number of categories of pathogenic *E. coli* isolates have been identified over the past few decades, which has led to the current situation in which there are now at least 11 recognized pathotypes of *E. coli* in humans (Kaper et al., 2004).

These pathotypes are defined by the presence of combinations of virulence and virulence-related genes; conversely, the pathotype of an uncharacterized strain can be inferred from its virulence gene profile (Kaper et al., 2004). Pathogenic *E. coli* strains are also divided into pathotypes on the basis of their distinct clinical symptoms of the host (Muhldorfer and Hacker, 1994). Three main types of clinical syndrome can result from infection with one of these pathotypes: enteric and diarrheal diseases, urinary tract infections, and sepsis/meningitis. The *E. coli* pathotypes responsible for intestinal infections include

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enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), entero-aggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli*, necrotoxic *E. coli*, and cell-detaching *E. coli*. Three additional *E. coli* pathotypes, collectively called extraintestinal pathogenic *E. coli* (Russo and Johnson, 2000) are responsible for extra-intestinal infections. Extraintestinal pathogenic *E. coli* is composed of uropathogenic *E. coli* (UPEC) isolates that cause urinary tract infections, neonatal meningitis-associated *E. coli* (MNEC), and *E. coli* strains that cause septicemia (Bekal et al., 2003). Diarrheal diseases continue to be a health problem worldwide (Avendan˜o et al., 1993; Kosek et al., 2003), especially in developing countries, where they are estimated to be responsible for 2.5 million infant deaths per year, with an annual mortality rate of 4.9 per 1,000 children and an incidence of 3.2 episodes per child per year among children under 5 years of age (Kosek et al., 2003). In South Africa it has been estimated that diarrheal diseases are the primary cause of death in infants that are younger than 5 years, leading to about 160 - 200 deaths per day (Nemarude et al., 2008).

Pathogenic *E. coli* bacteria are known to be associated with food-borne diseases; contamination of drinking or recreational waters with some pathotypes has resulted in water borne disease outbreaks and associated mortality. Outbreaks of pathogenic *E. coli* in Canada (O'Connor, 2002) have been associated with contaminated drinking water obtained from rural catchments. In 2000, in the town of Walkerton, Ontario, Canada, an estimated 2300 people became ill and 7 died from exposure to EHEC contaminated drinking water (Hrudey et al., 2003) and a recreational water outbreak in 2001 at a beach in Montreal, Quebec, resulted in the hospitalization of 4 children (Brunneau et al., 2004).

During October 1992, a large outbreak of bloody diarrhea affecting thousands of individuals, some of whom died, occurred in South Africa and Swaziland (Isaacson et al., 1993). *E. coli* O157 was isolated from 22.5% of 89 stool samples and epidemiological investigations implicated water borne spread. In some areas, cases were mainly men, who drank surface water from fields. *Escherichia coli* O157 was also isolated from 14.3% of 42 samples of cattle dung confirming that agricultural animals can serve as a vector for *E. coli* 0157:H7 (Fujioka, 2002; Moe, 2002).

Uropathogenic *E. coli* strains are frequently isolated from biofilms formed in the lumen of catheters, where they resist antibiotic treatment and shear forces (Nicole, 2005). Pathogenic *E. coli* is found in aquatic ecosystems physically isolated from any source of faecal contamination (Alam et al., 2006). Studies have been performed to determine their distribution in environmental surface waters including ponds (Alam et al., 2006), recreational waters (Lauber et al., 2003), lagoons (Chern et al., 2004), rivers (Obi et al., 2004a), streams (Higgins et al., 2005) and lakes (Hamelin et al., 2006). Isolation of EHEC from municipal sewage has been reported (Vernozy-Rozand et al., 2002). Prevention of the spread of strains of diarrheagenic *E. coli* depends on ensuring appropriate sewage treatment and disposal (Okoh and Osode, 2008). Marine research has also provided news about the health aspects of pathogens living in association with plankton. Previous studies have demonstrated strong relationships between abundance of both free-living and plankton-associated *E. coli* (Dixon, 2005) and indicating that potentially pathogenic organisms living in close association with zooplankton have considerable epidemiological and ecological implications. In this paper, we report the prevalence of potentially pathogenic *E. coli* as free-living and plankton-associated entities in the final effluents of a waste water treatment facility in a typical peri-urban community of the Eastern Cape Province of South Africa, as well as their relationship with some physiochemical variables.

MATERIALS AND METHODS

The study area

The waste water treatment plant located in a sub-urban settlement in the Eastern Cape Province within the geographical coordinates 32°51'274''S and 27°14'167''E, accepts municipal domestic sewage and wastewater containing a heavy industrial contribution. The plant is designed to treat an average dry weather flow of 7,000 m3/day and an average wet weather flow of 21,000 m3/day. The plant accounts for large daily inflow due to the high number of industries located in the area and high population of residents. The waste water treatment system is of a basic design, the inlet works comprises of two screens, three grit channels and a flow recorder. The plant has two aeration tanks, each equipped with three vertically mounted mechanical aerators, two anaerobic tanks and two sedimentation tanks. There is a return activated sludge (RAS) pump station which lifts the recycle sludge from the sedimentation tanks to the aeration tanks. A splitter box controls the flow of the raw sewage and RAS to the aeration tank. The plant has a waste mixed liquor pump station which pumps the waste mixed liquor from the aeration tank to two sludge lagoons. Chlorine contact is carried out by means of a water pressure operated, wall mounted, gas chlorinator in a baffled reinforced concrete contact tank. Thereafter the final effluent is pumped to a pair of final effluent reservoirs and into Tembisa sewerage dams.

Treatments of samples

All samples were collected between August, 2007 and July, 2008 from the sampling site aseptically using sterile 1L Nalgene bottles and transported on ice to the laboratory for analyses. The samples were dechlorinated by adding 0.5 ml of sterile concentrated sodium thiosulphate solution to give a final concentration of 100 mg/l. Samples were stored at 4°C until analyses were complete. All samples were processed after 24 h of collection. The procedure was as described by Alam et al. (2006) one liter of waste water was filtered successively through 180, 60 and 20 µm nylon nets (Millipore Corp., Bedford, MA), sequentially arranged in that order to a collection base. After filtration, each nylon nets and its content were suspended in 25 ml physiological-buffered saline containing sterile glass beads (0.1 mm BioSpec Products) and homogenized for 2 min in a glass homogenizer at 3,000 × g to dislodge the

Table 1. Primer sequences and expected size of PCR-amplified gene targets of the pathogenic strains of *E. coli*.

Target strain	Gene target	Primer sequence (5' → 3')	Amplicon size (bp)
E. coli	*uidA*	AAA ACG GCA AGA AAA AGC AG ACG CGT GGT TAA CAG TCT TGC	147
EAEC strain	*aap*	G CTT GGG TAT CAG CCT GAA TG AAC CCA TTC GGT TAG AGC AC	232
EHEC strain	*rfbE*$_{O157}$	CTA CAG GTG AAG GTG GAA TGG AATT CCT CTC TTT CCT CTG CGG	328
EHEC strain	*fliC*$_{H7}$	TAC CAT CGC AAA AGC AAC TCC GTC GGC AAC GTT AGT GAT ACC	247
EIEC strain	*ial*	CTG GAT GGT ATG GTG AGG GGA GGC CAA TTA TTT CC	320

attached bacteria and the homogenates used for direct plating.

Physicochemical analysis

The measurement of sample pH, temperature, turbidity, salinity and dissolved oxygen has been described elsewhere (Osode and Okoh, 2009). The concentrations of free chlorine residual in the final effluents were determined using a multi-parameter ion-specific meter (Hanna BDH-laboratory) and analysis was carried out in triplicate.

Estimation of *E. coli* densities

For direct plate count analyses of free-living samples, the samples were serially diluted and appropriate aliquots used to inoculate Eosine Methylene Blue agar (Merck, South Africa) (EMBA) agar and incubated at 37°C for 24 h. For the plankton-associated samples, the *E. coli* densities were obtained using the same agar and in accordance with the description of Alam et al. (2006). Colonies showing greenish metallic sheen in transmitted light were considered total presumptive *E. coli* and counted as described elsewhere (Obi et al., 2004b).

Identification of *E. coli*

Aliquots of the free-living and plankton-associated samples were inoculated into Nutrient Broth (NB) and incubated aerobically at 37°C for 18 - 24 h. Turbid cultures were streaked onto EMBA and incubated at 37°C for 24 h. Five to ten isolated colonies per plate were randomly picked from each sample and subsequently sub-cultured on fresh EMBA plates. The pure isolates were subjected to gram staining and oxidase test. Only gram-negative, oxidase-positive isolates were selected for biochemical identification using API 20E kits. The strips were then read and the final identification was secured using API LAB PLUS computer software (BioMerieux, Marcy l'Etoile, France). Only excellent identification reports were accepted.

Molecular characterization of *E. coli* using the polymerase chain reaction (PCR) DNA isolation

DNA was extracted from identified *E. coli* colonies and from a positive control strains for *E. coli* (ATCC 8739) (SABS No ESC 20) purchased from the South African Bureau of Standards (SABS), Pretoria, South Africa. The extraction was done following the method of Maugeri et al. (2004) and Torres et al. (2003). Briefly, a loop-full of overnight culture of *E. coli* colonies was suspended in 200 µl of sterile nuclease free water (Fermentas Life Sciences, SA), vortexed using a MS2 Minishaker (IKA Works Incorporation) and the cells were lysed using a Dri-Block DB.2A (Techne, SA) for 15 min at 100°C. The cell debris was removed by centrifugation at 10,000 rpm for 5 min using a MiniSpin microcentrifuge (Eppendorf).

Amplification of uidA, fliCH7, rfbEO157, aap, and ial genes

Oligonucleotide primers targeting the uidA structural gene, fliCH7 gene encoding for Enterohemorrhagic *E. coli* structural flagella antigen H7, rfbEO157 gene encoding Enterohemorrhagic *E. coli* somatic antigen, aap gene encoding for antiaggregation protein (dispersin) of Enteroaggregative *E. coli* and ial gene encoding for invasion-associated locus of Enteroinvasive *E. coli* was used in the polymerase chain reaction (PCR). The primers (Southern Cross Biotechnology, SA) sequences that were used to identify the target genes and the expected amplifications sizes are listed on Table 1. The PCR assays were carried out in a 25 µl reaction volume. The PCR master mix (2X) which was composed of 0.05 units/µl Taq DNA polymerase in reaction buffer, 4 mM MgCl2, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP (Fermentas Life Sciences, SA). Nuclease free water (Fermentas Life Sciences, SA) was included in each PCR assay as a negative control. The cell lysates (10 µl) were used as template in the PCR assays immediately after extraction. The PCR reaction was carried out in the Eppendorf model AG 22331 Thermocycler (Merck, SA).

The PCR was used to confirm the identities of the *E. coli* strains using the specific primers targeting the uidA structural gene. The following PCR conditions for uidA gene optimized in our laboratory were similar to those previously used by Tsai et al. (1993) . The thermal cycling profile was as follows: a 2 min denaturation at 94°C followed by 25 cycles at 94°C for 1 min, 58°C for 1 min and 72°C for 1 min and final extension at 72°C for 2 min. The amplified products were held at 4°C after completion of the cycles. The following PCR conditions for fliCH7, rfbEO157 genes optimized in our laboratory were similar to those previously used by Wang et al. (2002). The PCR condition consisted of initial denaturation at 95°C for 8 min followed by 30 cycles of heat denaturation at 95°C for 30 s, primer annealing at 58°C for 30 s and DNA extension at 72°C for 30 s. After the last cycle, the samples were kept at 72°C for 7 min to complete the synthesis of all strands. The aap genes optimization were similar to those previously used by Samie et al.

Table 2. Profile of some physicochemical parameters of the final effluent of the wastewater treatment facility over a period of twelve months (extracted from Osode and Okoh, 2009).

Variables	Season [a]	Final effluent	F-value	Pr > F
pH	Autumn	6.88 ± 0.19	75.98	0.0001
	Spring	7.05 ± 0.29	1154.20	<0.0001
	Summer	7.11 ± 0.18	3516.90	<0.0001
	Winter	7.05 ± 0.47	957.59	<0.0001
Temperature (°C)	Autumn	18.82 ± 0.25	5876.36	<0.0001
	Spring	19.91 ± 0.14	804.79	<0.0001
	Summer	21.65 ± 1.28	4558.87	<0.0001
	Winter	16.91 ± 1.91	113.66	<0.0001
Salinity (psu)	Autumn	0.12 ± 0.01	598.85	<0.0001
	Spring	0.15 ± 0.01	2839.89	<0.0001
	Summer	0.12 ± 0.02	389.55	<0.0001
	Winter	0.13 ± 0.02	768.93	<0.0001
Turbidity (NTU)	Autumn	10.42 ± 1.91	16584.6	<0.0001
	Spring	8.51 ± 0.75	857.21	<0.0001
	Summer	7.68 ± 1.65	998.80	0.001
	Winter	19.45 ± 12.37	14965.85	<0.0001
Dissolved oxygen (mg/l)	Autumn	5.33 ± 0.79	674.58	0.0001
	Spring	5.11± 0.20	1434.70	0.0001
	Summer	4.90 ± 0.32	529.20	0.0001
	Winter	4.91 ± 0.27	1349.85	0.0001

Values are means of triplicate determination ± standard deviations (SD), [a] Summer (November to March), autumn (April to May), winter (June to August), spring (September to October).

(2007), the PCR condition consisted of 1 cycle for 2 min at 50°C, 1 cycle for 5 min at 95°C, 40 cycles for 45 s at 95°C, 45 s at 55°C and 45 s at 72°C, and a final extension step for 10 min at 72°C. The ial genes optimization was similar to those previously used by Presterl et al. (2003). The PCR condition consisted of 1 cycle for 2 min at 50°C, 1 cycle for 5 min at 95°C, 40 cycles for 45 s at 95°C, 45 s at 55°C and 45 s at 72°C, and a final extension step for 10 min at 72°C to complete the synthesis of all strands. Amplifications were carried out using a Bio-Rad MyCycler thermal cycler with the specified conditions.

DNA electrophoresis

The PCR products (10 µl aliquots) were resolved in 1.8% agarose gel (Merck, SA) containing 0.5 µg/Ethidium bromide (EtBr) (Merck, SA) in 1 X TAE buffer (40 mM Tris–HCl, 20 mM Na-acetate, 1 mM EDTA, pH 8.5) before being visualized and photographed under the BioDoc-It System (UVP Upland, CA 91786, USA). A 100-bp DNA ladder (Promega, White Head Scientific) was included on each gel as a molecular size standard. The electrophoresis was carried out at 76 V for 1 h.

Statistical analysis

In assessing the relationship between *E. coli* abundance in the final effluent and physicochemical variables of the environment, linear regression was performed on the collected data. *E. coli* abundance was natural log transformed to achieve normal distribution before use as the dependent variable. All other measured environmental factors were used as independent variables in regression analysis. The relationship between independent variables was examined by analysis of variance (ANOVA). All statistical analyses were performed using SAS (SAS version 8, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Physicochemical analysis

In our previous study (Osode and Okoh, 2009) we reported the temperature of the final effluents to range from (13.8 - 22.0°C), while the pH, turbidity, salinity and dissolved oxygen varied from 6.9 - 7.8, 7.7 - 9.4 NTU, 0.12 - 0.15 psu and 4.90 - 5.33 mg/l respectively, (Table 2). Also water temperatures of the effluent samples were high in February and the lowest was in July which was characterized with low *E. coli* density. A positive correlation between *E. coli* densities and water temperature was observed ($r = 0554$, $p < 0.001$). The highest pH was

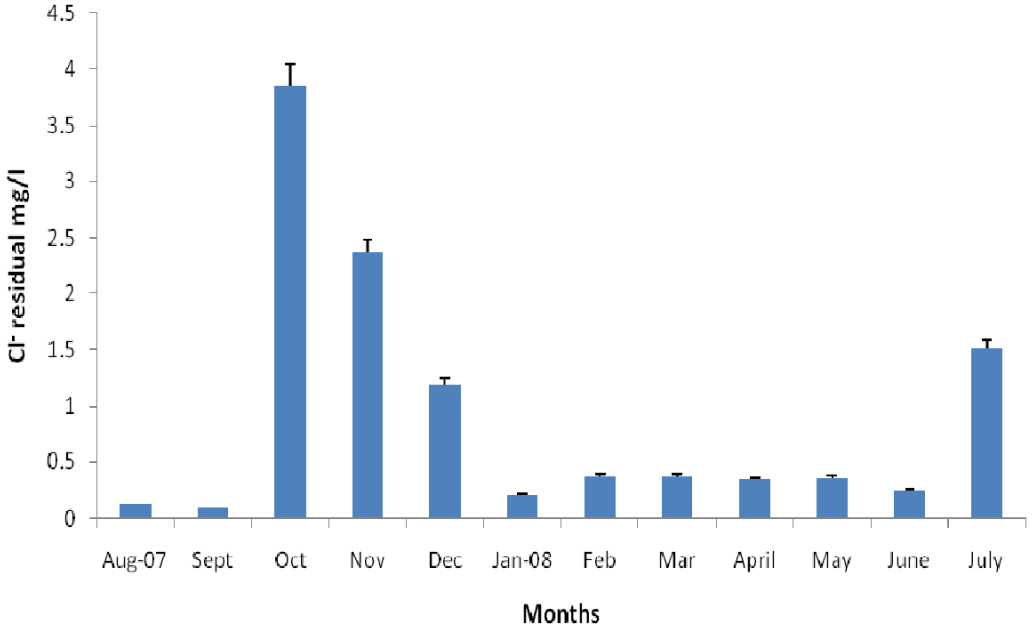

Figure 1. Residual chlorine profile of the final effluents of the wastewater treatment plant.

observed in the month of August which had high density of *E. coli*. There was an association between log10 *E. coli* density and pH. The lowest pH was observed in July. The highest turbidity value was observed in the month of August and the lowest in the month of January and there was a similar trend with pH during the same period. Also, increasing turbidity was associated with increasing *E. coli* abundance, suggesting a positive correlation.

The highest salinity value was observed in the month of September and the lowest in the months of January to April and June to July. Salinity negatively correlated with *E. coli* density ($r = -0.982$, $p < 0.001$) over the entire sampling period. Also, dissolved oxygen levels were highest in summer, and lowest in winter with the exception of the autumn season final effluents. Dissolved oxygen negatively correlated with *E. coli* densities in the final effluent and water temperature correlated the most with *E. coli* abundance.

Free chlorine residual

The profile of the chlorine residual in the final effluents is as shown in Figure 1. Chlorine residual varied significantly ($P < 0.05$) from 0.10 in the month of September to the highest level of 3.85 mg/L in the month of October. In this study, free chlorine residual range for domestic water (0.3 to 0.6 mg/l) (Moojiman et al., 2001) was considered as standard, since the South African guidelines do not specify any standard for final effluents in sewage treatment plants.

Abundance of *E. coli*

The abundance of presumptive *E. coli* in the effluents varied appreciably between sampling period and plankton sizes and is presented in Figure 2. Presumptive *E. coli* associated with 180 µm plankton size ranged from 0 to 4.30×10^1 cfu/ml. The highest density was observed in September while low densities were observed from October to February, April, May and July (Figure 2). In the 60 µm plankton size category *E. coli* densities ranged between 0 and 4.2×10^1 cfu/ml being highest in September and low in the months of October to May and July. Also in the 20 µm plankton size category presumptive *E. coli* density varied from 0 to 5.0×10^1 cfu/ml with the highest counts in September and low counts in the months of October to December and July. The free-living presumptive *E. coli* density ranged between 0 and 3.13×10^1 cfu/ml with the highest in January and lower counts in the months of October to December and July. With regards to season, low densities of presumptive *E. coli* was observed in summer (October to February) as well as the later end of spring (July) and were found to be more associated with 20 µm plankton size. There was no significant correlation between presumptive *E. coli* abundance and seasons either as free-living or plankton-associated cells. The densities of free-living *E. coli* in summer varied significantly with those of spring ($P < 0.05$), but not with other seasons. In general, the presumptive *E. coli* was most predominant as 20 µm plankton associated entities (40%) followed by the 180 µm (25%), 60 µm (20%) plankton associated entities

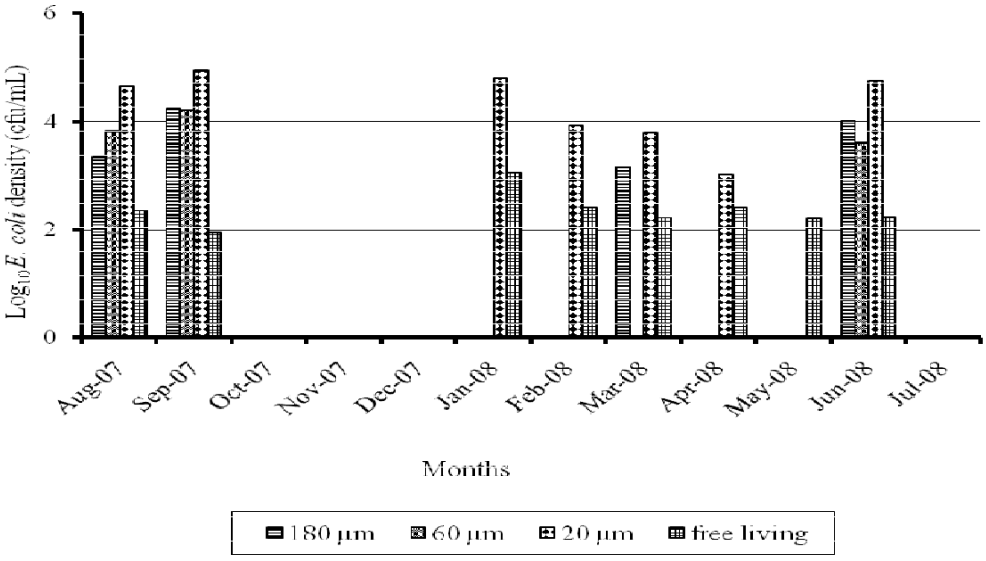

Figure 2. Abundance of *E. coli* in the final effluents of the wastewater treatment plant.

respectively, while the free-living *E. coli* constituted only 15 %.

E. coli found in domestic sewage come predominantly from human fecal material (Sturtevant and Feary, 1969). Thus, sewage isolates may serve as representatives of the strains of *E. coli* present within the human population in a given locale. A major goal of waste water treatment facilities is to reduce pathogen loads in order to decrease public health risks associated with exposure. The effectiveness of pathogen control is indirectly assessed through routine monitoring of the final effluent by using grab samples to detect standard indicator bacteria such as total or faecal coliforms. In this study we used *E. coli* as an indicator of the presence of pathogens from faecal contamination. The final effluent outfalls in October, November and December, 2007 and July, 2008 only complied with the South African General and Special Standards which stipulate that treated sewage effluents must have a standard of 0 faecal coliforms/100 ml (Act 96 of 18 May, 1984 No. 9225, Regulation 991), and according to (DWAF, 1998) the maximum limit for no risk of faecal coliforms is 0 cfu/100 ml. The standard of The World Health Organization for drinking water is no *E. coli* in a 100 ml sample (WHO, 2003). High *E. coli* counts suggest inefficiency of the waste water treatment plant in removing the bacteria. Crops such as vegetables can become contaminated with human and animal pathogens when irrigated with water containing this organism (DWAF, 1996).

Disinfection is a common final step in waste water treatments. In recent years, disinfection by ultra violet radiation or micro-filtration has been proposed because of the sanitary and ecological risks associated with using chemical compounds. However, in South Africa, disinfection by chemical agents, mainly chlorine, is still the most widespread process (Samie et al., 2009). In this study we considered as standard, the free chlorine residual range of 0.3 to 0.6 mg/l for domestic water and 0.6 to 0.8 mg/l as good free chlorine residual concentration with insignificant risk of health effects (Moojiman et al., 2001) since the South African guidelines do not specify any standard for final effluents in sewage treatment plants. Based on this concentration, the free chlorine residual in the effluents complied with the regulatory standard in about 33.3% of the sampling periods. The higher densities of *E. coli* in August and September, 2007 as well as in January and June, 2008 were due to chlorination activity which was under dosed at the wastewater treatment plant. Chlorine overdosing was observed in the October to December, 2007 and July, 2008 sampling periods, consequently resulting in lower densities of presumptive *E. coli*. It is evident from the result that there is a relationship between chlorine residual and densities of *E. coli*. The waste-water treatment plant contained high densities of *E. coli* in the final effluent which was supposedly disinfected. However, densities of *E. coli* remained high after treatment due to the inefficiency of the waste-water treatment plants in removing the bacteria. For sewage treatment plants to meet national and international standards, there is a need to improve treatment processes and to adopt stringent policies in terms of monitoring and control of the quality of the final effluent. This includes the use of effective methods for the detoxification and disinfection of the sewage effluent. The abundance of *E. coli* in the final effluent was been found to be associated with temperature. Water temperature fluctuated through the seasons thereby affecting *E. coli* abundance and corroborating previous studies by (Pfeffer et al., 2003) which reported correlations between *E. coli* abundance and water

temperature. However, the fact that plankton-associated *E. coli* were more abundant in the final effluent compared to free-living *E. coli*, suggests that bacterial attachment may play a role in the indecisive effect of the chlorine residual on *E. coli* populations in the final effluent. Some other factors that may affect the efficiency of disinfectants such as chlorine include contact time, temperature and pH (Obi et al., 2008). There is little or no report in literature with regards to the occurrence and distribution of *E. coli* as free-living and /or plankton-associated cells in the waste water final effluents and its receiving watershed.

The dynamics observed in the sizes of the most numerous plankton-associated cells describes a system that alternates between populations dominated by large cells (20 µm, possibly cryptophytes or diatoms). Bacteria associated with particles have been shown to survive in aquatic environments for longer times than suspended forms (Burkhardt et al., 2000). This includes bacterial species of concern to public health such as Vibrio and Enterococcus (Davies and Bavor, 2000; Jin et al., 2004; Signoretto et al., 2004) . Particle attachment of pathogens can play an important role in the ultimate fate of these microbes and the dynamics of the fraction attached may be as important as the total population numbers. For example, aggregation and particle settling presents an efficient mode of bacterial removal from surface waters (Characklis et al., 2005), potentially altering the exposure of local populations using the affected water body (Maugeri et al., 2004).

E. coli abundance correlated significantly ($P < 0.01$) with seasons in this study either as free-living or plankton-associated cells. Similar observations have been reported by other authors (Maugeri et al., 2004). The counts of free-living *E. coli* during spring varied significantly with those of summer ($P < 0.01$), but not with other seasons or plankton sizes. The findings of this study suggests that bacterial discharge into aquatic systems through inadequately treated waste water posses a potential health hazard to communities depending on such receiving watershed for domestic and other uses. Moreover, even when most of the *E. coli* cells are destroyed or at least injured by chlorination to make them non-culturable, evidence have shown that after chlorination, non-culturable cells of enteropathogenic strains of *E. coli* retain their enterotoxigenic activity and can recover and express pathogenicity *in vivo* (Singh et al., 1986). The molecular analysis using uidA-specific primer confirmed that a genetic region homologous in size to the *E. coli* uidA structural gene, including the regulatory region, was present in 10 of the *E. coli* isolates from Dimbaza. Out of the 10 isolates, 4 were positive for fliCH7 genes, 6 were for the rfbEO157 genes and 1 was positive for the aap genes. The results of this study confirm the poor microbiological quality of the effluents that is produced by many of the waste water treatment plants in the Eastern Cape Province. The PCR assays successfully amplified the target genes (fliCH7, rfbEO157) which are characteristic of the Entero-haemorrhagic *E. coli* O157:H7; (ial) characteristic of the Enteroinvasive *E. coli*; and (aap) characteristic of the Enteroaggregative *E. coli*. *E. coli* O157:H7 has been associated with water related outbreaks. It has been isolated from surface and ground waters (Hamner et al., 2007) and it is capable of survival in water for days or weeks. Enteroinvasive *E. coli* (EIEC) cause dysentery, however, it is less widely reported than other etiological agents in studies of diarrhea worldwide. EIEC has principally been associated with contaminated food and water (Gordillo et al., 1992) although cases of person- to-person transmission of EIEC have been noted (Harris et al., 1985). Enteroaggregative *E. coli* is an emerging diarrheagenic pathogen associated with diarrheal illnesses among patients in developed and developing countries. Enteroaggregative *E. coli* has been increasingly isolated and characterized around the world from human clinical, animal and environmental samples (Kahali et al., 2004; Falcao et al., 2004). However, frequencies of Entero-aggregative *E. coli* among patients with diarrhea in the Eastern Cape Province, South Africa, are not known. The outcome of the findings of Samie and co-workers (2007) highlights the need for the design of surveillance strategies to control Enteroaggregative *E. coli* diarrhea and to prevent the transmission of Entero-aggregative *E. coli* in the region through water or food contamination. Considering the microbiological quality of the effluent outfalls in the Eastern Cape Province in general and the Buffalo City District in particular and the profile of the molecular results of the present study, monitoring and strict compliance with effluent standard should be re-enforced.

The finding of this study further reaffirms the existence of association between planktons and potentially pathogenic *E. coli*. Association of general, pollution-indicator and pathogenic bacteria with zooplankton is a common feature (West and Lee, 1982; Hansen and Bech, 1996; Islam et al., 2001; Signoretto et al., 2004; Dixon, 2005). Also, the survival of *E. coli* at densities outside acceptable range suggest that the final effluents as veritable sources of pathogens in the watershed. This observation and ensuing inferences of this study are useful for managing effluent outfall into coastal ecosystems and demonstrated the necessity for regular monitoring of effluent quality prior to discharge into the environment. We conclude that when evaluating disinfection efficiency, the effect of disinfected waste water effluents on the self depuration process and bacterial survival in receiving waters should be considered and this is a subject of ongoing investigation in our group.

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