Journal of Public Health and Epidemiology Vol. 3(8), pp. 371-381, August 2011
Available online at http://www.academicjournals.org/jphe
ISSN 2141-2316 ©2011 Academic Journals

Full Length Research Paper

Multiresistant faecal indicator bacteria in stream and well waters of Ile-Ife City, Southwestern Nigeria: Public health implications

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Accepted 6 May, 2011

This study was carried out to determine the prevalence of multiple antibiotic-resistant faecal indicator bacteria in streams and wells which serve as major sources of water for inhabitants of Ile-Ife city in Southwestern Nigeria. Water samples from 2 streams and 10 wells situated at different parts of the city were collected over a 6-month period. The total heterotrophic bacteria, faecal coliform and enterococci counts were performed using standard procedures, and the sensitivity of the isolates to antibiotics was tested. The study indicated high faecal indicator concentrations exceeding quality standards for drinking and recreational waters according to World Health Organization (WHO) and United States Environmental Protection Agency (USEPA). All the faecal coliforms and enterococci isolates presented multiple antibiotic resistances. The water sources pose a threat to human health due to the danger of waterborne diseases and potential for the transfer of antibiotic resistance genes to pathogens. Effective public health education aimed at creating awareness of the implications of consumption of contaminated and untreated water is imperative. Antibiotics should only be administered based on physicians' prescription.

Key words: Antibiotic resistance, faecal indicator bacteria, Southwestern Nigeria, stream water, waterborne diseases, well water.

INTRODUCTION

Water is essential for man and other life forms. It is required for various human daily activities such as drinking, cooking, toothbrushing, bathing, washing utensils and also for agricultural and industrial purposes (McFeeters, 1989; Centre for Environmental Health, 2005). However, poor water quality continues to be a leading cause of health problems especially in developing countries where it is estimated that 80% of all illnesses are linked to water and sanitation and 15% of all child deaths under the age of 5 years result from diarrhoeal diseases (Thompson and Khan, 2003; WHO and UNICEF, 2004). Currently, an estimated 884 million people worldwide do not use improved sources of drinking water and 2.6 billion are not provided with adequate sanitation. The majority of these are in Southern Asia (25%) and sub-saharan Africa (37%) (WHO and UNICEF, 2010). In Nigeria, increasing population and infrastructural breakdown have made municipal pipe borne water to be inadequate in quantity and quality (Adesunkanmi and Ajao, 1996). Today, less than 30% Nigerians have access to safe drinking water due to these inadequacies and most of the populations have to resort to drinking water from wells and streams especially in the rural and suburban communities. These water sources are largely untreated and might harbour water-borne and vector-borne diseases such as cholera, typhoid fever, diarrhoea, hepatitis and guineaworm (Rahman et al., 2001; Adekunle, 2004; Fenwick, 2006). These diseases are caused by pathogenic bacteria, viruses, protozoa and other microbes which are shed in human faeces and pollute water supplies which people utilize for drinking and washing purposes. Many rivers, streams and wells worldwide are affected by faecal contamination leading to increased health risks to persons exposed to the water, degradation of recreational and drinking water quality (Simmons, 1994; Gregory and Frick,

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1995; Center for Watershed Protection 1999, Harrisson, 2003; Horman, 2005; Ganoza et al., 2006; Zvidzai et al., 2007; Obiri-Danso et al., 2009).

Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *Escherichia coli*, *Campylobacter*, *Salmonella* species, *Shigella* species and *Vibrio cholerae*. In addition to these organisms causing human diseases, resistance to antibiotics has made treatment of the diseases they cause more difficult (Lamikanra and Okeke, 1997; Hart and Kariuki, 1998; Okeke et al., 2007).

Antibiotic resistant bacteria have previously been isolated from surface waters such as streams (McArthur and Tuckfield, 2000; Ash et al., 2002; Roe et al., 2003; Yang and Carlson, 2003) and underground water sources such as wells (Oyetayo et al., 2007).

Bacteria vary widely in their response to antibiotic-induced stresses (Hawkey, 1998). There could be intrinsic resistance determined by chromosomal genes which are not transferable to other organisms (Rice et al., 2003). Bacterial resistance to antibiotics can also be acquired through mutation in the chromosomal genes or through acquisition of new genes responsible for antibiotic resistance. Genes responsible for acquired antibiotic resistance are often carried on genetic elements that can easily be transferred among bacteria. These could be plasmids, bacteriophages or transposons (Roy, 1999; Walsh, 2003; Levy and Marshall, 2004). In many cases, these genetic elements carry several antibiotic resistance genes, thus transferring multiple antibiotic resistances to other organisms.

Antibiotic resistance mechanisms utilised by bacteria include the production of enzymes that degrade the antibiotic (Davies, 1994; McAllister et al., 2001; Levy and Marshall, 2004). Some bacteria can rapidly pump the antibiotic out of the cell before it has chance to interact within the cell (Chopra et al., 1992). Also some bacteria can produce enzymes that inactivate the antibiotic by adding additional chemical structures onto the antibiotic. Bacteria may also change their cell surface to reduce the affinity of the antibiotic to its target site. Bacteria may express more than one type of mechanisms to resist one antibiotic (Levy and Marshall, 2004). As a result of the fact that many organisms typically found in surface waters have intrinsic antibiotic resistance (Farmer, 2003) or may not be pathogens, the identification of the resistant bacteria is required to interpret the health significance. Pathogenic bacteria with resistance to an antibiotic used to treat the infection caused by that organism are an obvious concern. Of equal importance but less obvious concern is the significance of the environmental (non pathogenic) bacteria with antibiotic resistances. This is because of the ability of environmental bacteria to transfer antibiotic resistance genes to human pathogens. In this study, the microbiological quality of water from streams and wells located in Ile-Ife City in Southwestern Nigeria was determined. The prevalence of antibiotic resistant faecal indicator bacteria in the stream and well waters were then investigated.

MATERIALS AND METHODS

Description of study area

Ile-Ife City with an estimated population of 500,000 lies on longitude 4° 69′E and latitude 7° 50′N in the rainforest region of Southwestern Nigeria (Figure 1). The city is surrounded by lots of suburban and rural communities whose inhabitants engage mainly in farming activities. Of the several streams that drain the city, the Esinmirin and Opa streams run through the city and flow past the outskirts and adjoining suburbs. The supply of municipal water has been inadequate in the city and is completely lacking in the adjoining towns and village communities. Most of the inhabitants therefore resort to the use of hand dug wells and streams as sources of water for drinking and other domestic purposes. Probability of contamination of the streams is high especially during the rainy seasons, from wastewater contaminations from urban and rural run offs and agricultural activities as the streams flow through the city and its suburbs.

Sample collection

Over a period of six months; June to November 2010, water samples were collected once monthly from eight sites on each of the two natural surface water sources. The two surface water bodies comprise of two streams located in and traversing Ile-Ife city in Osun State, Nigeria. These are the Esinmirin stream and the Opa stream. Each sample was taken from an area of the water source where the local communities usually make use of or fetch their water thereby making direct contact with the water sources. Water samples were also collected from ten wells sited in various locations of the city. Water samples were collected as 1 L grab samples in sterile glass bottles and transported in cold boxes containing freezer packs to the laboratory where they were processed within six hours of collection (Anon, 1992).

Bacterial counts

All bacterial counts were done in triplicates for each water sample.

Total heterotrophic bacteria plate counts (HPC)

The HPC in the water samples were obtained using the pour plate technique according to Anon (1994). Dilutions of water samples in buffered peptone water were inoculated in 1 ml aliquots into each of 10 ml molten plate count agar (Oxoid, England) in MacCartney bottles. After thorough mixing, these were poured into sterile Petri dishes and incubated for 48 h at 22°C. Dishes from dilutions containing 50 discrete colonies were counted and the results expressed as the numbers of bacteria colonies per millilitre.

Faecal coliform bacteria counts

Faecal coliform bacteria were isolated and quantified from all samples by means of membrane filteration techniques (APHA, 1998; US Environmental Protection Agency, 2000). 100 ml water samples were poured through individual sterile 0.45 µm pore size cellulose acetate membrane filters (Corning, England). The membrane filters were then placed aseptically, with the grid side up, on Petri dishes of fecal coliform membrane filters (mFC) agar medium (Fluka, USA). The Petri dishes were first incubated for 4 h

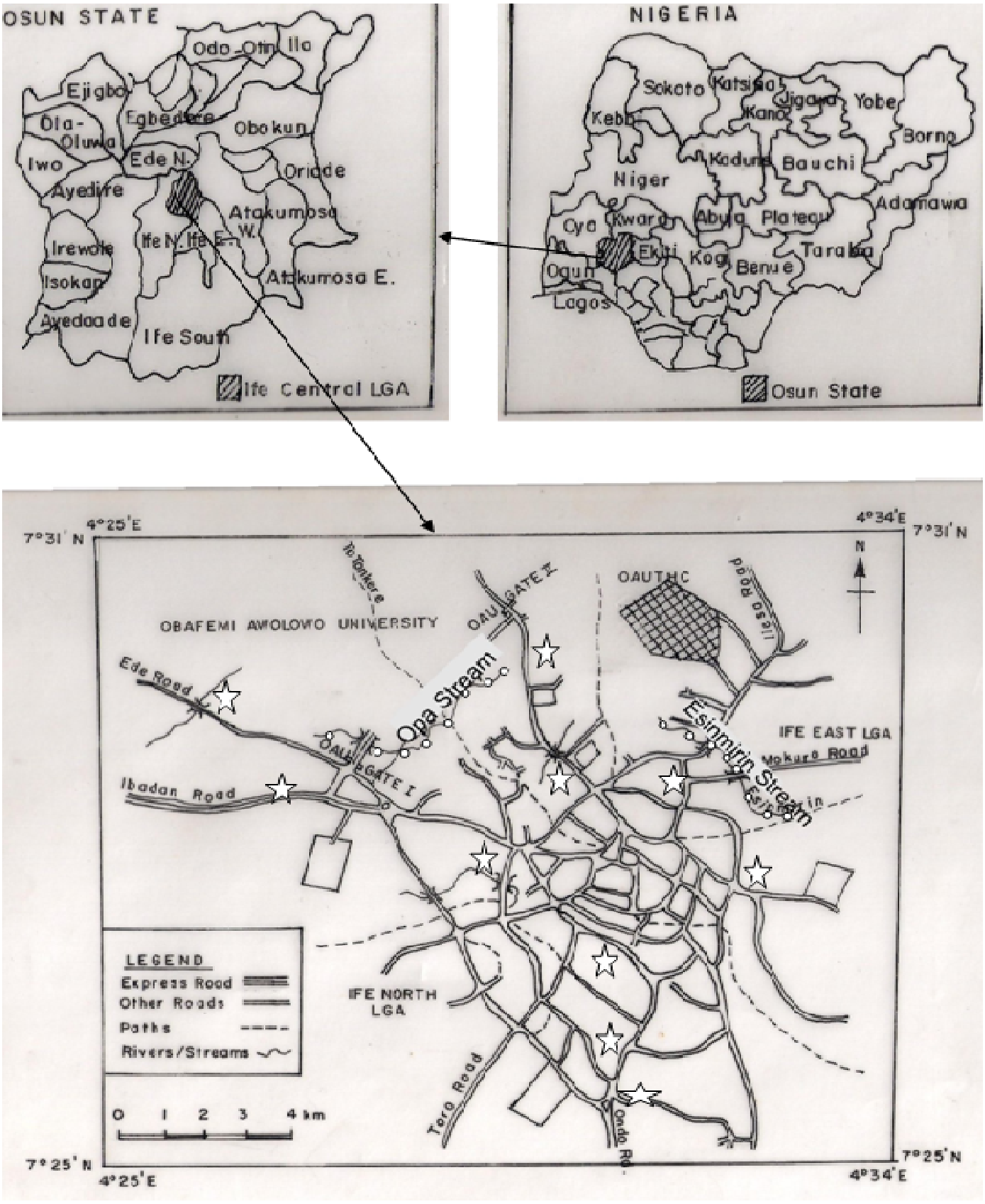

Figure 1. Map of Ile-Ife area showing the sub-urban sampling points with an insert of Nigeria map showing Ile-Ife in relation to the rest of the country. ☆ The Well water sampling points; ☆ Stream water sampling points.

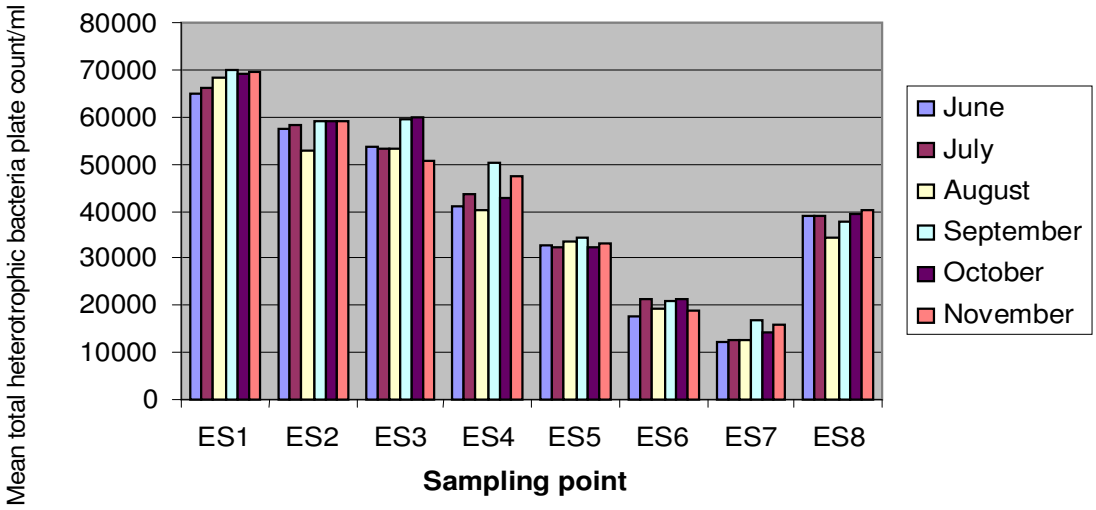

Figure 2a. Mean total heterotrophic bacteria plate counts (ml^{-1}) in water samples collected in June to November from different points along the Esinmirin Stream. Counts are means of three replicate determinations.

at 37°C and then transferred to an incubator maintained at 44.5°C for 24 h. Purple or violet colonies were counted as presumptive faecal coliform bacteria. Presumptive coliform bacteria were further tested on eosin methylene blue agar (Fluka, USA) for characteristic colour and identified and confirmed as *E. coli* by biochemical test results: Positive catalase reaction, negative oxidase reaction, a positive indole reaction, a positive O-Nitrophenyl-β-D-galactopyranoside (ONPG) reaction and a Gram negative morphology (Holt et al., 2004).

Faecal enterococci counts

Faecal enteroococci were isolated and quantified from all samples by means of membrane filteration technique (Anon, 1994). 100 ml water samples were filtered through individual sterile 0.45 µm pore size cellulose acetate membrane filters (Corning, England). The membrane filters were then placed with the grid side upwards on Petri dishes of enterococcus selective agar (Fluka, USA) and incubated at 37°C for 4 h before being taken to 44°C for 45 h. Red or pink colonies were counted as presumptive enterococci. All counts were expressed as colony forming units per 100 ml. Presumptive colonies were confirmed by bile aesculin hydrolysis on bile aesculin agar, growth in brain heart infusion broth containing 6.5% NaCl, negative catalase reaction, Gram positive reaction and cell morphology. These were then speciated by the API 20 STREP system (Biomereux, France).

Antimicrobial susceptibility test

The antimicrobial susceptibilities of faecal indicator bactera isolates were evaluated by the agar diffusion method on Mueller Hinton agar (CLSI, 2008). Antibiotics were chosen to define those more frequently used in human medical practices. The antibiotics used for faecal coliform baceria are ampicillin (10 µg), gentamycin (10 µg), streptomycin (10 µg), ceftriazone (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg) and sulphamethoxazole-trimethoprim (23.5 µg/1.25 µg). Antimicrobials used for faecal enterococci are ampicillin (10 µg), penicillin (10 units), vancomycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg) and chloramphenicol (30 µg). The plates were incubated for 18 to 24 h at 37°C. *E. coli* ATCC 25922 was used as control in microbial susceptibility tests on faecal coliform bacteria while for enterococci analysis, *Enterococcus faecalis* ATCC 29212 was used as control.

Retrospective study of incidence of water borne infections

One hundred case notes and log books were randomly selected from the two largest hospitals in the city and assessed for diagnosis of various waterborne infections within the period of two years, January, 2009 to December, 2010.

RESULTS AND DISCUSSION

Stream water

High faecal indicator bacteria concentrations were present in all samples from the sixteen stream water sampling points in all sampling months from June to November, 2010. In the Esinmirin Stream, the mean total HPC counts ranged from 12,143 (ES7 in June) to 69,967 CFU ml^{-1} (ES1 in September). Mean faecal coliform bacteria counts ranged from 1,607 (ES5 in July) to 4,923 CFU 100 ml^{-1} (ES4 in November). Mean enterococci concentrations ranged from 89 (ES5 in August) to 410 CFU 100 ml^{-1} (ES1 in July) (Figures 2a to c). In the Opa stream, the mean total HPC counts ranged from 4,870(OP1 in July) to 12,390 (OP5 in September). Mean faecal coliform bacteria counts ranged from 195 (OP4 in June) to 938 CFU 100 ml^{-1} (OP3 and OP5 in November). Mean faecal enterococci counts ranged from 39 (OP1 in July and November) to 297 CFU 100 ml^{-1} (OP5 in November) (Figures 3a to c). It is observed that the level of

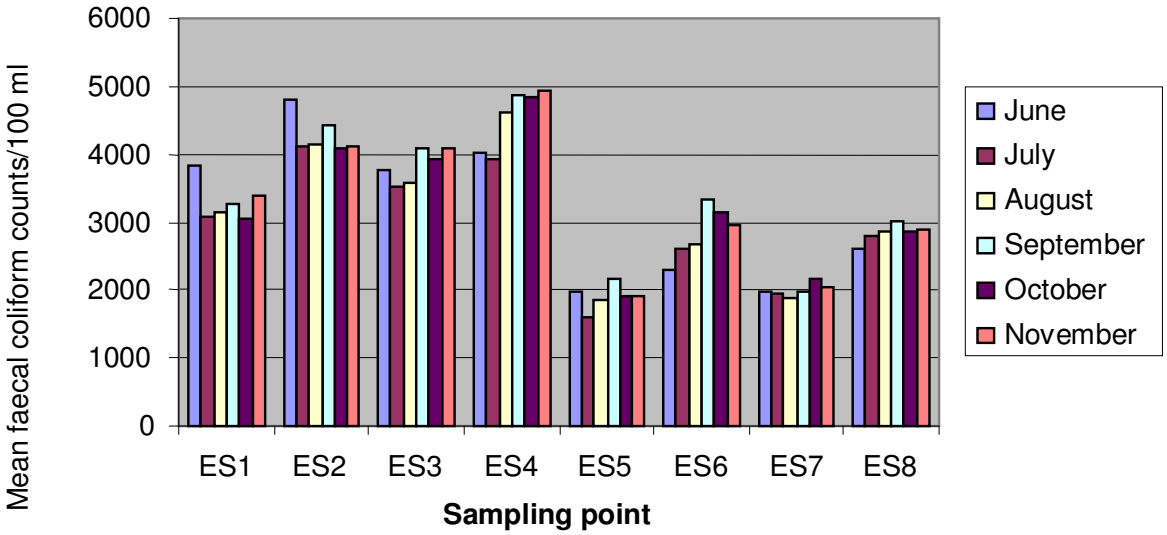

Figure 2b. Mean faecal coliform concentrations (100 ml^{-1}) in water samples collected in June to November from different points along the Esinmirin stream. Counts are means of three replicate determinations.

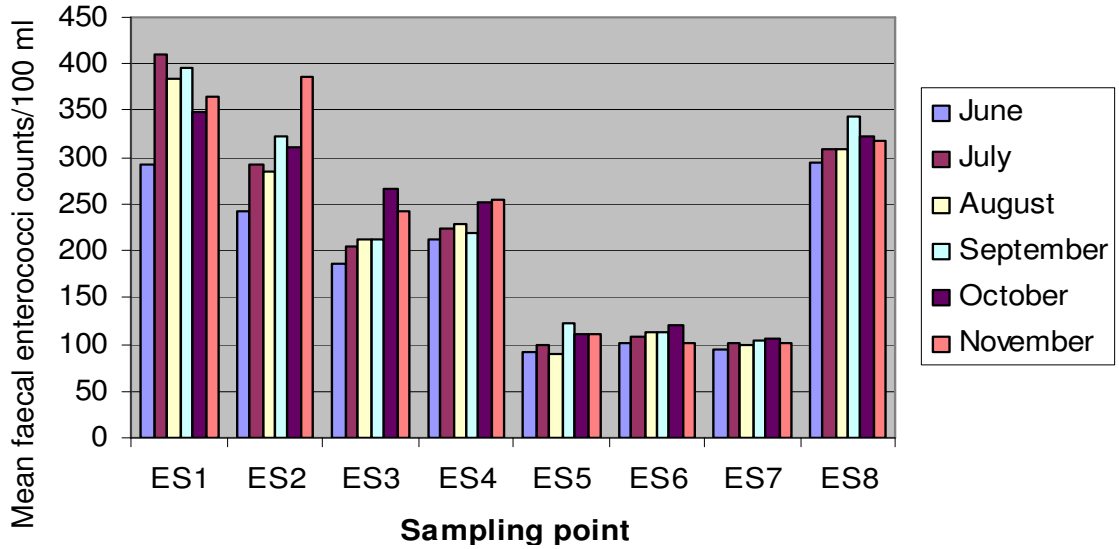

Figure 2c. Mean faecal enterococci concentrations (100 ml^{-1}) in water samples collected in June to November from different points along the Esinmirin stream. Counts are means of three replicate determinations.

contamination is lower in the Opa Stream compared to the Esinmirin Stream which is longer and passes through more communities along its course.

The World Health Organization (1997) recommends the absence of faecal coliform bacteria in any 100 ml of drinking water. The high counts obtained underline the unsuitability of these water sources for consumption purposes. Also none of the sources met the European Community and USEPA standards of maximum 100 HPC/ml of drinking and recreational waters.

High faecal indicator bacteria counts obtained could be attributed to faecal contamination by domestic sewage consistently disposed into the streams by villagers living along the streams. Located along the streams were horticultural gardens; the runoffs of which flow directly into the stream. The runoffs contained high level organic pollutants from the fertilizer which is locally produced from cow dung. As the inhabitants of several communities operate free range system in rearing domestic animals which roam the communities daily in search of food and

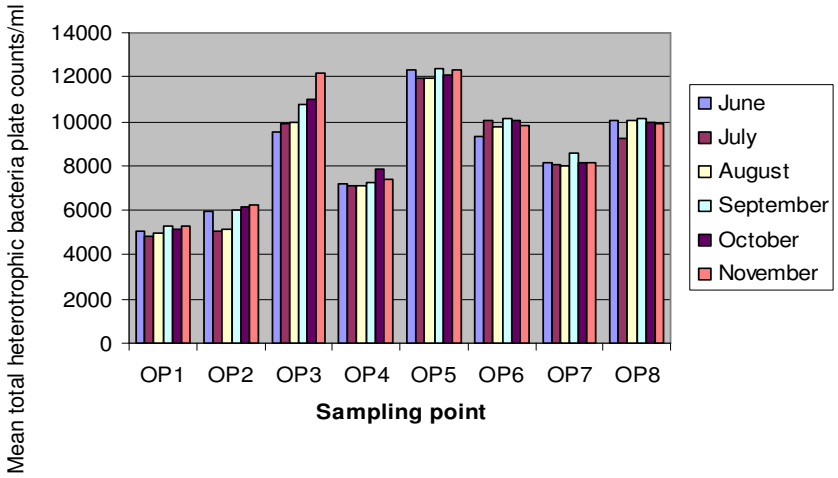

Figure 3a. Mean total heterotrophic bacteria plate counts (ml^{-1}) in water samples collected in June to November from different points along the Opa Stream. Counts are means of three replicate determinations.

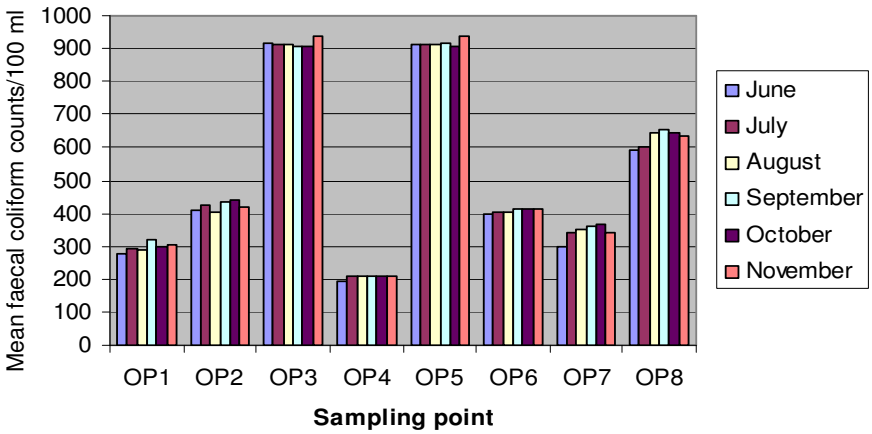

Figure 3b. Mean faecal coliform concentrations (100 ml^{-1}) in water samples collected in June to November from different points along the Opa Stream. Counts are means of three replicate determinations.

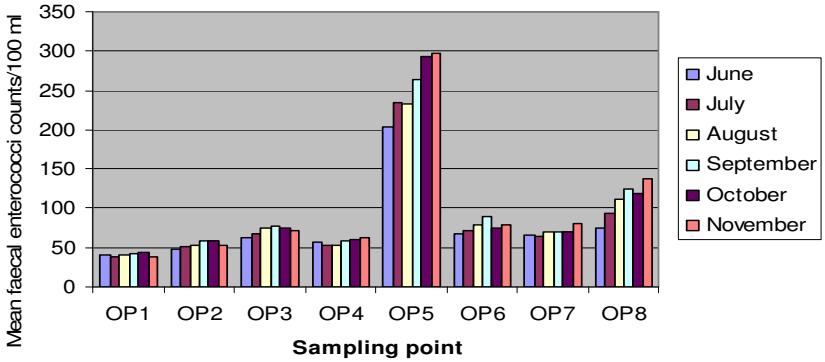

Figure 3c. Mean faecal enterococci concentrations (100 ml^{-1}) in water samples collected in June to November from different points along the Opa Stream. Counts are means of three replicate determinations.

water, there is a high probability of faecal coliform bacteria contamination (Meadows, 1995). The animals thereby indiscriminately contaminate the stream environments with their droppings.

Well water

High faecal indicator bacteria concentrations were also found to be present in all water samples from the ten wells studied although these were at much lower level compared to the stream waters (Figures 3a to c). The mean total HPC counts in the well waters ranged from 45 (WW6 in June and July) to 3,487 CFU ml^{-1} (WW1 in November). Mean faecal coliform bacteria counts ranged from 5 (WW6 in September) to 1,703 CFU 100 ml^{-1} (WW1 in September) while the mean enterococci concentrations ranged from 2 (WW6 in June) to 95 CFU 100 ml^{-1} (WW1 in November) (Figures 4a to c). Although none of the ten well water samples met the zero tolerance level for faecal coliforms according to WHO standard, two of them – WW5 and WW6 were observed to fall within the European Community and USEPA standard of maximum 100 HPC per milliliter drinking and recreational water.

Oyedeji et al. (2010) had observed that several sachet packed drinking water manufacturers in Nigeria utilised well water as their source of water with little or no prior treatment. Sanitary survey of wells sited at various locations in the city revealed a variety in the levels of their hygienic environmental qualities. This could be linked with the differences in the level of contamination of the wells studied. Contaminant seepage through defective well cap-seals, holes in the well casing and improperly sealed wells have been reported to allow water that has not been filtered through soil to enter the well (Ungate, 1996; Besner et al., 2002). The indiscriminate use of buckets for other purposes apart from drawing water from wells could also be a potential source of contamination as these may have had contact with human faecal matter. Rain water can also pick harmful bacteria and other pollutants on the land surface. If this water pools near the wells and seeps through, it could pose potential health problems (US Environmental Protection Agency, 2002, 2010). All the studied wells were also observed to be amenable to encroachment by roaming domestic animals which step on and litter the well environment with their droppings thereby reducing the hygienic conditions of the wells. Well waters should therefore be subjected to adequate disinfection treatments and buckets used for drawing water must be kept clean and retained specifically for the purpose. Wells should not be sited close to pit latrines while drainages around wells should be sanitized frequently.

Antibiotic resistances

High level and widespread antibiotic resistance were found among the faecal coliforms and enterococci isolates from the well and stream water (Table 1).

Although no ciprofloxacin resistant faecal coliform isolate was found, nevertheless, those nalidixic acid resistant isolates must be considered as strains with decreased susceptibility for ciprofloxacin (CLSI, 2005). Resistances were generally higher in stream water isolates compared to well water isolates. High level resistance were found to ampicillin, ceftriazone, chloramphenicol and tetracycline in the faecal coliform isolates. Many faecal coliform bacteria isolates in this study showed resistance patterns indicative of acquired resistance, such as multiple resistances to antibiotics in different groups and resistance patterns indicative of extended spectrum beta lactamase production. The high rate of resistance to ampicillin found in faecal coliform isolates is consistent with the work of Alhaj et al. (2007) in Malaysia and Junco-Diaz et al. (2006) in Cuba.

Faecal enterococci isolates from both well and stream water also showed high level resistance to tetracycline, penicillin, and chloramphenicol. Resistance to vancomycin was detected in three of the isolates from well water and eleven of the forty eight isolates from stream water. Generally multiple antibiotic resistances were exhibited by the faecal enterococci isolates with resistances being higher for stream water isolates compared with those from well water. *Enterococcus* species are intrinsically resistant to several antibiotics, such as cephalosporins, β-lactams, sulphonamides and low levels of aminoglycosides while they have the ability to develop or acquire resistance to chloramphenicol, erythromycin, aminoglycosides, tetracycline, β-lactams, fluoroquinones and glycopeptides such as vancomycin (Franz et al., 2003; Larson et al., 2008).

Incidence of waterborne infections

The results of the study about the number of patients with reported complaints and diagnosis with waterborne infections at the hospitals for the period of 2009 and 2010 are presented in Table 2. The waterborne infections that were consistently reported and diagnosed are typhoid fever, dysentery and diarrhoea. The cases of cholera diagnosis during this period occurred especially during rainy seasons. Of the total 80 laboratory requests made for typhoid fever, 40 diagnosed cases were found to be positive. Fifty laboratory requests for dysentery revealed 5 diagnosed cases while 75 laboratory requests made for diarrhoea revealed 20 confirmed cases. Thirty cases of cholera infections were diagnosed out of a total of 60 laboratory requests made.

Public health implications

Results of this study indicated high faecal indicator bacteria concentrations, antibiotic resistances of clinical

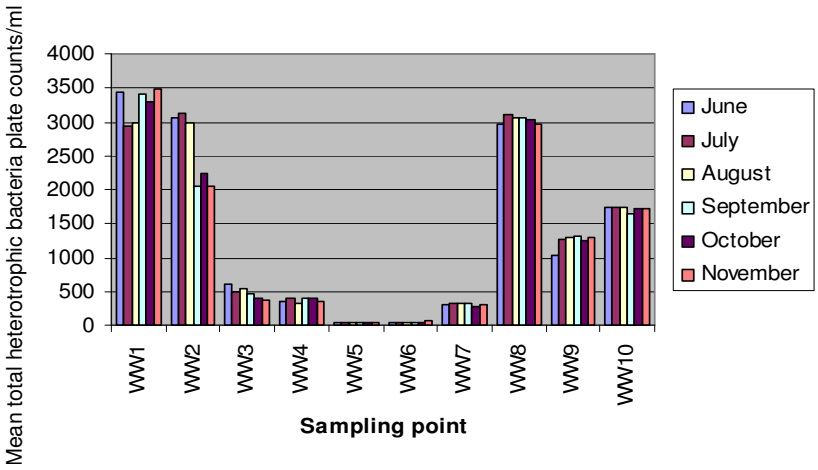

Figure 4a. Mean total heterotrophic bacteria plate counts (ml^{-1}) in well water samples collected in June to November from different locations. Counts are means of three replicate determinations.

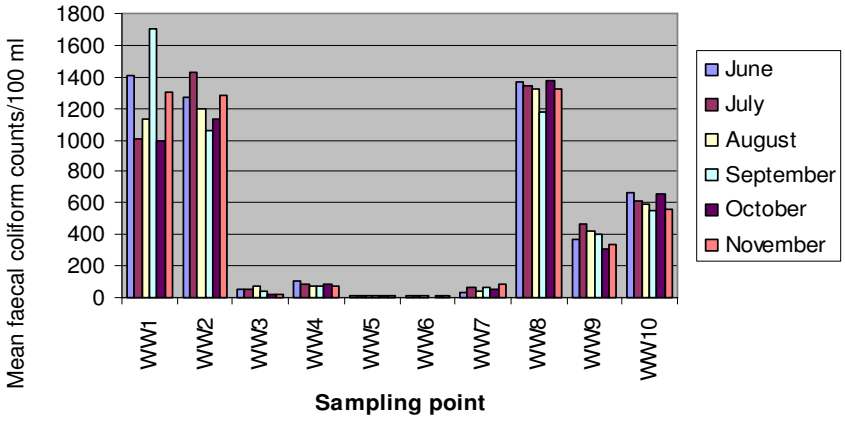

Figure 4b. Mean faecal coliform concentrations (100 ml^{-1}) in well water samples collected in June to November from different locations. Counts are means of three replicate determinations.

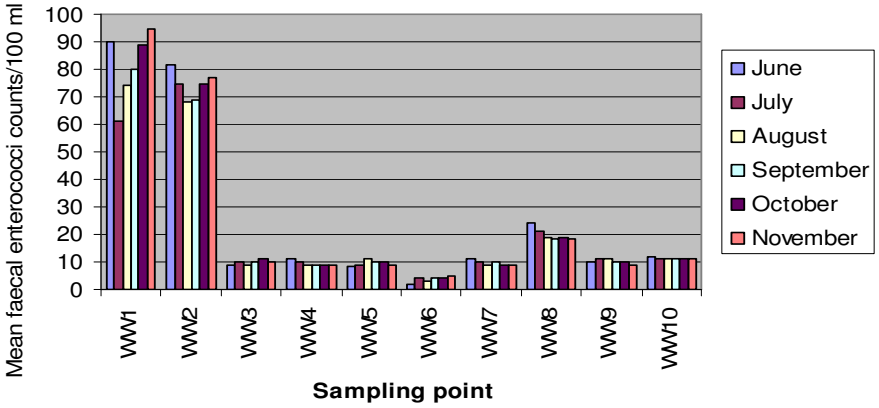

Figure 4c. Mean faecal enterococci concentrations (100 ml^{-1}) in well water samples collected in June to November from different locations. Counts are means of three replicate determinations.

Table 1. Antibiotic resistance of faecal coliforms and faecal enterococci isolates recovered from well and stream waters in Ile-Ife city, Southwestern Nigeria.

Antibiotic (Concentration)	Faecal coliform		Faecal enterococci	
	Well water n = 40	Stream water n = 64	Well water n = 30	Stream water n = 48
Ampicillin (10 µg)	34(85.0%)	64(100%)	14(46.7%)	38(79.2%)
Gentamycin (10 µg)	13(32.5%)	32(50.0%)	ND	ND
Streptomycin (10 µg)	24(60.0%)	58(90.0%)	ND	ND
Ceftriazone (30 µg)	34(85.0%)	61(95.3%)	ND	ND
Tetracycline (30 µg)	27(67.5%)	62(96.9%)	24(80.0%)	48(100.0%)
Ciprofloxacin (5 µg)	0(0.0%)	0(0.0%)	20(66.7%)	36(75.0%)
Chloramphenicol (30 µg)	34(85.0%)	51(79.7%)	27(90.0%)	48(100.0%)
Nalidixic acid (30 µg)	27(67.5%)	48(75.0%)	ND	ND
Nitrofurantoin (300 µg)	26(65.0%)	19(30.0%)	10(33.3%)	34(71.0%)
Sulphamethoxazole/trimethoprim (23.75µg/1.25 µg)	25(62.5%)	34(54.7%)	ND	ND
Penicillin (10 units)	ND	ND	30(100.0%)	489100.0%)
Vancomycin (30 µg)	ND	ND	3(10.0%)	11(23.0%)
Erythromycin (15 µg)	ND	ND	14(46.7%)	48(100.0%)
> 2 RES†	100	100	100	100

† Percentage of isolates resistant to two or more antibiotics. ND Not determined.

concern and potentials for acquired antibiotic resistances in the water sources commonly used by the inhabitants.

The World Health Organization (1997) recommends the absence of faecal coliform bacteria in any 100 ml of drinking water. The high counts obtained underline high level faecal contamination of these water sources which serve as major sources of water for consumption purposes by the inhabitants of Ile-Ife city. The public is therefore at a high risk of acquiring waterborne diseases. The proportions of waterborne infections for the period of study might be higher than reported due to self medication and several traditional practices such as use of local herbal remedies.

The ability of environmental bacteria to transfer antibiotic resistance genes to human pathogens can have grave consequences for human health. The presence of antibiotic-resistant faecal indicator bacteria in streams and wells used as sources of water for human consumption may pose a threat to human health because of the potential for transfer of antibiotic resistance genes to pathogens and the environment in that they may act as reservoirs contributing to the maintenance and spread of antibiotic resistance genes (Goni-Urriza et al., 2000; Van den Bogard and Stobberingh, 2000; Guardabassi et al., 2004; Constanzo et al., 2005).

Antibiotic resistance in bacteria is a serious problem facing society today and one of the reasons responsible for this problem is overuse of antibiotics in humans (Okeke et al., 2007). According to Sayah et al. (2005), the source of water contamination plays significant role in determining the extent of antimicrobial resistance as contaminating bacteria could come from domestic, wild animals or human sewage. Antibiotic resistance poses a threat to everyone most especially the children and the immunocompromised who are more vulnerable to bacterial illnesses. For the general public as a whole antibiotic resistance limits the number of effective drugs available leading to fewer treatment options for the sick.

There is therefore the need to control faecal pollution of water supply to avert the occurrence of waterborne diseases outbreak. Through effective public health education by relevant Government agencies, the people should be educated on the implications associated with the consumption of contaminated water for drinking and other domestic purposes. Public health education aimed at improving personal, household and community hygiene is imperative. For instance water needs to be adequately and appropriately treated or disinfected before

Table 2. Laboratory requests made and diagnosed cases of water borne infections in hospitals from January 2009 to November 2010.

Waterborne infection	Number of laboratory requests made	Confirmed/diagnosed cases
Cholera	60	30
Typhoid fever	80	40
Dysentery	50	5
Diarrhoea	75	20

consumption and well environments kept in hygienic conditions. To stem the tide of antibiotic resistance, infected persons should avoid self medication but seek proper medical attention so that appropriate antibiotic can be administered rather than using drugs indiscriminately which can lead to development of resistance by organisms. The importance of continuing with surveillance studies to follow the evolution of antimicrobial resistance in saprophytic bacteria of the intestinal tract recovered from water sources in order to detect possible reservoirs of antimicrobial resistance genes and antimicrobial-resistant bacteria should be emphasised.

ACKNOWLEDGEMENTS

The authors wish to appreciate the assistance of the laboratory staff of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria during the course of this study. The permission granted by owners for the sampling of water in their wells is also well appreciated.

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