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Status of contamination and antibiotic resistance of bacteria from well water in Ago- Iwoye, Nigeria

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ABSTRACT

Objective: In developing nations, majority of people depend on untreated surface and shallow groundwater that is subject to contamination from different sources. A cross-sectional study was conducted on wells that supply water for domestic uses in Ago-Iwoye, Nigeria to determine their microbiological quality.

Methodology and results: A total of 200 samples of water were collected from 50 wells over a period of three months. The samples were analyzed for total coliforms, faecal coliforms, faecal streptococci and heterotrophic bacterial count. The predominant bacteria recovered were screened for susceptibility to various antibiotics which are commonly used in the community. The total coliform counts in majority of the wells (86%) ranged from 1.2×10^2 - 1.9×10^2 cfu/ml, which exceeded the WHO standard of 10/100ml for drinking water. In addition all the samples had faecal coliform count ranging from 65-91 per ml which is above the WHO guideline of less than 1/100ml. The calculated faecal coliform/faecal streptococci (FC/FS) ratio revealed that contamination was essentially of human faecal origin. The organisms isolated belonged to seven genera, i.e. *Escherichia, Klebsiella, Pseudomonas, Bacillus, Proteus, Staphylococcus* and *Alcaligenes*. The predominant species was *Escherichia coli*. Higher level of resistance to the antibiotics tested was more prominent in *E. coli* than in *Klebsiella* spp. and *Staphylococcus aureus*.

Conclusions and application of findings: Although the susceptibility of the strains of organisms encountered to some commonly used antibiotics was high, those that were resistant to some antibiotics pose a serious public health risk especially with the increasing rate of transfer of resistant genes from one bacterium to another. There is therefore need to treat water obtained from wells in the community to make it safe for domestic use.

Key words: Bacterial contamination, well water, antibiotic resistance

INTRODUCTION

In Nigeria, especially in the rural and sub-urban communities, water for drinking and other domestic uses is mostly obtained from wells dug by inhabitants (Oyetao *et al*., 2007). This is in addition to the water available in streams and rivers in rural communities. The reliance on untreated ground water (wells) and streams is due to the lack of piped water. Such wells and streams are subject to contamination with pathogenic bacteria because of their proximity to human activities. Also poor wastewater and solid waste management, poor construction and inadequate protection of the wells and presence of latrines close to the wells predispose them to contamination.

The objectives of the present study were to examine the bacteriological quality of water from selected wells in the Ago-Iwoye community.

MATERIALS AND METHODS

Study area: The study area, Ago-Iwoye, is a town in Ijebu-North Local Government of Ogun State, Nigeria. Residents of this area comprise mostly of local inhabitants and students of the Olabisi Onabanjo University. They depend on water from dug wells and partly on tap water periodically available from public supply system, and mobile water tankers for their domestic needs. The study area was divided into 10 sampling regions, each about 1 km from the other. All selected wells had raised edges at ground level with cemented surfaces and depth ranged from 5.50 to 12.50 meters. Some of the wells were properly covered but left opened at times after use while some were partially covered, with a few of them situated not far from pit latrines, human septic tanks and farmland areas (Figure 1, Figure .2). Water was drawn from the wells mainly using plastic/rubber receptacles (Figure 3).

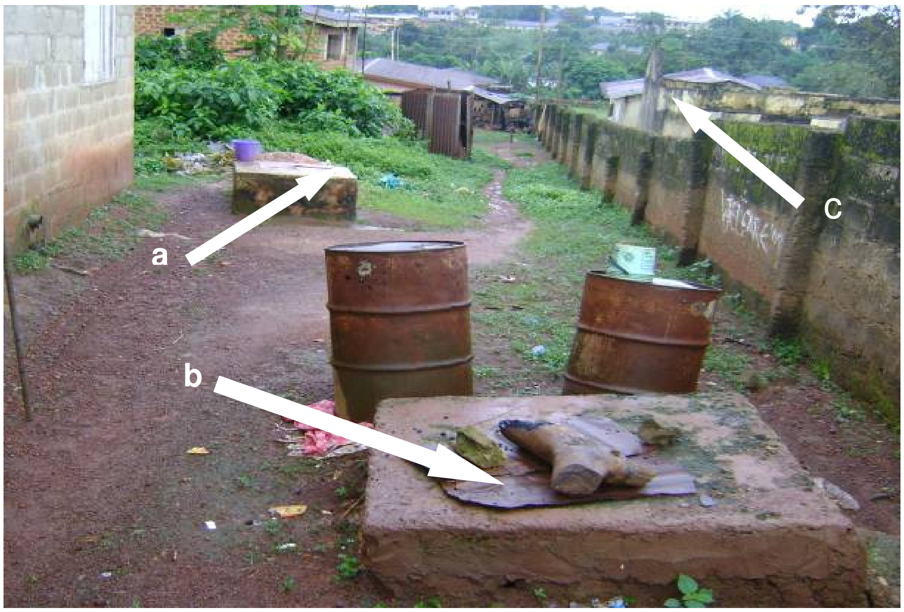

Figure 1: A well (a) situated very close to a septic tank (b) and a pit latrine (c).

Figure 2: A well close to a farm land and refuse dump

Figure 3: A well showing the black rubber receptacle for drawing water.

Collection of water samples: Water samples were collected from 50 groundwater wells using sterile 500ml universal bottles. Samples were placed on ice immediately after collection. Collection was done for each well at 3 weeks intervals. Four samples were collected from each well over a period of 3 months. Samples were analyzed within 2 hours of collection.

Isolation and identification of microorganisms: A three tube most probable number (MPN) method was used for the isolation of *E. coli* using lactose broth (Oxoid, U.K) as medium. Samples of 10ml, 1ml and 0.1ml were inoculated into respective dilution tubes containing inverted Durham's tubes and incubated at 45^{o}C for 24 hours. Tubes were observed for growth and gas production and the MPN determined. Contents of tubes showing growth were streaked on Eosin Methylene Blue (EMB) agar for isolation of *E. coli* and incubated at 37^{o}C for 24hours. Colonies showing typical *E. coli* characteristics (green metallic sheen) were selected, purified; Gram stained and identified further using indole, methyl red, Voges- Proskauer and citrate utilization tests. To determine whether *E. coli* 0157:H7 was present among the *E. coli* strains, the isolates were cultured on CT-Sorbitol MacConkey agar medium (Zadik et al., 1993) and incubated at 37^{0} C for 24 hours.

Non-selective media used for the recovery of heterotrophic bacteria included Tryptone Soy Agar and Blood agar and organisms were identified using standard procedure outlined in Bergey's Manual (Holt *et al*., 1994). Faecal streptococci were enumerated according to the most probable method using azide dextrose broth. The identity of faecal streptococci was confirmed on Bile Aesculin agar (APHA, 1992)

Antimicrobial Susceptibility Testing: The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. The antimicrobial agents tested included chloramphenical (30μg), tetracycline (30μg), gentamycin (10μg), ciprofloxacin (5μg), nalidixic acid (30μg), ampicillin (10μg), nitrofurantoin (300μg), vancomycin (30μg), pefloxacin (5μg), ofloxacin (5μg), amoxicillin (10μg), streptomycin (10μg) (Oxioid, U.K), cotrimoxazole (25μg) and augmentin (30μg). The medium used was Mueller Hinton (MH) agar. Pure cultures of organisms were enriched in nutrient broth and incubated at 37^{0}C to a turbidity of 0.5 Macfarland standards. The MH agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotics and the plates were incubated inverted at 37^{0}C for 24 hours. Results were recorded by measuring the zone of inhibition and comparing with the NCCLS interpretive performance standard for antimicrobial disk susceptibility testing (NCCLS, 2004).

RESULTS AND DISCUSSION

In the 50 hand-dug wells sampled, a total of 96 bacterial isolates were recovered from 200 water samples collected over a period of 3 months. The isolates recovered belonged to seven genera, i.e. *Escherichia, Bacillus, Proteus, Klebsiella, Pseudomonas, Staphylococcus,* and *Alcaligenes (Table 1).* Three isolates could not be identified with certainty. *Escherichia coli* was the predominant species recovered, constituting 36.5% of all isolates. The second most common bacterium was *Klebsiella aerogenes,* 26.0% followed by *Staphylococcus aureus,* 14.6% and *Pseudomonas aeruginosa* 7.3%. Other isolates identified were *Proteus* spp., *Bacillus cereus* and *Alcaligenes faecalis*. Of the 35 strains of *E. coli* isolated only 2 grew on Sorbitol MacConkey medium, indicating that they were *E.coli* 0157:H7 strains.

Table 1: Organisms isolated from the various well water sampling sites.

Sampling location	Average cfu/ml* x 10^3	Organisms isolated and number of strains								
		E. coli	*Proteus spp.*	*Bacillus cereus*	*Klebsiella spp.*	*Pseudomones aeruginosa*	*Staphylococcusaureus*	*Alcaligenes faecalis*	Unidentified spp	FC/FS Ratio
Campus Road	37	5	-	1	3	2	-	2	-	4.3
Iyalaje/ Mariam	27	5	-	-	2	-	5	-	-	4.1
Itamerin	19	-	1	-	4	2	-	-	-	4.1
Adesegun/Abobi	33	2	-	1	1	-	2	-	-	4.3
Olopomerin	41	4	1	-	2	-	1	-	1	0.7
Garage	39	7	-	-	2	-	1	-	-	0.2
Onabamiro/Idode	32	4	3	-	1	-	-	-	1	4.4
Igan Road	46	5	-	-	5	-	2	-	-	0.6
Ijesa Road	15	-	-	-	3	2	3	2	1	4.1
Ayegbami	29	3	-	-	2	1	-	1	-	4.1
Total		35	5	2	25	7	14	5	3	

**The total viable count was enumerated on TSA. cfu/ml=colony forming unit per milliliter*

All the isolates of *E. coli* were resistant to augmentin, cotrimoxazole, amoxycillin and nitrofurantion but susceptible to ciprofloxacin and pefloxacin. The resistance to streptomycin, tetracycline, ampicillin and vancomycin was 71.4, 80, 82.9, and 88.6% respectively. The least resistance was recorded to ofloxacin (8.6%). All the wells tested were positive for total coliform bacteria. *E. coli* was recovered from 80% of the wells sampled. Bacterial pathogens especially *E. coli* from these sources therefore pose a major threat to public health. *E. coli* can be life-threatening for infants, children, the elderly and those who are immunocompromised, leading to disease, gastrointestinal illnesses, urinary tract infections (UTIs), bacteraemia and sepsis (Kaper et al., 2004). A related study, Oyetao *et al.* (2007) had reported the isolation of *E. coli* from well water in Akure, Ondo State, Nigeria. The calculated faecal coliform/faecal streptococci (FC/FS) ratio in seven of the studied regions was between 4.1 and 4.4 while it was 0.2, 0.6 and 0.7 in Garage, Igan Road and Olopomerin locations, respectively. This means that while in Garage, Igan Road and Olopomerin region pollution from non-human faecal sources were responsible for water contamination, this was not so for the other seven sampling regions. Majority of the wells in these seven sampling regions were located close to potential sources of bacteria such as septic tanks and active animal rearing yards. Some of the wells are already very old, with their wooden caps already rotten and dropping into the wells or their metal covering already rusted (Figure 4, Figure 5). The majority of the wells were 7.5 metres deep. Shallow groundwater wells are the predominant type in rural areas of Nigeria, and are easily inundated by surface run-off and flood, thus increasing contamination with faecal coliform bacteria. Although the wells are dredged periodically, there has been no study to ascertain the microbiological quality of the well-water after dredging.

Figure 4: A well with rotten wood slab

Figure 5: A well which metal cover had rusted and fallen off.

The results of this study revealed that majority of the *E. coli, Klebsiella* spp. and *Staphylococcus aureus* were resistant to more than three antibiotics. The number of antibiotics to which they were resistant ranged from 3 to 8 (Table 2). Cardonha *et al*. (2004) also isolated *E. coli* strains from water which were resistant to more than one antibiotic. An earlier report (Sokari *et al*., 1988) showed that 80% of *E. coli* strains from water bodies around Port Harcourt in Nigeria were resistant to antibiotics. Recently, Oyetao *et al*. (2007) reported the sensitivity of *E. coli* strains from well water in Ondo State, Nigeria to norfloxacin, gentamicin, ciprofloxacin and chloramphenical. The % resistance to these antibiotics was also low, similar to the findings of the present study. Kaspar *et al.* (1990) and Mckeon *et al*. (1995) reported a lower level of antibiotic resistance than that reported in this study. The sensitivity patterns to the antibiotics used in our investigation were in consonance with the observation of Akharaiyi *et al*. (2007) who investigated *E. coli* isolated from rain water. Resistance pattern of the *Klebsiella* spp in this study was higher for amoxycillin, nitrofurantoin and gentamicin and lower for augmentin and ciprofloxacin than those obtained by Dash *et al.* (2008) with their isolates of *Klebsiella* spp. from urinary tract infections.

Table 2: Resistance of three predominant bacterial organisms isolated from water wells in Nigeria to various antibiotics.

Antibiotic	% Resistance		
	E. coli **(n=35)**	*Klebsiella spp.* **(n=25)**	*Staphylococcus. aureus* **(n=14)**
Ampicillin	82.9	100	92.9
Amoxycillin	100	100	85.7
Chloramphenical	17.1	12	14.3
Ciprofloxacin	2.9	4.0	21.4
Gentamicin	11.4	28	21.4
Co-trimoxazole	100	16	71.4
Ofloxacin	8.6	0	0
Pefloxacin	0	0	0
Tetracycline	80	36	28.6
Streptomycin	71.4	72	50.0
Vancomycin	88.6	16	14.3
Augmentin	100	28	0
Nalidixic acid	25.7	32	0
Nitrofurantoin	100	16	7

Groundwater, particularly private and public hand-dug wells supply drinking water for more than half of the Nigerian population. It is clear from our findings that the populations of people obtaining drinking water from these wells are at risk of waterborne diseases. The isolation of *Pseudomonas, Bacillus, Staphylococcus and Alcaligenes* from the wells water is of public health significance as opportunistic pathogens.

This study has shown that wells water in the community studied harbor bacteria that are resistant to multiple antibiotics. The high prevalence of enteric bacteria in the water could be due to poor sanitation of members of the community. The presence of bacteria that are resistant to antibiotic poses a serious health hazard especially since such organism can serve as reservoir for antibiotic resistant genes that could be transferred to potentially pathogenic bacteria in the ecosystem.

The prolonged survival of high density of coliform bacteria in aquatic environment in the absence of human faecal sources has been reported (Santiago-Mercado *et al*., 1987). The fluoroquinolones appeared to be potent against most of the bacterial isolates investigated *in vitro*. This could be attributed to their recent introduction and rational use through prescription which reduces frequency of use by the public at the moment.

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