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ANTIMICROBIAL RESISTANCE PATTERN OF COLIFORM BACTERIA ISOLATED FROM SACHET AND BOREHOLE WATERS SOLD IN ABAKALIKI METROPOLIS OF EBONYI STATE, NIGERIA

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ABSTRACT: This study investigated the susceptibility pattern of coliform bacteria isolated from sachet and borehole waters sold in Abakaliki metropolis. This was done by determining the presence of *Escherichia coli, Klebsiella spp* and *Enterobacter spp* and their antibiotic susceptibility profile using commercially prepared antibiotic discs. A total of Five hundred (500) samples of water comprising 250 each from selected brands of sachet water and boreholes were obtained from water dispensers in Abakaliki metropolis, Ebonyi State, Nigeria. Results of biochemical analysis revealed that out of the 311 confirmed isolates, 138 (55.2%) were from sachet waters while 173 (69%) were from borehole waters. The susceptibility profile of the isolates to ten antimicrobial agents indicated that majority of the isolates showed little susceptibility and was highly resistant to the following antimicrobial agents (nitrofurantoin, amoxycillin, ampicillin, ciprofloxacin, tetracycline, norbactin/norfloxacin, ofloxacin, cefuroxime and gentamicin). This showed that they exhibit multi-drug resistance pattern which is a common feature of medically important coliform bacteria. None of the water sources met the WHO microbiological standards for drinking water and thus pose a serious health risk to its consumers and users if not properly treated. We therefore report the presence of multi-drug resistant coliform bacteria in sachet and borehole waters sold in Abakaliki metropolis, Ebonyi State, Nigeria.

KEYWORDS: Microbiological standard, coliform, Bacteria, sucesptibility, sachet water, borehole water, Abakaliki.

1 INTRODUCTION

Following abstraction and treatment, water becomes vulnerable and perishable. It is vulnerable in that the integrity of the system used for the storage and distribution of water can be damaged and contamination through ingress can occur. It is perishable in that its microbial quality can deteriorate due to the bacteria remaining after treatment growing on the residual nutrient in the water. Water can therefore be regarded as having finite life (Robertson *et al.*, 2003).

Water is said to endanger health and life when it contains pathogenic microorganisms (Pelczar *et al.*, 1993). Free from contamination with faecal matter is the most important parameter of water quality because human faecal matter is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens (Scott *et al.*, 2003). Talaro and Talaro (1996) also reported that the coliform bacteria are known to be exclusively transmitted through faecal contaminated water and these are responsible for massive epidemics of enteric diseases like cholera and typhoid fever.

Since water is universally consumed in large quantity, it is important to know the types and number of microbes taken in by drinking water (Geldreich, 1990). In developing countries including Nigeria, where the majority of the people live in rural areas, rivers, streams, well and more recently boreholes, serve as the main sources of water for drinking and domestic use

(Ibe and Okplenye, 2005). The underground water supplies are usually consumed safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water (WHO, 1971).

Water has played a significant role in the transmission of human diseases and indicator organism (i.e. coliform) (Brock, 1991). Potential health risk may exist due to the microbial content of sachets/table water and borehole waters since water is one of the vehicles for the transmission of pathogenic organisms (Prescott *et al.,* 2008).

Worldwide record has it that, 1.1billion people lack access to sufficient quantities of safe drinking water. Unsafe drinking water, combined with insufficient water supply for sanitation and hygiene, is responsible for an estimated 4 billion cases of diarrhoeal diseases, 1.79 million diarrhoeal death and 3.7% of the global burden of disease (WHO, 2002). Water-borne diseases are associated with inadequate provision of water and sanitary services and the effect of these diseases vary in severity from stomach upsets to death. Most of the victims' age is young children especially from the developing world. An estimated number of more than 34 million people die annually worldwide as a result of these water related disease and death around the world (Sonu *et al*., 2007 and WHO 2005).

As a matter of fact, many aquifers and isolated waters are high in water quality and may be pumped from the supply and transmission network directly for human consumption, irrigation, industrial purposes, fire control, etc. However, such clean waters are not universally available. Thus, area of dense population or heavy agriculture may lack adequate supply of water. In this case, water supply must receive varying degrees of treatment prior to distribution (Uchegbu, 2002).

It is well known that the quality and safety of the drinking water continues to be an important public health issue (Hrudey and Hrudey, 2007), because its contamination has been frequently described as responsible for the transmission of infectious diseases that have caused serious illness and associated mortality worldwide (Marshal , 2006; Jones *et al.,* 2007). Clearly, point-of-use water quality is a critical public health indicator (Reynold *et al.,* 2007).

Over the past decades, there has been a markedly increase in the consumption of water derived from different sources in place of tap water for drinking purposes in many regions of the world. International drinking water- quality monitoring programs have been established in order to prevent or to reduce the risk of contracting related infections (Giorgio *et al.,* 2010).

Coliform bacteria have been used to evaluate the general quality of water. Their presence in water makes them indicator organisms for drinking water quality. Some waterborne coliform not normally considered to be of faecal origin are able to ferment lactose at 44.5^{0}C (Geldreich *et al.,* 1958; Geo *et al.,*2004; Mishra *et al.,* 1968), and under certain conditions these organisms may be present in relatively high numbers in water samples. Coliform bacteria are rod-shaped bacteria usually found in the intestinal tracts of animals, including humans. Coliform bacteria do not require but can use oxygen, and they do not form spores. They produce acid and gas from the fermentation of lactose sugar. Their presence in the water supply indicates recent contamination by human or animal faeces. Coliform bacteria are group of anaerobic, lactose-fermenting bacteria, of which *Escherichia coli* is the most important member. Most coliform are not harmful, but since they arise from faeces, they are useful as a test of faecal contamination, and particularly as a test for water pollution. Typical genera include *Enterobacter, Escherichia,and Klebsiella*. (Bender, 2005).

E. coli and thermotolerant coliform have been indicated by WHO as the parameter of choice for monitoring drinking water quality (WHO, 1993). Diseases that are transmitted through water i.e. waterborne diseases are cholera, typhoid fever, dysentery, diarrhoeal, schistosomiasias, dracunculiasis, trachoma, ascarisias, trichuriasis, and hookworm disease (Madema *et al.,* 2003).

Coliform bacteria are known to be exclusively transmitted through faecal contaminated water and these are responsible for massive epidemics of enteric diseases like cholera, diarrhoea, typhoid fever and dysentery e.t.c (Talaro and Talaro, 1996). Coliform bacteria are regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*,*Haffnia* and *Serratia*. Although coliform organisms may not always be directly related to the presence of faecal contamination, or pathogens in drinking water, the coliform test is still useful for monitoring microbial quality of treated piped borne water supplies (WHO, 1993).

2 MATERIALS AND METHODS

2.1 COLLECTION OF WATER SAMPLES

Five hundred samples of water comprising two hundred and fifty sachet water from different companies (Aqua Rapha, Bejoy, Gospel, Nene, and Rock Tama) and two hundred and fifty bore hole samples from bore hole water dispensers

Metropolis were collected. They were transported in cool boxes to the Laborayroy of the Deparatment of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria for immediate analysis.

2.2 MICROBIAL ANALYSIS

Five hundred water samples were collected. Fifty samples each were collected from five different batches of sachet waters and different locations of borehole waters. They were analyzed for the presence of coliform bacteria using standard Microbiological techniques. The Multiple Tube Fermentation Technique (MPN) presumptive test which involves adding the water sample to a set of test tubes was used each of which contains a triple strength lactose broth with a bromcresol purple indicator and an inverted Durham tube. The tubes were then incubated at 35±0.5°C for 24 to 48 hours for total coliform and at 44.5±0.2°C for faecal coliform. Gas productions were observed in an inverted tube after incubation for the presence of total coliform. The MPN confirmatory test was carried in brilliant green bile lactose broth and MPN completed test using eosine methylene blue (EMB) agar. Further characterization was carried out by using biochemical reactions viz: indole, methyl red, Voges-Proskauer and citrate utilization test (IMViC), motility and Gram staining (Cheesbrough, 2002).

2.3 ANTIBIOTIC SUSCEPTIBILITY STUDIES

Sensitivity testing of *E.coli*, *Klebsiella* sp and *Enterobacter* sp isolates to different antibiotics was performed by disc diffusion method. Sterile nutrient agar was prepared and a 0.5MacFarland equivalent standard of the test organisms were streaked with a sterile non cotton swab that was dipped into the standardized inocula on the surface of the agar and allowed to pre-diffuse for 15-20minutes.

The following antibiotics discs Nitrofurantoin (100µg), Ciprofloxacin (100µg), Tetracycline (50µg), Norfloxacin/Norbactin (10µg), Amoxycillin (30µg), Ofloxacin (5µg), Chloramphenicol (10 µg), Cefuroxine (30 µg), Ampicillin(30µg), Gentamicin(10µg) were aseptically placed on the surface of the agar plates with a sterile forceps. These were incubated at 35^{0}C for 18-24hours, after which the inhibition zone diameter (IZD) in mm was taken and interpreted using CLSI standard (Mills-Robertson *et al.,* 2003).

3 RESULTS

Of the 250 samples of sachet water brands examined, the findings revealed a high level of faecal contamination in Gospel sachet water 36 (72%), Aqua Rapha 30 (60%) and Bejoy 18 (36%). Low level of contamination was observed in Rock Tama 6(12%) and Nene 13 (26%). This could be as a result of poor sanitary standard of operation in the production line of the sachet water brands, also the presence of a particular type of faecal bacteria revealed a common source of contamination in all the batches of the water brands examined. But Nene and Rock Tama had a different contamination pattern; this could be as a result of some level of sanitary standard and proper treatments employed in the production line of these sachet waters (Table 1). Also, out of the 250 samples of borehole waters examined, from different locations, the results revealed a high level of faecal contamination from all the samples examined from seven different locations in Abakaliki. The results revealed that Aboffia had 27 (76.93%) samples contaminated with faecal bacteria while Azugwu 11 (28.5%), Azuiyiokwu 18 (50%), Azuiyiudene 29(80%), Kpirikpiri 24 (66.63%), Presco/Ntezi 16(46.15%) and Udensi 22 (61.54%). This was as a result of very poor environmental sanitary protection, seepage of faecal materials due to leakage of water pipes and poor sanitary procedure in drilling and dispensing of bore-hole water sold in Abakaliki metropolis as revealed in our result (Table 1 & 2).

Susceptibility studies showed that 62% of the test strain of *E.coli* from both sachet and borehole waters were resistant to gentamicin, 85% to cefuroxime, and chloramphenicol, 83% to ofloxacin, 80% to ciprofloxacin, 95% to norbactin/norfloxacin and 96% to nitrofurantoin, while the test strain of the *E.coli* were completely resistant to amoxycillin, tetracycline and ampicilin. 64% of the test strain of *Klebsiella* spp was resistant to gentamicin, 83% to cefuroxime, 85% to ofloxacin, 75% to chloramphenicol, 92% to amoxycillin, 93% to tetracycline, 66% to ciprofloxacin, while the test strain of *Klebsiella* spp was completely resistant to nitrofurantoin, norbactin/norfloxacin and ampicillin. 62% of the test strain of *Enterobacter* spp were resistant to gentamicin, 87% to cefuroxime, 81% to chloramphenicol, 93% to orfloxacin, 93% to norbactin/norfloxacin, 81% to ciprofloxacin, while the test strain of *Enterobacter* spp was completely resistant to nitrofurantoin, tetracycline, amoxycillin and ampicillin (Table 3).

Table 1: Percentage of Sachet Water Brand Contaminated with Coliform Bacteria

Sachet Water brands	Percentage/Number of Positive Samples	Percentage/Number of Negative Samples	Faecal coliform isolated
Aqua Rapha	30(60%)	20 (40%)	*E. coli, Klebsiella* spp, *Enterobacter* spp
Bejoy	18 (36%)	32 (64%)	*E. coli, Klebsiella* spp, *Entrobacter* spp, *Pseudomonas* spp
Gospel	36 (72%)	14 (18%)	*E. coli, Klebsiella* spp, *Enterobacter* spp
Nene	13 (26%)	37 (74%)	*E. coli, Klebsiella* spp, *Enterobacter* spp
Rock Tama	6 (12%)	44 (88%)	*E. coli, Klebsiella* spp

Table 2: Percentage of Borehole water Samples Contaminated with Faecal Coliform Bacteria.

Borehole Water Samples (Location)	Percentage of Positive Sample (%)	Percentage of Negative Sample (%)	Faecal Organism Isolated
Aboffia	76.93% (27)	23.07% (9)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp
Azugwu	28.59% (11)	71.43% (25)	*E.coli*, *Klebsiella* spp
Azuiyiokwu	50% (18)	50% (18)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp
Azuiyiudene	30% (29)	20% (7)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp
Kpiri kpiri	66.63% (24)	33.37 (12)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp
Presco/ntezi	46.15% (16)	53.85% (19)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp
Udensi	61.54% (22)	38.46% (13)	*E.coli*, *Klebsiella* spp, *Enterobacter* spp

Table 3: Percentage Resistance of Bacteria Isolate from Sachet Water to Different Antibiotics

Antibiotics/Disc conc [ug/ml]	Resistance		
	Escherichia coli	***Klebsiella*** **spp**	***Enterobacter*** **spp**
N (100)	96%	100%	100%
CIP (5)	80%	66%	81%
TE (50)	100%	93%	100%
NB (10)	95%	100%	93%
AX (30)	100%	92%	100%
OF (5)	83%	85%	93%
C (10)	85%	75%	81%
CF (30)	85%	83%	87%
AM (30)	100%	100%	100%
G (10)	62%	64%	62%

N = Nitrofurantoin, CIP = Ciprofloxacin, TE = Tetracycline, NB = Norfloxacin (Norbactin), Ax = Amoxycillin, OF = Orfloxacin, C = Chloramphenicol, CF = Cefuroxine, Am = Ampicillin, G = Gentamicin.

4 DISCUSSION

The bacteriological quality of sachet-packed water and borehole waters offered for sale in Abakaliki metropolis was investigated in this study. The results of findings revealed that the popular products that are sold to the public under a wide variety of brand names and borehole waters were unsafe for human consumption except for the sachet packed water with the brand name Rock Tama and Nene that showed low level of contamination. This suggests that the sanitary procedures in the production lines of these commercially sold products did not meet the World Health Organization and the United States Environmental Protection Agency standard for faecal coliform in drinking water which is zero faecal coliform per 100ml

(WHO, 1971; WHO 1986; USEPA, 2001). The borehole water sold in Abakaliki Metropolis for human consumption showed high level of contamination with faecal coliform. Among all the sachet water brands, Rock-Tama and Nene showed a very minimal level of contamination with both *E.coli* and other isolates. This study supports the result of other researchers in the case of faecal contaminations in sachet water brands produced in the South Eastern States of Nigeria and bacteriological analysis of bore-hole waters in some parts of South Eastern Nigeria (Afiukwa *et al.*, 2010a; Ezeugwunne *et al.*, 2009; Ibe and Okplenye, 2005).

Epidemiological investigations have proved that diarrhea has a world-wide occurrence and accounts for 4% deaths and 5% of health loss to disability. It was also recorded that there are about 4 billion cases of diarrhea on a yearly basis (Madema *et al*., 2003). Bacteria especially the family Enterobacteriaceae which are agents of water-related diarrhea are very different and significant in checking the microbiological quality of water meant for human use, as they are potentially present in contaminated water. Since an outbreak of disease from drinking water has been reported world-wide and that it has been estimated that water-borne disease such as diarrhoeal diseases, schistosomiasis, trachoma, dysentery, ascariasis, typhoid diseases might account for one-third of the intestinal infections globally (Madema *et al.*, 2003; WHO 2003; Afiukwa *et al*., 2010a; Prüss *et al*., 2002).

E.coli was found to be the most contaminant of the water samples examined in this work at the rate of 62.2%. This result followed a similar pattern of bacterial load from other results obtained from other South Eastern States, South Western and North-Western part of Nigeria which correlated with the report given by the experts in a communiqué at the 20th Regular Meeting of the National Council on Water Resources (NCWR) in Jos, Plateau State which the Council observed with regret that Nigeria performs abysmally low in water sector development when compared with global sector indices and that Nigeria currently has the highest prevalence of water-borne diseases in the world (Emejor, 2010). This, they added, has resulted in high infant mortality in the country and this clearly indicates poor quality of water products in Nigeria as a nation (Afiaukwa *et al.,* 2010b; Edema *et al,* 2011; Ezeugwunne *et al.,* 2009; Garba *et al.,* 2010; Ibe and Okplenye, 2005; Oyedeji *et al.,* 2010). The contamination rate with *Klebsiealla* spp and *Enterobacter* spp were 15.4% and 13.8%, respectively and that the water contamination was of faecal source.

Escherichia coli has been a central organism in water microbiology for decades as an indicator of faecal pollution, and its role as a pathogen rather than an indicator, in drinking water is been stressed in recent studies. This interest in the role of *E.coli* as a cause of diarrhoeal disease has increased because of the emergence of *E.coli* 0157:H7 and other enterohaemorrhagic *E.coli* due to the severity of the related disease (Paul, 2003). From the above report according to Paul (2003), it is obvious that the presence of *E.coli* in sachet water brands and borehole waters sold in Abakaliki metropolis could pose a serious threat to public health.

The high faecal coliform content observed in the water samples analysed was indicative of the likely presence of other pathogenic organisms such as *Aeromonas* spp or *Pseudomonas* spp. For instance, about 6(2.4%) isolates of *Pseudomonas* spp were identified in Bejoy sachet water product, and this may pose a health risk in the consumption of the water and its use for domestic purposes.

The bacterial isolates from the water samples of sachet water brands and borehole waters showed a moderately susceptibility to gentamycin and ciprofloxacin and were highly resistant to chloramphemical, nitrofurantion, ofloxacin, norfloxacin (norbactin), cefuroxine, amoxycillin and ampicillin. This result was similar with the recent studies obtained by Garba *et al.* (2010) at lower percentage but Sevanan *et al.,* (2011), in their studies was in direct correlation with the findings which revealed the resistance pattern of *E. coli* to be 55.5% to antimicrobials that have wide Gram-negative coverage.

5 CONCLUSION/RECOMMENDATION

This study suggests that more check should be put in place in order to maintain the WHO standards for borehole water drilling which recommends that boreholes should be located at least 30m away from latrines and 17m from septic tanks as most of the faecal contamination of pipe waters is caused by the seepage of faecal materials into leaking pipes since most sachet water products are processed from borehole waters. This is because the presence of faecal organisms in treated water for human consumption should pose a significant health concern and quality improvement to health workers such as National Food and Drug Administration Control (NAFDAC), and the manufacturers as to make necessary investigation to identify the point of entry and get it rectified as the public health importance of safe drinking water is inevitable. There should be a serious reassessment of water treatment methods in Nigeria especially for borehole and sachet packed waters since they major sources of water used for domestic purposes and drinking. This is because high load of faecal coliform in drinking water pose a serious health and economic problem in a society.

Also government should put more effort in establishing technologies that will enhance a greater understanding of coliform in recurrent gastrointestinal tract and urinary tract infections which will help in the development of new and more effective treatment of these problematic diseases.

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