Studies on Antibiotic Resistance of Some Bacterial Isolates from Sachet Water Samples in Nsukka, Nigeria

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Abstract

Sachet water samples of different brands were collected from several retail outlets in Nsukka, Nigeria and analysed microbiologically to determine their bacteriological quality and antimicrobial susceptibility of the isolates. A brand of the sachet water samples tested negative for the presence of bacteria while a total of 61 bacterial strains isolated from the other sachet water samples. Using the disc diffusion method, the 61 bacterial isolates were screened for susceptibility to 12 commonly used antimicrobial agents including amoxycillin, chloramphenicol, ciprofloxacin, cotrimoxazole, streptomycin and tetracycline. The isolates showed 91.8% susceptibility to chloramphenicol and 85.2%, 80.3% and 77.0% susceptibility to ciprofloxacin, nalidixic acid and nitrofurantoin, respectively. About 79% of the isolates were resistant to cotrimoxazole and rifampicin, respectively followed by gentamicin (69%). These results suggest that a large proportion of the bacterial flora in sachet water samples is resistant to a variety of antibiotics, and that resultant water-related infections will be more difficult to treat.

Keywords: Antibiotic resistance, Bacterial isolates, Sachet water samples

Introduction

Water is one of the most abundant and essential commodities of man and occupies about 70% of the earth's surface, yet a greater percentage of the world's population live without access to safe drinking water, especially in the developing countries (Hazen and Toranzos, 1990; Adriano and Joana, 2007). Water of good quality is of basic importance to man and his continued existence depends very much on its availability (Lamikanra, 1999; FAO, 1997). In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko *et al*., 2006).

The provision of potable water to the rural and urban population is necessary to prevent health hazards (Nikoladze and Akastal, 1989). Unsafe water is a global public health threat, placing persons at risk for a host of diseases as well as chemical intoxication (Hughes and Koplan, 2005). It has been reported by Kosek *et al*. (2003) and Parashar *et al*. (2003) that more than two million persons, mostly children less than five years of age, die of diarrheal diseases. Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation (WHO, 2004). An estimated 1.1 billion persons (one sixth of the world's population) lack access to clean water and 2.6 billion to adequate sanitation (WHO, 2005; Hughes and Koplan, 2005).

Nigeria is located in coastal West Africa where water is abundant, yet most of the population lacks adequate and safe drinking water. As a result, individuals who can afford it now sink boreholes and sell water, without any major form of treatment, to the ever-growing population. Many individuals and corporate bodies in Nigeria now engage in packaging water, popularly called "pure water" in polythene sachets of about 50 – 60 cl which they sell to the public. Thus, drinking water is commercially available in such easy-to-open sachets (Umeh *et al*., 2005). The production, marketing and consumption of sachet water have increased tremendously. There are now several brands of this sachet water marketed in Nigeria and other developing countries (Ogan, 1992; Kassenga, 2007).

Water in sachets is readily available and the price is affordable, but there is concern about its purity. The safety of the sachet water is still questionable because many who are engaged in its production do not follow strictly the standards set by FEPA (1999) and WHO (2006) for safe drinking water. Conformation with microbiological standards is of special interest because of the capacity of water to spread diseases within a large population. Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water-borne diseases to the barest minimum in addition to being pleasant to drink, which implies that it must be wholesome and palatable in all respects (Edema *et al*., 2001; Okonko *et al*., 2008).

In Nigeria, public drinking water supply is unreliable (Egwari and Aboaba, 2002) thereby encouraging the sell of drinking water in polythene sachets. The integrity of the hygienic environment and the conditions under which the majority of the water in sachets are produced are questionable.

The microbiological analysis of different water samples has revealed the presence of such bacterial species as *Staphylococcus aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Bacillus* spp., *Klebsiella* spp., *Flavobacterum* spp. and *Acinetobacter* spp. (Okonko *et al*., 2008; Chatterjee *et al*., 2007; Olayemi, 1999; Adekunle *et al*., 2004).

Antimicrobial resistance in bacteria associated with food and water has been a global concern. It is now widely accepted that there is a relationship between the use of antimicrobial agents and the occurrence of resistance (Kumar *et al*., 2005). Antimicrobials exert a selective pressure on microorganisms and therefore their use is considered a key issue in epidemiological studies (McGeer, 1998). The disease threat from antibiotic

resistant strains of pathogens has increased with time (Williams and Heymann, 1998). Antimicrobial resistance can spread through horizontal transfer of resistance genes from one type of bacteria to another. The presence of resistance, together with the acquisition of virulence genes can lead to clonal expansion and spread of a particular disease-causing agent (Kumar *et al*., 2005). Therefore, it is considered important to study the antimicrobial resistance in pathogenic as well as indicator bacteria associated with food and water (OIE, 1999).

In Nigeria, water-borne diseases are one of the main problems in rural and urban communities. These diseases are as a result of bacterial, fungal and other microbial infection of water. Thus, contaminated water may serve as potential source of transmission of pathogenic organisms from product to consumer. Moreover, the presence of antibiotic resistant microbial isolates in sachet water could lead to transfer of antibiotic resistance traits to hitherto sensitive gut or oral microflora to consumers. The present study attempts to isolate, identify and determine the susceptibility-resistance profile of bacteria isolated from sachet water.

Materials and Methods

Sachet water samples: A total of 50 sachet water samples, ten from each of five different brands, were randomly obtained from retail outlets in Nsukka, Nigeria. The different brands were coded A, B, C, D and E. The samples were transported to the laboratory immediately and analysed within four hours of collection.

Isolation and identification of bacterial contaminants: One ml of each sample was introduced into a test tube containing 9.0 ml sterile distilled water. The contents of the test tube were thoroughly mixed together after which ten-fold serial dilutions were made with sterile distilled water. Sterile Petri dishes of the appropriate media were spread-inoculated with 0.1 ml of the appropriate dilution. The media used for the inoculation included nutrient agar, MacConkey agar and mannitol salt agar. Duplicate plates were prepared and incubated at 37 ^{0}C for 24 hr. Discrete colonies with different morphological appearances on the plates were repeatedly subcultured on nutrient agar medium to obtain pure cultures. The pure isolates were characterized based on their morphological, physiological and biochemical characteristics and were identified based on the standard schemes of Barrow and Feltham (1993) and Holt *et al*. (1994).

Antibiotic susceptibility testing: The isolated bacterial strains were tested for antibiotic susceptibility by standard agar disc diffusion technique (Bauer *et al*., 1966) on nutrient agar using commercial discs. The following antibiotics with the disc strength in parenthesis were used: amoxycillin (25 μg), augmentin (30 μg), chloramphenicol (30 μg), ciprofloxacin (10 μg), cotrimoxazole (25 μg), erythromycin (30μg), gentamicin (10 μg), nalidixic acid (30 μg), nitrofurantoin (20 μg), rifampicin (10 μg), streptomycin (10 μg) and tetracycline (30 μg). The plates were incubated inverted at 37^{0}C for 24 hr and the corresponding inhibition zone diametres (IZD) that developed were measured and recorded. The strains were classified as susceptible or resistant to a given antibiotic on the basis of the diameters of growth inhibition zones.

Results

A summary of the bacterial species isolated in this study is presented in Table 1. A total of 61 bacterial strains were isolated from the sachet water samples. Of the total of 50 sachet water samples tested, ten which belonged to the same brand consistently did not give any bacterial growth. However, in all the other samples, at least five different bacterial genera were isolated. From Table 1, *Enterobacter aerogenes* (29.5%) and *Pseudomonas aeruginosa* (21.3%) were the most frequently isolated. *Bacillus* spp. (9.8%) and *Staphylococcus* spp. (8.2 %) were of the least frequencies.

Table 1: Bacterial species identified in sachet water sample

Bacteria	Number	%
Enterobacter aerogenes	18	29.5
Pseudomonas aeruginosa	13	21.3
***Micrococcus* spp.**	10	16.4
***Flavobacterum* spp.**	9	14.8
***Bacillus* spp.**	6	9.8
Staphylococcus aureus	5	8.2
Total	61	100.0

The bacterial strains isolated from the different brands of sachet water investigated are presented in Table 2. The sachet water samples belonging to the brand coded D tested negative for the presence of bacterial growth as no bacterial colony was identified during the period of study. Six genera of bacteria (*Enterobacter*, *Pseudomonas*, *Micrococcus*, *Klebsiella*, *Bacillus* and *Staphylococcus*) were isolated from brands coded C and E whereas brands A and B had five genera each.

Table 2: Bacterial species isolated in different sachet water samples investigated

Bacteria	Water sample					Total
	A	B	C	D	E	
E. aerogenes	8	3	5	-	2	18
P. aeruginosa	2	4	6	-	1	13
***Micrococcus* spp.**	3	3	1	-	3	10
***Klebsiella* spp.**	2	2	2	-	3	9
***Bacillus* spp.**	-	1	2	-	3	6
***Staphylococcus* spp.**	2	-	1	-	2	5
Total	17	13	17	-	14	61

The percentages of strains susceptible or resistant to individual antibiotic types are shown in Table 3. Resistance to rifampicin was observed in 78.7% of the strains followed by cotrimoxazole (70.5%) and gentamicin (68.9 %). Susceptibility to chloram-phenicol was observed in 91.8% of the isolates while it was 85.2% to ciprofloxacin, and 80.3% to nalidixic acid.

Table 3: Percentage of bacterial strains susceptible/resistant to antibiotics tested in this study

Antibiotic used	Number of strains susceptible	Number of strains resistant
Amoxycillin	21	40
Augmentin	28	33
Chloramphenicol	56	5
Ciprofloxacin	52	9
Cotrimoxazole	13	48
Erythromycin	38	23
Gentamycin	19	42
Nalidixic acid	49	12
Nitrofurantoin	47	14
Rifampicin	13	48
Streptomycin	38	23
Tetracycline	34	27

Thus the bacterial isolates were most susceptible to chloramphenicol followed by ciprofloxacin. The least susceptibility was shown towards rifampicin followed by cotrimoxazole. On the other hand, twenty one of the 61 bacterial strains isolated and analysed during this study were resistant to at least two antibiotics tested and no strain was sensitive to all the antibiotics tested. However, 14 strains were resistant to one antibiotic, 12 to three antibiotics, seven to four antibiotics, one was resistant to five, two were resistant to six and one was resistant to eight antibiotics used. The eight antibiotics to which one strain was resistant included amoxycillin, cotrimoxazole, erythromycin, gentamycin, nalidixic acid, nitrofurantoin, streptomycin and tetracycline.

Discussion

All the sachet water samples tested, except one brand, were found to contain at least five different genera of bacteria. The sachet drinking water, which is affordable and consumed by many Nigerians, is generally regarded as or termed “pure water”. However, according to Akunyili (2005), the question that remains is: how pure is our “pure water”?

Six different genera of bacteria were isolated from the sachet water samples tested. This indicated that these waters were not well treated, if treated at all. In the present study, 61 bacterial strains belonging to six different genera were obtained from the sachet water samples tested. The isolates included *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Bacillus* spp., *Micrococcus* spp., *Klebsiella* spp., and *Staphylococcus aureus*. Among the sachet water samples tested in this study, each of samples A and C recorded 17 different bacterial strains with sample C recording the presence of the representatives of the six bacterial genera encountered.

The isolated bacteria species are among those commonly encountered in water and aquatic environments as reported by Okonko *et al*. (2008). Though the most frequently isolated index of water quality and indicators of faecal contamination such as *Escherichia coli* and *Streptococcus faecalis* were not isolated in the present study, the presence of some indicator and other organisms is of special concern. The presence of enteric bacteria, namely, *Micrococcus* sp. and *E. aerogenes* as reported in this study suggests unhygienic handling or improper treatment. The presence in “pure water” of enteric bacteria associated with faecal contamination which include *S. faecalis*, *Citrobacter* sp., *Proteus mirabilis*, *Providencia* sp., *Micrococcus* sp., *E. coli*, *Shigella* sp., *E. aerogenes*, *Serratia* sp. and *Klebsiella* sp. has been reported by Umeh *et al*. (2005). While E. coli was not identified in the present study, its absence may not justify the purity of the water samples.

Antimicrobial resistance in bacteria associated with food and water has been a global concern. Large amounts of antibiotics used for medical, veterinary and agricultural purposes have resulted in the contamination of the environments with antibiotics (Huyeke *et al*., 1998; Kummerer, 2003; Wise *et al*., 1998).

The bacteria isolated from the sachet water samples were reasonably sensitive to ciprofloxacin (85.2%), nalidixic acid (80.3%) and nitrofurantoin (77.0%). Resistance to chloramphenicol was observed in only 8.2% of the strains. On the other hand, 78.7% of the strains were resistant to cotrimoxazole and rifampicin, respectively, and 68.9% to gentamicin, some of the widely used drugs. The isolates showed wide resistance to penicillins, that is amoxycillin and augmentin (amoxycillin-clavulanic acid combination), suggesting that many of the isolated bacterial strains could be penicillinase-producers. The high rate of resistance to the penicillins and cotrimoxazole renders these antimicrobial agents inappropriate for empirical therapy. Resistance to these antimicrobial agents is likely to be related to their widespread use as it has occurred with quinolones in countries such as Spain, where quinolones are used widely (Aguiar *et al*., 1992). Prudent use of these antimicrobial agents is advised to prevent or minimize the development of resistant strains.

Although several new antimicrobial drugs have recently been introduced and additional ones are forthcoming, experience suggests that, as new drugs become widely deployed, resistance to these agents will emerge and spread as well. Successful control of antibiotic resistance will require both the continued development of new drugs and the judicious use of our current arsenal of antibiotics.

Many authors have suggested that antimicrobial cycling, in which the empiric use of two or more classes of antibiotics is alternated over a time scale of months to years, may slow the evolution and spread of resistant and multiply resistant bacterial strains (Gerding *et al*., 1991; John, 2000; McGowan, 2000). The motivation is straightforward. Should resistance to one class of drugs reach high frequency, a scheduled switch of antibiotic classes would soon follow, leaving most of the bacterial strains susceptible to the new therapy (Moss *et al*., 2002). Moreover, fluctuating patterns of antimicrobial use may reduce the rate at which drug-sensitive strains can acquire resistance to single or multiple antibiotics. In addition to the intuitive appeal of these arguments, more than two decades of experience have shown that a one-time formulary shift can effectively control an epidemic of

antibiotic resistant strains (White *et al*., 1997; Rahal *et al*., 1998), as can a single rotation through a series of alternative drugs (Raymond *et al*., 2001).

The problem of increasing antimicrobial resistance is becoming serious worldwide (ASM, 1994). There is widespread misuse of antibiotics and this poses a serious health risk to the public and may complicate the treatment of human infections by stimulating the emergence of microbial resistance. It is, therefore, recommended that the use of antimicrobial agents, especially those with dual animal and human applications, be restricted. In addition regular monitoring of bacterial resistance to antimicrobial drugs is needed. The information should be made available in a national database in order to help health professionals optimize the use of antimicrobial agents.

References

Adekunle, L.V., Sridhar, M.K.C., Ajayi, A.A., Oluwade, P.A. and Olawuyi, J.F. (2004). An assessment of the health and social economic implications of sachet water in Ibadan, Nigeria: A public health perspective. Afr. J. Biomed. Res. 7: 5 – 8.

Adriano, A.B. and Joana, S.B. (2007). The quest for safe drinking water: An example from Guinea-Bissau (West Africa). Water Res. 41: 2978 – 2988.

Aguiar, J.M., Chacon, J., Canton, R. and Baquero, F. (1992). The emergence of highly fluoroquinolone-resistant *Escherichia coli* in community-acquired urinary tract infection. J. Antimicrob. Chemother. 29: 349 -350.

Akunyili, D. (2005). Good potable water in the society. NAFDAC Information Bulletin 4: 5 – 6.

American Society for Microbiology (ASM) (1994). Report of the ASM task force on antibiotic resistance. Washington DC.

Barrow, G.I. and Feltham, R.K.A. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edition. Cambridge University Press, Cambridge.

Bauer, A.W., Kirby, W.M.W, Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. 45: 493 – 496.Chatterjee, S.N., Das, D., Roy, M., Banerjee, S., Dey, P., Bhattacharya, T. and Chandra, G. (2007). Bacteriological examination of drinking water in Burdwan, India with reference to coliforms. Afr. J. Biotechnol. 6: 2601 – 2602.

Edema, M.O., Omemu, A.M. and Fapetu, O.M. (2001). Microbiology and physicochemical analysis of different sources of drinking water in Abeokuta, Nigeria. Niger. J. Microbiol. 15: 57 – 61.

Egwari, L. and Aboaba, O. (2002). Environmental impact on the bacteriological quality of domestic water supplies in Lagos, Nigeria. Rev. Saude Publica. 36(4): 513 – 520.

Food and Agricultural Organization (FAO) (1997). Annual Report on Food Quality Control 1: 11 – 13.

Federal Environmental Protection Agency (FEPA) (1999). Nigerian standards on drinking water. Bull. Natl Policy Environ., 2: 12 – 13.

Gerdin, D.N., Larson, T.A., Hughes, R.A., Weller, M., Shanholzter, C. and Peterson, L.R. (1991). Aminoglycoside resistance and aminoglycoside usage: ten years of experience in one hospital. Antimicrob. Agents Chemother. 35: 1284 – 1290.

Hazen, T.C. and Toranzos, G. A. (1990). Tropical source water. In: Drinking water microbiology, McFeters, G.A. (Ed.). Springer, New York. Pp. 32 – 52.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., and Williams, S.T. (1994). Bergey's Manual of Determinative Bacteriology. 9th Edition. William and Wilkins, Baltimore.

Hughes, J.M. and Kaplan, J.P. (2005). Saving lives through global safe water. J. Emerg. Infec. Dis. 11(10):1636 – 1637.

Huyeke, M.M., Sahm, D.F. and Gilmore, M.S. (1998). Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. Emerg. Infec. Dis. 4: 239 – 249.

John, J.F., Jr. (2002). Antibiotic cycling: Is it really for prime time. Infec. Contr. Hosp. Epidemiol. 21: 9 – 11.

Kassenga, G.R. (2007). The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. J. Water Health. 5(1): 179 – 185.

Kosek, M., Bern, C. and Guerrant, R.L. (2003). The global burden of diarrhoeal diseases as estimated from studies published between 1992 and 2000. Bull. World Health Org. 81: 197 – 204.

Kumar, H.S., Parvathi, A., Karunasagar, I. and Karunasagar, I. (2005). Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. World J. Microbiol. Biotechnol. 21: 619 – 623.

Kummerer, K. (2003). Significance of antibiotics in the environment. J. Antimicrob. Chemother. 52: 5 – 7.

Lamikanra, A. (1999). Essential Microbiology for Students and Practitioners of Pharmacy, Medicine and Microbiology. 2nd Ed. Amkra Books Lagos. P. 406.

McGeer, A. (1998). Agricultural use of antibiotics and resistance in human pathogens: villain or scapegoat. Can. Med. Assoc. J. 159: 1129 -1136.

McGowan, J.E., Jr. (2000). Strategies for study of the role of cycling on antimicrobial resistance. Infec. Control Hosp. Epidemiol. 21: S36 – S43.

Moss, W.J., Beers, M.C., Johnson, C., Nicols, D.G., Perl, T.M., Dick, J.D., Veltri, M.A. and Willoughby, R.E., Jr. (2002). Pilot study of antibiotic cycling in a paediatric intensive care unit. Crit. Care Med. 30: 1877 – 1882.

Nikoladze, G.D.M. and Akastal, S. (1989). Water treatment for public and industrial supply. MIR Publisher, Mosoul. p. 163.

Ogan, M.T. (1992). Microbiological quality of bottled water sold in retail outlets in Nigeria. J. Appl. Bacteriol. 73: 175 – 181.

Office Internationale des Epizotics (OIE) (1999). The use of antibiotics in animals – ensuring the protection of public health. Proc. Sci. Conf., Paris, France.

Okonko, I.O., Adejoye, O.D., Ogunnusi, T.A., Fajobi, E.A. and Shittu, O.B. (2006). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. Afr. J. Biotechnol. 7(3): 617 – 621.

Okonko, I.O., Ogunjobi, A.A., Adejoye, O.D., Ogunnusi, T.A. and Olasoja, M.C. (2008). Comparative studies and microbial risk assessment of different water samples used for processing frozen sea foods in Ijora-olopa, Lagos State, Nigeria.

Olayemi, A.B. (1999). Microbial potability of bottled and packaged drinking waters hawked in Ilorin metropolis. Int J. Environ Health Res. 9(3): 245 – 248.

Parashar, U., Bresee, J.S. and Glass, R.I. (2003). The global burden of diarrhoeal disease in children. Bull. World Health Org. 81: 236.

Rahal, J.J., Urban, C., Horn, D., Freeman, K., Segal-Maurer, S., Maurer, J., Manano, N., Marks, S., Burns, J.M., Dominic, D. and Lim, M. (1998). Class distinction of cephalosporin use to control total cephalosporin resistance in nosocomial *Klebsiella*. J. Am. Med. Assoc. 280: 1233 - 1237.

Raymond, D.P., Pelletier, S.J., Crabtree, T.D., Gleason, T.G., Hamm, L.L., Pruett, T.L. and Sawyer, R.G. (2001). Impact of rotating empiric antibiotic schedule on infectious mortality in an intensive care unit. Crit Care Med. 29: 1101 – 1108.

Umeh, C.N., Okorie, O.I. and Emesiani, G.A. (2005). Towards the provision of safe drinking water: The bacteriological quality and safety of sachet water in Awka, Anambra State. In: Book of abstracts of the 29th Annual Conference and General Meeting of the Nigerian Society for Microbiology, University of Agriculture, Abeokuta. p. 22.

World Health Organization (WHO) (2004). Water, sanitation and hygiene links to health: Facts and figures updated November, 2004.

World Health Organization (WHO) (2005). The WHO report 2005 - make every mother and child count, Geneva.

World Health Organization (WHO) (2006). WHO guidelines on drinking water quality. First addendum to 3rd edition, vol. 1. p. 132.

White, A.C., Jr., Atmar, R.L., Wilson, J., Cate, T.R., Stager, C.E. and Greenberg, S.B. (1997). Effects of requiring prior authorization for selected antimicrobials: expenditures, susceptibilities, and clinical outcomes. Clin. Infec. Dis. 25: 230 – 239.

Williams, R.J. and Heymann, D.L. (1998). Containment of antibiotic resistance. Science 279: 1153 – 1154.

Wise, R., Hart, T., Cars, O., Streulens, M., Helmuth, R., Huovinen, P. and Sprenger, M. (1998). Antimicrobial resistance is a major threat to public health. Br. Med J. 317: 609 – 610.