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River Opa - A potential agent for the dissemination of multiple-antibiotic resistant bacteria

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In the process of determining the bacteriological quality of water from the Opa River, samples were taken from the river at five different points on five separate occasions. These samples were analysed by the most probable number method and by filtration, and the bacterial isolates obtained were identified by standard methods. The isolates obtained were tested for their resistance to thirteen antibiotics using the disc agar diffusion method, and four of the isolates were further tested for their ability to transfer their antibiotic resistances by conjugation. The microbial load at the different sampling points ranged between 0.01 × 10^2 and more than 300 × 10^2 while the most probable number of coliforms in 100 ml of samples were in excess of 1,800 cfu. The organisms most frequently isolated from the samples included those of the Genera *Enterobacter, Enterococcus, Klebsiella, Flavobacteria, Proteus* and *Streptobacillus.* The Opa River was found to be contaminated along its length by multiply antibiotic resistant organisms, some of which had the ability to transfer their resistance to another organism. Antibiotics to which the isolates were resistant included tetracycline (100%), ampicillin (98.9%), cephalothin (95.5%), doxycycline (92.4%), chloramphenicol (25.8%), tobromycin (24.2%) and spectionomycin (21.1%). Approximately, 30% of the isolates were resistant to norfloxacin while 6.1% were resistant to ciprofloxacin. The transferable resistances included those to tetracycline, cephalothin, trimethoprim and erythromycin. The Opa River is a source of antibiotic resistant organisms, and the presence of such organisms in this body of water which is used for many purposes within the area suggests a means whereby these organisms and the antibiotic resistances which they carry can spread through the populations that come in contact with the river.

Key words: Antibiotic resistance, contamination, Opa River, South-Western Nigeria.

INTRODUCTION

Water is essential to life, as all biochemical reactions which initiate and maintain life take place in the aqueous environment of the cell so that, as pointed out by Nester et al. (2004) and Chaplin (2007), there can be no life in the absence of water. Water is a physiological requirement but more than this, it is also required for a plethora of activities, some of which have been identified by Conway et al. (1996) as drinking, culinary purposes, bathing, washing, laundering, agricultural purposes, fire protection, fishing, swimming and other recreational activities, as well as navigation. By far, the most important use of water is drinking, and water used for this

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purpose has to be of good quality. According to The World Health Organization (1996, 2001, 2003, 2008), whilst water intended for human consumption should be pleasant to drink, coolness, absence of turbidity, colour and any disagreeable taste or smell must also be regarded as being desirable. In addition, such water must be free of chemical impurities as well as microorganisms, especially the coliforms which are associated with diarrhoeal diseases.

In developing countries such as Nigeria, most of the people have very restricted access to potable water supplies and rely primarily, if not solely on surface waters from rivers, streams, lakes, reservoirs, ponds and shallow wells for their daily water supplies. Nester et al. (2004), Environmental Protection Agency (EPA) (2007) and Tarver (2008) have however identified these bodies of water as habitats for a large number of microorganisms some of which may be pathogenic to humans. The most important sources of contamination in such bodies of water are human beings and animals which defecate in water meant for human consumption. Water may also be contaminated by the discharge of sewage and sediments into rivers and other water sources, which as pointed out by Atlas and Bej (1990), may also be contaminated with human and animal waste.

Organisms found in water, even when they are not pathogenic, may be carriers of antibiotic resistances and this issue, as pointed out by Ash et al. (2002) and American Public Health Association (APHA) (2004) has become one of global concern. The most frequently encountered antibiotic resistant microorganisms in fresh water are species of *Actinobacter*, *Alcaligenes*, *Citrobacter, Enterobacter, Pseudomonas* and *Serratia*, and various studies have reported the isolation of multiply antibiotic resistant organisms from fresh water sources (French et al., 1987; Ogan and Nwiika, 1993; Young, 1993; Ash et al., 2002), thus suggesting that such waters could be associated with the dissemination of antibiotic resistant organisms within communities which have access to waters in which these organisms are found.

Since water has been reported to be a source of antibiotic resistant organisms, the high and steadily increasing incidence of antibiotic resistant organisms which has been reported by Okeke et al. (2000) among others may be at least partly derived from organisms present in rivers, streams and other bodies of water within the study environment. This being the case, it was thought expedient to isolate organisms from the Opa River flowing through the semi-urban community of Ile-Ife and subject such organisms to antibiotic testing.

MATERIALS AND METHODS

Study site

River Opa serves a large population in some parts of Western Nigeria, including the Obafemi Awolowo University (OAU), Ile-Ife, where the river has been dammed and the water treated to provide a source of pipe-borne water for drinking, domestic and other purposes within the university. The river's source is in Esa-Oke in Osun-state and flows through many towns and villages (Figure 1) before emptying into the Osun River at Asejire which has been dammed to supply water to Ibadan, a large and expanding city with a population in excess of 2 million people.

Collection, isolation and identification of bacteria

Samples of water from the Opa River were collected at five different points around Ile-Ife on five different occasions over a period stretching from 8th December, 2003 to 12th February, 2004 into sterile sample-bottles, and bacteriological examinations were made as described by the modified protocols of Ash et al. (2002). In collecting the samples, the bottle cover cap was aseptically removed from the sterile bottle which was clamped to the end of a stick. With the bottle mouth facing upstream, it was plunged downwards (about 30 cm) below the water surface, and then tilted slightly upwards to allow it to be filled completely before carefully replacing the cap. The bottle was labelled, placed in an insulated cold box and immediately transported to the laboratory for analysis.

In the laboratory, the detection and enumeration of thermo-tolerant organisms and presumptive *Escherichia coli* were performed by the modified standardized multiple tube (most probable number) methods described by Talaro and Talaro (2002), APHA (1998) and the International Organization for Standardization (ISO; 9308-2: 1990). On each sampling occasion, 105 ml quantities of water were distributed (five 10 ml double strength, five 1-ml single strength and one 50-ml double strength amounts) in bottles of sterile MacConkey broth purple (Oxoid, England) to which were added inverted Durham tubes for gas collection. Samples were incubated at 37°C for 24 ± 2 h. A positive test resulted in the production of acid (a yellow colour) and the number of tubes showing this result was counted and referred to the McCrady's probability table. Simultaneously, a positive presumptive test which constitutes a modified standardized method in microbiological examination of water, as described by APHA (1998) was also employed. Water samples were filtered through a 0.45 µm cellulose ester membrane and filters were placed on the surface of sterile dried MacConkey and Eosine Methlylene Blue (EMB) agar media (Oxoid, England). Plates were incubated at 37°C for 18 h. All colonies that were cultured from the membrane filters were sub-cultured for pure isolates and identification of isolates was by conventional characterization (Ewing, 1986; Holt et al., 1994; Farmer, 1995).

Antimicrobial susceptibility tests

The standard disk agar diffusion method according to guidelines recommended by Clinical Laboratory Standards Institute (CLSI) (2006) was used for susceptibility testing with iso-sensitest (Oxoid, England) agar. The antibiotic disks used were: ampicillin (10 µg), chloramphenicol (30 µg), cephalothin (30 µg), ciprofloxacin (10 µg), doxycycline (30 µg), erythromycin (15 µg), nalidixic acid (30 µg), sulfisomidine (250 µg), streptomycin (30 µg), spectinomycin (25 µg), tobramycin (30 µg), trimethoprim (5 µg) and tetracycline (30 µg), all obtained from AB Biodisk, Sweden. Susceptibility break points were defined according to manufacturer's recommendations. An isolate was considered resistant if it had either intermediate or high-level resistance to an antibiotic. *E. coli* NCTC 10418 and K-12

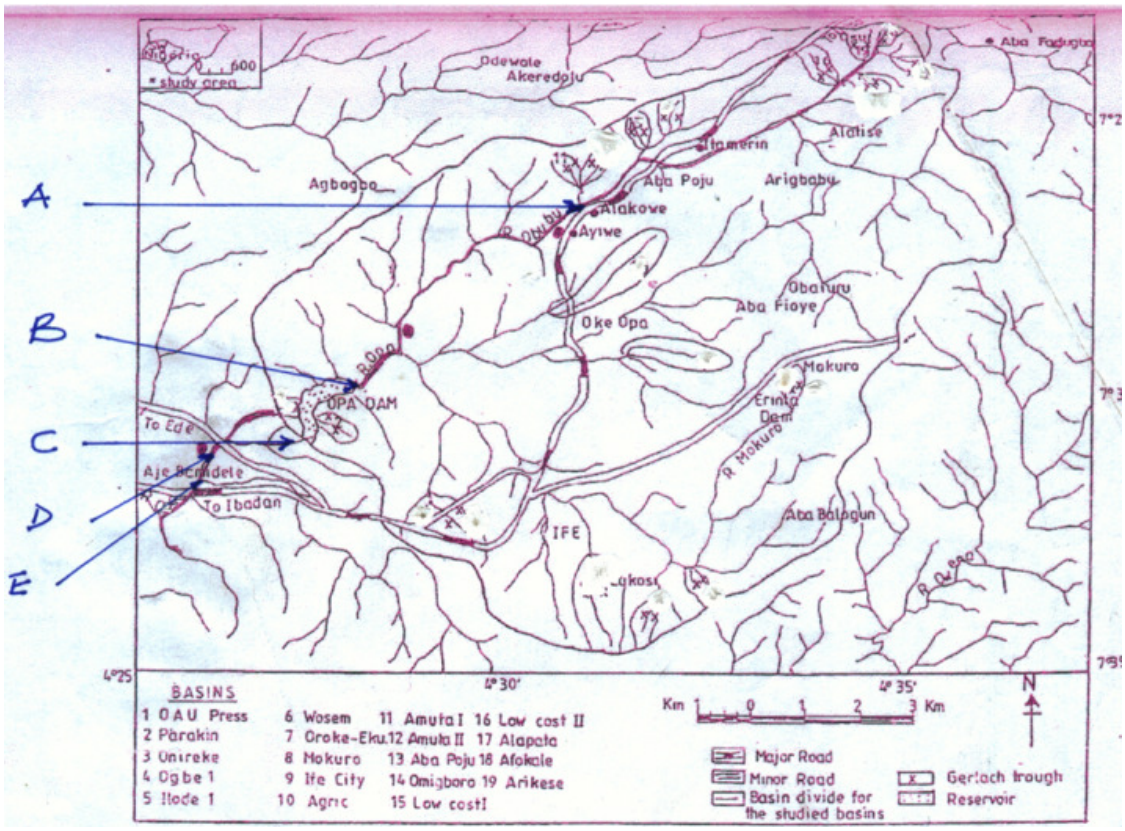

Figure 1. The study area and basins in Opa reservoir catchment. A: Alakowe (Opa); B: Road 7 Junction (OAU); C: campus gate, Ede Rd., immediately after the University dam; D: Ajebamidele before abattoir; E: after the abattoir.

C600 were used as controls.

In vitro transconjugation

The protocol described by Sundström et al. (1987) was used to determine the ability of the isolates to transfer their antibiotic resistance to C600, a plasmidless strain of *E.coli*. Each isolate that was resistant to tetracycline was mated with *E. coli* C600. A 0.20 mL portions of the overnight cultures of both the donor and recipient were transferred onto surface of over-dried Iso-sensitest agar plates and the conjugation mixtures were incubated at 37°C for 24 h. Growth was harvested into a sterile test-tube and washed down with 3.00 mL portions of freshly prepared phosphate buffered saline (pH 7.2). The suspensions obtained were then streaked out on recovery plates (Iso-sensitest agar containing 40 mg/L of nalidixic acid and 40 mg/L tetracycline. Colonies growing after incubation at 37°C for 24 h were further sub-cultured for pure isolates on freshly prepared recovery plates. Authenticated trans-conjugants were then subjected to antibiotic susceptibility patterns in accordance to protocols described above (CLSI, 2006).

RESULTS

Twenty-five water samples were taken from the Opa River at five different locations during the period under study Table 1. The organisms most frequently isolated in the course of this exercise were of the Genera; *Enterobacter, Enterococcus, Klebsiella, Flavobacteria, Proteus* and *Streptobacillus* (Table 2). Of the 66 bacterial isolates from the water samples tested for antimicrobial resistance, all (100%) isolates showed resistance to at least one of the antibiotics (Table 3). As shown, all the isolates were found to be resistant to tetracycline. Resistance to ampicillin was encountered in 65 (98.9%) of the isolates, whilst 95.5% of the isolates were resistant to cephalothin. Resistances to doxycycline (92.4%), erythromycin (89.4%), nalidixic acid (87.9%), trimethoprim (69.7%), sulfisomidine (36.4), chloramphenicol (25.8%), tobromycin (24.2) and spectionomycin (21.1%) were also observed. Approximately, 30% of the isolates were resistant to norfloxacin; however, only 4 (6.1%) of 66 were resistant to ciprofloxacin. The four isolates which were resistant to tetracycline but sensitive to nalidixic acid were able to transfer their resistance to C600, a plasmidless, nalidixic acid resistant strain of *E. coli*. All four isolates transferred

Table 1. Microbial load of River Opa at five (5) sampling locations during the period December, 2003 through February, 2004.

Date	Sampling location	CFU/ml (×10^2)	Coliform (MPN/100 ml)
	A	0.01	>1800
	B	41	>1800
8/12/03	C	16	>1800
	D	3.5	>1800
	E	>300	>1800
	A	73	>1800
	B	>300	>1800
22/12/03	C	2.1	>1800
	D	3.2	>1800
	E	12	>1800
	A	36	>1800
	B	>300	>1800
14/01/04	C	14.4	>1800
	D	2.8	>1800
	E	>300	>1800
	A	37	>1800
	B	120	>1800
14/02/04	C	14.8	>1800
	D	3.4	>1800
	E	>300	>1800
	A	49	>1800
	B	180	>1800
18/02/04	C	12.4	>1800
	D	4.4	>1800
	E	164	>1800

A: Alakowe (Opa); B: Road 7 Junction (OAU); C: Campus gate, Ede Rd., immediately after the University dam; D: Ajebamidele before abattoir; E: after the abattoir.

tetracycline resistance along with cephalothin, while two of them transferred both trimethoprim and erythromycin resistances along with tetracycline resistance. One of the isolates was able to transfer resistances to five different antibiotics.

DISCUSSION

Easy access to potable water is an important public health factor given the ability of water to encourage the spread of diarrhoeal diseases. This is of special importance in developing countries where water of good quality is often very difficult to find. Even in developed countries however, the availability of potable water can no longer be taken for granted given the report of Ramirez and Williams (2004) that water supplies are becoming increasingly difficult to find all over the world. This difficulty is deemed to have been caused by increases in human population, increased per capita consumption of water; especially in developed countries and the impact of human activities on the global environment. The absence of appropriate technology in the developing countries is an added complication to this problem in those countries.

Even when there are facilities for water purification, the problem of adequate water supplies is still of pivotal importance as water meant for purification should not be grossly contaminated with coliforms. The work of Guardabassi et al. (1998), Sánchez-Pérez et al. (2000),

Table 2. Microbial isolates from River Opa at five (5) sampling locations during the period December, 2003 through February, 2004.

Date	Sampling location				
	A	B	C	D	E
8/12/03	*Klebsiella* spp., *Enterobacter* spp.	*Hafnia, Enterobacter* spp.,	*Xanthomonas, Enterococcus* spp., *Megasphoera, Actinomyces.*	*Cedecca* spp., *Streptobacillus* spp., *Enterobacter* spp.	*Enterobacter* spp., *Enterococcus* spp
22/12/03	*Proteus* spp., *Flavobacterium* spp., *Enterobacter* spp.	*E. coli, Klebsiella* spp.	*Proteus* spp., *Staphylococcus* spp., *Enterococcus* spp.	*Cedecca* spp., *Streptobacillus* spp.	*Staphylococcus* spp., *Proteus* spp.
14/01/04	*Flavimonas* spp., *Flavobacterium* spp.	*Enterobacter* spp.	*Enterobacter* spp., *Enterococcus* spp., *Staphylococcus* spp.	*Streptobacillus* spp., *Norcardia* spp.	*Pseudomonas* spp.
14/02/04	*Enterobacter* spp., *Flavobacterium* spp., *Pseudomonas* spp.	*Enterobacter* spp., *Klebsiella* spp.	*Pseudomonas* spp., *Enterobacter* spp.	*Streptobacillus* spp., *Norcardia* spp.	*Xanthomonas* spp.
18/02/04	*Serretia* spp., *Enterobacter* spp., *Klebsiella* spp., *Actinobacillus* spp., *Flavobacterium* spp.	*Klebsiella* spp., *Enterobacter* spp.	*Enterobacter* spp.	*Cedecca* spp., *Streptobacillus* spp., *Norcardia* spp.	*Enterobacter* spp., *Actinomyces* spp.

A: Alakowe (Opa); B: Road 7 junction (OAU); C: Campus gate, Ede Rd., immediately after the University dam; D: Ajebamidele before abattoir; E: After the abattoir.

Table 3. Percentage of organisms resistant to the antibiotics used in study.

Antibiotic	Percentage resistant organisms
Ampicillin	98.8
Cephalothin	95.5
Tetracycline	100
Doxycycline	92.4
Nalidixic acid	87.9
Norfloxacin	30.3
Ciprofloxacin	6.1
Chloramphenicol	25.8
Spectinomycin	21.1
Tobramycin	24.2
Erythromycin	89.4
Sulfisomidine	36.4
Trimethoprim	69.7

Ensink et al. (2004) and Mazari-Hiriart et al. (2008) suggest that the variety as well as densities of human pathogens present in water are related to the population from which they originate, the waste water and treatment system, the diseases prevalent in the human population as well as contributions from agriculture, animal husbandry and industry. The isolation of various organisms derived from the gastro-intestinal tract in the course of this study suggests that the Opa River is contaminated along its length by faecal matter derived from the people and animals that have access to the river.

Workers in other parts of the world have made a similar observation in respect of organisms such as *Enterobacter, Enterococcus, Klebsiella, Proteus, Streptobacillus, Staphylococcus, E. coli, Pseudomonas* and *Serratia* (Niemi et al., 1983; French et al., 1987; Roszak and Colwell, 1987; Ogan and Nwiika, 1993; Quintiliani et al., 1999; Ash et al., 2002) which have been isolated from various rivers in different parts of the world. The isolation of *E. coli* from the Opa River also suggests that the contamination of this river is probably continuous so that water from the Opa River should be regarded as a source of potentially pathogenic organisms which furthermore carry multiply antibiotic resistances and therefore are a danger to the people who live along the river. The danger posed by these organisms is exacerbated by the fact that many of these people do not have a source of treated water and must use the river water for many purposes, including drinking, thus the opportunity exists for acquiring water borne infections from the river.

Opportunistic pathogens such as those isolated in this study are naturally present in the environment and are not formally regarded as pathogens but they are able to cause disease in people with impaired local or general defence mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS). Should water used by such groups of people contain large numbers of these organisms for drinking or bathing, it can lead to various infections of the skin and the mucous membranes of the eye, ear, nose, and throat. Examples of such agents are *Pseudomonas aeruginosa* and various species of *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Serratia*, *Aeromonas*, and certain "slow-growing" mycobacteria.

An important aspect of water contamination in this environment is the fact that the organisms isolated were all found to carry resistances to antibiotics. Similar findings have been reported from a number of natural and man-made environments (Pillai et al., 1997; Goni-Urriza et al., 2000; Roe et al., 2003; Oyedeji et al., 2011) and these resistant organisms have been said to be either indigenous to, or introduced through natural or anthropogenic causes within the natural environments (Wegener et al., 1999; American Academy of Microbiology, 1999). It is particularly noteworthy that some of the resistant organisms isolated in the course of this study were found to transfer their resistance to a recipient organism so that even if the organisms themselves are not pathogenic, they are easily capable of transferring their resistances to frank pathogens within the gastrointestinal tract, this may be regarded as being one way through which antibiotic resistances are acquired by organisms infecting people living within this environment.

According to Okeke et al. (2000), the incidence of bacterial resistance within the study environment is large and growing, and even though antibiotics use is prevalent within the study environment, the volume of consumption is not large enough to explain the widespread incidence of antibiotic resistances. As the results of this study suggest, there are environmental sources of antibiotic resistant organisms within the study environment and the presence of such organisms in the water, which is used for many purposes within the area, suggests a means by which these organisms can spread through the populations that come in contact with the water. These results highlight the need for the provision of treated water to the people living along the banks of the Opa River. The distribution of adequately treated water within the study environment should be helpful in curtailing the spread of antibiotic resistant organisms within the area of study and indeed in other localities which suffer the same level of exposure to antibiotic resistant organisms.

Conclusion

The results of this study show that the River Opa which runs through many rural and semi-urban communities in Osun State in South-Western Nigeria is contaminated along its length with multiply antibiotic resistant bacteria, some of which are capable of transferring their resistances to other organisms. Infections with these antibiotic resistant organisms are likely to be difficult to treat and their antibiotic resistances when transferred into other pathogens or commensals, increase the reservoir of antibiotic resistances within the host, and by extension the community in which public health facilities are practically non-existent. The results of this study highlight the need for the provision of treated water to the communities along rivers like the Opa, and in the absence of such facilities, the need to educate the people about the dangers of using untreated water from rivers for drinking and other domestic purposes.

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