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Full Length Research Paper

Antibiotic resistant pattern of isolated bacteria from Obere River in Orile-Igbon, Oyo State, Nigeria

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Good quality water must be odourless, colourless, tasteless and free from pathogens. This is adversely affected when it is polluted beyond certain limits. Potable water for domestic use should however be free of pathogenic organisms. Water samples were collected from Obere River in Orile-Igbon, Oyo state, Nigeria in order to study the antibiotic resistance pattern of isolated bacteria. The bacteria genera detected were *Pseudomonas*, *Bacillus*, *Proteus* and *Flavobacterium*. The isolates were subjected to antibiotic sensitivity test with about 90% showing multiple antibiotic resistance. All the isolates have a high resistance rate (100%) against amoxicillin, augmentin (100%), streptomycin (95%), chloramphenicol (95%) and ceftriaxone (90%); while, the least resisted antibiotics was ciprofloxacin (35%). It was concluded that Obere River in Orile-Igbon, Oyo State is polluted with pathogenic bacteria showing multiple antibiotic resistance. Thus, there is need for proper treatment of water in this study area before distribution for consumption and general domestic usage.

Key words: Water samples, Obere River, antibiotic sensitivity test, multiple antibiotic resistance.

INTRODUCTION

In the present era, man has made flowing water a medium for getting rid of his wastes from anthropogenic sources thus introducing noxious substances into them consequently polluting the water bodies. These wastes adversely affect the physico-chemical properties as well as the microbial component of the water bodies which then affect lives in it. Obere River is located in Orile igbon, Ogbomoso, Oyo State, Nigeria, with poultry farm, cement industry, market, cassava processing sites and arable crop farms located along its course. The river serves as a source of water for nearby dwellers; it is used for drinking, cooking, swimming, etc.

Contaminated water bodies often serve as natural habitats of pathogenic coliforms, thereby playing a role in disease process (Ademola et al., 2009). Antibiotic resistance then sets in as a result of insurgent sub-therapeutic concentration of antibiotics in wastewater discharged into natural water bodies (Diwan et al., 2010). In developing countries, high population growth has led to increased human activities. These activities have cumulated to indiscriminate dumping of refuse, waste disposal, etc (Akubugwo and Duru, 2011) in water bodies hence, making accessibility and availability of clean and uncontaminated water difficult (Oladipo et al., 2011; Ogbonna et al., 2011).

Studies have been done by various researchers to measure chemical and microbial contaminants in ground water and surface water such as streams, lakes and rivers. Chukwu (2008) reported on the ground water pollution from abattoir waste in Minna state. Garba et al. (2010) reported a mean arsenic concentration of 0.34 mg/l in drinking water from hand dug wells, boreholes and taps of Karaye Logal Government area, Kano state. Olaoye and Onilude (2009) have documented varying

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levels of microbial contaminations in drinking water from western parts of Nigeria. Indeed, the microbial quality of potable water should not exceed the limits specified in the water quality guideline (APHA, 1998). Before the abusive antimicrobial usage, only a slight resistance level had been detected among enteric bacterial pathogens. Nowadays susceptibility to antimicrobials has changed and resistant patterns have been used as epidemiological markers (Bechtlufft et al., 2008). The indiscriminate usage and subsequent release of residual antibiotics in wastewater is considered an important factor for the emergence, selection and dissemination of antimicrobial resistant bacteria. Drinking water must meet specific criteria and standards to ensure that water supplied to the public is safe and free of pathogenic micro organisms as well as hazardous compounds. Therefore, this research work is meant to microbiologically analyze Obere River which is consumed by Orile-Igbon residents in Oyo State and determine if its portability is justified.

MATERIALS AND METHODS

Sample collection

Water samples were aseptically collected from Obere River, Orile-Igbo, Ogbomoso, Oyo state. For the purpose of this study, the river was divided into three sampling points. Samples were collected into 250 ml sterile plastic container from the three sampling points between 9:00 am and 10:00 am. The samples were collected once in a month for a period of three months.

Media preparation

Nutrient Agar powder weighing 14 g was dispensed in 500 ml of distilled water in conical flask. After mixing the solution, it was heated gently to dissolve and then autoclaved. The agar was allowed to cool to about 45°C then poured into sterile disposable Petri dishes.

Isolation of microorganisms

Serial dilution was carried out on the samples and then inoculated unto nutrient agar plate using the spread plate method. The plates were incubated at 35°C for 24 h. The colonies observed on the plates were then subcultured and stored on slants at 4°C for further use.

Identification of isolated micro organisms

Biochemical and morphological characterization of the isolates were done using the manual of Berger and Petz (1991).

Antibiotic sensitivity test

The *in vitro* antibiotic susceptibility test of the bacterial isolates was performed using the standardized disc diffusion method described by Bauer et al. (1996). Sterile Petri dishes of Mueller Hinton agar were prepared. 0.1 ml of each organism was then seeded into Mueller-Hinton agar plates and allowed to stand for 45 min. It was investigated using Fondoz laboratory antibiotic sensitivity disc for both Gram positive and Gram negative discs containing: Augmentin- 30 µg, Amoxicillin - 25 µg, Erythromycin- 5 µg, Tetracycline- 30 µg, Nitrofurantoin- 200 µg, Gentamycin-10 µg Cotrimoxazole- 25 µg, Oxofloxacin- 5 µg, Pefloxacin- 5 µg, Amoxicillin- 25 µg, Chloramphenicol-30 µg, Ceftriaxone-30µg, Gentamycin-10 µg, Pefloxacin-5 µg, Cotrimoxazole- 25 µg, Ciprofloxacin-30 µg, Erythomycin- 3 µg. The commercial antibiotic discs were placed on the prepared plates previously seeded with 18 hours broth culture of the test organisms. The plates were incubated at 37°C, 48 h after which the zones of inhibition were measured in millimeters and interpreted accordingly considering the appropriate break point (Andrew, 2008).

Table 1. Distribution and proportion of bacteria in Obere River.

Isolates identified	Prevalence
Pseudomonas spp.	10 (50%)
Proteus spp.	4 (20%)
Flavobacterium spp.	4 (20%)
Bacillus spp.	2 (10%)

RESULTS

A total number of 20 bacterial isolates belonging to 4 genera were obtained. *Pseudomonas*, *Flavobacterium*, *Bacillus* and *Proteus,* their distribution are as shown in Table 1. A high occurrence was obtained in *Pseudomonas* species 10 (50 %), followed by *Proteus* species 4 (20%), *Flavobacterium* species 4 (20%) and *Bacillus* species 2 (10%). The number of antibiotics to which resistance was shown is as presented in Table 2, while Figure 1 reflects the percentage resistance of the isolates to each antibiotics. Augmentin and Amoxicillin was 100% resisted, with Ciprofloxacin being the least resisted antibiotics.

Table 3 presents the antibiotic resistance profile for all the isolates. *Pseudomonas* and *Bacillus* species exhibited the highest number of resistance against the antibiotics.

DISCUSSION

All the isolates have a high resistance rate (100%) against Amoxicillin, Augmentin (100%), Streptomycin (95%), Chloramphenicol (95%) and Ceftriaxone (90 %). Moreover, from the antibiotic sensitivity profiles in Table 3, each bacterium showed resistance to minimum of seven antibiotics, indicating the multiple resistance pattern characteristic of the isolated bacterial. This could probably result from a recent contamination of the water with sewage; this is a submission reached by Ajayi and Akonai (2005). Adebayo et al. (2012) also reported 96% resistance to Augmentin by most isolates from salad.

Table 2. Distribution of antibiotic resistance among bacterial isolates.

Isolates identified	Total number	Number of resistance exhibited to antibiotics												
		Aug	CRO	NIT	GEN	COT	OFL	AMX	CPX	TET	PFX	STR	CHL	ERY
Pseudomonas species	10	10	9	6	8	7	6	10	4	5	5	10	10	7
Proteus species	4	4	4	2	2	2	2	4	0	2	0	4	4	2
Flavobacterium species	4	4	4	4	4	2	0	4	2	2	2	4	4	2
Bacillus species	2	2	1	2	1	2	2	2	2	1	1	1	1	1

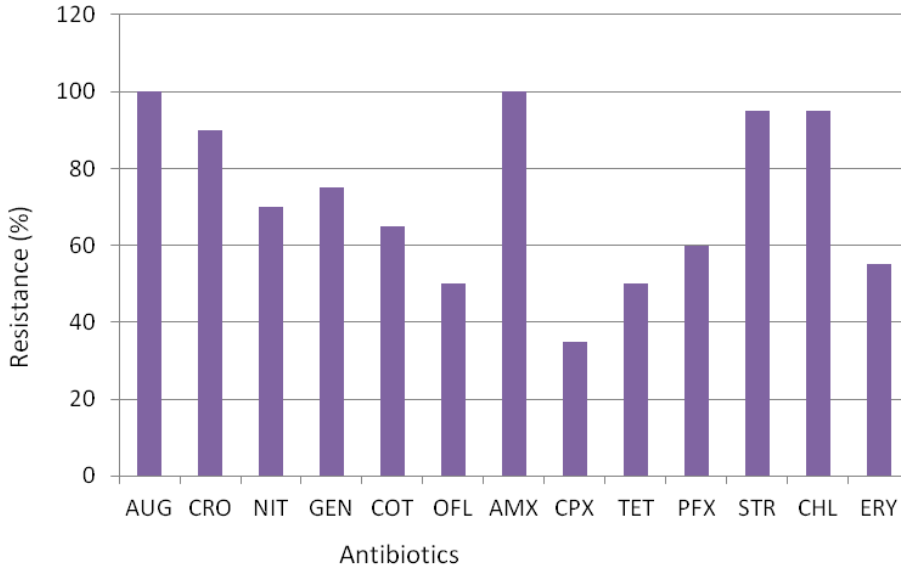

Figure 1. Antibiotics resistance pattern in percentage. AUG: Augmentin, NIT: nitrofurantoin, OFL= ofloxacin; TET: tetracycline, CPX: ciprofloxacin AMX: amoxicillin, STR: streptomycin; CHL: chloramphenicol; CRO: ceftriaxone, GEN: gentamycin, PFX: pefloxacin, COT: cotrimazole, CPX: ciprofloxacin, ERY: erythromycin.

This implies that either these isolates have been able to circumvent the active ingredient in these antibiotics or probably fake drugs are in circulation. One of the ways to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing anti-microbial agents (Adebayo and Adegoke, 2008; Akinjogunla et al., 2009a).

As shown in this study, the isolates showing resistance to the greatest number of antibiotics was identified in *Bacillus* and *Pseudomonas* strains. This result correlates with the work of Foti et al. (2009) where it was concluded that the highest rate of multiple resistant to antibiotics by Gram negative isolates from the Mediterranean water was found in *Pseudomonas* strains where antibiotics such as Amoxicillin, Tetracycline and Gentamicin were used. Al-Bahry et al. (2009) reported that the source of multiple antibiotic resistant bacteria could be from polluted effluents. Suman et al. (2013) revealed that antibiotic resistance and metal tolerance traits are conjugative plasmid borne, as they could be transferred to sensitive strains through the conjugation process. The contamination of Obere River with antibiotic resistant bacteria could be as a result of contamination with sewage or effluents from various sources such as poultry farm, cement industry, market, cassava processing sites and arable crop farm.

Conclusion

Obere River is obviously polluted with antibiotic resistant microorganisms, as shown in this research. The highest multiple antibiotic resistant was mainly demonstrated by *Bacillus* spp. and *Pseudomonas* spp. Most bacterial isolates demonstrated resistance to most antibiotics tested, but the least resistance was observed with ciprofloxacin (35%).

Multiple antibiotic resistance is significant health wise, thus, there is need for proper treatment treatment of water in this study area before distribution for consumption and general domestic usage. Authorities concerned should therefore increase surveillance of water bodies, intro-duction of new multi-active antibiotics and eradication of fake drugs from circulation.

Table 3. Antibiotic resistance profile of bacterial isolates.

Isolate	Number of isolates that demonstrated resistance	Antibiotic resistance pattern	Number of antibiotics resisted
Pseudomonas species	2	AUG, AMX, CRO, STR, CHL, GEN, ERY, COT,	8
	1	AUG, AMX, CRO, STR, OFL, CPX, CHL, GEN, PFX, COT, ERY, NIT, TET	13
	1	AUG, AMX, CRO,STR, OFL, CHL, GEN	7
	2	AUG, AMX, CRO, STR, OFL, CPX, CHL, GEN, PFX, ERY, NIT, TET	12
	1	AUG, AMX, CRO, STR, CPX, CHL, GEN, COT, ERY, NIT.	10
	1	AUG, AMX, CRO, STR, OFL, CPX, CHL, GEN, PFX, COT, ERY, NIT, TET	13
	1	AUG, AMX, STR, OFL, CHL, PFX, COT, ERY, NIT, TET	10
	1	AUG, AMX, CRO, STR, CHL, COT, ERY.	7
Flavobacterium species	2	AUG, AMX, CRO, STR, CHL, GEN, PFX, COT, ERY, NIT, TET.	11
	2	AUG, AMX, CRO, STR, CPX, CHL, GEN, COT, NIT, TET	10
Proteus species	2	AUG, AMX, CRO, STR, CHL, GEN, COT, NIT.	8
	2	AUG, AMX, CRO, STR, OFL, ERY, TET	7
Bacillus species	1	AUG, AMX, CRO, STR, OFL, CPX, CHL, GEN, PFX, COT, ERY, NIT, TET.	13
	1	AUG, AMX, OFL, CPX, COT, ERY, NIT, TET	8

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