Original Research Article

AN ASSESSMENT OF MULTIDRUG RESISTANT BACTERIAL STATUS OF OGANE-AJI RIVER, ANYIGBA, KOGI STATE

Abstract

Surface aquifers can be useful for different purposes however they can act as fomites. The multidrug resistance pattern of bacteria from Ogane-Aji River was assessed. Samples were taken from the river at two different points (Point A with a depth of 10 cm and point B with a depth of 45 cm). These samples were analyzed by the most probable number (MPN) of which point B had more MPN index/ml (180) than point A which had 79 MPN/index/ml. The bacterial isolate obtained were identified by standard microbiological methods. The isolates obtained were tested for their resistance to nine imported antibiotics and 20 indigenous antibiotics using the disk agar diffusion method. The organisms isolated from the samples included those of the genera *Staphylococcus, Bacillus, Enterobacter, Pseudomonas, Escherichia, Klebsiella, Streptococcus* and *Salmonella*. Percentage resistance to all the foreign antibiotics by the isolates were obtained {*Staphylococcus* sp.(55.5%), *Bacillus* sp. (66.6%), *Enterobacter* sp.(77.8%), *Streptococcus* sp. (88.8%), *Pseudomonas* sp. (66.6%), *E. coli, Klebsiella* (77.8%), *Streptococcus* sp.(88.8%) and *Salmonella* sp. (88.8%)while percentage resistance to all theindigenous antibiotics were *Staphylococcus* sp.(30%), *Streptococcus* sp.(20%), *Bacillus* sp.(70%), *Enterobacter* sp. (60%), *Pseudomonas* (50%), *E. coli* (50%), *Klebsiella* (40%) and *Salmonella* (20%)}. All the isolates were found to be multidrug resistant and the presence of such organisms in this body of water which is used for many purposes within the area suggest a means whereby these bacteria and the multidrug resistance properties can spread through the population in contact with the river water.

Key words: agar diffusion, antibiotics, bacteria, multidrug resistant.

Introduction

The emergence of multidrug-resistant bacteria limits the clinical use of antibiotics and as resistant bacteria become more prevalent, there is increasing concern that existing antibiotics would become ineffective against these pathogens and more expensive. Multidrug-resistant

genes conferring resistance to a wide variety of organisms have been identified in a large range of water environments including drinking water in both developed and developing countries. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The potential of drinking water to transport microbial pathogens to a greater number of people, causing subsequent illness, is well documented in countries at all levels of economic development (Argerso, 2007).

According to the World Health Organization, 80% of all diseases are attributed to unsafe water. Developing countries in particular, a/re plagued with water-related diseases such as diarrhoea which a/3ccount for 10% of the disease burden in such countries.

Antimicrobial resistance (AMR) presents a major and growing threat to effective treatment of bacterial infections. For almost a century antimicrobials have been used to control bacterial infections and disease in humans and animals. However, with increasing microbial resistance to these drugs, despite current interventions, we face a return to nineteenth century levels of morbidity (Hawkey, 2008; Chan, 2011). Hitherto, investigation and policy development for the control of AMR using surveillance data have focused largely on patterns of resistance to individual antimicrobials (Skjøt-Rasmussen *et al.,* 2009) Quantitative studies have concentrated on theoretical frameworks using simulated and in vitro experimental data (Smith *et al.*, 2002; Bergstrom *et al.*,2004), while much of our current understanding of the impacts of individual antimicrobials has been derived from small scale clinical epidemiological studies (Jiang *et al.*, 2006).

Only recently, has the broader ecological landscape occupied by the bacteria and their hosts been considered (Singer *et al.,* 2006, Martınez, 2008). Novel approaches may bring new perspectives on the origins and spread of AMR, or assist in the development of new or revised targeted interventions. The use of antimicrobials in agriculture as a major driver of AMR in pathogenic bacteria of significance to humans is an issue over which opinions are

divided (Angulo *et al.,* 2004; Wassenaar, 2005). The prophylactic and metaphylactic use in animal populations has been a particular concern (Aarestrup *et al.,* 2001), especially when the drug classes are the same as or related to the pharmaceuticals used in the control of human infections. Exposure of microbial populations to antimicrobials evidently selects for resistance; however, the critical and unresolved issue is t/he relative contribution to resistance in these populations from the different hostcommunities.

The multi-drug resistance mechanism employed by bacteria has resulted in a serious public health threat today. River water is the major source of water for household use in most rural communities in Kogi State. It is used for various purposes such as drinking, cooking, bathing and laundry; hence it serves as a perfect medium for spreading these bacteria. River contamination occurs when waste and different other pollutants are discharged into river without being properly treated (Abraham, 2010).

Multidrug resistance is rising to dangerously high level in all parts of the world. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A growing list of infections – such as pneumonia, tuberculosis, blood poisoning, gonorrhoea and food borne diseases are becoming harder and sometimes impossible, to treat as antibiotics become less effective (WHO, 2010).

Materials and methods

The samples were obtained from different points of Ogane-aji River in Anyigba, Kogi State, using sterile glass bottles. Thereafter, a ten-fold serial dilution of the samples was prepared and the enumeration of bacterial colonies was done using the pour plate method. All the inoculated plates were replicated and incubation was done at 37^{o}C for 24hrs. After moderate growth, the cfu/ml on each plate was counted and recorded with the aid of a colony counter. Then, distinct colonies were subculture until pure isolates were achieved.

The bacteria isolates were characterized using the methods described by Nester *et al.*,(2007) and thebiochemical characterization that were carried out include the catalase test describe by Chessbourg (2005), methyl red test, indole test, Voges Proskauer test, oxidase test, citrate utilization test, and urease test. The Total coliform count was estimated using the most probable number (MPN) method.

Antibiotic Susceptibility Testing of the Isolates

The Kirby-Bauer method was adopted for the antimicrobial susceptibility using the Mueller-Hinton agar. Then, identification of Multidrug Resistant Isolates was recorded through the zone of inhibition measured to the nearest millimetres.

Results

Table 1: Gram reaction and biochemical characterization of pure bacterial isolates

Isolates	Gram Reaction	Cell shape	Biochemical Tests							Probable organisms
			CAT	INDO	CIT	MR	UR	OXI	VP	
A_1	+	Cocci	+	-	+	+	-	-	+	*Staphylococcus* sp.
A_2	-	Rod	+	-	+	+	-	-	-	*Bacillus* sp.
A_3	-	Rod	+	+	+	-	-	-	+	*Enterobacter* sp.
A_4	-	Rod	+	-	+	-	-	+	-	*Pseudomonas* sp
B_1	-	Rod	+	+	-	+	-	-	-	*E. Coli*
B_2	-	Rod	+	-	+	-	+	-	+	*Kelbsiella* sp
B_3	+	Cocci	-	-	-	+	+	-	+	*Streptococcus* sp.
B_4	-	Rod	+	+	-	-	-	-	-	*Salmonella* sp

KEY: CAT = Catalase OXI = Oxidase INDO = Indole URE = Urease CIT = Citrate MR = Methyl Red VP = Voges-Proskauer

The Gram reaction and biochemical characteristics of the pure bacterial isolates from Ogane-Aji River water is shown in Table 1. A total of eight isolates were isolated and characterized. Table 2 showed the results of antibiotic sensitivity test carried out on the pure bacterial isolates of the Ogane-Aji River water using the imported antibiotic disks while Table 3 showed the antibiotic sensitivity of the Gram positive isolates to the indigenous disks.

Table 2: Antibiotic susceptibility test of isolates (imported disks)

Chemical class of antibiotics	**Antibiotics**	***Staphylococcus* sp.**	***Bacillius* sp.**	***Enterobacter* sp.**	***Pseudomonas***	***E. coli***	***Klebisella***	***Streptococcus* sp.**	***Salmonella sp***
β-lactam	AMP(10µg)	I	R	R	R	R	R	R	R
	OX(1µg)	R	R	R	R	R	R	R	R
	AUG(30µg)	R	R	R	R	S	R	R	S
Macrolide	VAN(30µg)	S	S	S	S	R	I	R	R
Cephalosporin 3 & 4 generation	CAZ(30µg)	R	R	R	R	R	R	R	R
	CTX(30µg)	R	R	R	R	R	R	R	R
Tetracycline	TE(30µg)	R	R	R	R	R	R	R	R
	DO(30µg)	S	S	R	R	R	R	R	R
Chloramphenicol	C(30µg)	S	S	I	S	S	I	S	R
	Susceptible	33.3%	33.3%	11.1%	22.2%	22.2%	0%	11.1%	11.1%
	Intermediate	11.1%	0%	11.1%	11.1%	0%	22.2%	0%	0%
	Resistant	55.5%	66.6%	77.8%	66.6%	77.8%	77.8%	88.8%	88.8%

KEY: AMP = ampicillin OX = oxacillin VAM = vancomycin AUG = amoxicillin/clavulanic acid C = chloramphenicol CAZ = ceftazidime CTX = cefotaxime TE = tetracycline DO = doxycyline S = susceptible I = intermediate R = resistant

Table 3: Antibiotic susceptibility of gram positive isolates (indigenous disk)

Chemical Class of Antibiotics	**Antibiotics**	***Staphylococcus* sp.**	***Streptococcus* sp.**
Fluoroquinolones	CPX(10µg)	S	S
	NB(10µg)	R	I
	LEV(20µg)	S	S
Aminoglycosides	GN(10µg)	S	S
	S(30µg)	I	S
Macrolide	E(30µg)	S	S
β-lactam	AML(20µg)	I	R
	APX(20µg)	I	R
Antitubercular	RD(20µg)	R	I
Chloramphenicol	CH(30µg)	R	I
	Susceptibility	40%	50%
	Intermediate	30%	30%
	Resistant	30%	20%

The range of resistance is between 20-30%

KEY:CPX =ciproflox NB = norfolaxinGN = gentamycinAML = amoxilS = streptomycin RD = rifampicin E = erythromycinCH = chloramhenicolAPX = ampiclox, LEV = levofloxacin

The antibiotic sensitivity of the Gram negative isolates from Ogane-Aji River is shown in

Table 4. The six genera of bacterial isolates from the river water were tested

Table 4: Antibiotic susceptibility of Gram negative isolates

Chemical Class of Antibiotics	***Antibiotics***	***Bacillus* sp.**	***Enterobacter* sp.**	***Pseudomonas* sp**	***E. Coli***	***Klebsiella* sp**	***Salmonella* sp**
Fluoroquinolones	OFX(10µg)	S	R	S	S	S	S
	CPX(10µg)	S	R	S	R	S	S
	NA(30µg)	R	I	R	R	R	I
β-lactam	AU(20µg)	R	I	S	R	I	S
	PN(20µg)	R	R	R	S	R	I
Aminoglycosides	GN(30µg)	R	S	R	R	R	R
	S(20µg)	R	R	R	S	I	I
Cephalosporin	CEP(30µg)	R	R	R	R	R	I
Sulfonamides	SXT(20µg)	S	R	S	I	I	S
Reflacine	PEF(10µg)	R	S	S	I	I	R
	Susceptibility	30%	20%	50%	30%	20%	40%
	Intermediate	0%	20%	0%	20%	40%	40%
	Resistant	70%	60%	50%	50%	40%	20%

KEY: OFX = tarivid PEF = reflacine CPX = ciproflox AU = augmentin GN = gentamycin S = streptomycin. CEP = ceporex NA = nalidixicacid SXT = septrin PN = ampicilin

Most Probable Number (MPN) results for the estimation of number of bacteria per 100ml of the water sample is shown in Table 5

Table 5: MPN of the isolates from Ogane-Aji river water

Sample site	No. of tubes giving			MPN index per 100ml	95% confidence limit	
	10ml	1ml	0.1 ml		low	High
A	5	3	0	27	9	80
B	5	3	3	33	11	93

Discussion

Water sample collected from Ogane-aji River in Anyigba, Kogi State was used to assess the multidrug resistance status of the river. Two organisms were Gram positive (Tables 3) while six organisms were Gram negative (Tables 4).

The antibiotic susceptibility and resistance pattern (Table 2) for imported antibiotic disk showed that all the isolates were resistant to not less than five out of the nine tested antibiotics , this is of grave public health concern. 33.3% sensitivity, 11.1% intermediate resistance and 55.5% resistance were observed for *Staphylococcus* sp., *Bacillus* sp. showed 33.3% sensitivity, 0% intermediate and 66.6% resistance. *Enterobacter* showed 11.1% sensitivity, 11.1% intermediate and 77.7% resistance. *Pseudomonas* showed 22.2% sensitivity, 11.1% intermediate resistance and 66.6% resistance.*E.coli*also showed 22.2% sensitivity, 0% intermediate resistance and 77.7% resistance. *Klebsiella* showed 0% sensitivity, 22.2% intermediate resistance and 77.8% resistance. *Streptococcus* showed 11.1% sensitivity, 0% intermediate resistance and 88.8% resistance. For *salmonella* 11.1% of antibiotics showed intermediate resistance and 88.8% resistance respectively. *Staphylococcus* showed resistance to oxacillin, Amoxicillin, Cefotaxime, Ceftazidime and Tetracycline. Intermediate resistance to Ampicilin and susceptibility to Vancomycin, chloramphenicol and Doxycycline. *Bacillus* sp. showed resistance to Ampicillin, Oxacillin, Amoxicillin, Ceftazidime, Cefotaxime and Tetracycline. Susceptible to vancomycin, chloramphenicol and Doxycycline. *Enteroacter* showed resistance to Ampicillin, Oxacillin, Amoxicillin, Ceftozidime, Cefotaxime, Tetracycline and Docycline. Intermediate resistance to only Chloramphenicol and susceptibility to only vancomycin. *Pseudomonas* showed resistance to Ampicillin, Oxacillin, Amoxicillin, Ceftazidime, cefotaxime and Tetracycline. Intermediate

resistance to only Doxycycline and susceptibility to Vancomycin and chloramphenicol. *E. coli* showed resistance to Ampicillin, Oxacillin, VancomycineCeftazidime, Cefotaxime, Tetracycline and Doxycycline. Susceptibility to Amoxillin and Chloramphemicol. *Klebsiella* showed resistance to Ampicillin, Oxacillin, Amoxicillin, Ceftazidime, Cefotaxime , Tetracycline and Doxycycline. It showed intermediate resistance to all the antibiotics used except chloramphenicol which it was susceptible to. Salmonella also showed resistance to all the antibiotics used except Augmentin which it was susceptible to.

The antibiotic susceptibility and resistance pattern (Table 4 and 5) for commercially available disk showed 50% susceptibility to ciproflox, gentamycin, Erythromycin, Chloramphenicol and Levofloxacin. 30% intermediate resistance to Amoxil, Streptomycin and Ampiclox. 20% resistance to Norfloxacin, and Rifampicin for *Staphylococcus* sp. *Streptococcus* sp. showed 50% susceptibility to ciprofolox, streptomycin, gentamiycin, erythromycin and Levofloxacin. 30% intermediate reisistance to Norfloxacin, Rifampicin and Ampiclox. 20% intermediate resistance to Amoxil and Chloramphenicol. 20% susceptibility to streptomycin and erythromycin. *Bacillus* sp showed 70% resistance to Reflacin, Augmentin, Gentamycin, Streptomycin, Ceporex, Nalidixic acid and Ampicllin, 0% intermediate resistance and 30% susceptibility to Tarivid, Ciproflox and septrin. *Pseudomonas* showed 50% resistance to Gentamycin, Streptomycin, ceporex. Nalidixic acid and ampicillin. 0% intermediate resistance and 50% susceptibility to Tarivid, reflacine, ciproflox and ampicillin. *Kelbsiella* showed 40% interemediate resistance to Reflacine, Augmentin, Streptomycin and Septrin. 20% susceptibility to tarivid and ciproflox. *Salmonella* showed 20% resistance to Reflacineand Gentamycin, 40% intermediate resistance to Streptomycin, Ceporex, Nalidixic acid and Ampicillin, 40% susceptibility to tarivid, ciproflox, Augmentin and septrin.

The pattern of resistance in this study to several key antibiotics commonly used in therapeutic treatment is considered a public health threat. The isolation of *E. coli* multidrug resistant bacteria from Ogane-aji River correlates with the work of Stephen and Kennedy (2018) that isolated multidrug resistant *E. coli* from water sources. Multidrug resistance patterns have been also detected in *E.coli* isolated from river water in Osun State Nigeria, (Titilayo *et al.*, 2015) and from Yamuna River in the holy city of Mathura, India (Amit *et al*., 2014). The isolation of multidrug resistant *Enterobacter, Klebsiella*, and *Streptococcus* from Ogane-aji River correlates with the work of Abdul-Rasheed and his co-workers (2013) that isolated similar multidrug resistant organisms from Opa River, South-Western Nigeria.

The incidence of multidrug resistant isolates from the *Enterobacteriaceae* family from Ogane-Aji River Anyigba correlates with the work of Graham *et al.* (2014) that identified multidrug resistant isolates from the *Enterobacteriaceae* family in Almendares River in Cuba.

As shown in this study, the organisms are more sensitive to the imported disks than the indigenous disks. Consequently, the foreign disk (Table 2) were with higher bacterial resistance range of 55.5% to 88.8% compared to the indigenous disk which showed a resistance bacterial range between 20.0 -70.0%. The study showed that there was no uniformity in potency of the imported and indigenous disks for same drugs and same concentration. Preparation and method of storage could have adverse effect on the disks potencies.

The presence of coliforms in water samples is an indication of faecal contamination (Agunwamba, 2000). The presence of *E. coli*, in the water sample used in this study indicates the possible presence of other intestinal pathogens. *Streptococcus* sp, *Staphylococcus* sp, *Salmonella* sp, *Pseudomonas* sp and *Bacillus* sp which were isolated and identified from the samples are

other pathogens of importance that have been linked to gastrointestinal disorders (Nwidu *et al*., 2008)

Overall, the results of the present study demonstrated that the antibiotic resistance patterns detected in the isolates collected from Ogane-aji River Anyigba, could lead to ineffective use of antibiotics in the treatment of gastrointestinal infections among users of Ogane-Aji River water and lower alternatives for therapeutic treatments of such infections. Considering the level of multidrug resistant bacteria in the water body caution must be taken by the inhabitants of this community in using the water for domestic purposes and drinking to prevent spread of resistant genes and traits. The MPN results showed that the water is not fit for consumption without further treatment.

Conclusion

The results of this study showed that there is a high level of multidrug bacteria in Ogane-Aji River water body. This water could serve as formite for water borne diseases and medium for spread of multidrug resistant genes in the communities in and around Ogane-Aji River.

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