Multidrug resistant (MDR) bacteria isolated from different Drinking Water Sources

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ABSTRACT: Bacterial load of different water samples was determined using standard bacteriological methods. Susceptibility of the bacteria isolated to commercial antibiotics was also assessed. The most probable number (MPN) for positive water samples ranged from 3 to 240 MPN/100ml and 2 to 17MPN total and faecal coliform respectively. Predominant bacteria isolated were *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Citrobacter* sp., *Proteus* sp., *Klebsiella* sp., *Vibrio sp., Bacillus* sp. *and Enterobacter* sp. The antibiogram carried out using the disc diffusion technique showed that all bacterial isolates were susceptible to gentamycin (100.0%) and streptomycin (77.8%) except for *Citrobacter* sp and *Klebsiella* sp which were resistant to streptomycin (22.2%). It also showed that all bacterial isolates were resistant to erythromycin (88.9%), augumentin (100.0%), and ciprofloxacin (100.0%), except for *Bacillus* sp which were inhibited by erythromycin (11.1%). *Klebsiella* sp showed the highest percentage resistance (87.5%) and lowest sensitivity (12.5%). This was followed by *Salmonella* sp, *Proteus* sp and *Citrobacter* sp showing sensitivity to only 2(25.0%) antibiotics and resisted 6(75.0%) antibiotics. *E. coli* and *Vibrio* sp showed senstivity to 3(37.5%) and resistance to 5(62.5%) antibiotics. The highest percentage sensitivity was exhibited by *Shigella* sp, *Bacillus* sp and *Enterobacter* sp (50.0%) and showed resistance to 4(50.0%) antibiotics. In term of the size of the zone of inhibition, *Shigella* sp was most sensitive to chloramphenicol, septrin and least to gentamycin. This was followed by *Escherichia coli*, which was also most sensitive to streptomycin, septrin and least to gentamycin. On the contrary, gentamycin, streptomycin and chloramphenicol was highly inhibitory to *Bacillus* species in the same way as gentamycin and tetracycline was to *Citrobacter* species. *Salmonella* species were highly sensitive to gentamycin and streptomycin, while the *Klebsiella* species was resistant to all the antibiotics tested except for gentamycin which is of public health concern. *Proteus* species was resistant to all the antibiotics tested except for gentamycin and streptomycin. The study showed the presence of multi-drug resistant (MDR) organisms in these drinking water sources and this calls for particular attention, as their presence indicate public health hazard and possible occurrence of water borne intoxication.

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1. Introduction

The increasing pollution of surface water with domestic and industrial wastes coupled with the alarming cost of construction of water treatment plants and distribution network for human use has made ground water an attractive and important option in the social and economical development of many communities (Inyang, 2009). In safeguarding public water supplies, public health authorities and engineers rely on information obtained from the results of frequent bacteriological tests (Inyang, 2009).

Many infectious diseases are transmitted by water through the fecal-oral route. Unsanitary water has particularly devastating effects on young children in the developing world. Each year, >2 million persons, mostly children <5 years of age, die of diarrheal disease (Kosek *et al*., 2003; Parashar *et al.,* 2003; Okonko *et al.,* 2008; Ibiene *et al.,* 2011). According to Shittu *et al.* (2008), water is vital to our existence in life and its importance in our daily life makes it imperative that thorough microbiological and physico-chemical examinations be conducted on water. The quality of water influence the health status of any populace, hence, analysis of for physical, biological and chemical properties including trace element contents are very important for public health studies (Shalom *et al*., 2011; Ibiene *et al.*, 2011).

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused sulphura demand for new antibacterial agents (Okonko *et al*., 2009). The effectiveness of currently available antibiotics

is decreasing due to the increasing number of resistant strains causing infections (Nawaz *et al*., 2009; Okonko *et al.,* 2010). Drug resistant strains have been reported among staphylococci, gonococci, pneumococci, enterococci, and gram negative bacteria including *Salmonella*, *Shigella*, *Klebsiella*, *Escherichia coli, Pseudomonas* as well as among Mycobacterium tuberculosis (Cheesebrough, 2006; Riboldi *et al.,* 2009; Inyang, 2009). In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystems (Chikwendu *et al.,* 2008; Okonko *et al*., 2010). In developing countries like Nigeria, antibiotics are used only when necessary, especially if the animals fall sick, and only the sick ones are treated in such cases (Chikwendu *et al.,* 2008; Okonko *et al.*, 2010).

This study was therefore carried out to ascertain the antimicrobial susceptibility pattern of the organisms contaminating different drinking water sources.

2. Materials and methods

2.1. Sample collection

Twenty one borehole water samples were collected across seven designated areas in Opuraja community of Okpe Local Government area, Delta State, Nigeria. Samples were collected into sterile 500ml bottle and transported to the microbiology laboratory and analysed within 6 hours of collection.

2.2. Bacteriological Analysis

The tube dilution technique was used to enumerate coliforms and fecal coliforms employing Mac Conkey broth and incubating at 37^0C and 44^0C, respectively. After enumeration, representative colonies were subcultured until pure isolates were obtained. Pure isolates were characterized using morphological, physiological and different biochemical tests according to the procedure of John *et al.* (1994) and Cheesebrough (2006). Further identification of isolates was done by comparing their characteristics with those of known taxa, as described by Jolt *et al*. (1994) and Oyeleke and Manga (2008). Following these tests, the isolates were identified (Sneath *et al*., 1986).

2.3. Antibiotic susceptibility of bacterial isolates

Disc diffusion method was used for the sensitivity test (Beathy *et al*., 2004). Actively growing young cultures of the bacterial isolates ≈ 108 cells /ml was streaked on Mueller –Hinton agar using sterile swab stick, allowed to dry for 5 min before placing multidisc antibiotics on the cultured plates. Contact between the antibiotic discs and the culture was ensured by gently pressing the disc with sterile forceps. Within 30 min of applying the discs, the plates were incubated at 37^OC for 18 h. Zones of inhibition were determined as mm diameter. The antibiotic discs used were chloramphenicol (30µg), ciprofloxacin (10 µg), erythromycin (10µg), streptomycin (30µg), Septrin (30µg), Gentamycin (10µg), Augumentin (30µg) and tetracycline (30µg).

3. Results Analysis

A total of 20 samples of water were examined. Samples A to L refers to well water samples from different locations in Opuraja community, samples M to R were water samples from taps while S and T were water samples collected from the stream.

3.1. Most probable number (MPN) for positive water samples

Table 1 shows the most probable number (MPN) for positive water samples. It showed that the MPN ranged from 2 to 17 MPN/100ml for faecal coliform and 3 to 240 MPN/100ml. For faecal coliform, water samples B, G, H, I and R had the highest MPN values of 17 MPN/100ml. This was closely followed by samples D, J, M, and N, all having 14 MPN/100ml. Samples A and O had 12 MPN/100ml. Samples C, E, and F had 9MPN/100ml. Sample P had 7MPN/100ml, Q had 6 MPN/100ml while K and T had 4MPN/100ml. However, water sample S had the lowest MPN value of 2MPN/100ml for faecal coliform count (Table 1). For the total coliform count, water samples G and I had the highest MPN values of 240 MPN/100ml. This was closely followed by samples B, H and R having 150MPN/100ml. Sample M had 93 MPN/100ml. Samples D, J, L and N had 75 MPN/100ml. Samples A and O had 29 MPN/100ml. Samples C, E, and F had 21 MPN/100ml. Sample P had 15 MPN/100ml and Q had 11 MPN/100ml while T and K had 7.3 and 6.2 MPN/100ml respectively. Sample S had the lowest MPN value of 3 MPN/100ml for Total coliform count (Table 1). The MPN values were higher than the recommended standard for these organisms (WHO,1984, 1995; FAO, 1997).

3.2. In- vitro antibiotic sensitivity pattern of the bacterial isolates

Tables 2 shows the results of the in- vitro antibiotic sensitivity pattern of the bacterial isolates. All bacterial isolates were susceptible to gentamycin (100.0%) and streptomycin (77.8%) except for *Citrobacter* sp and *Klebsiella* sp which were resistant to streptomycin. In the same vein, all bacterial isolates were resistant to erythromycin (88.9%), augumentin (100.0%), and ciprofloxacin (100.0%), except for *Bacillus* sp which were inhibited by erythromycin (11.1%).

Klebsiella sp showed the highest percentage resistance in this study, it was inbited by only 1(12.5%) antibiotics and it resisted 7(87.5%) of the antibiotics tested. This was followed by *Salmonella* sp, *Proteus* sp and *Citrobacter* sp showing sensitivity to only 2(25.0%) antibiotics and resisted 6(75.0%) of the antibiotics tested. *E. coli* and *Vibrio* sp showed senstivity to 3(37.5%) and resistance to 5(62.5%) of the antibiotics tested. The highest percentage sensitivity was exhibited by *Shigella* sp, *Bacillus* sp and *Enterobacter* sp. They were inhbited by 4(50.0%) of the antibiotics tested, though they also showed resistance to 4(50.0%) of the antibiotics tested.

In this study, tetracycline inhibited only *Citrobacter* sp and *Enterobacter* sp. *Escherichia coli* were inhibited by only 3(37.5%) antibiotics (septrin, streptomycin and gentamycin) tested and was resistant to 5(62.5%) of the antibiotics tested. *Proteus* sp and *Salmonella* sp were susceptible to gentamycin and streptomycin (25.0%) but resistant to all other antibiotics (75.0%). This This in variance with what was reported by Mordi and Momoh (2009) and Okonko *et al.* (2010), who reported *Proteus* sp to be susceptibile to ofloxacin and ciprofloxacin. Sensitivity of *Proteus* sp to gentamicin, and its resistance to tetracycline reported by Mordi and Momoh (2009) and Okonko *et al.* (2010) is similar to this present finding. According to Mordi and Momoh (2009) and Okonko *et al.* (2010), literature reports indicated that most strains of *Proteus* are susceptible to septrin and almost all species are sensitive to gentamicin. Here in this present study, *Proteus* sp was also resistant to septrin. However, the *in vitro* sensitivity in this study did show gentamicin and streptomycin to be the drug of choice for *Proteus* infections.

Citrobacter sp followed same pattern with Salmonella and Proteus, but was resistant to streptomycin. It was inhbited by 2(25.0%) of the antibiotics tested and resisted 6(75.0%) antibiotics. Chloramphenicol inhibited 3(33.3%) isolates (*Bacillus* sp, *Shigella* sp. and *Vibrio* sp), but was resisted by other bacterial isolates 6(66.7%). Septrin also inhibited 3(33.3%) isolates (*E. coli*, *Shigella* sp and *Enterobacter* sp), but was resisted by other bacterial isolates 6(75.0%). Erythromycin inhbited only 1(11.1%) isolate and was resisted by 8(88.9) others. Only *Bacillus* sp was inibited by erythromycin. *Bacillus* sp showed resistance to half (50.0%) of the tested antibiotics. This deviated from 100% resistivity reported by Inyang (2009). *Bacillus* sp was inhibited by erythromycin and chloramphenicol in this study. This is in agreement with 100% susceptibility reported for *Bacillus* sp to erythromycin and chloramphenicol (Umar *et al.,* 2006). The variation in susceptibility and resistance of the isolates to different antibiotics could be attributed to the difference in the concentration of antibiotics (Tables 2), source of isolates and drug resistance transfer (Shewmake and Dillon, 1998; Inyang 2009; Okonko *et al.,* 2009, 2010).

Also, in this study, high percentage resistance rate of 62.5% was observed for *E. coli*. This has satisfied multidrug resistant (MDR) pattern of resistance to >3 antibiotics (chloramphenicol, tetracycline, erythromycin, augumentin and ciprofloxacin). This deviate from the findings of Okonko *et al.* (2010), who reported *E. coli* resistance to gentamycin, but the MDR pattern were the same as E. coli was resistatnt to 5(62.5%) of the test antibitotics. The MDR pattern reported on *E. coli* in this study is comparable to previous studies (Dolejska *et al*., 2007; Sjölund *et al.,* 2008). However, gentamicin senstive *E. coli* observed in this study is in agreement with the zero gentamicin resistance reported by Sjölund *et al.* (2008). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Karlowsky *et al.,* 2004; Okonko *et al.,* 2010). This findings on *E. coli* showed close resemblance to those of a recent study of ciprofloxacin-resistant *E. coli* from humans and chickens in the late 1990s in Barcelona, Spain reported by Johnson et al. (2007) as ciprofloxacin-resistant *E. coli* was reported in this study.

In this study, *Salmonella* sp was susceptible to only 2(25.0%) antibiotics tested (gentamycin and streptomycin) but resistant to all other antibiotics, 6(75.5%). *Salmonella* spp. were among the most common causes of human bacterial gastroenteritis worldwide, and food animals were important reservoirs of the bacteria (Skov *et al*., 2007). It is recognized worldwide as important pathogens in the intestinal tracts of both animals and humans (Okonko *et al.,* 2010). In recent years, an increase in the occurrence of

antimicrobial drug–resistant *Salmonella* spp. has been observed in several countries (Skov et al., 2007; Okonko *et al*., 2010). Mbuko *et al*. (2009) in a study conducted in Zaria Nigeria, reported 18.4% fowl typhoid (FT) cases among chickens, a disease usually following the ingestion of food or water contaminated by the fecal. *Salmonella* sp was resistant to 6(75.0%) out of the 8 antibiotics tested *in vitro* (septrin, chloramphenicol, augumentin, erythromycin, ciprofloxacin, and tetracycline). This indicated that a large proportion of the *Salmonella* isolates were resistant to a variety of the drugs tested particularly tetracycline. This agrees favourably with the findings of Okonko *et al.* (2010). The resistance obtained with these test antibiotics were comparable with those reported in other studies (Abdellah *et al.,* 2009; Okonko *et al.,* 2010). Ineffectiveness of chloramphenicol, ciprofloxacin, and tetracycline against *Salmonella* sp has been previously reported (Adachi *et al.,* 2005; Oteo *et al.,* 2005; Filioussis *et al*., 2008; Okonko *et al.*, 2010).

Emergence of multiple resistances to antibiotics by organisms has also been documented (Cheesebrough, 2006; Chikere *et al.,* 2008; Okonko *et al.,* 2009, 2010). According to Suchitra and Lakshmidevi (2009), intensive medical therapies and frequent use of antimicrobial drugs are capable of selection of resistant microbial flora. This also points to the fact that the prevalence of such multidrug resistant organisms should be checkmated since their economic implication cannot be over emphasized (Okonko *et al.,* 2010). A prominent reason for concern with regard to these MDR isolates is the recognized emergence of antimicrobial resistance among key species. However, a number of studies in the literature indicated a gradual increase in the emergence of antibiotic-resistant microorganisms especially in hospitals (Suchitra and Lakshmidevi, 2009). Many factors apart from antibiotic exposure can contribute to the development of antibiotic resistance in bacterial isolates.

Table 1: Most Probable Number (MPN) for positive water samples

Samples	Faecal coliform count (MPN/100ml)	Total coliform count (MPN/100ml)		Faecal coliform count (MPN/100ml)	Total coliform count (MPN/100ml)
A=well water	12	29	K=well water	4	6.2
B=well water	17	150	L=well water	14	75
C=well water	9	21	M=tap water	14	93
D=well water	14	75	N=tap water	14	75
E=well water	9	21	O=tap water	12	29
F=well water	9	21	P=tap water	7	15
G=well water	17	240	Q=tap water	12	11
H=well water	17	150	R=tap water	17	150
I=well water	17	240	S=stream	2	3
J=well water	14	75	T=stream	4	7.3

Tables 2: In- vitro antibiotic sensitivity pattern of the bacterial isolates

Isolates	Antibiotics Zone of Inhibition (mm diameter)								Percentage (%)	
	SEP (30µg)	CHL (30µg)	TET (30µg)	STR (30µg)	GEN (10µg)	ERY (10µg)	AUG (30µg)	CIP (10 µg)	Sensitive	Resistance
Escherichia coli	12	0	0	13	11	0	0	0	3(37.5)	5(62.5)
Salmonella sp	0	0	0	10	10	0	0	0	2(25.0)	6(75.0)
Shigella sp	18	20	0	10	9	0	0	0	4(50.0)	4(50.0)
Proteus sp	0	0	0	11	10	0	0	0	2(25.0)	6(75.0)
Bacillus sp	0	16	0	16	17	13	0	0	4(50.0)	4(50.0)
Klebsiella sp	0	0	0	0	11	0	0	0	1(12.5)	7(87.5)
Citrobacter sp	0	0	10	0	14	0	0	0	2(25.0)	6(75.0)
Enterobacter sp	10	0	9	14	15	0	0	0	4(50.0)	4(50.0)
Vibrio sp	0	10	0	15	14	0	0	0	3(37.5)	5(62.5)
No. Senstive (%)	3(33.3)	3(33.3)	2(22.2)	7(77.8)	9(100.0)	1(11.1)	0(0.0)	0(0.0)	6(75.0)	2(25.0)
No. Resistant (%)	6(66.7)	6(66.7)	7(77.8)	2(22.2)	0(0.0)	8(88.9)	9(100.0)	9(100.0)	7(87.5)	1(12.5)

Key: Disc size = 8mm; 0 = No zone of inhibition; CHL=chloramphenicol (30µg), CIP=ciprofloxacin (10 µg), ERY=erythromycin (10µg),

STR=streptomycin (30µg), SEP=Septrin (30µg), GEN=Gentamycin (10µg), AUG=Augumentin (30µg), TET=tetracycline (30µg).

4. Conclusion

The most common multidrug resistance (>3 drugs) patterns included resistance to septrin, chloramphenicol, erythromycin, augumentin, ciprofloxacin and tetracycline. The presence of multidrug resistant organisms such as *Bacillus sp*., *E. coli, Proteus* sp, *Salmonella sp., Klebsiella* sp, *Citrobacter* sp, *Enterobacter* sp, *Shigella* sp, and *Vibrio* sp encountered in these drinking water sources is alarming. The presence of these organisms in these water sources should receive particular attention, because their presence indicate public health hazard and gives warning signal for the possible occurrence of food borne intoxication (Kabir, 2009). The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Alim *et al.,* 2009; Okonko *et al*., 2010).

In conclusion, the study has revealed the non conformity of drinking water sources in Opuraja community in Delta State, Nigeria to WHO recommended standards for drinking water. Adequate treatment is hereby advocated before use and any case of water borne disease or food poisoning resulting from use of these contaminated drinking waters could be treated with sensitive antibiotics indicated in this study such as streptomycin and gentamycin. And the isolation of these organisms in this especially *E. coli, Salmonella* sp. and *Vibrio* sp. is an indication that if not check, an outbreak could occur in the near future. This calls for urgent and appropriate public health measures in this community under study.

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