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Prevalence of Multidrug Resistant Bacteria Isolated from Biomedical Waste Generated in Makurdi Metropolis, Benue State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VTO and OAM designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors VTO, OAM and SOA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Multiple drug resistance bacteria in solid and liquid wastes in public and private hospitals were investigated between December, 2013 and February, 2014. Solid and liquid wastes were collected from discharged sites from different wards of both hospital environments. The antibiotic susceptibility patterns of the different isolates to commonly used antibiotic using multi-antibiotic disc at different concentrations were determined. A hundred and twenty-four bacterial isolates were identified from the collected samples, 36 (29.0%) and 88 (70.9%), of which were from liquid and solid wastes respectively. The bacteria isolated were *Escherichia coli* (*E. coli*) 23 (18.6%), *Staphylococcus aureus* 20 (16.1%), *Enterobacter sp* 18 (14.5%), *Pseudomonas sp* 15 (12%),

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Proteus sp 12 (9.7%), *Shigella sp* 11 (8.9%), *Klebsiella sp* 8 (6.5%), *Salmonella sp* 7 (5.7%), *Bacillus sp* 5 (4.0%), *Citrobacter sp* 3 (2.4%), and *Serratia sp* 2 (1.6%). The results showed that *E. coli* had the highest percentage of 18.6%; followed by *Staphyloccocus aureus* (16.1%) from the total number of bacterial species identified from the solid and liquid wastes. *Serratia* sp had the least percentage of 1.6% and *Citrobacter sp* with 2.4%. *E. coli* were 100% resistant to ciproflox and ampicillin while *Klebsiella pneumoniae* and *Shigella* sp were highly resistance to ampicillin with 87.5% and 81.8%, respectively. Among the gram-positive isolates, *Staphylococcus aureus* was found to be resistant to floxapen (70%) followed by erythromycin (60%) while *Bacillus* sp was resistant to erythromycin (80%), norfloxacin (60%) and floxapen (60%). The studied area showed high occurrence of multiple drug resistance patterns in both the public and private hospitals to the commonly used antibiotics tested on the isolated organisms.

Keywords: Biomedical waste; multidrug resistance; prevalence.

1. INTRODUCTION

Efficient bio-medical waste management is a major challenge in Africa, and Nigeria in particular [1]. Bio-medical wastes refers to all waste, biological or non-biological from hospitals, that is discarded and not intended for further use [2] and these include: stock cultures, blood and blood products, pharmaceutical wastes, needles, syringes, scalpels, radioactive wastes, hazardous chemicals, pressurized containers, batteries, plastics, low level radioactive wastes, disposable and other sharp items. These are in addition to food wastes, clinical bandages, gauze, cotton and other miscellaneous wastes. Other types of waste include toxic chemicals, cytotoxic drugs, flammable and radioactive wastes that can often be considered infectious [3]. Hospital waste can be hazardous to public health and ecological balance since it can contain various kinds of pollutants such as radioactive, chemical and pharmaceutical wastes and also pathogenic microorganisms [4]. Uncontrolled and excessive use of antibiotics by human may cause an increase in the prevalence and distribution of resistance genes in the environmental samples such as bio-medical waste [5]. According to a previous study, 5.2 million people (including 4 million children) die each year from waste-related diseases [6]. Globally, the amount of municipal waste generated will be doubled by the year 2000 and quadrupled by year 2025 [7]. Hospital waste has contributed to the high rates of resistant bacteria that are being discharged in the natural environment [8]. As demonstrated by Colomer-Lluch et al. [9], the occurrence of bacteriophages from samples of human fecal wastes could be environmental vectors for the horizontal transfer of antibiotic resistance genes.

Higher numbers of resistant bacteria occur in polluted habitats [10]. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance [11]. The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens [12]. The volume of antibiotics used in hospitals and released into effluent and municipal sewage provide a selective pressure on bacteria [13]. Effluent waste from hospitals contains high numbers of resistant bacteria and antibiotic residues at concentrations able to inhibit the growth of susceptible bacteria [14]. Until recently, hospital wastes were unmanaged and simply 'disposed off'. The disposal of hospital waste can be very hazardous particularly when it gets mixed with municipal solid waste and is dumped in uncontrolled or illegal landfills such as vacant lots in neighboring residential areas and slums. This can lead to a higher degree of environmental pollution, apart from posing serious public health risks such as hepatitis, plague, cholera, etc.

As a result, hospital waste effluents could increase the number of resistant bacteria in the recipient sewers by both mechanisms of introduction and selection of resistant bacteria [15]. As reported by Ngwuluka [16], postoperative surgical site infections in the hospital were due contaminated medical equipment, environmental surfaces, air and hands of health personnel.

In Nigeria, especially in North-Central Nigeria, there is no data on resistance profiles concerning multi-drug resistance profile of bacteria isolated from biomedical waste. This study is therefore, an attempt to generate original local data and

examine the magnitude of drug resistant pathogens in General hospital and a private hospital, in Makurdi Metropolis.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 36 samples of solid and liquid wastes were collected from selected public and private hospital in Makurdi Metropolis. Solid waste samples were collected from the waste bins of laboratory unit, medical ward, and surgical ward (containing stock cultures, blood and blood products, plasters, pharmaceutical wastes, needles, syringes, scalpels, cotton wool, disposable and other sharp items) while the effluents from each of these sources served as liquid waste samples. All the samples were collected 3 times from both hospitals at one month interval over a period of three months. This study was carried out between December, 2013-February, 2014.

After collection, all samples were transported to the laboratory within 1 hour for further work [17].

2.2 Cultivation and Isolation of Organism

A 10-fold serial dilution of all the samples collected were prepared in physiological saline and 0.1 aliquots of the 1×10^{-8} dilutions were streaked on each agar plates containing mannitol salt agar, eosin methylene blue, nutrient agar, MacConkey agar and Salmonella-Shigella agar (Oxoid microbiological media, UK). All prepared media were prepared according to oxoid specification. All inoculated plates were incubated for 24 hours at 37℃ after which presumptive identifications of target colonies were performed.

All identified colonies were subcultured unto freshly prepared nutrient agar medium to obtain pure culture of the organisms. The pure cultures obtained were Gram stained and examined microscopically, after which they were subjected to different biochemical tests [17]. The biochemical tests include catalase, coagulase, citrate, indole, methyl red-Voges proskrauer, motility, ornithine decarboxylase, oxidase, urease and triple sugar iron agar test.

2.3 Antibiotic Susceptibility Testing

All the isolates obtained were standardized to 10^8 bacteria/ml using 0.5 McFarland turbidity standards. The peptone water was inoculated with test organism and incubated at 37℃ for 18 hrs. The antibiogram of the bacterial isolates were carried out by Kirby Bauer's disk diffusion method [18].

Antibiotic susceptibility testing were done using the following antibiotics: tarivid 10 mcg, peflacine 10 mcg, ciproflox 10 mcg, augumentin 10 mcg, gentamycin 10 mcg, streptomycin 30 mcg, nalidixic acid 30 mcg, septrin 30 mcg, ampicillin 30 mcg, ceporex 10 mcg (gram-negative discs). The Gram positive antibiotic disc contained the following concentration: ciprofloxacin 10 mcg, norfloxacin 10 mcg, gentamycin 10 mcg, lincocin 20 mcg, streptomycin 30 mcg, rifampicin 20 mcg, erythromycin 30 mcg, chloramphenicol 30 mcg, ampliclox 20 mcg, and floxapen 20 mcg.

Four impregnated antibiotic discs were carefully and aseptically placed on the inoculated agar plates. The antibiotic susceptibility testing for each isolate was carried out in duplicate plates.

All plates were then incubated at 37℃ for 24 hours after which they were examined for evidence of zone of inhibition, which appeared clearly around the discs. The diameter of the zone of inhibitions was measured in millimeters using a transparent meter rule. The test organisms were classified as resistant or susceptible according to the criteria recommended by the guidelines of CLSI on antibiotic susceptibility test [19].

3. RESULTS

The occurrence of bacterial isolates generated from different wards and effluent sites at the private hospital is revealed in Table 1. The frequency of isolates from laboratory unit was high compared to other wards. The isolate with the least frequency is seen in effluent site C.

The occurrence of bacterial isolates generated from different wards and effluent sites at the General Hospital is revealed in Table 2. The frequency of isolate from medical ward was high compared to other wards and this was followed closely by the laboratory unit. The frequency of isolates from effluent site A, site B and site C were all equal. However, no species of *Salmonella* and *Serratia* were isolated from the liquid wastes collected from public hospital.

Table 3 shows the total isolates of both solid and liquid waste from both hospitals. The result

indicated higher frequency of isolates in solid wastes that in liquid waste. *E. coli* in solid waste was higher compared to that of liquid waste while the occurrence of *Klebsiella sp* in liquid waste was higher compared to that of solid waste. *Serratia sp* was not isolated from liquid waste.

Table 4 showed the percentage of all the bacterial isolates in solid and liquid waste generated in both hospitals and *E. coli* had the highest percentage of 18.6%, followed by *Staphyloccocus aureus* with 16.1%. *Serratia* sp had the least percentage of 1.6% and was followed by *Citrobacter sp* at 2.4%.

The antibiotic susceptibility patterns of different isolates to commonly used antibiotic using multi-antibiotic disc at different concentrations were determined. The Gram negative isolates showed highest resistance to ciproflox (69.1%), ceporex (68.1%), nalidixic acid (72.3%) and ampicillin (84%), while they showed the highest sensitivity to gentamycin (77.7%), streptomycin (77.7%), and augumentin (65.9%). Whereas, Gram positive isolates showed the highest resistance to floxapen (68%), erythromycin (64%) and norfloxacin (56%) and showed highest sensitivity to streptomycin (96%), gentamycin (84%), ampiclox (72%) and rifampicin (68%).

Table 1. Occurrence of bacterial isolates from wastes generated in different wards and effluent sites in private hospital

Isolates	Total no. of Isolates (%)					
	Solid waste			Effluent sites		
	MW	SW	LB	SITE A	SITE B	SITE C
Escherichia coli	2(20)	3(30)	4(40)	-	-	1(10)
Enterobacter sp	2(20)	2(20)	3(30)	2(20)	-	1(10)
Proteus sp	3(42.9)	-	2(28.6)	1(14.3)	1(14.3)	-
Shigella sp	-	-	2(50)	-	2(50)	-
Pseudomonas sp	1(20)	3(60)	-	-	1(20)	-
Serratia sp	-	1(100)	-	-	-	-
Staphylococcus sp	1(14.3)	2(28.6)	2(28.6)	1(14.3)	1(14.3)	-
Klebsiella sp	-	-	1(20)	2(40)	1(20)	1(20)
Bacillus sp	-	-	-	-	1(50)	1(50)
Salmonella sp	-	-	-	-	2(66.7)	1(33.3)
Citrobacter sp	1(100)	-	-	-	-	-
Total	10	11	14	6	9	5

Key = MW = Medical ward, SW = Surgical ward, LB = Laboratory unit, Site A = Effluent from Medical ward, Site B = Effluent from Surgical ward, Site C = Effluent from Laboratory unit

Table 2. Occurrence of bacterial isolates from wastes generated in different wards and effluent sites in general hospital (public)

Total no. of isolates in (%)	Solid waste			Effluent sites		
	MW	SW	LB	SITE A	SITE B	SITE C
Escherichia coli	4(30.8)	3(15)	4(22.2)	1(16.7)	1(16.7)	-
Enterobacter sp	1(7.7)	2(10)	3(16.7)	1(16.7)	-	1(16.7)
Citrobacter sp	-	1(5)	-	1(16.7)	-	-
Salmonella sp	2(15.4)	-	2(11.1)	-	-	-
Shigella sp	2(15.4)	-	4(22.2)	-	-	1(16.7)
Klebsiella pneumonia	-	2(10)	-	-	-	1(16.7)
Serratia sp	1(7.7)	-	-	-	-	-
Proteus sp	-	-	2(11.1)	2(33.3)	1(16.7)	-
Pseudomonas sp	2(15.4)	5(25)	1(5.6)	-	1(16.7)	1(16.7)
Staph. Aureus	1(7.7)	6(30)	2(11.1)	1(16.7)	2(33.3)	1(16.7)
Bacillus sp	-	1(3.3)	-	-	1(16.7)	1(16.7)
Total	13	20	18	6	6	6

Key = MW = Medical ward, SW = Surgical ward, LB = Laboratory unit, Site A = Effluent from Medical ward, Site B = Effluent from Surgical ward, Site C= Effluent from Laboratory unit

Table 3. Percentage occurrence of bacterial isolates in solid and liquid wastes generated from both hospitals (private and public)

Isolates	Liquid waste (%)	Solid waste (%)
Escherichia coli	3(13.0)	20(86.9)
Enterobacter sp	5(27.8)	13(72.2)
Citrobacter sp	2(66.7)	1(33.3)
Salmonella sp	3(42.9)	4(57.1)
Shigella sp	1(9.09)	10(90.9)
Klebsiella pneumonia	5(62.5)	3(37.5)
Serratia spp	-	2(100)
Proteus sp	5(41.7)	7(58.3)
Pseudomonas sp	2(13.3)	13(86.7)
Staphylococcus aureus	6(30)	14(70)
Bacillus sp	4(80)	1(20)
Total	36(29.0)	88(70.9)

Table 4. Percentage occurrence of the total number of bacterial isolates from wastes generated from both hospitals (private and public)

Isolates	Total no. of isolates	Percentages (%)
Escherichia coli	23	18.6
Enterobacter sp	18	14.5
Citrobacter sp	3	2.4
Salmonella sp	7	5.7
Klebsiella pneumonia	8	6.5
Serratia sp	2	1.6
Proteus sp	12	9.7
Pseudomonas sp	15	12.1
Staphylococcus aureus	20	16.1
Bacillus sp	5	4.0
Shigella sp	11	8.9
Total	124	100

4. DISCUSSION AND CONCLUSION

4.1 Discussion

Antibiotics exert a selection in favour of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance [11]. The main risk for public health is the transfer of resistance genes from environmental bacteria to human pathogen [12]. The volume of antibiotics used in hospitals released into effluent and municipal sewage indicates a selective pressure on bacteria [13]. In this study, the pathogenic bacteria isolated from the biomedical wastes were *E. coli* (18.6%), *Enterobacter sp* (14.5%), *Shigella* sp (8.9%), *Proteus* sp (9.7%), *Pseudomonas sp* (12%), *Serratia sp* (1.6%), *Staphylococcus aureus* (16.1%), *Klebsiella* sp (6.5%), *Citrobacter* sp (2.4%), *Bacillus* sp (4.0%) and *Salmonella* sp (5.7%). Some of the samples however, did not show any growth on the various media used. From the result, more gram-negative organisms (especially members of the enteriobacteriaceae) were isolated than gram-positive organisms. *E. coli* (amongst the gram-negative) and *Staphylococcus aureus* (amongst the gram-positive) were isolated in higher concentrations from the samples collected for this study. This is in agreement to similar study by Sintayehi [20], who reported some potential pathogenic bacteria such as *Salmonella* sp*, Shigella* sp*, Staphylococcus aureus* and *E. coli* in hospitals' waste effluents. In a study carried out in Erbil city, Rhizgari by Aziz et al. [21] revealed that *E. coli* was mostly isolated (100%) from a hospital wastewater. This study also conforms to the work of Anitha and Jayraaj, [22], who reported *E. coli* as the predominant organism in hospital wastes and the presence of gram-positive isolates such as *Bacillus subtilis* and *Staphylococcus aureus* in biomedical wastes collected in a public and private hospital in

Coimbatore, India. In the study conducted by Oyeleke and Istifanus [23], the most predominant pathogens isolated from hospital wastes were *Bacillus* and *Staphylococcus* species (80-90%). However, findings by Oviasogie et al. [24] showed that *Pseudomonas aeruginosa* was the highest Gram negative organism isolated from hospital waste accounted for 25.00% overall of all the isolates. Several researchers have also reported similar bacterial isolates from hospital wastes [25,26].

In the present study, similar isolates were reported as possible nosocomial organisms in a hospital environment [27]. *Salmonella* species were not isolated from the solid wastes and liquid wastes collected in public and private hospitals' biomedical wastes respectively. This may probably due to the nature of the organism; viable but non-culturable or the effect of possible pre-treatment given to wastes. Dudley et al. [28] also reported variety of pathogenic bacteria in sewage sludge. However, *Shigella* species were not detected in their study due to low sensitivity of enrichment procedure and high temperature which decreased its survival in their study. The high frequency of pathogenic bacteria in this study may be due to high admission of cases with bacterial infections, which is common in developing countries like Nigeria.

The result of this study revealed more bacterial isolates in the solid wastes generated in the public hospital environment 88 (70.9%) than in the liquid wastes 36 (29%) in the duration of three months with *E. coli* accounted for the highest percentage of 18.6%, followed by *Staphylococcus aureus* (16.1%). This was closely followed by *Pseudomonas aeruginosa* and *Enterobacter* spp (14.5%). Our findings also showed higher frequency of bacterial isolates from public hospital than in private hospital environment. However, these may be due to the fact that the majority of people are low income earners in this part of the country and ordinary working citizens who tend to patronize the public hospital because of lesser medical charges compared to the private hospital where charges are higher.

The study showed that Gram negative bacteria were more resistant to the tested antibiotics than the Gram positive organisms. The result concurs with the study carried out by Levy [29], who reported the prevalence of antibiotic resistance among Gram negative bacteria than Gram positive bacteria [29]. Gram-negative bacteria are of particular concern because these organisms are inherently resistant to many hydrophobic antibiotics [30,31]. However, Gram negative bacteria are the most common causes of hospital and community acquired infections [32]. Several factors that influence the action and effectiveness of antimicrobial agents on bacterial cells are known, however, it is the remarkable difference in structure and composition of the cell wall's murein layer between the gram negative and the gram positive bacteria that is responsible for this trend [33].

In Table 5 and Table 6, most of the tested gram-negative and gram-positive organisms accounted for 90% resistance to all commonly used antibiotics. *E. coli* was 100% resistant to ciproflox and ampicillin *while Salmonella* showed 100% and 57.1% susceptibility to ciproflox and ampicillin respectively. The result also revealed that *Klebsiella pneumoniae* and *Shigella* sp were highly resistance to ampicillin with 87.5% and 81.8%, respectively. Among the Gram-positive isolates, *Staphylococcus aureus* was resistant to floxapen (70%) followed by erythromycin (60%) while *Bacillus* sp was resistant to erythromycin (80%), norfloxacin (60%) and floxapen (60%). Streptomycin showed 100% sensitivity to *Staphyloccous aureus* and *E. coli.* Similar studies have been carried out and showed resistance of commonly used antibiotics on pathogens isolated in a hospital environment [27,34,35]. In the study carried out by Chikere et al. [27], the Gram positive isolates were more resistant to Norfloxacin (71.1%). This was closely followed by Floxapen (57.8%), Ciprofloxacin (51.1%) and Erythromycin (44.4%) respectively. They were sensitive to Gentamycin, (93.3%), Rifampicin (93.3%), Streptomycin (82.2%) and Lincocin (80%). While the Gram negative isolates were more resistant to Ampicillin (81.8%), Ceporex (72.7%), Nalidixic acid (72.7%) and Augumentin (63.6%). They were very sensitive to Tarivid (100%), Peflacin (100%), Gentamycin (100%), Streptomycin (90.9%) and Ciproflox (63.6%). *E. coli* accounted for the highest resistance to the antibiotics. Odeyemi [36] opined that high level of resistance has been associated with members of the family Enterobacteriaceae which could caused an increased in the incidence of pathogenic strains of bacteria with acquired antibiotics resistance [36].

Table 5. Antibiogram of Gram-negative isolates from biomedical wastes generated from both private and public hospitals

	TAR		PEF		CIP		AU		GN		SN		CEP		NA		S		AMP	
	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)
A1	12(52.2)	11(47.6)	16(69.6)	7(30.4)	23(100)	0(0)	15(65.2)	8(34.8)	6(26.1)	17(73.9)	0(0)	23(100)	14(60.9)	9(39.1)	14(60.9)	9(39.1)	15(65.2)	8(34.8)	23(100)	0(0)
B2	9(50)	9(50)	7(38.9)	1(61.1)	10(55.6)	8(44.4)	6(33.3)	12(66.7)	8(44.4)	11(55.6)	4(22.2)	14(77.8)	13(72.2)	5(27.8)	12(66.7)	6(33.3)	5(27.8)	13(72.2)	14(77.8)	4(22.2)
C3	1(33.3)	2(66.7)	1(33.3)	2(66.7)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	0(0)	3(100)	1(33.3)	2(66.7)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	2(66.7)	1(33.3)
D4	3(42.9)	4(57.1)	2(28.6)	5(71.4)	0(0)	7(100)	2(28.6)	5(71.4)	3(42.9)	4(57.1)	3(42.9)	4(57.1)	5(71.4)	2(28.6)	4(57.1)	3(42.9)	2(28.6)	5(71.4)	4(57.1)	3(42.9)
E5	2(25)	6(75)	3(37.5)	5(62.5)	5(62.5)	3(37.5)	1(12.5)	7(87.5)	2(25)	6(75)	3(37.5)	5(62.5)	6(75)	2(25)	5(62.5)	3(37.5)	4(50)	4(50)	7(87.5)	1(12.5)
F6	0(0)	2(100)	1(50)	1(50)	1(50)	1(50)	0(0)	2(100)	0(0)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	2(100)	0(0)
G7	6(50)	6(50)	4(33.3)	8(66.7)	7(58.3)	5(41.7)	5(41.7)	7(58.3)	3(25)	9(75)	4(33.3)	8(66.7)	8(66.7)	4(33.3)	9(75)	3(25)	5(41.7)	7(58.3)	6(50)	6(50)
H8	7(46.7)	8(33.3)	6(40)	9(60)	11(73.3)	4(26.7)	5(33.3)	10(66.7)	3(20)	12(80)	7(46.7)	8(53.3)	8(53.3)	7(46.7)	13(86.7)	2(13.3)	7(46.7)	8(53.3)	12(80)	3(20)
I9	5(45.5)	6(54.5)	4(36.4)	7(63.6)	6(54.5)	5(45.5)	2(18.2)	9(81.8)	1(9.1)	10(90.9)	3(27.3)	8(72.7)	7(63.6)	4(36.4)	8(72.7)	3(27.3)	1(9.1)	10(90.9)	9(81.8)	2(18.2)
Total	45(47.9)	54(57.4)	44(46.8)	55(58.5)	65(69.1)	34(36.2)	37(39.4)	62(65.9)	26(27.7)	73(77.7)	26(27.7)	73(77.7)	64(68.1)	35(37.2)	68(72.3)	31(32.9)	41(43.6)	58(61.7)	79(84)	20(21.3)

Key = A1= E.coli, B2= Enterobacter sp, C3=Citrobacter sp, D4=Salmonella sp, E5=Klebsiella sp, F6=Serretia sp, G7=Proteus sp, H8=Pseudomonas sp, I9=Shigella sp TAR= Tarivid, PEF= Peflacine, CIP= Cipflox, AU= Augumentin, GN= Gentamycin, SN= Streptomycin, CEP = Ceporex, NA = Nalidixic acid, S = Septrin, AMP = Ampicillin

Table 6. Antibiogram of Gram-positive isolates from biomedical wastes generated from both private and public hospitals

	NOR		LIN		CIP		RIF		GN		SN		E		AMP		CH		FLX	
	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)	R(%)	S(%)
N1	11(55)	9(45)	10(50)	10(50)	8(40)	12(60)	6(30)	14(70)	2(10)	18(90)	0(0)	20(100)	12(60)	8(40)	4(20)	16(80)	9(45)	11(55)	14(70)	6(30)
N2	3(60)	2(40)	2(40)	3(60)	2(40)	3(60)	2(40)	3(60)	2(40)	3(60)	1(20)	4(80)	4(80)	1(20)	2(40)	3(60)	1(20)	4(80)	3(60)	2(40)
Total	14(56)	11(44)	12(48)	13(52)	10(40)	15(60)	8(32)	12(68)	4(16)	21(84)	1(4)	24(96	16(64)	9(36)	6(24)	18(72)	10(40)	15(60)	17(68)	8(32)

Key = N1= Staphylococus aureus, N2= Bacillus sp, NOR= Norfloxacin, LIN= Lincocin, CIP= Ciprofloxacin, RIF= Rifampicin, GN= Gentamycin, SN= Streptomycin, E= Erythromycin, AMP= Ampiclox, CH= Chloramphenicol, FLX= Floxapen

Similar findings revealed high antibiotic resistance of clinical isolates from hospital equipments and sewage [15,27]. The extent of resistance to an antibiotic may be associated with the extent of antibiotic usage. *E. coli* isolated from the hospital waste was highly resistant to ciprofloxacin and ampicillin, which could be the result of unmetabolized antibiotics released from the hospitals in low concentration [17,37,38] and repeated prescription of antibiotics by the medical practitioners, can lead to resistant organisms, which is common practice in Nigeria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [39]. In developing countries like Nigeria, self-medication is a common practice and could be major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable to treat themselves [27].

Multidrug resistance is a function of R-plasmid transfer within a bacterial population [40]. Some other mechanisms of antimicrobial drug resistance include inability of antibiotic to penetrate cell wall of bacteria because of alterations in plasma membrane, decreased intracellular availability of drugs, production of plasmid-or chromosomally encoded enzymes that hydrolyze the drugs [41-43]. The presence of these resistant bacteria from wastes collected in the hospital environments may be transmitted to humans and could result in disease that cannot be treated by conventional antibiotics [44].

5. CONCLUSION

From this present study, it can be concluded that solid and liquid wastes in the study area are potential source and reservoir of multiple drug resistance organisms. The presence of high number of pathogenic organisms resident in the disposed wastes is a treat to the populace in such environment and has a serious public health implication especially due to high resistance to commonly used antibiotics in our hospitals. It is imperative that all hospital wastes should be incinerated and treated properly before discharge into the environment and there should be an efficient waste management practices such as landfill where wastes could be channelled in both public and private hospitals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fongwa MN. International health care perspectives: The Cameroon example. J. Transcultural Nursing. 2002;13(4):325-330.
2. USEPA. Standards method for the treating and management of medical water: Interim final rule and request for comments. 1989; 7-111.
3. Caltivelli EG. In medical waste treatment Ispracourses; 1990, Waste Treatment Manage. 1973;1-12.
4. Sharpe M. High on pollution: Drugs as environmental contaminants. J Environ Monit. 2003;5:43–46.
5. Iversen A, Kuhn I, Franklin A, Mollby R. High prevalence of vancomycin resistant Enterococci in Swedish wastewater. Appl Environ Microbiol. 2002;68:2838–2842.
6. Nasima A. Medical waste management: A review. Environmental Engineering Program. School of Environment, Resources and Development. Khlongluang, pathum Thani, Thailand: Asian Institute of Technology (ATI); 2000.
7. Akter N, Kazi NM, Chowdhury AMR. Environmental investigation of medical waste management system in Bangldesh with special reference to Dhaka City. BRAC, Research and Evaluation Division, 75 Mohakhali, Dhaka 1212, Bangladesh; 1999.
8. Yang CM, Lin MF, Liao PC, Yeh HW, Chang BV, Tang TK, Cheng C, Sung CH, Liou ML. Comparison of antimicrobial resistance patterns between clinical and wastewater strains in a regional hospital in Taiwan. Lett Appl Microbiol. 2009;48:560–565.
9. Colomer-Lluch M, Imamovic L, Jofre J, Muniesa M. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrob Agents Chemother. 2011;55:4908–4911.
10. Pathak SP, Bhattacherjee JW, Ray PK. Seasonal variation in survival and antibiotic resistance among various bacterial populations in a tropical river. J Gen Appl Microbiol. 1993a;39:47–56.
11. Kruse H. Indirect transfer of antibiotic resistance genes to man. Acta Vet Scand. 1999;92:59–65.
12. Wegener H, Aarestrup F, Gerner-Smidt P, Bager F. Transfer of resistant bacteria from animals to man. Acta Vet Scand. 1999; 92:51–58.
13. Kummerer KH. A promoting resistance by the emission of antibiotics from hospitals

and household into effluent. Clinical Microb Infection. 2003;12:1203.
14. Grabow W, Prozesky O. Drug resistance of coliform bacteria in hospital and city sewage. Antimicro Agents Chemother. 1973;3:175–180.
15. Linton KB, Richmond MH, Bevan R, Gillespie WA. Antibiotic resistance and R factors in coliform bacilli isolated from hospital and domestic sewage. J Med Microbiol. 1974;7:91–103.
16. Ngwuluka N. Waste management in healthcare establishments within Jos Metropolis, Nigeria. Afr J Environ Sci Technol. 2009;3(12):459-465.
17. Guardabassi L, Petersen A, Olsen JE, Dalsgaard A. Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. Appl Environ Microbiol. 1998;64(9):3499-3502.
18. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard disk diffusion method. Am J Clin Pathol. 1996;45(4):493-6.
19. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement. Wayne, PA, USA: CLSI: M100-S22; 2012.
20. Sintayehi F. Adis Abba School of graduate studies, M.sc. thesis, Assessment of disinfectant resistant Bacteria in Hospital wastewater, South Ethopia; 2011.
21. Aziz RJ, Al-Zubaidy FS, Al-Mathkhury HJ, Resul B, Musenga J. Antibiogram of *Escherichia coli* isolated from different hospitals wastewater in Erbil City, Iraq. Iraqi J Sci. 2014;55(2):341-351
22. Anitha, J, Jayraaj IA. Isolation and identification of bacteria from biomedical waste (BMW). International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(5):1-3.
23. Oyeleke SB, Istifanus N. The microbiological effects of hospital wastes on the environment. African Journal of Biotechnology. 2009;8(22):6253-6257.
24. Oviasogie FE, Ajuzie CU, Ighodaro UG. Bacterial analysis of soil from waste dumpsite. Archives of Applied Science Research. 2010;2(5):161-167.
25. Bolaji AS, Akande IO, Iromini FA, Adewoye SO, Opasola OA. Antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria. European Journal of Experimental Biology. 2011;1(4):66-71.
26. Vichal R, Pooja R, Shalini B. Bacteriological profile of biomedical waste: Management guidelines. J Indian Acad Forensic Med. 2011;33 (2):145-14.
27. Chikere CB, Chikere BO, Omoni VT. Antibiogram of clinical isolates from a hospital in Nigeria. Afri J Biotechnol. 2008; 7(24):4359-4363
28. Dudley DJ, Guentzel NM, Ibarra MJ, Moore BE, Sagik BP. Enumeration of potentially pathogenic bacteria from sewage sludges. Appl Environ Microbiol. 1980;118-126.
29. Levy SB. The challenge of antibiotic resistance. Scientific American. 1998;278: 46–53.
30. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67(4):593-656.
31. Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev. 1992;56(3):395–411.
32. Ayse Bastopcu, Halil Yazgi, M. Hamidullah Uyanik, Ahmet Ayyildiz. Evaluation of quinolone resistance in Gram negative bacilli isolated from community and hospital acquired infections. The Eurasian J Medicine. 2008;40:58-61.
33. Hancock RE. Peptide antibiotics. Lancet. 1997;349:418–422.
34. Shahriar M, Hossain M, Kabir S. A survey on antimicrobial sensitivity pattern of different antibiotics on clinical isolates of *Escherichia coli* collected from Dhaka City, Bangladesh. J. Appl. Sci. Environ. Manage. 2010;14(3):19–20.
35. Sharma DR, Pradhan B, Mishra SK. Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. Kathmandu University Medical Journal. 2010;8(1):40-44.
36. Odeyemi AT. Antibiogram status of bacterial Isolates from air around dumpsite of Ekiti State Destitute Centre at Ilokun, Ado-Ekiti, Nigeria. J Microbiol Resear. 2012;2(2):12-18.
37. Munoz-Aguayo J, Lang KS, LaPara TM, González G, Singer RS. Evaluating the effects of chlortetracycline on the proliferation of antibiotic-resistant bacteria in a simulated river water ecosystem. Appl Environ Microbiol. 2007;73(7):5421-5425
38. Thomas S, Holger V, Slike K, Wolfagang K, Katja S, Bernd J, Ursula O. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface

water, and drinking water biofilms. FEMS Microbiol. Ecol. 2007;43(3):325-335.
39. Prescott LM, Harley JP, Klein DA. Microbiology. 6th ed. McGraw-Hill, New York. 2005;833-842.
40. Grohmann E, Muth G, Espinosa M. Conjugative plasmids transfer in Gram positve bacteria. Microbiol Rev. 2003; 67(2):277-301
41. Pelczar MJ, Chan ECS, Krieg NR. Microbiology. 5th Edition., Tata McGraw-Hill, New Delhi, India. 1993;900.
42. Cloete TE. Resistance mechanisms of bacteria to antimicrobial compounds. Int BiodeteriolBiodegrad. 2003;51:277-282.
43. Courvalin P, Weber JT. Antimicrobial drugs and resistance. Emerg Infect Dis. 2005; 11:791-797.
44. Khachatourians GG. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association J. 1998;159(9):1129-36.

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