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Occurrence and Antibiogram of Bacteria Isolated from Effluent and Waste Dump Site Soil of Selected Hospitals in Calabar Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigated the antibiogram of bacteria isolated from effluent and waste dump site soil of selected hospitals in Calabar Metropolis, Nigeria. The bacterial isolates were identified on the basis of standard cultural, morphological and biochemical characteristics. Antibiotic susceptibility pattern of the bacterial isolates was carried out according to Kirby-Bauer's disc diffusion method. The results revealed that one hundred and seventy nine bacterial isolates were identified from the collected samples, of which 85(47.5%) and 94(52.5%), were from effluent and waste dump site soil respectively. The bacterial isolates were *Staphylococcus aureus* (22.9%), *Escherichia coli* (20.7%), *Pseudomonas aeruginosa* (12.8%), *Streptococcus* sp (11.7%), *Salmonella* sp (7.8%), *Klebsiella pneumonia* (6.7%), *Providencia* sp (5.0%), *Enterobacter aerogenes* (5.0%), *Proteus* sp (2.8%), *Chryseobacterium* sp (1.7%), *Bacillus cereus* (1.7%) and *Serratia marcescens* (1.1%). *Bacillus cereus* was the only isolate that showed susceptibility to all the antibiotics tested against. However, *Chryseobacterium* sp showed 100% resistance to all the antibiotics tested against. The resistance observed in both samples (Effluents and waste dump site soil) were not statistically significant

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(p>0.05). The high level of antibiotic-resistant bacteria with various degrees of resistance observed in this study could be a potential public health risk. Therefore effective waste management practice should be put in place so as to control the wide spread of these antibiotic resistant bacteria in the environment.

Keywords: Antibiogram; resistance; bacteria; effluent; genes.

1. INTRODUCTION

Microorganisms constitute an essential part of the biosphere and play an important role in the sustainability and maintenance of the ecosystem. They exhibit the greatest genetic and metabolic diversity [1,2]. In order to survive, they have evolved mechanisms that enable them to respond to selective pressure exerted by various environment and competitive challenges [3]. The pathogenic microorganisms are particularly vulnerable to man's selfishness for survival who has sought to deprive them of their habitat using antimicrobial agents such as antibiotics [4].

Antibiotics are biologically-active compounds produced by bacteria and fungi which are capable of killing or inhibiting competing microbial species [5]. The first antibiotic penicillin, produced by *Penicillium notatum* was discovered by Alexdander Fleming in 1928 [6]. However, soon after the discovery of pencillin, a number of treatment failures and occurrence of some bacteria such as *Staphylococci* which were no longer sensitive to pencillin started being noticed. The observation of *Staphylococci* species that could still grow in the presence of penicillin was the beginning of the era of antibiotic resistance [7].

The indiscriminate and irrational use of antibiotic for therapeutic and non-therapeutic purpose has led to the development and dissemination of microbial resistance determinant both in clinical and non-clinical settings [8,9]. The use of antibiotics and spread of antibiotic resistance in clinical setting is a well-recognised problem, but antibiotic and antibiotic resistance as environmental problem and pollutants have largely been overlooked. As a result, the increasing incidence of resistance to a wide range of antibiotic agents by a variety of microorganisms is a major concern facing medical practice [10].

Resistant bacteria in the environment can lead to changes in the composition of microbial communities, with potential toxic effect on the balance of natural ecosystem. Studies carried out by Pei [11] and Wright [12] highlights that soil and water environment are recipients, reservoirs and sources of antibiotic resistant genes of clinical concern. Similarly, soil and water environment receive inputs of antibiotic and antimicrobials, which can serve to amplify antimicrobial resistant genes (ARGs) [13,14]. The ARGs associated with the bacterial contaminants can multiply in the hosts, transfer to other bacterial populations and be subject to further development and progression in the bacterial community. As such antibiotic resistant bacteria (ARB) that occur in the environment present potentially serious risks to human health and the sanctity of the environment.

The major problem associated with the ARB is the emergence of antimicrobial resistance among pathogenic bacteria to human and animals which makes treatment difficult for some life-threatening infections. The wide spread of ARGs and methods of acquiring resistance by clinically relevant bacteria is associated with the increased environmental pollution which constitutes a serious challenge for health and wellbeing of humans. These increasing wide spread of ARGs among environmental bacteria has led environmental scientist to consider ARB and ARGs as emerging pollutants or contaminant in the natural environment [15,16].

Proper hospital waste management is a major challenge in Africa and Nigeria in particular. Hospital waste (both biological and non-biological) are waste generated from hospitals that are discarded and not intended for further use. It includes stock cultures of microorganism, blood and blood products, scalpels, radioactive waste, needles, syringes, hazardous chemical, pharmaceutical waste, clinical bandages, miscellaneous waste etc. Hospital waste can be hazardous to public health and ecological balance since it contains various kinds of pollutants such as chemicals, radioactive substances and pharmaceutical waste and also pathogenic microorganisms [17].

Improper disposal of untreated hospital waste into the environment especially in developing

country such as Nigeria create a major problem on public health and is of major concern, if hospital effluent are not treated, concentrated forms of infectious agents and antibiotic resistant organisms are shed into the environment resulting in the spread of ARGs in the environment. More so, trace amount of antibiotics have been detected in waste water effluent of hospital, this has the ability to select and develop antibiotic resistance in organisms when they are exposed to it for a long time. Thus when such selective pressure contributes to persistence and dissemination of resistant gene, the natural environment become reservoir of resistant bacteria and resistant genes [18].

Hospitals were meant to protect the health of the community, but they however produce wastes that convey high potentials for infections and injuries. The waste produced by hospitals, if disposed of improperly can pose a more serious threat than the original disease.

2. MATERIALS AND METHODS

2.1 Description of Study Site

This research was carried out in three major hospitals located within Calabar Metropolis. Calabar (also referred to as "Canaan city") is a city in Cross River State, South Southern Nigeria. The original name for Calabar was Akwa Akpa from Efik language (Joseph et al. 2010). Calabar is the capital of Cross River State. It lies between latitudes 04 45' 30" North and 05 08'30" North of the Equator and longitudes 8 11' 21" and 8°27'00" East of the Meridian. For the purpose of administration, the city is divided into Calabar Municipality and Calabar South local Government Areas. Table 1 shows the coordinates of the hospitals investigated.

2.2 Collection of Samples

Hospitals effluents were collected from each of the hospitals investigated from the outermost chambers before discharging into drainage systems. The waste dump site soils were collected using sterile trowel. All the samples were collected into sterile containers. Samples were transported immediately after collection to microbiology post graduate laboratory in University of Calabar.

2.3 Microbiological Analysis

Serial dilution of samples: 10-fold serial dilutions of water and soil samples were carried out. Samples were serially diluted following standard serial dilution method.

Inoculation and incubation: After serial dilution, 1 ml of 10^{-4} and 10^{-5} for water and soil samples respectively, were plated in duplicates by pour plate technique using freshly prepared nutrient agar plates and were incubated aerobically at 23°C for 24 hours.

Enumeration and isolation of pure culture: After 24 hours of incubation, bacterial colonies were counted and colonies differing in size, shape and colour in different plates were selected and further sub-cultured on nutrient agar. The pure culture were then transferred to and maintained on agar slants.

Characterisation and identification of bacterial isolates: The isolates were characterised based on colonial and cell morphology, growth on differential selective media and biochemical test. The bacterial isolates were then identified by comparing their characteristics with those of known taxonomy using the schemes of Cowan and Steel [19].

Susceptibility testing: The susceptibility pattern of the bacterial isolates from effluent and waste dump site soil ere assayed according to Kirby-Bauer disc diffusion method [20], on Muller Hinton agar plates following the procedures described by Clinical and Laboratory Standard Institute (CLSI). The antibiotic disc used were Imipenem (10 µg), ceforuxime (30 µg), Augmentin (30 µg), Levofloxacin (5 µg), Gentamicin (10µg), Ramicef (5 µg), Cefoxitin (30 µg), Grazone (30 µg), Vancomy (30 µg) and Ofloxacin (5 µg).

Table 1. Coordinates of the hospitals investigated

Hospitals	Coordinates
H1	Longitude: 8.3510994 Latitude: 4.9553919
H2	Longitude: 8.3360793 Latitude: 4.9536581
H3	Longitude: 8.3175682 Latitude: 4.9491021

2.4 Statistical Analysis

Data obtained in this research were analysed using Microsoft excel 2010. Replicate readings were subjected to one way analysis of variance (ANOVA). Student T-test was used to compare paired mean readings.

3. RESULTS AND DISCUSSION

Hospital waste (biochemical waste) is any kind of waste containing infectious (or potentially infectious) materials. It may also include waste associated with the generation of biomedical waste that visually appears to be of medical or laboratory origin (e.g packaging, unused bandages, infusion kits etc) as well as research laboratory waste containing biomolecules or organisms that are restricted from environmental release. The main risk of hospital waste to public health is the transfer of resistant gene from environmental bacteria to human pathogens [21]. The management of hospital waste in Nigeria is an issue of great concern and importance in view of potential public health risk associated with such waste and also the volume of antibiotics used in hospitals released into hospital waste indicates a selective pressure on bacteria [22,23].

Table 2 shows the biochemical characterisation of bacterial isolates from effluent and waste dump site soil of the hospitals investigated. A total of 179 bacterial isolates were isolated. They were *Staphylococcus aureus* 41(22.9%), *Escherichia coli* 37(20.7%), *Pseudomonas aeruginosa* 23(12.8%), *Streptococcus* sp 21(11.7%), *Salmonella* sp 14(7.8%), *Klebsiella pneumoniae* 12(6.7%), *Providencia* sp 9(5.0%), *Enterobacter aerogenes* 9(5.0%), *Proteus* sp 5(2.8%), *Chryseobacterium* sp 3(1.7%), *Bacillus cereus* 3(1.7%) and *Serratia marcescens* 2(1.1%). From the result, more gram negative bacteria, especially members of the enterobacteriaceae, were isolated than gram positive bacteria. Among the gram negative bacteria, *Escherichia coli* had the highest percentage of isolation. This is because *E.coli* is able to withstand competition from other indigenous microorganisms with higher growth rates, while in the genera of gram positive bacteria isolated, *Staphylococcus aureus* had the highest percentage of isolation, and this may also be as a result of the ubiquity of *S. aureus* in the environment and as normal flora humans.

Antibiotics exert a selection in favour of resistant bacteria by killing or inhibiting the growth of susceptible bacteria; resistant bacteria can adapt to varying environmental conditions and serve as vectors for the spread of antibiotics resistant gene [24]. Table 3 shows the antibiotic susceptibility pattern of bacteria species isolated from samples used in this study. All the bacterial species isolated from effluent and waste dump site soil of the hospitals were subjected to susceptibility test with ten (10) antibiotics. All the isolates show various percentages of susceptibility and resistance to the entire antibiotics used. Among the antibiotics used, the bacterial species showed high level of susceptibility to Ofloxacin 169(94.4%) and Imipenem 165(92.2%) and high level of resistant to Ranicef 130(72.6%), Ceforuxaime 116(64.8%) and Graxone 111(62.0%).

Table 4 shows the percentage susceptibility of bacterial isolates to antibiotics used. From the result obtained, out of the 179 isolates that were subjected to susceptibility test, the isolates showed resistance rate of 7.8% to Imipenem; 46.8% to Ceforuxine; 25.7% to Argumentin; 10.6% to Levofloxacin; 21.8% to Gentamicin; 72.6% to Ramicef; 53.6% to Cefoxitin; 62.0% to Graxone; 49.2% to Vancomycin and 5.6% to Ofloxacin. All isolates were resistant to at least four or more antibiotics. It was observed that gram negative bacteria were more resistant to the tested antibiotics than the gram positive bacteria. This may be due to their unique outer membrane which excludes certain antibiotics from penetrating the cell. Also the porin channels in gram negative bacterial outer membrane can also prevent the entry of relatively large hydrophillic antibiotics. Moreso, gram negative bacteria also have a high transformation rate, i.e they have a great facility for exchanging genetic material (DNA) among stains of same species and even among different species. The gram positive bacteria do not have outer membrane, so there are more susceptible to antibiotics.

Figs. 1 and 2 shows the percentage resistance to antibiotics among bacterial isolates from hospitals effluent and waste dump site soil of hospitals respectively. From the results, it was observed that *Chryseobacterium* sp, *pseudomonas aerugenosa, Providencia* sp, *Staphylococcus aureus* and *Klebsiella pneumonia* showed 100% resistant to some of the antibiotics used. The result obtained also showed that isolates from waste dump soil were more resistant to antibiotics than those isolated from the effluent. *Chryseobacterium* sp isolated from waste dump site soil showed 100% resistant to all the antibiotics used. *Providencia* sp showed 100% resistant to six (6) antibiotics and other bacterial spp shows various percentage of resistant to the antibiotics as presented in the figure. Resistant was not observed in *Bacillus cereus* as it did not show any level of resistant to any of the antibiotics used.

Table 2. Biochemical characterisation of bacterial isolates from effluent and waste dump site soil of the hospitals investigated

Numbers of Isolates showing similar reaction	Gram's reaction	Catalase	Motility	Oxidase	Citrate	Indole	MR	VP	Coagulase	Gas	H2S	Manitol	Glucose	Sucrose	Lactose	Probable organism
2	-	+	+	-	+	-	-	+	+	-	-	+	+	+	-	*Serratia marcescens*
3	**-**	**+**	**-**	**-**	**+**	**+**	**+**	**-**	NA	**-**	**-**	**+**	**+**	**-**	**+**	*Chryseobacterium* sp
3	+	+	+	+	+	-	-	+	NA	-	-	+	+	+	+/-	*Bacillus cereus*
5	-	+	+	-	-	+	+	-	NA	+	+	-	+	-	-	*Proteus* sp
9	-	+	+	+	+	+	-	-	NA	+	+	+	+	+	-	*Providencia* sp
9	-	+	+	-	+	-	-	+	NA	+	-	+	+	-	-	*Enterobacter aerogenes*
12	-	+	-	-	+	-	-	+	NA	+	-	+	+	+	+	*Klebsiella pneumoniae*
14	-	+	+	-	-	-	+	-	NA	+	+	+	+	-	-	*Salmonella* sp
21	+	-	-	-	-	-	-	+	NA	+	-	-	+	+	+	*Streptococcus* sp
23		+	+	+	+	-	-	+	NA	-	-	+	-	-	-	*Pseudomonas aeruginosa*
37	-	+	+	-	-	+	+	-	+	+	-	+	+	+/-	+	*Escherichia coli*
41	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	*Staphylococcus aureus*

Table 3. Antibiotic susceptibility pattern of bacteria species isolated from samples

Susceptibility pattern	Imipenem	Ceforuxime	Augmentin	Levofloxacin	Gentamicin	Ranicef	Cefoxitin	Graxone	Vancomycin	Ofloxacin
***Klebsiella pneumoniae* (n=12)**										
Sensitive (%)	12(100)	0(0.0)	7(58.3)	8(66.7)	12(100)	9(75.0)	8(66.7)	3(25.0)	11(91.7)	12(100)
Resistant (%)	0(0.0)	12(100)	5(41.7)	4(33.3)	0(0.0)	3(25.0)	4(83.3)	9(75.0)	1(8.3)	0(0.0)
***Pseudomonas aeruginosa* (n=23)**										
Sensitive (%)	17(73.9)	14(60.9)	20(87.0)	21(91.3)	18(78.3)	3(13.0)	8(34.8)	2(8.7)	1(4.3)	19(82.6)
Resistant (%)	6(26.1)	9(39.1)	3(13.0)	2(8.7)	5(21.7)	20(87.0)	15(65.2)	21(91.3)	22(95.7)	4(17.4)
Escherichia coli (n=37)										
Sensitive (%)	37(100)	3(8.1)	28(75.7)	31(83.8)	29(78.4)	2(5.4)	7(18.9)	8(21.6)	11(29.7)	35(94.6)
Resistant (%)	0(0.0)	34(91.9)	9(24.3)	6(16.2)	8(21.6)	35(94.6)	30(81.1)	29(78.4)	26(70.3)	2(5.4)

Susceptibility pattern	Imipenem	Ceforuxime	Augmentin	Levofloxacin	Gentamicin	Ranicef	Cefoxitin	Graxone	Vancomycin	Ofloxacin
***Staphylococcus aureus* (n=41)**										
Sensitive (%)	39(95.1)	4(9.8)	24(58.5)	38(92.7)	27(65.9)	5(12.2)	14(34.1)	11(26.8)	14(34.1)	39(95.1)
Resistant (%)	2(4.9)	37(90.2)	17(41.5)	3(7.3)	14(34.1)	36(87.8)	27(65.9)	80(73.2)	27(65.9)	2(4.9)
***Chryseobacterium* sp (n=3)**										
Sensitive (%)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(66.7)
Resistant (%)	3(100)	3(100)	3(100)	2(66.7)	3(100)	3(100)	3(100)	3(100)	3(100)	1(33.3)

Table 3. Antibiotic susceptibility pattern of bacteria species isolated from samples (contd.)

Susceptibility pattern	Imipenem	Ceforuxime	Augmentin	Levofloxacin	Gentamicin	Ranicef	Cefoxitin	Graxone	Vancomycin	Ofloxacin
***Streptococcus* sp (n=21)**										
Sensitive (%)	21(100)	18(85.7)	20(95.2)	20(95.2)	17(81.0)	2(9.5)	11(52.4)	18(83.7)	16(76.2)	21(100)
Resistant (%)	0(0.0)	3(14.3)	1(4.8)	1(4.8)	4(19.0)	19(90.5)	10(47.6)	3(14.3)	5(23.8)	0(0.0)
Bacillus cereus (n=3)										
Sensitive (%)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
Resistant (%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
***Proteus* sp (n=5)**										
Sensitive (%)	5(100)	3(60.0)	4(80.0)	5(100)	5(100)	2(40.0)	5(100)	5(100)	5(100)	5(100)
Resistant (%)	0(0.0)	2(40.0)	1(20.0)	0(0.0)	0(0.0)	3(60.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
***Enterobacter aerogenes* (n=9)**										
Sensitive (%)	9(100)	5(55.6)	9(100)	9(100)	9(100)	6(66.7)	9(100)	9(100)	9(100)	9(100)
Resistant (%)	0(0.0)	4(44.4)	0(0.0)	0(0.0)	0(0.0)	3(33.)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
***Providencia* sp (n=9)**										
Sensitive (%)	6(66.7)	0(0.0)	2(22.2)	8(88.9)	4(44.4)	7(77.8)	2(22.2)	0(0.0)	5(55.6)	8(88.9)
Resistant (%)	3(33.3)	9(100)	7(77.8)	1(11.1)	5(55.6)	2(22.2)	7(77.8)	9(100)	4(44.4)	1(11.1)
***Salmonella* sp (n=14)**										
Sensitive (%)	14(100)	12(85.7)	14(100)	14(100)	14(100)	9(64.3)	14(100)	8(57.1)	14(100)	14(100)
Resistant (%)	0(0.0)	2(14.3)	0(0.0)	0(0.0)	0(0.0)	5(35.7)	0(0.0)	6(42.9)	0(0.0)	0(0.0)
***Serratia mercescens* (n=2)**										
Sensitive (%)	2(100)	1(50.0)	2(100)	2(100)	2(100)	1(50.0)	2(100)	1(50.0)	2(100)	2(100)
Resistant (%)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)

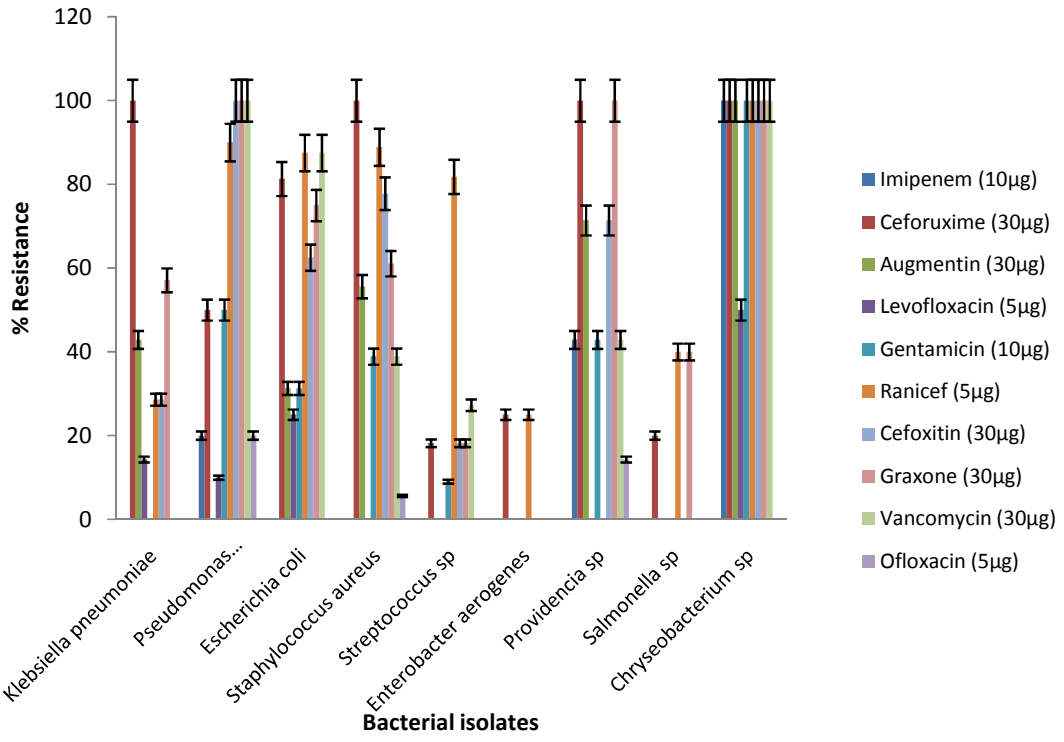

Fig. 1. Percentage resistance to antibiotics among bacterial isolates from hospitals liquid waste

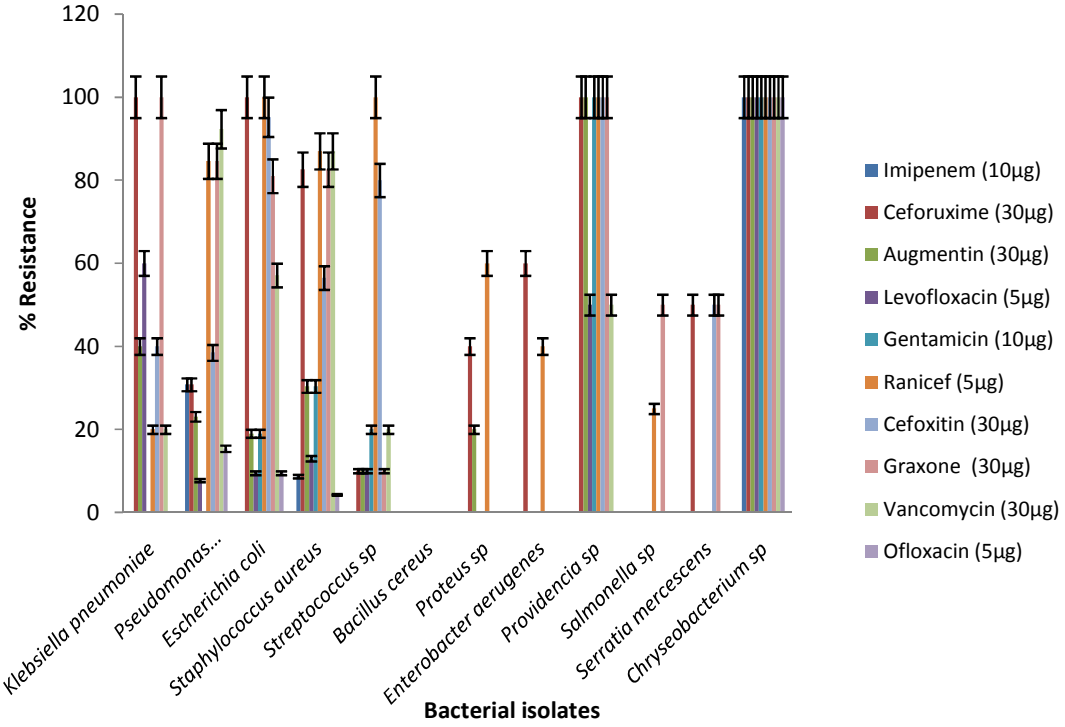

Fig. 2. Percentage resistance to antibiotics among bacterial isolates from hospitals waste dump site soil

Table 4. Percentage susceptibility of bacterial isolates to antibiotics used

Antibiotics	Sensitive (%)	Resistant (%)
Imipenem (10 µg)	165 (92.2) [a]	14(7.8) [b]
Ceforuxime (30 µg)	63 (35.2) [a]	116(64.8) [b]
Augmentin (30 µg)	133(74.3) [a]	46(25.7) [b]
Levofloxacin (5 µg)	160(89.4) [a]	19(10.6) [b]
Gentamicin (10 µg)	140(78.2) [a]	39(21.8) [b]
Ranicef (5 µg)	49(27.4) [a]	130(72.6) [b]
Cefoxitin (30 µg)	83(46.4) [a]	96(53.6) [b]
Graxone (30 µg)	68(38.0) [a]	111(62.0) [b]
Vancomycin (30 µg)	91(50.8) [a]	88(49.2) [b]
Ofloxacin (5 µg)	169(94.4) [a]	10(5.6) [b]

Superscripts a and b represents non-significant paired Student t-test with p values (p > 0.05) across the various rows

The extent of resistance to the antibiotic by bacterial isolates in this study may be associated with the extent of antibiotic usage. *E. coli* isolated from this study was highly resistant to some of the antibiotic used, which could be as a result of un-metabolised antibiotics released from the hospital in low concentration and repeated prescription of antibiotics by the medical practitioners can lead to resistant bacteria, which is commonly practiced in Nigeria. Self-medication, counterfeit drugs and inadequate hospital control measures can as well promote the development of resistance in clinical isolates [25]. In developing countries like Nigeria, self-medication is a common practice and could be a major cause of antibiotics resistance in clinical isolates since patients only thinks of going to the hospitals when they are unable to treat themselves.

4. CONCLUSION

Hospital effluents and waste dump site soil in the study areas are potential source and reservoir of antibiotic resistant bacteria. The proliferation of these antibiotic resistant bacteria in the disposed wastes is a threat to the public health and has a negative impact on the populace of such environment. On the basis of the aforementioned consequences, hospital wastes should be treated properly before discharging into the environment and efficient waste management technique should be employed in our hospitals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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