

Open Journal of Bioscience Research (OJBR) ISSN: 2734-2069 Article Details: DOI: 10.52417/ojbr.v5i1.577 Article Ref. No.: OJBR0501003-577 Volume: 5; Issue: 1, Pages: 21 – 33 (2024) Accepted Date: $31st$ May, 2024 © 2024 Bale *et al.*

RESEARCH ARTICLE OJBR0501003-577

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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA ASSOCIATED WITH CLINICAL WASTE MATERIALS IN GOKANA LOCAL GOVERNMENT AREA OF RIVERS STATE.

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ABSTRACT

A significant issue in world healthcare today is the rise in illnesses brought on by bacteria that have developed resistance to widely used antibiotics. The goal of this study was to determine the pattern of antibiotic susceptibility of bacteria that were isolated from five clinical wastes that were gathered from approved health facilities in Rivers state's Gokana Local Government. Fifty (50) samples of clinical waste were collected for six months from two different health centers. Samples were subjected to Standard microbiological analysis. Total heterotrophic count, total Staphylococcal count, coliform count, and feacal coliform count were estimated. Five (5) bacteria species isolated during the study and their percentage occurrence were *Staphylococcus* sp. was the most frequently isolated bacteria 15 (55.6%), *Bacillus* sp. 8(29.6%), *Klebsiella* sp. 2(7.4%), *Pseudomonas* sp. and *Escherichia coli* 1 (3.7%). Susceptibility of bacteria to antibiotics revealed that the Gram-positive bacteria were 77.3% resistant to Cefexime, 68.2% resistant to Cefotaxine and 63.6% resistant to Augmentin and Cefuroxine while the Gram-negative bacteria were 100% resistant to Nalidixic acid, 80% resistant to Ampiclox, 60% resistant to Augmentin, Imipenem/Cilastatin, Cefuroxine and Nitrofurantoin. Out of the twenty-seven (27) isolates, 74% had a multidrug resistance index \geq 0.2, 14.9% had \geq 0.5, and 11.1% had <0.2 Multidrug Resistance Index value. This study revealed that azithromycin, ciprofloxacin, levofloxacin, imipenem/cilastatin, and ofloxacin can be used as a drug of choice for treatment of Gram-positive bacteria associated with hospital wastes while levofloxacin, ofloxacin, ceftriaxone sulbactam, gentamycin, and cefixime could be used for Gram-negative bacteria associated with hospital wastes.

Keywords: *Clinical wastes, Antibiotic susceptibility, Fecal heterotrophic bacteria, Fecal coliform, Staphylococus.*

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INTRODUCTION

Clinical wastes are a very dangerous kind of waste that, if improperly managed, can cause major issues with the environment and human health (Alemu *et al*. (2015). Any waste produced during a human diagnosis, treatment, or vaccination, as well as during study, is considered biomedical waste (Al-Mutair *et al,* (2004). The garbage generated during activities related to health care has a larger prospective for contamination and injury than other types of rubbish Engda *et al.* (2018). The two main categories of clinical waste produced in hospitals are non-hazardous and biohazardous. Non-contaminated plastic, cardboard, packing materials, paper, etc. are components of non-hazardous trash Al Laham *et al.* (2012). There are two categories of biohazardous waste: (a) infectious waste, which includes sharps and non-sharps as well as plastic disposables and liquid waste Abor *et al.* (2008) (b) Non-infectious waste includes burned trash, glass that has been thrown away, chemical waste, radioactive waste, and cytotoxic waste. Like any other kind of municipal waste, between 75 to 90 percent of clinical waste is non-hazardous and harmless, according to Bakkali *et al.* (2015). The remaining 10–25% are dangerous and may harm people or animals as well as the environment. Large hospitals make up a significant portion of the biological waste produced. A significant portion is also contributed by smaller hospitals, clinics, nursing homes, pathology labs, and blood banks Beima *et al.* (2002).

The bacteria in the waste have the potential to seep out and contaminate the surrounding area. Medical waste can be divided into several categories, including aerosol containers, gas, and open sources used in nuclear medical therapy or in vitro diagnosis, pathological waste (body fluids from surgeries), infectious waste from labs, pharmaceutical waste (old pharmaceutical products), chemical waste (used solvents, disinfectants, pesticides, and diagnostic chemicals), and so on Bisma (2011).

Hospital sewage, also known as clinical waste, is a specific type of waste that includes all biological and non-biological wastes that are disposed of in hospitals and healthcare facilities and are not meant for reuse Chaoui *et al.* (2019). Due to the extensive use of water in hospitals, large amounts of waste containing radioactive elements, heavy metals, toxic compounds, and microbes the common of which are pathogenic are produced Alemu *et al.* (2015). Hospital effluent contains a significant amount of antibiotics, which puts choice stress on microorganisms. Disinfectants are a series of chemical compounds that, in addition to medications, are widely utilized in hospitals and have sparked worries about possible environmental damage Beima *et al.* (2002). Because resistant bacteria operate as a vector or reservoir for the resistance gene and contain the transmissible gene, its release into the receiving environment may affect public health Chen *et al.* (2017). This study's objective was to detect, categorize, and isolate the microbial populations present in biomedical wastes in light of these particulars.

MATERIALS AND METHODS

Study Area

The study area was Gokana Local Government Area in Rivers State. Two sampling locations were chosen from the study area; B-Dere Model Primary Health Center (4°40'23.9" N 7°15'49.4" E) and Kpor Model Primary Health Center $(4^{\circ}40^{\circ}24.6^{\circ}$ N $7^{\circ}14^{\circ}39.4^{\circ}$ E). Five (5) sampling points in each hospital were chosen for this study, the samples were transferred to the Department of Microbiology for analysis. Sakpenwa-Bori Road, which is located off the Port Harcourt Eket segment of the East-West Expressway, provides access to the area by car. The hospitals' environmental conditions played a factor in the selection of the two (2) sample locations.

Sample Collection and Processing

Clinical waste samples used for this study were: Colton wool, Drip set, Canula, scalp vein, and Syringe. These were collected from two different sampling locations (B-Dere Primary Health Center and Kpor Primary Health Center in Gokana Local Government Area in Rivers state). The clinical waste samples were collected by rinsing method Davies *et al.* (2010). Each clinical waste was sorted separately from both locations after being appropriately labeled and rinsed with sterile distilled water, the samples were aseptically transferred in an ice box to the Department of Microbiology laboratory at Rivers State University for bacteriological investigation within two hours after collection. Preparation of the stock analytical unit was done by weighing 10 g of clinical waste (Colton wool, Drip set Canula, scalp vein, and Syringe) samples and rinsing in 100ml of the diluent (Normal saline) to give 10-1 dilution Al Laham *et al*. (2012).

Bacteriological Analysis

Enumeration and Isolation of Bacteria

Tenfold Serial dilution was done from the homogenized samples of the waste 10^{-1} dilution, up to 10^{-2} . On plates of Mannitol salt (MSA), Eosin Methylene Blue (EMB), and Nutrient Agar (NA), an aliquot (0.1 ml) of the suitable dilutions were spread out in duplicate. The plates were incubated at 37°C for 24 hours. The Colonies on the plates were counted and described morphologically. Total heterotrophic bacteria were counted using the colonies on nutrient agar plates. Colonies on Eosin Methylene Blue (EMB) agar were used to estimate faecal coliform counts (FCC) and colonies on Mannitol salt agar were used to estimate total Staphylococcal counts (TSC). To generate pure cultures, representative different colonies were sub-cultured on newly prepared sterile nutrient agar plates and cultured at 37°C for 24 hours.

Characterization and Identification of Bacteria

Colonial/morphological characteristics and Chemical tests were conducted on the uncontaminated isolates for identification of the bacteria. Gram Staining and Motility test, and Biochemical tests such as salt tolerance, starch hydrolysis, coagulase, catalase, indole, methyl red, Voges Proskauer, sugar fermentation test (glucose, lactose, mannose, and sucrose), and citrate utilization tests were carried out to characterize the Bacteria isolates.

Antimicrobial Susceptibility Testing

Agar Disk Diffusion Method (Kirby Bauer Disk Diffusion)

The bacterial suspension in the tube, which was standardized to 0.5 McFarland Turbidity Standard, was immersed using a germ-free swab stick Cheesbrough (2003). Thereafter, the prepared Mueller Hinton agar was evenly spread across the surface of the petri dish, and the plates were rotated by roughly 60 degrees to guarantee that the organism was dispersed equally. The agar plates were allowed to stand for three to five minutes to dry. The impregnated antimicrobial discs were equally distributed across the surface of the inoculation plate using germ-free forceps, 15 mm from the plate's edge. Using the forceps head, each disc was gently pulled down to make contact with the agar. After the discs were applied, the plates were incubated aerobically for $16-18$ hours at 35° C in an inverted orientation. The test plates were checked after incubation to make sure confluence growth had occurred. The diameter of each inhibitory zone on the underside of the plate was measured in millimeters as a point of reference CLSI, (2017).

Determination of Multiple Antibiotic Resistance Index (MAR)

Bacteria isolates that are resistant to three or more drugs are said to have multiple antibiotic resistance Chen *et al.* (2017). Each isolate's multiple antibiotic resistance (MAR) index was calculated using the formula MAR = a/b , where "a" denotes the number of medications to which the test isolate has shown resistance and "b" denotes the total number of medicines to which the test isolate has been evaluated for sensitivity Dallolio (2018).

Data Analysis

A statistical analysis of the number of bacteria found in clinical waste samples was computed. The Duncan Multiple Range Assess (DMRT) and Analysis of Variance (ANOVA) were engaged to test for significance and mean separation between the locations, respectively. SPSS version 25, a computer application, was utilized for this purpose.

RESULTS

Bacterial Population of Clinical Waste Samples

The bacterial population of Colton wool from various locations sampled is presented in Table 4.1. The result of the analysis displayed that the mean total heterotrophic bacterial count ranged from 8.6 ± 77.5 x10⁴ to 1.41 ± 116.5 x10⁵ CFU/g. Faecal coliform count ranged from $9+13.1 \times 10^2$ to $1.2+15.2 \times 10^3$ CFU/g. Total Staphylococcal count ranged from $6.3\pm85.1 \times 10^3$ to $9.1\pm112.0 \times 10^3$ CFU/g.

The bacterial population of the Drip set from various locations sampled is presented in Table 4.2. The result of the analysis displayed that the mean total heterotrophic bacterial count ranged from $4.2\pm51.0x10^4$ to 1.30 ± 102.8 x10⁵ CFU/g. Faecal coliform count ranged from $7 + 8.5 \times 102$ to $1.1 + 13.4 \times 103$ CFU/g. Total Staphylococcal count ranged from 2.9+18.7 x103 to 5.6+68.9 x10³ CFU/g.

The bacterial population of Cannular from various locations sampled is presented in Table 4.3. The result of the analysis displayed that the mean total heterotrophic bacterial count ranged from 1.8 ± 10.7 x10⁴ to $3.2\pm33.1x10^4$ CFU/g. Faecal coliform count ranged from $1.6\pm 15.6 \times 10^3$ to $1.7 \pm 16.1 \times 10^3$ CFU/g. Total Staphylococcal count ranged from $4.2\pm68.0 \times 10^3$ to $7.1\pm33.1 \times 10^3$ CFU/g.

The bacterial population of Scalp vein from various locations sampled is presented in Table 4.4. The result of the analysis displayed that the mean total heterotrophic bacterial count ranged from $3.8\pm9.0 \times 10^4$ to $3.8\pm43.3 \times 10^4$ CFU/g. Feacal coliform count ranged from $2 \pm 2.4 \times 10^2$ to $7 \pm 16.1 \times 10^2$ CFU/g. Total Staphylococcal count ranged from $3.5+32.4 \times 10^3$ to $3.6+62.5 \times 10^3$ CFU/g.

The bacterial population of Syringe from various locations sampled are presented in Table 4.5. Mean total heterotrophic bacterial count ranged from $6.2\pm32.4 \times 10^4$ to $9.8\pm93.5 \times 10^4$ CFU/g. Faecal coliform count ranged from $4\pm6.9 \times 10^2$ to 3.2 $\pm65.8 \times 10^3$ CFU/g. Total Staphylococcal count ranged from $5.1\pm49.8 \times 10^3$ to $5.2\pm59.1 \times 10^3$ CFU/g.

Table 1: Bacterial Population of Colton wool sample from various locations sampled

Key: THBC (Total Heterotrophic Bacteria Count), FCC (Faecal coliform count), TSC (Total Staphylococcal count). MPHC (Modern primary health center) *Mean with the same superscript along the column is not significantly different $(p \le 0.05)^*$

Table 2: Bacterial Population of Drip set sample from various locations sampled

Key: THBC (Total Heterotrophic Bacteria Count), FCC (Faecal coliform count), TSC (Total Staphylococcal count). MPHC (Modern primary health center) *Mean with the same superscript along the column is not significantly different $(p \le 0.05)^*$

Table 3: Bacterial Population of Cannular sample from various locations sampled

Key: THBC (Total Heterotrophic Bacteria Count), FCC (Faecal coliform count), TSC (Total Staphylococcal count). MPHC (Modern primary health center) *Mean with the same superscript along the column is not significantly different $(p \le 0.05)^*$

Table 4: Bacterial Population of Scalp vein sample from various locations sampled

Key: THBC (Total Heterotrophic Bacteria Count), FCC (Faecal coliform count), TSC (Total Staphylococcal count). MPHC (Modern primary health center) *Mean with the same superscript along the column is not significantly different $(p \le 0.05)^*$

Table 5: Bacterial Population of Syringe sample from various locations sampled

Key: THBC (Total Heterotrophic Bacteria Count), FCC (Faecal coliform count), TSC (Total Staphylococcal count). MPHC (Modern primary health center) *Mean with the same superscript along the column is not significantly different $(p \le 0.05)^*$

Table 6: Frequency distribution of bacteria isolated from clinical waste samples

Antibiotic	Conc. (μg)	Resistant	Intermediate $n\frac{6}{6}$	Susceptible $n\frac{6}{6}$
		$n\left(\frac{6}{6}\right)$		
$\mathbf{A}\mathbf{U}\mathbf{G}$	30	14(63.6)	6(27.3)	2(9.1)
CTX	25	15(68.2)	3(13.6)	4(18.2)
ZEM	5	17(77.3)	0(0.00)	5(22.7)
CRO	45	9(40.9)	6(27.3)	7(31.8)
LBC	5	1(4.5)	2(9.1)	19(86.4)
IMP	10	2(9.1)	2(9.1)	18(81.8)
CXM	30	14(63.6)	2(9.1)	6(27.3)
OFX	5	1(4.5)	3(13.6)	18(81.8)
ERY	15	6(27.3)	10(45.5)	6(27.3)
GN	10	6(27.3)	0(0.00)	16(72.7)
AZN	15	2(9.1)	0(0.00)	20(90.9)
CIP	5	0(0.00)	2(9.1)	20(90.9)

Table 7: Susceptibility pattern of gram positive organism isolated from clinical waste samples

Key. AUG (Amoxilin clavulanate), CTX (Cefotaxine), ZEM (Cefixine), CRO (Ceftriaxone sulbactan), LBC (Levofloxacin), IMP (Imipenem/Cilastatin), CXM (Cefuroxine), OFX (Ofloxacin), ERY (Erythromycin), GN (Gentamycin), AZN (Azithromycin), CIP (Ciprofloxacin)

Key. AUG (Amoxilin clavulanate), CTX (Cefotaxine), ZEM (Cefixine), CRO (Ceftriaxone sulbactan), LBC (Levofloxacin), IMP (Imipenem/Cilastatin), CXM (Cefuroxine), OFX (Ofloxacin), NA (Nalidixic acid), GN (Gentamycin), ACX (Ampiclox), NF (Nitrofurantoin)

Table 9: MAR Indices of Organism (N=27)

Key: Multiple Antibiotic Resistance (MAR)

DISCUSSION

The study's findings demonstrated that the bacterial strains that were isolated had developed multi- and multi-resistant resistance to the antibiotics that had been tested, making these medications resistant as first-line treatments for infections brought on by these pathogens. The percentage of isolates that were resistant to every antibiotic that was tested made this clear. Bacteria counts revealed that the total heterotrophic bacterial counts in Colton wool samples were higher in samples obtained from B-Dere Health Center than Kpor Health Center. The faecal coliform counts were higher in samples obtained from Kpor Health Center than B-Dere Health Center while the total Staphylococcal counts were higher in samples obtained from B-Dere Health Center than Kpor Health Center. The study found a higher level of bacterial contamination, which possibly will be primarily ascribed to the use of an unsuccessful disinfectant through exterior cleaning, as well as the improper application of common precautions like hand cleanliness and contact precautions and the organism's migration through airflow. Hospitals that show a reluctance to commit financial resources to the control of contamination, such as ventilation systems; hospitals that are ignorant of the level of impurity and the usefulness of generally used antiseptics; and hospitals that use unsuitable waste management techniques are closely linked to this situation.

The total heterotrophic bacterial counts in Drip set samples were higher in samples obtained from B-Dere Health Center than Kpor Health Center. The faecal coliform counts were higher in samples obtained from B-Dere Health Center than Kpor Health Center while the total Staphylococcal counts were higher in samples obtained from B-Dere Health Center than Kpor Health Center. The results of this investigation showed that clinical wastes were considerably polluted by a wide range of bacterial species, including both Gram-positive (81.5%) and Gram-negative (18.5%) bacteria. The fact that certain Gram-positive bacteria's outer membranes naturally maintain their viability in an abiotic hospital environment for several days to months may help to explain why Gram-positive bacteria predominate Eshetie *et al.* (2016). Nonetheless, research carried out in Zimbabwe and Morocco revealed that Gram-negative bacteria constituted the majority of clinical waste bacteria, which runs counter to our findings. These differences could be caused by a variety of circumstances, including different sample times, the use of various sample procedures and culture techniques, the inclusion of patients who have already been colonized or infected, and modifications to sampling sites Eichenberger et al. (2015).

The total bacterial counts in Scalp vein samples were highest in both locations. The feacal coliform counts were higher in samples obtained from B-Dere Health Center than Kpor Health Center while the total Staphylococcal counts were higher in samples obtained from Kpor Health Center than B-Dere Health Center. The total heterotrophic bacterial counts in Syringe samples were higher in samples obtained from Kpor Health Center than B-Dere Health Center. The feacal coliform counts were higher in samples obtained from B-Dere Health Center than Kpor Health Center while the total Staphylococcal counts were higher in samples obtained from Kpor Health Center than B-Dere Health Center.

Overall, *Staphylococcus* sp. was the most frequently isolated bacteria 15(55.6%) followed by *Bacillus sp* 8(29.6%), *Klebsiella sp* 2(7.4%), *Pseudomonas sp* and *Escherichia coli* 1(3.7%) across the sampling location which is in line with the results of several study conducted in Africa and outside (Ford *et al*., 2015). One common component of the flora that inhabits the skin and mucous membranes is *Staphylococcus aureus*.

Patients and medical staff constantly shed the bacteria into the hospital environment, where it continues to exist. These isolates were also resistant to standard disinfection techniques, which made it easier for them to proliferate and infect hospital patients by colonizing the laboratory and surrounding areas.

In the twenty-first century, serious infections brought on by bacteria resistant to widely used antibiotics have emerged as a major worldwide health concern. The rise and spread of antibiotic resistance in pathogenic bacteria, which makes them resistant to previously effective treatment strategies, poses the biggest danger to the use of antibiotics. From the result it was observed that the Gram-positive isolates were 77.3% resistant to Cefexime, 68.2% resistant to Cefotaxime, and 63.6% resistant to Augmentin and Cefuroxine while the Gram-negative organism was 100% resistant to Nalidixic acid, 80% resistant to Ampiclox, 60% resistant to Augmentin, Imipenem/Cilastatin, Cefuroxine, and Nitrofurantoin. The results indicated that these organisms had good exposure to the antimicrobials under test and had evolved defense mechanisms against them (Table 4.7-8). It has been reported that clinical and hospital waste from all around the world contains germs resistant to antibiotics. The use of antibiotics in veterinary and medical treatment has sparked worries about the occurrence and spread of antibiotic resistance in bacterial populations. Antibiotic use in veterinary or medical care has led to the selection of resistant bacteria, which has unavoidably resulted in the introduction of these germs into the natural environment. This is especially true in settings like hospitals where human life is in danger during transfers Endalafer *et al.* (2011). Antimicrobial resistance in bacteria is caused by the overuse, abuse, and underuse of antibiotics globally Dancer *et al.* (2004). Lateef (2004) posits that in poor countries, the availability of drugs to the general people may encourage the practice of self-administration of antibiotics, hence elevating the risk of drug-resistant strains becoming more common. The comparatively high level of resistance to antimicrobial drugs identified in this study is indicative of environmental overuse or abuse of these compounds. Multiple drug resistance has been associated with the establishment of global epidemics, which is a critical public health concern (Ekrami *et al.*, 2011). Therefore, the diverse medication resistance exhibited by these pathogens is concerning and should raise concerns for public health. Additionally, it has been noted that if terminal cleaning is ineffective, patients admitted to rooms that had previously been inhabited by individuals colonized or infected with MDR strains of bacteria may have a threefold increased risk of contracting healthcare-associated infections (HCALs) from contaminated environmental surfaces or equipment. Antimicrobial resistance (AMR) is becoming more common, which raises the morbidity and mortality rates linked to infections that are related to healthcare settings (HCALs).

Bacteria organisms are becoming resistant to more antibiotics which is of serious issue for public health. The presence of multi-drug resistant variants demonstrates how organisms are evolving new ways to resist antibiotics, limiting and raising the cost of therapy choices. Consequently, the analysis of the Multiple Antibiotic Resistance (MAR) index of the bacterial isolates employed in this study revealed that 11.1% of the isolates exhibited a MAR score below 0.2. See Table 4.9. It is crucial to remember that MAR index values larger than 0.2 signify sources of contamination with a significant risk of contamination and frequent usage of antibiotics (Ford *et al*., 2015). According to Lenzi *et al.* (2020), a MAR index of more than (>) 0.5 indicates the presence of an isolate from a high-risk contamination source that often uses antibiotics. Seventy-seven percent (77.3%) of the organism isolated in this study showed multiple resistance to

antibiotics, probably due to indiscriminate use of antibiotics arising from healthcare-associated infections acquired from this source (Chung *et al.,* 2003) So, the presence of bacteria in this study is concerning rather than surprising. As a result, several antibiotic-resistant pathogenic bacteria in this study are consistent with the findings of Toroglu *et al.* (2005) and represent a well-known phenomenon that has a detrimental effect on public health.

CONCLUSION

The outcome demonstrated that these organisms have had ample exposure to the antimicrobials under test and have evolved defense mechanisms against them. This study demonstrated that hospitals, where patients seek treatment for diseases, harbor microorganisms resistant to antibiotics. This demonstrated how microorganisms resistant to antibiotics are already commonplace. These isolates' pattern of resistance is consistent with the kind of antibiotics that these hospitals typically utilize. Consequently, all levels of government should make an effort to fund studies on the creation of novel antibiotics that may be useful in the management of serious diseases brought on by bacteria resistant to existing antibiotics. The source of nosocomial infections may have been hospital settings, healthcare personnel's hands, and clinical specimens from admitted patients, according to many studies that used molecular type to establish the clonal relationship between clinical wastes. This demonstrated the widespread presence of microorganisms resistant to antibiotics. These isolates' resistant patterns match the kinds of antibiotics that are often administered in these facilities.

The findings indicated that the best drugs to treat Gram-positive organisms linked to hospital diseases are Azithromycin, Ciprofloxacin, Levofloxacin, Imipenem/Cilastatin, and Ofloxacin; for Gram-negative organisms linked to hospital health care infections, the best drugs to use are Levofloxacin, Ofloxacin, Ceftriaxone sulbactan, Gentamycin, and Cefixine. It is consequently concerning rather than surprising that this investigation has found numerous antibiotic-resistant microorganisms. As a result, the study's discovery of many antibiotic-resistant pathogenic bacteria illustrates a well-known phenomenon that is detrimental to public health.

CONFLICT OF INTEREST

The authors of the manuscript declare that they have no competing interests in having this work published.

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