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Bacteria Associated with Hospital Waste of Federal University Teaching Hospital, Owerri

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ABSTRACT

Introduction: Hospital waste management is critical for minimizing health risks and environmental damage, particularly in developing countries where inadequate training and resources exacerbate these issues. The Federal University Teaching Hospital, Owerri, serves as a case study to determine the bacteria associated with hospital waste. Objectives: This study aimed to determine the bacterial contamination in hospital waste.

Materials and Methods: An experimental study design was employed. The hospital waste samples were analysed for bacterial contamination via microbiological standards.

Results: The infectious waste had the highest total viable count (9.86±0.28 CFU/g), indicating a high level of microbial presence, whereas the sharps waste presented a much lower total viable count of 5.40±0.52 CFU/g. The highest number of infectious waste isolates was 45 (30.2), followed by general waste 41 (27.5) and chemical waste 8 (5.4). Among the infectious waste, *Enterobacter cloacae* (50%) was the most common bacteria, followed by *Staphylococcus Scuri* ssp. *Lentus* (32%). Among the sharps waste, *Staphylococcus gallinarum* (12%) was the most common, followed by *Staphylococcus xylosus* and *Escherichia coli* (11%). In general, waste, *Pantoea ananatis* (100%) was the most prevalent bacteria, followed by *Escherichia coli* (38.9%), and the least prevalent bacterium was *Staphylococcus Scuri* ssp. *Lentus* (20%).

Conclusion: This study highlights the need to mitigate the spread of resistant pathogens. Continuous monitoring and updated waste management strategies are essential to protect public health and the environment.

Keywords: Hospital waste, bacteria, susceptibility, distribution and susceptibility

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INTRODUCTION

Hospitals are non-replaceable institutions for mankind's health and well-being with universal provision of services that ensure round-the-clock attention to intricate health problems ¹. However, waste management in hospitals has become a real issue due to the generation of obvious health risks and environmental issues ².

It remains a critical issue, largely in developing countries where many healthcare centres are confronted with technological, economic development and social constraints; and the lack of proper training of healthcare workers responsible for waste disposal ³. Poor handling and disposal methods embraced in the disposal and handling of medical waste are increasing

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health and environmental hazards due to the infectious nature and pungent smell of the waste⁴. Nigeria hospital waste management has not received the attention it should have due to insufficient economic resources, poor policy regimes, inadequate public infrastructure, and insufficient other key capacities³. Approximately 15% of waste from hospitals is hazardous; 85% of the waste is non-hazardous⁵.

Disease-causing microorganisms in unprocessed medical waste can percolate as well as contaminate soil and surface waters 6. It was revealed through a WHO/UNICEF joint 2015 survey of 24 sampled countries that fewer than a simple majority (58%) were reporting adequate management of medical waste 7. Dispose of improperly shredded medical trash with no treatment, resulting in percolation into the ground as well as contaminating soil and aquifers with toxic chemicals or drugs. Failure in burn or incineration releases harmful substances such as furans and dioxins into the environment 8. Hospital waste management is now an essential issue globally, especially in light of antimicrobial resistance (AMR), which has been recognized as one of the most pressing global public health concerns.

According to the Geneva-based World Health Organization (WHO), antibiotic resistance is increasing to unprecedented levels around the globe and contributing to increased rates of morbidity and mortality ⁹. There had already been 929,000 directly AMR-related deaths in the year 2019 alone and an overall related impact due to AMR amounting to an estimated 3.57 million lives. The six major pathogens responsible for these deaths are Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa ¹⁰. According to the World Health Organization, by 2050 it may

increase to 10 million. This study was therefore aimed at determining the bacteria associated with hospital waste of Federal University Teaching Hospital, Owerri hospital waste.

MATERIALS AND METHODS

Study Design

The study employed an experimental design. The experimental design was used to study the resistant pathogens associated with the waste at Federal University Teaching Hospital, Owerri.

Area of Study

The Federal University Teaching Hospital is located in Owerri city, Imo State, southeastern Nigeria. In 1903, Federal University Teaching Hospital, Owerri was founded as a colonial dispensary¹¹. It was promoted to a district hospital, then a general hospital, before finally becoming a Federal Medical Centre in 1995 ¹².

Collection of hospital waste sample

The waste was received from color-coded and labeled containers that indicated the type of waste that they contained. Such waste was traditionally sorted at source into categories including infectious, pathological, sharps, chemicals, pharmaceuticals, and general waste. It was facilitated through staff that had been trained on how to dispose of waste properly. 24 samples were taken in total. 4 samples were taken on average from each based-on categories like infectious waste, pathological waste, sharps, chemical waste, pharmaceutical waste, and general waste. Personal protective gear (PPE; i.e. gloves and laboratory coats) was worn before taking samples to provide a clean environment and safety from danger. The samples were aseptically collected using a sterile collection vessel to avoid further contamination by the environment while collecting. Each sample was labeled with the correct data, which included name of waste collected, where sampled and collection day.

Microbiological analysis

Enumeration and identification of bacteria were carried out aseptically in the Laboratory of Infectious Disease and Molecular Epidemiology at the Department of Public Health, Federal University of Technology, Owerri, Imo State, Nigeria. The waste samples were initially separated in a Biosafety Cabinet II. One gram of hospital solid waste was placed in a 5 ml sterile Stuart transport medium tube, and the sample was allowed to wet completely. The tube was tightly closed to prevent leakage and contamination. Vortexing was performed on the samples, and 90 µL of the eluate was used to inoculate the culture for each plate/test condition 13. The samples were inoculated in triplicate in the following culture media using the spread plate method: MacConkey agar with cefotaxime (Hardy), Nutrient agar (Hardy), mannitol salt agar (Hardy), and blood agar (Hardy). The plates were incubated at 37°C for up to 48 hours in an air incubator 14. Once growth on the culture media was obtained, plates were counted using an electronic colony counter, and all isolates were subcultured and purified and then stored at -80°C in Tryptic Soy Broth containing a glycerol concentration of 20% (v/v). Isolated microbes were identified based on a combination of morphological, biochemical, and microscopic examination of colony morphology, cell shape, and size, which provided initial clues for identification ¹⁵.

Antimicrobial susceptibility testing (AST)

The susceptibility of the pathogens isolated was tested against a series of antibiotics. It was carried out using Kirby-Bauer Disk Diffusion Susceptibility Test ¹⁶, according to the Clinical and Laboratory Standards Institute guidelines ¹⁷. A fresh subculture of each isolate was prepared

on nutrient agar. A sterile straight wire was used to touch 5 colonies of every isolate, and they were then suspended into a sterile Bijou bottle containing 5 mL peptone water (Lab M), which was subsequently incubated overnight at 37°C. Overnight broth cultures were diluted in sterile saline to 10⁶ colony-forming units/mL by comparing the inoculum turbidity with 0.5 McFarland turbidity standards. A sterile cottontip applicator was brought into contact with a standard inoculum and used to inoculate air-dried plates of Mueller-Hinton agar (Oxoid, England). Individual sterile antibiotic discs were inoculated onto each plate and incubated aerobically for 24 h at 37°C. The antibiotics used were levofloxacin (LEV, 30 µg), cefalexin (30 µg, CN) ciprofloxacin (10 μg, CIP), erythromycin (10 μg, E), ampicillinsulbactam (30 µg, SAM), sulfamethoxazole (125 μg, SXT), cefotoxine (10 μg, CTX), tetracycline (30 µg, TE), ampicillin (10 µg, AMP) and amikacin (30 µg, AK). The diameter of the zone of inhibition of every isolate to the disc was measured with a calibrated ruler according to the standardized CLSI 2021 guidelines ¹⁸.

Method of Data Analysis

The data were analysed via the Statistical Package for Social Sciences (SPSS) computer software version 25. Frequencies and percentages were used to analyse the variables.

RESULTS

Table 1 presents the mean bacterial counts of various types of hospital wastes, i.e., the total viable count, coliform count, and total selective bacterial count, expressed as colony-forming units per gram (CFU/g). Infectious waste contained the greatest total viable count (9.86±0.28 CFU/g) with a significant presence of microbes. It has a coliform count of 3.40±0.31 CFU/g and a total bacterial count of 4.60±0.20 CFU/g, showing high microbial contamination and infection risk. The pharmaceutical waste had

a lower total viable count of 2.40 ± 0.22 CFU/g. It has a coliform count of 1.10 ± 0.20 CFU/g and a total bacterial count of 2.10 ± 0.10 CFU/g, showing relatively low levels of microbial contamination.

Cultural Morphology and Biochemical Characteristics of the Bacterial Isolates

Table 2 provides detailed information on the cultural morphology, microscopic characteristics, biochemical characteristics, and carbohydrate utilization profiles of various bacterial isolates found in the hospital waste. The following bacteria were isolated: Raoultella ornithinolytica, Staphylococcus gallinarum, Enterococcus cloacae, Staphylococcus Scuri ssp. Lentus, Escherichia coli, Staphylococcus xylosus, Klebsiella pneumonia and Pantoea ananatis

Distribution of bacterial isolates among the various types of hospital waste

Table 3 shows the distribution of bacterial isolates among the various types of hospital waste; infectious waste had the highest number of isolates at 45 (30.2), followed by general waste at 41 (27.5) and chemical waste at 8 (5.4). Among the infectious waste, *Enterobacter cloacae* (50%) was the most common bacteria, followed by

Staphylococcus Scuri ssp. Lentus (32%), and the least common species was Pantoea ananatis, which was absent. Among the sharps waste, Staphylococcus gallinarum (12%) was the most common, followed by Staphylococcus xylosus and Escherichia coli (11%). In general waste, Pantoea ananatis (100%) was the most prevalent bacteria, followed by Escherichia coli (38.9%), and the least prevalent bacterium was Staphylococcus Scuri ssp. Lentus (20%). Among the bacteria subjected to antibiotic susceptibility testing (AST), the highest number of Staphylococcus gallinarum (n=25) were found to be sensitive to amikacin (24) but resistant to other antibiotics used. All 30 isolates of Raoultella ornithinolytica were resistant to antibiotics, except for amikacin, for which Raoultella ornithinolytica was sensitive. The isolates of Klebsiella pneumoniae (n=20) were sensitive to the entire antibiotic used. The isolates of Pantoea ananatis (n=2) were sensitive to all the antibiotics except for ampicillin, which produced resistance, whereas the Escherichia coli isolates (n=18) were resistant to trimethoprim and ampicillin. The bacterial isolates were highly resistant to ampicillin, with the exception of Klebsiella pneumoniae, followed by tetracycline and trimethoprim (Table 4).

Table 1: Mean bacterial counts of the various types of hospital waste

Waste Type	Total viable count $(cfu/g) \times 10^2$	Coliform count $(cfu/g) \times 10^1$	Selective bacteria count (cfu/g) × 10 ²
Infectious Waste	9.86 ± 0.28	3.40±0.31	4.60±0.20
Sharps	5.40 ± 0.52	1.20 ± 0.22	2.15 ± 0.14
Pathological Waste	6.45 ± 0.18	2.20 ± 0.33	3.31 ± 0.40
Chemical Waste	4.10±0.52	0.0	1.0 ± 0.20
Pharmaceutical Waste	2.40 ± 0.22	1.10 ± 0.20	2.10 ± 0.10
General Waste	7.86 ± 0.28	2.40 ± 0.32	4.50±0.24

Table 2: Cultural morphology and biochemical characteristics of the bacterial isolates from hospital waste

	MICROSCOPY	7				BIO	CHE	MIC,	BIOCHEMICAL REACTIONS	EAC	LION	SI			ARB	OH)	CARBOHYDRATE UTILIZATION	N I		
COLONY FEATURES	Cell Arrangement	sbore	Motility Capsule	Catalase	Oxidase	Coagulase	əlopuI	Nitrate	MethylRed	$\mathrm{d} \Lambda$	Urease	$S^{z}H$	citrate	Glucose	Sucrose	Гастоѕе	maltose	lotinsm	xλjose	ORGANISM
Smooth circular colonies creamy and butyrous and translucent on Nutrient Agar (NA)	gram-positive group of oval cells			+	1	1	+	+	+	+	+	+	1	+	+	+	+	+	+	Staphylococcus gallinarum
Small dark red colonies that ferment lactose on MacConkey Agar (MA) Circular smooth colonies with	gram-positive cocci in pairs and some in short chains			1	1	1	1	1	+	+	1	1	1	+	+	+	+	+	+	Enterococus cloacae
light-yellow pigments on Mannitol Salt Agar (MSA).	gram-positive group of oval cells, some clustered			+	ı	1	+	+	+	+	+	+	ı	+	+	+	+	+	+	Staphylococcus Scuri ssp. Lentus
Small pink shiny smooth colonies on MacConkey Agar (MA).	gram-negative short rods in singles	1		+	1	1	+	İ	+	1	1	1	ī	+	+1	+	+	+	+	Eseberichia coli
Circular smooth colonies with light-yellow pigments on Mannitol Salt Agar (MSA).	gram-positive group of oval cells, some clustered			+	1	1	+	+	+	+	+	+	i .	+	+	+	+	+	+	Staphylococcus xylosus
Large mucoid pink colonies on MA	gram-negative short rod in singles		+	1	1	1	1	+	1	+	+		+	+		+		1	1	Klebsiella pneumonia
Slight pink colonies on MA	gram-negative rods in singles	1	+	+	1	1	1	+	1	+	+	1	1	+	+	1	1	+	1	Pantoea ananatis.
Large mucoid colonies, pink on MacConkey Agar (MA)	Gram-negative short rods in singles	'	+	+	1	1	ı	+	+	+	+	1	+	+	+	+	+	+	+	Raoultella ornithinolytica

KEY: + = Positive, - = Negative, V.P = Voges-Proskauer, NA = Nutrient Agar, MA = MacConkey Agar, BA = Blood Agar.

Table 3: Distribution of bacterial isolates among the various types of hospital waste

Waste →	Infectious Waste	Sharps	Pathological Waste	Chemical Waste	Pharmaceutical Waste	General Waste
Bacteria						
Raoultella ornithinolytica (n=30)	9 (30.0)	3 (10)	6 (20)	1 (3.3)	4 (13.3)	7 (23.3)
Klebsiella pneumonia (n=20)	5 (25.0)	2 (10.0)	3 (15.0)	2 (10.0)	3 (15.0)	5 (25.0)
Staphylococcus gallinarum (n=25)	7 (28.0)	3 (12.0)	3 (12.0)	3 (12.0)	2 (8.0)	7 (28.0)
Enterobacter cloacae (n=12)	6 (50.0)	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	4 (33.3)
Staphylococcus Scuri ssp. Lentus (n=25)	8 (32.0)	2 (8.0)	4 (16.0)	1 (4.0)	2 (8.0)	5 (20.0)
Staphylococcus xylosus (n=17)	5 (29.4)	2 (11.8)	3 (17.6)	1 (5.9)	2 (11.8)	4 (23.5)
Escherichia coli (n=18)	5 (27.8)	2 (11.1)	3 (16.7)	0 (0.0)	1 (5.5)	7 (38.9)
Pantoea ananatis (n=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
Total=149	45 (30.2)	14(9.4)	24 (16.1)	8 (5.4)	14 (9.4)	41 (27.5)

Table 4: Antibiotic susceptibility pattern of the bacteria isolates from the hospital wastes

Bacteria	LEV	(30	CN (30 μg)	CIP ((5 μg)	E (30) μg)	SAM	(30		(125	CTX	(30	TE (30 μg)	AK (30 μg)	AMP	(10
	μg) S	R	S	R	S	R	S	R	μg) S	R	μg) S	R	μg) S	R	S	R	S	R	μg) S	R
Raoultella ornithinolytica (n=30)	0	30	0	30	0	30	0	30	0	30	0	30	0	30	0	30	20	10	0	30
Klebsiella pneumonia (n=20)	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	20	0	19	1
Staphylococcus gallinarum (n=25)	0	25	0	25	0	25	0	25	0	25	0	25	0	25	0	25	24	1	0	25
Enterobacter cloacae (n=12)	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	12	0	9	3
Staphylococcus Scuri ssp. Lentus (n=25)	20	5	20	5	22	3	0	25	0	25	0	25	24	1	0	25	22	3	0	25
Staphylococcus xylosus (n=17)	17	0	17	0	17	0	17	0	17	0	17	0	17	0	17	0	17	0	0	17
Escherichia coli (n=18)	18	0	18	0	18	0	18	0	18	0	0	18	18	0	18	0	18	0	0	18
Pantoea ananatis (n=2)	2	0	2	0	2	0	2	0	2	0	2	0	2	0	0	2	2	0	0	2

Levofloxacin (LEV), cefalexin (CN) ciprofloxacin (CIP), erythromycin (E), ampicillin-sulbactam (SAM), sulfamethoxazole (SXT), cefotoxine (CTX), tetracycline (TE), ampicillin (AMP) and amikacin (AK). The interpretative standard for S= Sensitive, R=Resistant is the Clinical and Laboratory Standards Institute.

DISCUSSION

The bacteria associated with hospital waste of Federal University Teaching Hospital, Owerri was studied.

The infectious waste demonstrated the greatest extent of microbial contamination. The high

microbial loading is to be anticipated owing to the composition of infectious waste, in which it typically contains bodily fluids, tissues, and other miscellaneous materials that might harbor and spread pathogen ¹⁹. The high microbial load indicates the significance of strict waste management practices, such as the use of

incineration and autoclaving, to lower the risk of infection among healthcare professionals and the public. Sharps, being a physical hazard, have much lower microbial contamination levels, which are single-use and discarded immediately upon use, limiting the potential for microbial growth ²⁰, ²¹. Nevertheless, the physical hazard posed by sharps necessitates rigorous disposal procedures, as seen in the use of incineration and encapsulation by the hospital. Pathological waste had viable bacteria, coliform, and bacterial, with moderate microbial contamination. This type of waste, body parts and human tissue, can be a source of pathogens if not handled. Autoclaving and incineration of pathological waste in the hospital are optimal practices because these will be efficient in reducing microbial loads and inhibiting infection transmission ²². Chemical waste had the least microbial contamination, and no detectable coliform or total bacteria were found. The low microbial counts show the effectiveness of chemical disinfection and the inhibitory effect of chemical agents towards microbial growth. This finding agrees with the fact that chemical waste, if disposed of properly, poses a reduced risk of biological contamination 23; 2. Pharmaceutical waste is relatively low in microbial contamination. The low microbial counts show that pharmaceutical waste, particularly when incinerated, is of low risk towards causing infection. However, improper wastage of medicinal waste can lead to environmental pollution and the development of antimicrobial resistance 24. The total waste had high to moderate level of microbial contamination. General waste is not hazardous; however, presence of high-level microbial contamination puts into prominence proper segregation and disposal of waste methods to prevent spread of infection both in the community and hospitals ^{25; 19}. The findings of this study point towards some of the areas of hospital practice of waste improvement. The high microbial load in infectious and general

wastes reflect the need for more training in health workers on waste handling and segregation ²².

These have isolated Staphylococcus gallinarum, Enterococcus cloacae, Staphylococcus Scuri ssp. Lentus, Escherichia coli, Staphylococcus xylosus, Klebsiella pneumonia and Pantoea ananatis. This is consistent with previous research that has identified these bacteria to be prevalent hospital contaminants ²⁶. The incidence of bacterial isolates in different classes of hospital wastes reflects significant variation in microbial contamination levels, and this is inversely proportional to the type of waste and management. Infectious waste, and the most prominent number of isolates is the most hazardous among them, of which Enterobacter cloacae is most prevalent. This finding is concerning, given the reported resistance of Enterobacter cloacae to a number of antibiotics and its association with hospital-acquired infection ²⁷. The isolation of *Staphylococcus Scuri* ssp from infectious waste also indicates the potential for transmission of resistant organisms, as this species is commonly linked to methicillin and other beta-lactam antibiotic resistance ²⁸. In sharp waste, Staphylococcus gallinarum and Escherichia coli were the most common isolates. The relatively lower degree of contamination in sharps waste can be attributed to the sterilization processes that are typically applied for sharp instruments before disposal. Nevertheless, the presence of Escherichia coli, a known pathogen with documented cause of urinary tract infection and other healthcare-associated infection, is still a significant risk, particularly where the sharps are not adequately sterilized 29. General waste, with a mix of materials, had the second-largest number of bacterial isolates. Pantoea ananatis was unexpectedly the most prevalent bacterium in general waste, followed by Escherichia coli as the second most prevalent. The widespread presence of Pantoea ananatis in general waste is especially concerning, given its recent designation as an emerging human pathogen with reported

antibiotic resistance ³⁰. The occurrence of *Escherichia coli* once again highlights the potential public health risk in the improper disposal and handling of general waste that can serve as a reservoir for resistant pathogens. Chemical waste was least contaminated. This was because of the inherent antimicrobial activity of most chemicals used in hospitals that prevents bacterial growth. However, the presence of any bacterial contamination in chemical waste remains justifiable, as it implies potential weaknesses in segregation or treatment procedures ³¹.

The antibiotic susceptibility testing (AST) findings point to an issue regarding the prevalence of antibiotic-resistant bacteria in hospital waste. Staphylococcus gallinarum, one of the most frequently isolated bacteria, was amikacin-sensitive but resistant to all other antibiotics. This is a concerning trend, as it indicates a narrow spectrum of effective treatment, highlighting the need for strict antibiotic stewardship and infection control practices within the hospital setting. Raoultella ornithinolytica was resistant to all but amikacin. This antibiotic profile further burdens treatments and suggests bacteria possess efflux pump mechanisms or antibiotic-modyfying enzymes providing them broad-range resistance 32. Klebsiella pneumoniae, on the other hand, was sensitive to all of the tested antibiotics and therefore, at least among tested isolates, this bacterium is not resistant to these treatments. This finding is opposite to global trends where Klebsiella pneumoniae has increasingly become resistant, particularly to carbapenems 33. Pantoea ananatis was ampicillin-resistant but sensitive to other drugs, a trend that can be attributed to the presence of beta-lactamase enzymes that are penicillin-class resistant ³⁴. Escherichia coli isolates are resistant to trimethoprim and ampicillin, as in global increases in resistance among E. coli bacteria, particularly in hospital environments where antibiotic pressure is significant 35. The

existence of resistance of these bacteria to antibiotics, particularly ampicillin, is evidence of the prevalent occurrence of resistance genes, most likely as a result of excessive and improper antibiotic usage. The prevailing trend towards resistance, particularly high resistance to ampicillin, tetracycline, and trimethoprim, indicates that these antibiotics are no longer effective in treating infections caused by these pathogens. This resistance can cause more severe healthcare-associated infections and more complicated treatment courses, which require the use of more powerful, often more toxic, or more expensive antibiotics.

CONCLUSION

The study found significant microbial contamination in certain waste types. *Staphylococcus gallinarum* was one of the most frequently isolated bacteria, it was amikacinsensitive but resistant to all other antibiotics while *Raoultella ornithinolytica* was resistant to all except amikacin. This is a concerning trend, as it indicates a narrow spectrum of effective treatment, highlighting the need for strict antibiotic stewardship and infection control practices within the hospital setting.

Limitations

By focusing exclusively on the Federal University Teaching Hospital in Owerri, the results bear relevance only to this institution and may not be generalized to other hospitals, even within the same locale, where waste management practices and bacterial resistance patterns may significantly differ.

The focus of the study is narrowed to bacteria within the hospital setting, but not the bigger environmental impacts of wastes affecting surrounding communities or ecosystems. More detailed environmental assessments might enhance the findings, but this would require different methods and resources.

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