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THE ANTIBIOTIC PROFILE OF BACTERIA ISOLATED FROM THE MEDICAL WASTES OF TWO HOSPITALS IN ILARO, OGUN STATE

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ABSTRACT

Hospital waste acts as store house of harmful infectious pathogens. Nosocomial infections have impacted great burden on hospitals and healthcare systems and have led to deteriorating health condition and deaths. For Ore-Ofe hospital, a total of 93.00 x 10^4 cfu/g (mean values) bacterial colonies of three different bacteria belonging to three genera were isolated (*Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae*), while a total of 77.67 x 10^4 cfu/g (mean values) bacterial colonies of also three different bacteria but belonging to two genera were isolated from Hosannah hospital (*Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella enterica*). The investigation, for Ore-Ofe hospital, showed that *Escherichia coli* had a higher bacterial load than *Staphylococcus aureus* and *Shigella dysenteriae*. It was also established that *Escherichia coli* and *Staphylococcus aureus* were both resistant to six out of the thirteen antibiotics used for the assay while *Shigella dysenteriae* was resistant to five of them. On the other hand, for Hosannah hospital, the investigation showed that *Staphylococcus aureus* had a higher bacterial load than *Staphylococcus epidermidis* and *Salmonella enterica*. It was also established that *Staphylococcus aureus* and *Staphylococcus epidermidis* were both resistant to six out of the thirteen antibiotics used for the assay while *Salmonella enterica* was resistant to five of them. The bacterial isolates all exhibited different antibiotic profiles to the antibiotics that were used for the assay. Medical wastes should be promptly and meticulously discarded from hospitals' premises to avert the inadvertent spread of particularly air-borne infectious agents.

Keywords: Harmful, nosocomial, health, assay, inadvertent spread

INTRODUCTION

Hospitals and health centers should be safe havens from pathogens and infections present in the immediate environment, however, the reverse is now the case as the increasing rate of antimicrobial resistance has become a global cause for concern. The main risk of public health is that resistant genes are transferred from environmental bacteria to human pathogens (Omoni, Makinde and Abutu, 2015). Evaluation of antimicrobial susceptibility profile of various bacterial pathogens in health facilities and waste is important to assess the potential risk of dissemination of resistant pathogens to the environment. The increasing emergent of antibiotic-resistant pathogenic bacteria has necessitated the need to seek alternatives or better orthodox medicines to curb their menace. The occurrence of nosocomial infections is also becoming

worrisome, hence, the need to improve on the sanitary practices and waste management in hospitals. The increasing emergence of antibiotic-resistant pathogenic bacteria is fast becoming a menace, hence, the need to implore effective sanitary practices in medical waste management in hospitals and to seek for alternative or better orthodox medicines against nosocomial infections.

The aim of this study is to profile the antibiotic-resistant bacteria isolated from the medical wastes of Ore-Ofe hospital and Hosannah hospital, Ilaro. Waste disposal is a worrisome predicament to most societies of the world. The microorganisms associated with medical wastes particularly make it a thing of public health concern and the medical wastes from Ore-Ofe and Hosannah hospitals are no exception because of their potentiality for myriad of microorganisms, particularly pathogenic bacteria.

MATERIALS AND METHODS

Sample Collection

The investigation was carried out in the Microbiology Laboratory of The Federal Polytechnic, Ilaro, Ogun State, Nigeria. The soil samples used were the sub-surface soil collected, using a hand trowel, from three different points at the medical waste dump sites of Ore-Ofe and Hosannah hospitals on the 18th of July, 2022.

Media Preparation

The media employed i.e. the nutrient agar (NA) and Mueller Hinton agar were prepared according to the manufacturer's instructions (Cheesbrough, 2000).

Isolation

The isolation of the bacteria was by pour-plate method. The method described by Chukwu *et al*. (2010) was employed to isolate the bacteria from the soil sample.

Cultural Identification

This was done according to Cheesbrough (2000) and Baker, Silverton and Pallister (2001).

Determination of Bacterial Population

The bacterial load was determined using the plate-count technique and expressed in cfu/g. The frequency of occurrence of the isolated and identified bacterial isolates was determined and then subsequently recorded in percentage.

Antibiotic Profile of the Isolates

The isolates' antibiotic profiles were determined by culture and sensitivity testing technique by agar-disc diffusion assay to ascertain their antibiotic susceptibility or resistance. The inocula for the test were prepared using 24hr-old cultures of the pure isolates. Fresh Mueller Hinton agar plates were prepared for the assay, the plates were inoculated with the axenic cultures respectively by spreading each inoculum on the entire surface of the Mueller Hinton agar using a sterile inoculating loop to produce a confluent growth. The susceptibility test was performed by placing the appropriate antibiotics paper disks on the different plates containing pure cultures of Gram positive and Gram negative bacteria accordingly. Each plate containing axenic cultures was then incubated at 35°C ± 2°C for 24hrs. The zones of inhibition were recognized around each antibiotic wafer and thereafter marked by using a pair of venier calipers to determine the clear distance between

the edge of each wafer and the inoculum (diameter of zone of inhibition). This was then placed on a transparent ruler to take the exact measurement or reading in mm (Jorgensen and Turnidge, 2015). A control experiment, without an antibiotic paper disk, was setup. The ability of the antibiotics wafers to inhibit the growth of the isolates was indicated by the appearance of clear zones within the test medium and recorded as sensitivity, otherwise, resistance vice-versa.

RESULTS

Tables 1 and 2 reveal the identities of the bacteria isolated from the medical waste samples (predominantly used and discarded syringes, plaster, bandages and cotton wools) after they were aseptically collected from the soil debris at three different dump site points at the two hospitals respectively, for bacteriological analysis. For Ore-Ofe hospital, the soil debris was observed to be light brown and slightly foul-smelling in colour and odour and with a total of 93.00 x 10^4 cfu/g (mean values) bacterial colonies of three different bacteria belonging to three genera were isolated (*Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae*), while a total of 77.67 x 10^4 cfu/g (mean values) bacterial colonies of also three different bacteria but belonging to two genera were isolated from Hosannah hospital (*Staphylococcus epidermidis*, *Staphylococcus aureus* and *Salmonella enterica*) and its soil debris was observed to be hazel and obnoxious in colour and odour. For Ore-Ofe hospital, the bacterial load for each respective bacterium isolated from the samples is shown in table 3. From the table, it can be deduced that *Escherichia coli* is more prevalent than *Staphylococcus aureus* and *Shigella dysenteriae*; with mean values of 41.33 x 10^4 cfu/g, 33.67 x 10^4 cfu/g and 18.00 x 10^4 cfu/g respectively. *Shigella dysenteriae* is the least prevalent bacterium. Table 4 shows the bacterial load for each respective bacterium isolated from the samples from Hosannah hospital and from the table, it can be deduced that *Staphylococcus aureus* is more prevalent than *Staphylococcus epidermidis* and *Salmonella enterica*; with mean values of 36.67 x 10^4 cfu/g, 26.67 x 10^4 cfu/g and 14.33 x 10^4 cfu/g respectively. *Salmonella enterica* is the least prevalent bacterium.

Table 1: Bacterial Strains Isolated from Ore-Ofe Hospital's Medical Waste

Characteristic test/Probable identity	***E. coli***	***S. aureus***	***S. dysentriae***
Gram stain	-	+	-
Morphology/shape	Rod	Coccus	Rod
Motility	+	-	-
Indole	+	-	+
Citrate	-	-	-
H_2S	-	+	-
Urease	-	-	-
Spore	-	-	-
Catalase	+	+	+
Oxidase	-	-	-
Methyl Red	+	+	+

Voges-Proskauer	-	-	-
Capsule	-	-	-
Coagulase	-	+	-
Starch hydrolysis	+	+	+
Nitrate reduction	+	-	-
		Sugar Fermentation Test	
Glucose	+	+	-
Fructose	-	+	+
Lactose	+	+	+
Sucrose	+	+	+
Raffinose	+	-	-
Maltose	-	+	-
Mannose	-	+	+
Xylose	-	-	+
		Sugar Alcohol Test	
Mannitol	+	+	+
Sorbitol	+	+	+
Inositol	-	+	-

Keys: + = Positive, - = Negative

Table 2: Bacterial Strains Isolated from Hosannah Hospital's Medical Waste

Characteristic test	***S. aureus***	***S. epidermidis***	***S. enterica***
Gram stain	+	+	-
Morphology/shape	Coccus	Coccus	Rod
Motility	-	-	+
Indole	-	+	-
Citrat.	-	-	-
H_2S	+	-	+
Urease	-	-	-
Spore	-	-	-
Catalase	+	-	+
Oxidase	-	-	-
Methyl red	+	+	+
Voges-Proskauer	-	-	-
Capsule	-	-	-
Coagulase	+	-	-
Starch hydrolysis	+	-	+
Nitrate reduction	-	-	-
		Sugar Fermentation Test	
Glucose	+	-	-
Fructose	+	+	+
Lactose	+	-	-
Sucrose	+	-	-
Raffinose	-	+	+
Maltose	+	+	+
Mannose	+	+	+
Xylose	-	-	-
		Sugar Alcohol Test	
Mannitol	+	+	+
Sorbitol	+	+	+
Inositol	+	-	-

Keys: + = Positive, - = Negative

Table 3: The Bacterial Population of Ore-Ofe Hospital's Medical Waste's Soil Samples

Bacterium	Site A (cfu/g)	Site B (cfu/g)	Site C (cfu/g)	Mean value (cfu/g)
E. coli	48.00×10^4	33.00×10^4	43.00×10^4	41.33×10^4
S. dysentriae	19.00×10^4	10.00×10^4	25.00×10^4	18.00×10^4
S. aureus	32.00×10^4	30.00×10^4	39.00×10^4	33.67×10^4
Total	99.00×10^4	73.00×10^4	107.00×10^4	93.00×10^4

Table 4: The Bacterial Load of Hosannah Hospital's Medical Waste's Soil Samples

Bacterium	Site A (cfu/g)	Site B (cfu/g)	Site C (cfu/g)	Mean value (cfu/g)
S. aureus	33.00×10^4	37.00×10^4	40.00×10^4	36.67×10^4
S. enterica	11.00×10^4	17.00×10^4	15.00×10^4	14.33×10^4
S. epidermidis	27.00×10^4	25.00×10^4	28.00×10^4	26.67×10^4
Total	71.00×10^4	79.00×10^4	83.00×10^4	77.67×10^4

From the investigation, as shown in table 3 for Ore-Ofe hospital, it was observed that the bacterial load from point C recorded the highest value of 107×10^4 cfu/g while that of point B is the lowest with a value of 73.00×10^4 cfu/g. The frequency of occurrence of its bacterial isolates is shown in table 5, as *Shigella dysentriae* recorded a 19.35% frequency of occurrence while *Staphylococcus aureus* recorded almost twice as much with a value of 36.20% frequency of occurrence. *Escherichia coli* recorded the highest frequency of occurrence with a value of 44.44%. For Hosannah hospital, table 4 shows that the bacterial load from point B recorded the highest value of 79×10^4 cfu/g while that of point A is the lowest with a value of 71.00×10^4 cfu/g. The frequency of occurrence of its bacterial isolates is shown in table 6. *Salmonella enterica* recorded an 18.44% frequency of occurrence while *Staphylococcus epidermidis* recorded almost twice as much with a value of 34.34% frequency of occurrence. *Staphylococcus aureus* recorded the highest frequency of occurrence with a value of 47.21%.

Table 5: The Frequency of Occurrence of the Bacteria from Ore-Ofe Hospital

Bacteria	Population (cfu/g)	% Occurrence
E. coli	4, 133	44.44%
S. dysentriae	1, 800	19.35%
S. aureus	3, 367	36.20%
Total	9, 300	100%

Table 6: The Frequency of Occurrence of the Bacteria from Hosannah Hospital

Bacterium	Population (cfu/g)	% Occurrence
S. aureus	3, 667	47.21%
S. enterica	1, 433	18.44%
S. epidermidis	2, 667	34.34%
Total	7, 767	100%

Table 7 shows the antibiotic profiles of the three isolated bacteria with their respective varying degrees of resistance or susceptibility to different types of antibiotics from antibiotic

paper discs/wafers placed in their culture plates after 24-hour incubation, for Ore-Ofe hospital. Their resistance or susceptibility was depicted as clear zones of inhibition recognized around each antibiotic wafer and recorded in millimetres (mm). A control experiment, without an antibiotic paper disk, that was setup for each bacterium, showed significant growth as there was no zone of inhibition. From the table, the three bacterial isolates were all resistant to amoxicillin, oxacillin and methicillin. Only *Staphylococcus aureus* was resistant to nalidixic acid and chloramphenicol. *Escherichia coli* and *Staphylococcus aureus* were both resistant to ampicillin and cefotaxime but only *Shigella dysenteriae* was resistant to tetracycline. *Escherichia coli* and *Shigella dysenteriae* were both resistant to vancomycin. On the average, *Shigella dysenteriae* appeared to be the most susceptible bacterium while *Staphylococcus aureus* seemed to be least susceptible bacterium to most of the antibiotics used for the assay. They were sensitive to other antibiotics at varied values in millimetres. For Hosannah hospital, table 8 shows the antibiotic profiles of the three isolated bacteria with their respective varying degrees of resistance or susceptibility to different types of antibiotics from antibiotic paper discs/wafers placed in their culture plates after 24-hour incubation. Their resistance or susceptibility was depicted as clear zones of inhibition recognized around each antibiotic wafer and recorded in millimetres (mm). A control experiment, without an antibiotic paper disk, that was setup for each bacterium, showed significant growth as there was no zone of inhibition. From the table, the three bacterial isolates were all resistant to amoxicillin, oxacillin and methicillin. Only *Staphylococcus aureus* was resistant to nalidixic acid and chloramphenicol. *Staphylococcus epidermidis* and *Staphylococcus aureus* were both resistant to ampicillin and cefotaxime but only *Salmonella enterica* was resistant to tetracycline. *Staphylococcus aureus* and *Salmonella enterica* were both resistant to vancomycin. On the average, *Salmonella enterica* appeared to be the most susceptible bacterium while *Staphylococcus epidermidis* seemed to be least susceptible bacterium to most of the antibiotics used for the assay.

Table 7: The Antibiotic Profiles of the Bacterial Isolates from Ore-Ofe Hospital

Antibiotic	*Escherichia coli*	*Staphylococcus aureus*	*Shigella dysenteriae*
Amoxicillin	R	R	R
Cotrimoxazole	33mm	13mm	26mm
Ciprofloxacin	29mm	18mm	16mm
Gentamycin	10mm	11mm	19mm
Nalidixic acid	10mm	R	11mm
Ampicillin	R	R	15mm
Oxacillin	R	R	R
Erythromycin	19mm	24mm	15mm
Methicillin	R	R	R

Tetracycline	12mm	20mm	R
Vancomycin	R	18mm	R
Chloramphenicol	14mm	R	17mm
Cefotaxime	R	R	12mm

Table 8: The Antibiotic Profiles of the Bacterial Isolates from Hosannah Hospital

Antibiotic	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Salmonella enterica*
Amoxicillin	R	R	R
Cotrimoxazole	30mm	15mm	22mm
Ciprofloxacin	26mm	10mm	18mm
Gentamycin	18mm	11mm	15mm
Nalidixic acid	10mm	R	17mm
Ampicillin	R	R	15mm
Oxacillin	R	R	R
Erythromycin	15mm	14mm	18mm
Methicillin	R	R	R
Tetracycline	15mm	24mm	R
Vancomycin	R	18mm	R
Chloramphenicol	12mm	R	19mm
Cefotaxime	R	R	20mm

Key: R = Resistant

DISCUSSION

The investigation, for Ore-Ofe hospital, showed that *Escherichia coli* had a higher bacterial load than *Staphylococcus aureus* and *Shigella dysenteriae*. It was also established that *Escherichia coli* and *Staphylococcus aureus* were both resistant to six out of the thirteen antibiotics used for the assay while *Shigella dysenteriae* was resistant to five of them. That suggests that the latter is more susceptible to those antibiotics than the former but between *Escherichia coli* and *Staphylococcus aureus*, *Escherichia coli* seems to be more susceptible than *Staphylococcus aureus*, clearly judging by their respective zones of inhibition. Although, *Shigella dysenteriae* was the most vulnerable, it is still quite potentially pathogenic. The result of this investigation is in agreement with Maina *et al*. (2018) who also recorded a high presence of *Escherichia coli* in their hospital waste in Kenya.

On the other hand, for Hosannah hospital, the investigation showed that *Staphylococcus aureus* had a higher bacterial load than *Staphylococcus epidermidis* and *Salmonella enterica*. It was also established that *Staphylococcus aureus* and *Staphylococcus epidermidis* were both resistant to six out of the thirteen antibiotics used for the assay while *Salmonella enterica* was resistant to five of them. That suggests that the latter is more susceptible to those antibiotics than the former but between *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Staphylococcus aureus* seems to be more susceptible than *Staphylococcus epidermidis*, clearly judging by their respective zones of inhibition. Although, *Salmonella enterica* was the most vulnerable, it

is still quite being regarded as a pathogenic bacterium. This study agrees with Prakasam *et al*. (2017) who also recorded the presence of both *Escherichia coli* and *Staphylococcus aureus* in their soil samples contaminated with hospital waste. The two bacterial strains were also resistant to some of the antibiotics they also used for their assay.

CONCLUSION

It can thus be concluded that the medical wastes from Ore-Ofe hospital and Hosannah hospital are potentially active inanimate reservoirs for habouring the isolated bacterial cultures by way of their substantially high bacterial load, from the investigation. The bacterial isolates; *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enterica* and *Shigella dysenteriae*, are typical and common bacterial pathogens and they all exhibited different antibiotic profiles to the antibiotics that were used for the assay. The six isolates did not seem to be coincidentally-equally susceptible to three of the antibiotics (amoxicillin, oxacillin and methicillin); that could be a major source of public health concern.

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