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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM PATIENTS' WOUNDS AND HOSPITAL ENVIRONMENTS OF MADONNA UNIVERSITY TEACHING HOSPITAL ELELE, RIVERS STATE NIGERIA

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Abstract: **This research was aimed at investigating the antibiotic susceptibility pattern of bacteria isolated from patients' wounds and hospital environment of Madonna University Teaching Hospital Elele, Rivers State. A total of 100 samples each comprising of 25 samples of wound swab, sink swabs, dirty waste water samples and sand samples. Samples were cultured on laboratory culture media and incubated at 37°C for 24 hours. Isolates were identified based on standard microbiological techniques. Thereafter, by disc diffusion methods, isolates were cultured on Muller Hilton agar impregnated with commercial antibiotic discs and incubated for 24hrs and zones of inhibition were recorded in millimeter (mm) to determine antibiotic sensitivity. The data obtained were subjected to Analysis of Variance (ANOVA) and T-test using SPSS version 15. A total of eight species of bacteria were recovered. These included *Staphylococcus* species 37(36.63 %), *Enterobacter* species 4(3.96 %), *Pseudomonas* species 3(2.97%), *Escherichia coli* 10(9.90 %), *Klebsiella* species 10(9.90 %), *Streptococcus* species 4(3.96%), *Salmonella* species 29(28.71%) and *Proteus* species 4(3.96%). *Staphylococcus* species had the highest prevalence ($p< 0.05$) while *Pseudomonas* species was significantly lower than others. For *Pseudomonas* species the most potent antibiotics were streptomycin, ampicillin, peflacine, augmentin and ciprofloxacin with percentage sensitivity 33.33 % while it (*Pseudomonas* species) was resistant to ceporex, tarivid, septrin and gentamycin. For *Staphylococcus* species was sensititive to erythromycin with 46.0 % sensitivity and the least potent were norfloxacin and ampiclox with 16.2 %. Generally isolated organisms showed high level resistance to most of the tested antibiotics. Based on the findings, it is therefore recommended that wounds be kept clean to avoid microbial infections and antibiotic prescriptions should be made after sensitivity has be evaluated.**

Keywords: **Antibiotic, Susceptibility, Wounds, Patients.**

1. INTRODUCTION

Wound is said to have occurred when there is disruption of anatomic structure and function of the skin. These wound is referred as cutaneous wound as they interfere with skin integrity. Cutaneous according to Irfan-Maqsood (2018), are defined as damaging skin integrity because of some external or internal factors. External factors are also termed as environmental factors damaging the skin e.g. accidental injuries, whereas internal factors are caused by de-regulations in metabolic

pathways e.g. diabetic wounds etc (Irfan-Maqsood, 2018). Cutaneous wounds are generally classified into two namely the acute wound resulting from knife cuts and chronic wound which are wounds resulting from metabolic disorder they take a lot of time to heal. These sort of wounds include venous/vascular ulcers, diabetic ulcers, and pressure ulcers (Mustoe, 2004; Moreo, 2005).

Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound. Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and in- convenience for the patient (Kotz *et al.,* 2009).

The prevalent organisms that have been associated with wound infection include *Staphylococcus aureus* (*S. aureus*) which from various studies have been found to account for 20-40% and *Pseudomonas aeruginosa* (*P. aeruginosa*) 5-15% of the nosocomial infection, with infection mainly following surgery and burns. Other pathogens such as Enterococci and members of the Enterobacteriaceae have been implicated, especially in immune compromised patients and following abdominal surgery (Taiwo *et al.,* 2002).

Wound healing needs a good healthy environment so that the normal physiological process will result in a normal healing process with minimal scar formation. One of the most important strategies to keep the process of healing ongoing is to sterilize damaged tissue from any microbial infection (Al-Waili *et al.,* 2011). Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains which in turn, has driven the continued search for new agents.

Cooper *et al.* (2002), opined that unfortunately, the increased costs of searching for effective antimicrobial agents and the decreased rate of new drug discovery has made the situation increasingly worrisome. It is in the light of this that this study was conceived to examine the antibiotic susceptibility pattern of bacteria isolated from patients' wounds and hospital environments of Madonna University Teaching Hospital Elele, Rivers State Nigeria.

2. MATERIALS AND METHOD

Sample Collection

With the aid of sterile swab stick, a total of 25 wound samples were collected from patients in Madonna University Teaching Hospital Elele campus in different wards (Medical ward, Emergency and Surgical ward). Also, 25 sink-swabs samples were collected. Using sterile universal containers, dirty water (i.e water after mopping the wards and the surrounding soil) were also collected. Collected samples were within 30 minutes following standard procedures transported to the microbiology laboratory Faculty of Science for analysis according to the method described by (Cheesbrough, 2000). The specimens were collected on a weekly basis in the month of May.

Specimen Preparation

Soil sample: the soil samples (1gram) weighed out, placed into a calibrated test tube, peptone water was added into the test tube up to the 10ml mark and incubated for 24 hours at 37°C.

Media Preparation

All the media (blood agar, nutrient agar, MacConkey Agar, Muller Hilton agar) used in this research were prepared based on manufacturer's instruction.

Media Inoculation

A loopful incubated peptone soil sample solution was inoculated on prepared isolating media. Also, by streak technique, wound swab samples and sinks swab samples where inoculated on the prepared isolating media. However, 0.1ml of the dirty water samples were inoculated by spread plate techniques. The inoculated plates were the incubated 37°C for 24hrs

Identification of Isolates

- **Colonial morphology:** This was based on pigmentation, shape, size, surface, elevation, edge and their ability to produce alpha, beta and gamma hemolysis on blood agar plates. (Cheesbrough, 2000).
- **Cellular Morphology:** This was based on gram reactions of the isolates.

➢ **Biochemical Identification:** this was based on reactions shown by individual isolates on treatment with reagents. Such tests included oxidase test, catalase test, urease test, Coagulase testm Indole test, Citrate utilization, Voges Proskauer, Methyl red, Citrate, Ribose, glucose. Sucrose tests.

Antibiotic susceptibility testing

The disc diffusion method was employed using commercially impregnated paper disc with a known concentration of antimicrobial agent which includes ampicillin (30 mcg), ampiclox (20 mcg) amoxicillin (20 mcg), rifampin (20 mcg), levofloxacin (20 mcg), augmentin (30 mcg), tarivid (10 mcg chloramphenicol (30 mcg), ceporex(10 mcg), erythromycin (30 mcg), gentamycin (10 mcg), streptomycin (30 mcg), septrin (30 mcg), nalixidic acid (30 mcg), nitrofurantoin (200 mcg), ciprofloxacin (10 mcg), norfloxacin (10 mcg) and peflacine (30 mcg).

An aliquot of sterile 0.1ml of peptone water containing the inoculated isolates were brought to correspond with the McFarland's turbidity standard. 0.1ml each of the standardized samples was by spread plate techniques, inoculated unto prepared Muller Hilton agar. The inoculated media was each impregnated with the various antibiotic discs and then incubated at 37°C for 24 hours. They were thereafter observed for zones of inhibition and results recorded in millimeter (mm). Zones measuring 18mm and above were regarded as indicative of sensitivity, the zone of inhibition between 13-17mm were regarded as intermediate and those less than 12mm resistant as described by Clinical Laboratory Standard Institute (CLSI (2011).

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Statistical Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) and T-test using SPSS version 15 at 0.05 level significance. Though infection was higher among male, the mean difference was not significantly different ($p > 0.05$) (Agwung-Fobellah, 2007).

3. RESULTS

The present study recovered eight special of both gram positive and gram negative bacteria. These included – *Escherichia coli*, *Pseudomonas* species, *Proteus* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Enterobacter* species and *Salmonella* species (tab. 1).

Age and sex distribution of isolated bacteria from wound infection indicates that isolates from males with 10 (40.0 %) were higher but not significantly different ($p > 0.05$) from females with 9 (36.0 %). Age range of 26-30 years with 6 (28.0 %) recorded significantly higher ($p < 0.05$) infection while aged grade 36-40 years with 1(4.0 %) had the least number of isolates (tab. 2).

Percentage occurrence of different organisms isolated from wound and the hospital environment indicates that, dirty water samples with 31 (30.7 %), had the highest isolates but not significantly different ($p > 0.05$) from sand with 28 (27.7 %) with the second highest isolates. Wound swab with 22(21.8 %) and sink swab with 20(19.8 %) were significantly lower ($p < 0.05$). However, *Staphylococcus* and *Salmonella* species had the highest percentage occurrence of among the isolates in terms occurrence in samples. *Staphylococcus* species had percentage occurrence of 13(59.1%) from wound and then

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8(25.81%) in sink, sand and dirty water respectively. Also, *Salmonella* sp. Had the highest percentage occurrence of 13(41.94%) in dirty water, sand 10(35.71%) and sink 6(30.0%) but was not isolated from wound. Percentage occurrence of *E. coli* was high in dirty water 4(12.90%), wound 3(13.64%), sand 2(7.14%) and sink 1(5.0%). Percentage occurrence of *Klebsiella* sp. was 3(13.64) and 3(9.68%) respectively in both wound and dirty water (table 3). *Enterobacter* sp. was only isolated from sand and dirty water with percentage occurrence of 3(10.71) and 1(3.23%) respectively. *Pseudomonas* sp. was not isolated from sand but was isolated from wound, sink and dirty water with percentage occurrence of 1(4.5%), 1(5.0%) and 1(3.23%) respectively. However, *Streptococcus* sp. was not isolated from dirty water but was recovered from wound, sink and sand with percentage occurrence of 1(4.5%), 1(5.0%) and 2(7.14) respectively. Moreover, *Proteus* sp. was recovered from wound, sink, sand and dirty water but at lower percentage occurrence of 1(4.5%), 1(1.50%), 1(3.57) and 1(3.57%) respectively (tab. 3).

Antibiotic susceptibility pattern of the Gram negative isolates and their percentage susceptibility shows that *Pseudomonas* species was resistant to almost all the antibiotics used. *Proteus* species had 50 % sensitivity to streptomycin, and 75 % sensitivity to tarivid. *Escherichia coli* had a sensitivity of 50 % to tarivid. *Enterobacter* species and *Proteus* species had 40 % sensitivity to gentamycin (tab. 4).

However, percentage susceptibility pattern of Gram positive isolates to commonly used antibiotics. *Staphylococcus* species showed 40.5 % sensitivity to gentamycin and *Streptococcus* species showed 50 % sensitivity to ciprofloxacin. *Streptococcus* species showed resistance to almost all the antibiotics used. There is no significant difference ($p > 0.05$) in the frequency of bacteria isolated in wounds and hospital environments (tab. 5).

Table 1: Biochemical features of isolates

G. Stain	Cat	Coag	Ind	VP	MR	Ure	Oxi	Cit	Rib	Fruc	Glu	Lac	Malt	Manno	Suc	Isolates
-	+	+	+	-	+	-	-	-	+	+	+	+	+	+	-	***E. coli***
-	+	-		-	+	-	-	+	-	-	+	-	+	+	-	*Shigella*
+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	*S.* sp.
-	+		-	+	-	-	-	+	-	-	+	-	-	-	+	***Enterobacter*** spp.
-	+	-	-	+	-	+	-	+	nd	-	+	+	nd	nd	nd	*Klebsiella* spp.
+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	*Pseudomonas* sp
-	+	-	-	-	+	+	-	+	+	+	+	-	-	-	-	*Proteus* sp.
+	-	+	-	+	+	-	+	-	+	+	+	-	+	-	+	*Streptococcus* sp.

KEY:

G. stain=Gram Stain, VP= Voges Proskauer, MR = Methyl red, Cit = Citrate, Rib = Ribose, Coag = Coagulase, Suc = Sucrose, Malt=Maltose, Ind = Indole, Cat = Catalase

Table 2: Age and Sex distribution of the isolates from wound infection and their percentage

Age range	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	55-60
no.of isolates	2	4	6	2	1	0	3	0	4
Male									
E. coli	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%)
*Pseudomonas*species	1(50.0 %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%)
Klebsiella species	0(0.0%)	0(0.0%)	0(0.0%)	1(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(25.0%)
Proteus species	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(25.0%)
Staphylococcus species	1(50.0 %)	1(25.0%)	0(0.0%)	1(50.0%)	1(100.0)	0(0.0%)	1(33.3%)	0(0.0%)	2(50.0%)

*Streptococcus*species	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(33.3%)	0(0.0%)	0(0.0%)
Female									
E. coli	0(0.0%)	0(0.0%)	2(33.3%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
*Pseudomonas*species	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%)
Klebsiella species	0(0.0%)	0(0.0%)	1(16.7%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Proteus species	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%)
Staphylococcus species	0(0.0%)	3(75.0%)	3(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Streptococcus species	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%)

Table 3: Percentage Occurrence of the organisms isolated from wounds and hospital environment

Isolate	Wounds N = 25	Sink N = 25	Sand N = 25	Dirty water N = 25
Escherichia coli	3(13.64 %)	1 (5.0 %)	2 (7.14 %)	4 (12.90 %)
Pseudomonas species	1 (4.5 %)	1 (5.0 %)	0 (0.0 %)	1 (3.23 %)
Proteus species	1 (4.5 %)	1 (5.0 %)	1 (3.57 %)	1 (3.23 %)
Klebsiella species	3 (13.64 %)	2(10.0%)	2 (7.14 %)	3 (9.68 %)
Staphylococcus species	13 (59.1 %)	8 (40.0 %)	8 (28.57 %)	8 (25.81 %)
Streptococcus species	1 (4.5 %)	1 (5.0 %)	2 (7.14 %)	0 (0.0 %)
Enterobacter species	0 (0.0 %)	0 (0.0 %)	3 (10.71 %)	1 (3.23 %)
Salmonella species	0 (0.0 %)	6 (30.0 %)	10 (35.71 %)	13 (41.94 %)
Total	22 (100.0 %)	20 (100.0 %)	28 (100.0 %)	31 (100.0 %)

KEY

N – Number of samples

Table 4: Percentage Antibiotic susceptibility pattern of the Gram Negative Isolates

Isolates	No. of Isolates	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
Pseudomonas species	3	33.3	33.3	0.0	0.0	0.0	33.3	0.0	33.3	33.3	0.0
Escherichia coli	10	40.0	10.0	40.0	50.0	10.0	14.3	40.0	20.0	40.0	20.0
Enterobacter species	4	25.0	25.0	0.0	25.0	0.0	25.0	50.0	25.0	25.0	0.0
Klebsiella species	10	40.0	20.0	10.0	30.0	0.0	40.0	40.0	30.0	40.0	20.0
Proteus species	4	50.0	0.0	25.0	75.0	25.0	25.0	50.0	0.0	25.0	25.0
Salmonella species	29	24.1	0.0	13.8	24.1	6.9	20.7	17.2	6.9	10.3	6.9

KEYS

OFX- Tarivid (10 mcg)

CPX- Ciprofloxacin (10 mcg)

CEP- Ceporex (10 mcg)

S- Streptomycin (30 mcg)

CN- Gentamycin (10 mcg)
AU- Augmentin (30 mcg)
NA- Nalixidic acid (30 mcg)
PEF- Peflacine (30 mcg)
SXT- Septrin (30mcg)
PN- Ampicillin (30 mcg)

Table 5: Percentage Antibiotic susceptibility pattern of Gram Positive Isolates

Isolates	No. of Isolates	CN	CPX	AMX	NB	APX	RD	S	E	LEV	CH
Staphylococcus species	37	40.5	29.7	16.2	16.2	16.2	18.9	32.4	46.0	48.6	29.0
Streptococcus species	4	0.0	50.0	25.0	25.0	0.0	0.0	25.0	25.0	25.0	0.0

KEYS

RD- Rifampin (20 mcg)
AMX- Amoxicillin (20 mcg)
E- Erythromycin (30 mcg)
CH- Chloramphenicol (30 mcg)
APX- Ampiclox (20 mcg)
CPX- Ciprofloxacin (10 mcg)
NB- Norfloxacin (10 mcg)
CN- Gentamycin (30 mcg)
LEV- Levofloxacin (20 mcg)
S- Streptomycin (30 mcg)

4. DISCUSSION

This study was aimed at evaluating the Antibiotic Susceptibility Pattern of Bacteria Isolated from Patients' Wounds and Hospital Environments of Madonna University Teaching Hospital Elele, Rivers State Nigeria. Study of the 100 samples (25 from each of the four sample sites) were collected.

Analysis of the collected samples showed the presence of eight (8) different species of both gram positive and gram negative bacteria. These included – *Escherichia coli*, *Pseudomonas* species, *Proteus* species, *Klebsiella* species, *Staphylococcus* species, *Streptococcus* species, *Enterobacter* species and *Salmonella* species (tab. 1). This indicates that this samples sites especially dirty water collected resulting from washing of hospital environments should be a sources of worry to the health care givers.

Analysis of Age and sex distribution of isolated bacteria from wound infection. Isolates from males with 10 (40.0 %) were higher but not significantly different ($p > 0.05$) from females with 9 (36.0 %). Age range of 26-30 years with 6 (28.0 %) recorded significantly higher ($p< 0.05$) infection while aged grade 36-40 years with 1(4.0 %) had the least number of isolates.

However, *Staphylococcus* and *Salmonella* species had the highest percentage occurrence of among the isolates in terms of occurrence. *Staphylococcus* species had percentage occurrence of 13(59.1%) from wound and then 8(25.81%). The high percentage occurrence of *Staphylococcus* sp. from wound samples recorded in this present study is in agreement with the study conducted by Mohammed *et al.* (2013). The high prevalence of *S.* sp. and the presence of *Pseudomonas* sp. in this present study agrees with the research conducted by Taiwo *et al.* (2002), when they opined that prevalent organisms that have been associated with wound infection include *Staphylococcus aureus* (*S. aureus*) which from various studies have been found to account for 20-40% and *Pseudomonas aeruginosa* (*P. aeruginosa*) 5-15% of the nosocomial infection, with infection mainly following surgery and burns. This may reflect the level of hygienic measures in the hospital, though infection was higher in males, the mean difference was not statistically significant ($p> 0.05$) (Agwung-Fobellah, 2007).

The high prevalence of *S.* sp. isolates from wound in this present study must not be unconnected with the fact that *S. aureus* is a normal commensal of the skin. Any break in the integrity of the skin will see this organism finding its way into the body thereby causing infection on the wound. Our position is in line with the view of Mama *et al.* (2014), who in this study noted that the high prevalence of *S. aureus* infection may be because it is an endogenous source of infection. They further noted that infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier *S. aureus*, which is a common bacterium on surfaces, easily find their way into wounds.

The antibiotic activity of *S.* sp. as recorded in this present study indicates that of all the ten antibiotics used, the *S.* sp. was sensitive to only three which are Gentamycin (40.5%), Levofloxacin (48.6%), Erythromycin (46.0%) and Streptomycin (32.4%). This result agrees slightly with the study conducted by Mama *et al.* (2014), in Ethiopia. They reported higher sensitivity against *S. aureus* by ciprofloxacin (96%) and gentamicin (96%). The result equally agrees with the result of other scholars in their independent studies noted. Bessa *et al*., (2013), Bibi S. *et al*. (2012), Shamsuzzaman *et al*. (2003) opined independently that *Staphylococci* sp. showed 100% sensitive to vancomycin and amikacin.

The high resistance offered against most of the antibiotics used in this present study also agrees with the result recorded of Mama *et al.* (2014) in their study on the determination of the susceptibility of *S. aureus* on fifteen selected antibiotics by disk diffusion technique. They showed that *S. aureus* tend to be resistant to a wider spectrum of antibiotics. In this studies *S. aureus* was highly resistance to ampicillin (95.7%), penicillin (91.5%) and tetracycline (51%). However, *Pseudomonas* species was resistant to almost all the antibiotics used. *Proteus* species had 50% sensitivity to streptomycin, and 75% to tarivid. But where resistant to the other antibiotics used in the study. *Proteus* sp. was resistant to ciprofloxacin in this present study. This result disagrees with that recorded by Mama *et al*. (2014), who recorded 83% sensitivity to ciprofloxacin.

Escherichia coli had a sensitivity of 50% to tarivid. *Enterobacter* species and *Proteus* species had 40% sensitivity to gentamycin. This in line with the results recorded by Mama *et al.,* (2014) who noted that Proteus species were sensitive gentamicin thought at a higher degree of sensitivity (74%). But the result of this present study was contrary to that of Mama *et al.,* (2014), who recorded sensitivity of *Proteus* sp. to ciprofloxacin (83%) while the present study recorded resistance against ciprofloxacin by *Proteus* sp. furthermore, *Streptococcus* species showed resistance to almost all the antibiotics used. There was no significant difference ($p > 0.05$) in the frequency of bacteria isolated in wounds and hospital environments.

The degree at which the isolates from this present study showed resistance to majority of the antibiotics used must not be unconnected with the length of time these antibiotics have been in circulation and also the level at which people use most of these antibiotics on self prescription basis. Our position in line with the view of most research such as Mama *et al.,* (2014), who opined in theirn study that most of the gram negative bacteria isolated were resistant to ampicillin, cephalothin, tetracycline and chloramphenicol. This may be due to the antibiotics having been in use for much longer time and their oral route of administration that affects their rate of absorption into blood stream.

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