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ANTIBIOGRAM PROFILE OF BACTERIA ISOLATED FROM WOUND INFECTION OF PATIENTS IN THREE HOSPITALS IN ANYIGBA, KOGI STATE, NIGERIA.

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ABSTRACT

Antibiogram profile of bacteria isolated from wound infections in patients attending three Hospitals in Anyigba; namely Kogi State Teaching Hospital, Grimard Catholic Hospital and Maria- Goretti Hospital were investigated. Seventy- two wound swabs were collected from male and female patients using sterile swab sticks. Based on morphological and biochemical characteristics of the isolates, *Staphylococcus aureus* (31.9%) was the most prevalent in wound infection in the study area in both male and females followed by *Escherichia coli* (27.8%), *Streptococcus* spp (16.7%), *Klebsiella* spp (13.9%) and *Proteus* spp (9.7%). Grimard Catholic Hospital had higher occurrence of isolates compared to Kogi State Teaching Hospital and Maria- Goretti Hospital. The pathogens were higher in males than females. The highest number of bacterial species were found in the age group 16-20 (17), followed by 51 years and above (15), while the age group 21-25 (4) had the least bacterial load. The antibiogram revealed the presence of multi-drug resistant organisms. However, ciprofloxacin, gentamicin, streptomycin, augmentin and ofloxacin should be considered as first line drug in the treatment of wound in the area as they were the most effective antibiotics. Prompt and timely treatment is therefore, recommended on the onset of wound infections.

Keywords: Antibiogram, Hospital, multi-drug resistant organism, wound swab

INTRODUCTION

The skin is a host to various transient bacteria contaminants. These are found in the openings of the hair follicles, sweat glands and sebaceous glands (Deurden *et al*., 2007). Any breach in the skin surface, whether accidental or surgical, provides an open door for bacterial infection (Dettenkofer *et al*., 2009).

The skin is a protective barrier that shields the body from contamination by viruses, bacteria and toxins (Kirketerp *et al*., 2008). Damage to the skin compromises the body's ability to fight off infections by providing an unobstructed route into the blood stream. According to Deurden *et al*. (2007), every individual carries a large resident microbial population on the skin surfaces, and in the openings of the hair follicles, sweat glands and sebaceous glands. *Propionibacterium, Corynebacterium, Pityrosporum* and *Staphylococcus* species have been reported to be part of the microbial population of the skin (Deurden *et al*., 2007, Hsiao *et al*., 2011).

The risk of wound infection increases with the degree of contamination and it has been estimated that about 50% of wounds contaminated with bacteria become clinically infected (Sahu *et al*., 2011). Hsiao *et al*. (2011) observed that S*taphylococcus aureus* accounts for 20-40% of hospital -acquired wound infection while Sahu *et al*. (2011) reported that *Pseudomonas aeruginosa* accounts for 5-15% of nosocomial wound infections. Other pathogens associated with nosocomial wound infections include *Staphylococcus* spp, *Klebsiella* spp, *Pseudomonas* spp, *Proteus spp* and *Escherichia coli* (Giacometti *et al*., 2000, Goswami *et al*., 2011 and Sahu *et al*., 2011).

According to Sahu *et al*. (2011), nosocomial wound infection tends to be associated with bacteraemia, septicaemia, shock and prolonged hospital stay in some patients.

A myriad of substances such as antibiotics, synthetic drugs are used in the hospitals for treatment (Akubuenyi *et al*., 2011) in addition to formulated drugs. Ruseel and Path (2001) are of the opinion that acquired resistance to antibiotics may arise by cellular mutation or by acquisition of genetic elements in the form of plasmids. According to Akubuenyi *et al*. (2011), the occurrence of strongly selective environments such as hospitals promotes not only the growth of resistant bacteria, but also leads to an increase in the frequency of resistance bacterial genes and genetic elements such as plasmids.

There is dearth of information on the antibiogram profile of bacteria isolated from wounds of patients visiting hospitals in Anyigba, a University town of over 130,000 populations (National Population Commission, NPC, 2006). Bacterial resistance creates clinical problems for physicians (Phol, 2007). The aim of this study therefore, was to identify the bacterial isolates found in wounds and establishes their antibiogram profile with a view to provide information for health workers, managers and administrators of health centres in Anyigba and environs.

MATERIALS AND METHODS

Description of Study area

Anyigba lies between latitudes 7^0 29^1 North and longitude 7^0 11^1 East and falls within the rain forest belt of Nigeria with an annual mean rainfall of about 1600mm. The average temperature of the study area is 25°C (Stephen and Egene, 2012). The people are mostly farmers, traders and civil servants.

Sample Collections

Samples were collected from three Hospitals in Anyigba namely, Kogi State University Teaching Hospital, Grimard Catholic Hospital and Maria-Goretti Hospital. Sterile swabs were used to swab the surfaces of patient's wounds. The swabs were taken to the Microbiology laboratory of Kogi State University, Anyigba, under aseptic conditions within 30 minutes of collection for bacteriological analysis. A total number of seventy- two (72) samples were collected for this study.

Isolation, Characterization and Identification of Isolates

The swab sticks containing the specimens were streaked on MacConkey agar, Mannitol salt agar and Blood agar and incubated at 37^0C for 24hours. Representative colony types were sub-cultured on their respective isolation media until pure cultures were obtained as confirmed by microscopy. The pure cultures were tentatively characterised and identified on the basis of their colonial morphology, Gram's reaction and biochemical tests (Schofield *et al*., 2007; Uppal *et al*., 2007).

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method as described by Akubuenyi *et al*. (2011) was used for this test. The commercially prepared antibiotics used were amoxil (20 µg/ml), gentamicin (10µg/ml), augumentin (30µg/ml), chloramphenicol (30µg/ml), streptomycin (30 µg/ml), ofloxacin (30 µg/ml), pefloxacin (10 µg/ml), ciprofloxacin (10 µg/ml), sparfloxacin (10 µg/ml), septrin (30 µg/ml) norfloxacin (10 µg/ml), rifampicin (20 µg/ml), erythromycin (30 µg/ml), ampidox (20 µg/ml), levofloxacin (20 µg/ml).

The various antibiotics discs were carefully placed on the surface of Muller-Hinton agar plates seeded with standardized suspensions of each purified isolate. The standardization of the bacterial suspensions was achieved using 0.5 Mcfarland solution (NCCLS, 2003). Inhibition zone diameters were measured after 18-24 hours of incubation at 37^0C.

Statistical analysis

Data observed were subjected to Chi-square and one way analysis of variance (ANOVA) using Minitab statistical software version 13.1 (2000).

RESULTS

Table 1 shows the shapes, Gram's reaction and biochemical characteristics of the bacterial isolates from wounds. The isolates were tentatively identified as *Staphylococcus aureus*, *Streptococcus* spp, *Escherichia coli*, *Klebsiella* spp and *Proteus* spp. The frequency of occurrence of the isolates was in the order of *Staphylococcus aureus* (31.9%), *Escherichia coli* (27.8%), *Streptococcus* spp (16.7%), *Klebsiella* spp (13.9%) and *Proteus* spp (9.7%) (Table 2).

Table 3 shows the occurrence of the bacterial isolates from the three Hospitals.

Staphylococcus aureus was frequently isolated in all the Hospitals followed by *Escherichia coli* while *Proteus* spp was the least isolated. Grimard Catholic Hospital had the highest bacterial species isolated followed Kogi State University Teaching and Maria Gorreti Hospital.

Table 4 shows the distribution of pathogen among male and female patients. In males, *Staphylococcus aureus* was frequently isolated (34.21%) followed by *Escherichia coli* (28.95%) while *Klebsiella* spp (10.53%) was the least isolated. The frequency of isolation In the females was in the order of *Staphylococcus aureus* (29.41%) > Escherichia *coli* (26.47%) > *Streptococcus* spp (20.59%) > *Klebsiella* spp (17.65%) > *Proteus* spp (5.88%). There were significant differences ($p<0.05$) in the distribution of pathogen among male and female patients.

The distribution of the bacterial isolates according to age group is illustrated in Table 5. The highest number of bacterial species was found in the age group 16-20 (17), followed by 51 years and above (15), while the age group 21-25 (4) had the least bacterial load. There were variations in the types of bacterial isolates among the age group. There were significant differences in the distribution of pathogen among the age groups at 5% probability level.

Table 6 shows the antibiogram profile of the bacterial isolates. All bacterial isolates from wound displayed multi-drug resistant having each proved resistant to at least three antibiotics from different classes (Table 6).

DISCUSSION

The bacteria isolated in the course of this study were *Staphylococcus aureus, Streptococcus* spp, *Escherichia coli, Klebsiella* spp and *Proteus* spp. This result is in agreement with those of Thanni *et al*. (2003) from wound swabs in Ibadan and Surucuoglu *et al*. (2005) in Turkey. In addition to these five organisms, *Peptostreptococcus* spp, *Bacillus* spp, *Clostridium* spp and *Pseudomonas aeruginosa* were also isolated by Thanni *et al*. (2003) and Surucuoglu *et al*. (2005) while working on wound infection of patients visiting hospitals in Ibadan and Turkey. This is an indication that different bacteria contaminate and colonize wound infections, depending on the type and location of wound in the body.

The most frequently isolated organism was *Staphylococcus aureus* followed by *Escherichia coli*. This is in agreement with earlier works by Giacometti *et al*. (2000), Thanni *et al*. (2003) and Surucuoglu *et al*. (2005). They reported that the sources of most wound infections are endogenous flora of the patient's skin or mucous membrane.

According to our findings, significant difference was found in the distribution of pathogens among males and females. This result confirmed previous report where the prevalence was higher in males than in female's accident victims (Akinjogunla *et al*., 2009). However, the reason for this difference is unclear as most of the patients were not accident victims.

The finding that age significantly affected the prevalence of wound infections agree with the reports of Torpy *et al*. (2005). People within the age group 16-20 may be involved in activities such as sports and farming which may expose them to injuries while older age (51 and above) is considered a risk factor for wound infections (Torpy *et al*., 2005). This may be due to decreasing trend of immune competence with age (Hakim and Gress, 2007).

Ciprofloxacin, gentamicin, streptomycin, augmentin and ofloxacin were the most effective antibiotics in *in vitro* testing followed by amoxicillin which was effective against all the pathogens except *S. aureus*. Similar results with aminoglycosides, betalactams and quinolones have been reported by other authors (Gupta *et al*., 1999; Goswami *et al*., 2011). Gupta *et al*. (1999) reported that quinolones were effective in treatment of wound infection caused by Gram negative bacteria. All isolates were resistant to septrin, rifampicin, erythromycin and chloramphenicol. This may be due to wide and frequent use of these antibiotics (Akoh *et al*., 2013), as many people in this area are in the habit of engaging in self medication in the treatments of wounds even before visiting hospitals for treatment.

Table 1: Gram's reaction and biochemical tests

Isolates	shapes	GR	Catalase	Coag	Indole	Mot	Cit	Urease	Glu	Suc	Man	lac	Probable isolate
A	cocci	+	+	+	-	-	-	-	+	+	+	-	*Staphylococcus aureus*
B	cocci	+	-	-	NA	-	-	-	+	+	-	+	*Streptococcus* spp
C	rods	-	+	-	+	+	-	-	+	+	-	+	*Escherichia coli*
D	rods	-	-	-	-	-	+	+	+	+	-	+	*Klebsiella* spp
E	rods	-	+	-	+	+	+	+	+	-	-	-	*Proteus* spp

GR: Gram's reaction, Coag: coagulase test, Mot: motility test, Cit: citrate test, NA: not applicable, Glu: glucose, Suc: sucrose, Man: Mannitol, Lac: lactose

Table 2: Frequency of occurrence of isolates

Isolates	Frequency	Percentage (%)
Staphylococcus aureus	23	31.9
Escherichia coli	20	27.8
Streptococcus spp	12	16.7
Klebsiella spp	10	13.9
Proteus spp	7	9.7
	72	100

Table 3: Frequency of occurrence of isolates from the three hospitals

Hospitals	*S.aureus*	*Streptococcus* spp	*E. coli*	*Klebsiella* spp	*Proteus* spp	Total
KSUTH	10 (43.5%)	4 (33.3%)	7 (35.0%)	2 (20.0%)	2(28.6%)	25
GCM	8 (34.8%)	6(50.0%)	8 (40.07%)	3 (30.0%)	4(57.1%)	29
MGH	5 (21.7%)	2(16.7%)	5 (25.0%)	5 (50.0%)	1(14.3%)	18
Total	23 (100%)	12(100%)	20(100%)	10 (100%)	7(100%)	72

KSUTH: Kogi State university Teaching Hospital, GCM: Grimard Catholic Hospital, MGH: Maria Goretti Hospital

Table 4: Distribution of pathogens among female and male patients

sex	*S.aureus*	*Streptococcus* spp	*E. coli*	*Klebsiella* spp	*Proteus* spp	Total
Male	13 (34.21%)^{a,b}	5 (13.16%)^{b}	11 (28.95%)^{b}	4 (10.53%)^{a,b}	5(13.16%)^{b}	38
Female	10 (29.41%)^{b}	7(20.59%)^{a,b}	9 (26.47%)^{b}	6(17.65%)^{b}	2(5.88%)^{b}	34

^{a,b} : Superscript with different alphabets in each column are significantly different
$X^2 = 2.396$, DF = 4, P-Value = 0.663

Table 5: Distribution of pathogens according to age of patients (Male and Female)

Age	*S.aureus*	*Streptococcus* spp	*E. coli*	*Klebsiella* spp	*Proteus* spp	Total
16-20	6 (35.29%)[a]	3 (17.64%)[a]	4 (23.52%)[a]	2 (11.76%)[a]	2(11.76%)[a]	17
21-25	2 (50%)[c]	0(0%)[c]	1 (25 %)[c]	0(0%)[c]	1(25%)[c]	4
26-30	3(60%)[c]	1(20%)[c]	0(0%)[c]	1(20%)[c]	0(0%)[c]	5
31-35	1(20%)[c]	1 (20%)[c]	0(0%)[c]	3(60%)[c]	0(0%)[c]	5
36-40	2(33.33%)[b,c]	1(16.66%)[b,c]	1(16.66%)[b,c]	2(33.33%)[b,c]	0(0%)[b,c]	6
41-45	2(22.22%)[a,b,c]	2(22.22%)[a,b,c]	2(22.22%)[a,b,c]	3(33.33%)[a,b,c]	0 (0%)[a,b,c]	9
46-50	3 (30%)[a,b,c]	2(20%)[a,b,c]	1(10%)[a,b,c]	3(30%)[a,b,c]	1(10%)[a,b,c]	10
51 & above	4 (28.57%)[a,b]	2 (14.28%)[a,b]	3(21.42%)[a,b]	4 (28.57%)[a,b]	1(7.14%)[a,b]	14

[a,b,c] : Superscript with different alphabets in each column are significantly different

Fcal = 3.302, DF = 7, P-Value = 0.01

.

Table 6: Antibiotic susceptibility test with inhibition zone diameters

Antibiotics	A		B		C		D		E	
Ciprofloxacin	17mm	S	18mm	S	19mm	S	21mm	S	20mm	S
Norfloxacin	22mm	S	17mm	S	0mm	R	0mm	R	0mm	R
Gentamicin	23mm	S	0mm	R	18mm	S	19mm	S	18mm	S
Amoxil	19mm	S	18mm	S	6mm	R	2mm	R	16mm	S
Streptomycin	20mm	S	22mm	S	28mm	S	27mm	S	20mm	S
Rifampicin	16mm	S	2mm	R	7mm	R	0mm	R	0mm	R
Erythromycin	2mm	R	5mm	R	3mm	R	0mm	R	0mm	R
Chloramphenicol	2mm	R	2mm	R	4mm	R	8mm	R	0mm	R
Ampidox	18mm	S	18mm	S	0mm	R	0mm	R	3mm	R
Levofloxacin	25mm	S	18mm	S	0mm	R	0mm	R	2mm	R
Co-trimoxazole (Septrin)	0mm	R	0mm	R	0mm	R	0mm	R	0mm	R
Sparfloxacin	0mm	R	20mm	S	0mm	R	24mm	S	0mm	R
Augmentin	26mm	S	17mm	S	17mm	S	18mm	S	3mm	S
Ofloxacin	18mm	S	18mm	S	18mm	S	25mm	S	18mm	S
Pefloxacin	0mm	R	21mm	S	0mm	R	30mm	S	6mm	R

A: *Staphylococcus aureus*, B: *Streptococcus* spp, C: *Escherichia coli*, D: *Klebsiella* spp, E: *Proteus* spp, S: susceptible (16mm and above), R: resistant (15mm and below).

CONCLUSION

This study revealed that *Staphylococcus aureus* was the most predominant etiologic agent of wound infections and also the presence of multiple drug resistant pathogens in the study area. It also shows a high level of resistance to septrin which implies that septrin is not suitable for treating cases of wound infection. As a matter of fact, culture and sensitivity testing of the isolates from wound swabs should be carried out prior to the administration of any treatment. However in this study, ciprofloxacin, gentamicin, streptomycin, augmentin and ofloxacin should be considered as first line drug in the treatment of wound in the locality.

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