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Bacteriological Study of Wound Infections in Patients Attending Specialist Hospitals Sokoto

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Abstract

Wound infection is the invasion and proliferation by one or more species of microorganisms sometimes resulting in pus formation. The aim of this research was to determine the bacterial profile and antibiotic susceptibility pattern of bacterial pathogens isolated from wound infections in patients attending some selected hospitals in Sokoto metropolis, Northwestern of Nigeria. One hundred and twenty-four (124) wound swabs were collected from patients of different ages between April to September 2018. The wound swab samples were cultured on MacConkey, blood and Chocolate agar. The isolates were gram stained and characterized using standard bacteriological procedure. Seventy-seven bacterial isolates (62.1%) were isolated, males had the highest prevalence of 77.9% than the female's counterparts (22.1%). *S. aureus* 27 (35.1%) was the most common isolated organism, followed by *Pseudomonas* spp 25(32.5%), *E. coli* 11 (14.3%), *Klebsiella* spp 7(9.1%) *Proteus* spp 6(7.8%), and CoNS 1(1.3%). Among the total isolates, 45(58.4%) of them were resistant to two or more antibiotics (multi-drug resistant), 11(14.3%) were sensitive to all drugs tested and 15(19.5%) were resistant to only one drug tested. National surveillance of antibiotic resistant organisms and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaigns and appropriate antimicrobials are recommended to limit increase growth of bacterial resistance.

***Keywords**: Bacteriological, wound, Infection and Patients*

Introduction

In addition to skin and soft tissue infections, which are primarily caused by breaks in the skin's surface, wound infections can also result from surgeries, trauma, bites, or diseases that disrupt the skin. Human skin is constantly being inundated with organisms from the environment, and it serves as the body's primary defense organ, preventing infection by forming an effective barrier that keeps the underlying tissue intact [1]. Loss of skin integrity or wound formation exposes the subcutaneous tissue, which provides a moist, nutritious environment, which encourages microbial colonization and proliferation. Even with the finest skin preparation before an incision, microbial infection is inevitable since every skin incision compromises the cutaneous barrier. Furthermore, wound colonization by Gram-negative bacteria is prevalent beyond the first 24 hours, despite the fact that a burn site is relatively sterile during that time. Such infections can have a detrimental effect on the patient's quality of life and the rate at which the wounds heal, making them a significant barrier to healing [2]. According to Khan *et al*. (2017), infected wounds are typically more painful, odorous, and hypersensitive, which causes the patient to be more uncomfortable. *S. aureus* and *Pseudomonas aeruginosa* are the most often found bacteria linked to wound infections; between them, they cause 20–40% of all nosocomial, post-surgery, and burn infections. Several infections, including Enterococci and Enterobacteriae, have also been linked, particularly in individuals with impaired immune systems following abdominal surgery [3]. Wound infections become more difficult to treat when there is antibiotic resistance, along with the concerns outlined above. Changes in microbial ecology, genetics, and the non-selective use of antimicrobial drugs are the main causes of antibiotic resistance [4]. Methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), and multidrug-resistant tuberculosis (MDR-TB) are examples of antimicrobial resistance that are medically significant [3]. Wound becomes infected when one or more types of germs invade it and multiply, occasionally causing pus to develop. Numerous microorganisms, including fungi, parasites, and bacteria, can infect a wound [3]. *Pseudomonas aeruginosa* infections mostly occur after surgery and burns, accounting for 5–15% of cases. Various bacteria, including Enterococci, *Escherichia coli*, Klebsiella species, and Proteus species, have been linked to infections, particularly in patients with impaired immune systems and after abdominal surgery. Candida species are among the fungi that cause wound infections [5]. Antimicrobial resistance is therefore becoming a global concern [3]. Globally, the threat posed by bacterial infections caused by multidrug-resistant (MDR) pathogens is increasing. In underdeveloped nations like Nigeria, they are a leading source of illness and death. A significant issue with hospital-acquired infections as well as community-acquired illnesses is antimicrobial resistance (AMR) [6]. A bacterium is referred to as multidrug resistant (MDR) if it possesses multiple mechanisms of resistance to different antimicrobial medicines from different chemical classes or subclasses. Because of the high frequency of infections, inappropriate use of antibiotics, availability of over-the-counter medications, and dearth of clinical microbiology facilities for antimicrobial susceptibility testing, the antimicrobial resistance (AMR) problem is particularly difficult in low-income countries. The development of resistant bacterium infections poses a significant challenge to future chemotherapies as they have a negative impact on treatment outcomes, costs, disease spread, and illness length. Furthermore, the struggle between bacteria and their drug susceptibility continues to be a source of concern for the general public, academics, physicians, and pharmaceutical corporations in their quest for efficient medications [7]. The indiscriminate use of antibiotics is contributing to the continuous spread of resistant microorganisms. Patients who are treated with empirical antimicrobial medications frequently develop antibiotic resistance. This is especially important in developing nations where antibiotics are widely administered inappropriately. Monitoring resistance profiles in healthcare facilities is necessary to get over these obstacles [7].

Materials and Methods

Study Population

The study population comprised of male and female patients of all age and sex attending Maryam Abacha Women and children Hospital and Specialist Hospital Sokoto.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. Patients with wound infections

2. Patients who agreed to participate and gave informed consent.

Exclusion Criteria

1. Patients who do not develop wound infection based on clinical examination.
2. Patients on antibiotic treatment.
3. Patients who did not consent to participate in the study.

Sample size determination

Sample size determination for this study would be calculated based on prevalence reported initial studies carried out around the country using the following formulae:

$$n = \frac{z^2\, p \times q}{d^2}$$ (Thrust field, 1997)

n=72

Ethical approval

Prior to the commencement of sample collection, ethical approval was obtained from ethical committee under the chairman medical advisory committee (CMAC) specialist hospital Sokoto (SHS/SUB/133/VOL 1).

Sample collection and technique

Sterile swab stick was used to obtain the sample from the site of wound by first cleansing the wound with sterile saline to irrigate any purulent debris. The swab was then rotated over a 1cm square with sufficient pressure to express fluid from within the wound tissue. The swab was transported immediately to the laboratory for processing.

Culture

The samples were cultured on MacConkey, Blood agar and Chocolate agar and incubated aerobically at 37°C for 24h; the Chocolate agar was incubated under microareophilic environment in a candle jar at 37oc for 48h. The isolates were gram stained and characterized using standard bacteriological procedure [8].

Gram Staining Procedure

The smear was stained with crystal violet for one minute and was washed with tap water. Lugol's iodine was applied for one minute and rinsed with tap water. The smear was decolorized briefly with acetone for 30 seconds and was washed with tap water immediately. Counter stain (Neutral red) was applied for two minutes and rinsed with tap water. The smear was allowed to air dry and examined microscopically under oil immersion x100 objective [9].

Culture identification

The Preliminary identification of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests. Namely: Kliger Iron Agar (KIA) [10], Indole [11], Simmon's citrate agar [12] and urease [12]. Gram-positive cocci were identified based on their gram reaction, catalase [13] and coagulase [14] test results.

Antibacterial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique according to criteria set by CLSI [15]. Susceptibility testing was performed by Kirby-Bauer disk diffusion technique. The inoculum was prepared by picking parts of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over the Mueller-Hinton agar (oxoid) surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic impregnated paper disk into the agar medium, and then incubated at 37°C for 16–18 hours. Diameters of the zone of inhibition around the discs was measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI [16]. The drugs tested for both gram negative and gram-positive bacteria was selected based on the availability and prescription frequency of these drugs in the study area [17].

RESULTS

Results obtained from this study area, showed that, seventy-two (72) wound samples collected from Specialist hospital, fiftyfour (75%) were infected and this is highlighted in Table 1. The result of this study shows that identification and prevalence of bacterial isolate with gram positive and gram-negative bacteria *staphylococcus aureus* (30.6%) was the most common isolated organism, followed by *Pseudomonas aeruginosa*. (11.1%), *Escherichia coli* (5.6%), *Klebsiella* spp. (11.1%) *Proteus* spp (8.3%) Table 2. The result of this study shows that susceptibility pattern on *Staphylococcus aureus* showed high sensitivity to piperacillin/tazobactam (96.3%) followed by ceftazidime (85.2%), gentamicin (85.2%), ciprofloxacin (74.1%), sulfomethoxazole/trimethoprim (74.1%), imipenem (63%), and tetracycline (63%). High resistance was showed to cefoxitin (85.2%). Table 3. Coagulase negative *Staphylococci* showed high sensitivity (100%) to all the antibiotics as depicted. Table 4 *Pseudomonas* spp showed high sensitivity to piperacillin/tazobactam (100%), piperacillin (96%), imipenem (88%), Ceftazidime (84%) ciprofloxacin (68%), gentamicin (64%), aztreonam (64%) and highly sensitive to colistin sulphate (64%). Table 5. *Klebsiella* spp showed high resistance to aztreonam (85.7%). High sensitivity was shown to piperacillin/tazobactam (100%), imipenem (100%), gentamicin (85.7%), ciprofloxacin (71.4%), colistin sulphate (71.4%), ceftazidime (57.1%) and piperacillin (57.1%). Table 6.

Escherichia coli showed high sensitivity to piperacillin/tazobactam (100%), piperacillin (90.9%) colistin sulphate (90.9%), imipenem (90.9%), gentamicin (81.8%), and ciprofloxacin (72.7%). The least resistance was showed to aztreonam (63.6%) and ceftazidine (63.6%), Table 7. *Proteus mirabilis* showed high sensitivity to piperacillin (100%), piperacillin/tazobactams (100%), gentamicin (100%), ciprofloxacin (83.3%), imipenem (83.3%). The least resistance was seen to Aztreonam, colistin sulphate and ceftazidime with 66.7% each. Table 8. The result of this study shows that the age distribution of patents with wound infections. Out of 72 cases, the patients of age group 11-20 and 31-40 had higher growth of organism, and the list (9.7%) was seen in < 10 years. (P=0.05). Table 9. Among the total of 72 samples, the result shows that the sex distribution 31 (43.05%) were from Outpatients, while 23 (31.9%) were from Inpatients. Among outpatient samples, 31 (43.05%) and inpatient samples, 23 (31.9%) were growth positive (P=0.5). The overall prevalence of bacterial wound infection in the present study was 75%. Table 10.

Table 1: Prevalence of bacterial wound infection in the study area by Hospital

Hospital	Infected (%)	Non-infected (%)	Total (%)
S.H.S	54 (75%)	18 (25%)	72(100)
Total	54 (75%)	18 (25%)	72(100)

Table 2: Identification of bacterial isolates of gram positive and gram negative.

Organism Isolated	No. of isolates (%)
Gram Positive	
Staph. aureus	**5(9.3)**
CoNS	**2(3.7)**
Strep. pneumoniae	**8(15)**
Strep. pyogenes	**7(12.9)**
Escherichia coli	**4(7.4)**
Klebsiella spp	**8(15)**
Salmonella spp.	**6(11.1)**
P. aeruginosa	**8(15)**
Proteus spp	**6(11.1)**
Total	**54(75)**

Key:
CoNS= Coagulase negative staphylococci

Table 3: Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotic	Sensitive (N)	%	intermediate (N)	%	Resistant (N)	%	Total
Ciprofloxacin	3	(60)	1	(20)	1	(20)	5
Gentamicin	2	(40)	2	(40)	1	(20)	5
Trimethoprim	3	(60)	0	(0.0)	2	(40)	5
Ceftazidime	3	(60)	1	(20)	1	(20)	5
Imipenem	4	(80)	0	(0.0)	1	(20)	5
Piperacillin/Tazobactam	3	(60)	1	(20)	1	(20)	5
Tetracycline	3	(60)	1	(20)	1	(20)	5
Cefoxitin	0	(0.0)	1	(20)	4	(80)	5

Table 4: Antibiotic susceptibility pattern of Coagulase negative *Staphylococcus*

Antibiotic	Sensitive No %	intermediate No %	Resistant No %	Total No
Ciprofloxacin	1(20)	1(20)	0(0.0)	2
Gentamicin	1(20)	1(20)	0(0.0)	2

Trimethoprim	1(20)	1(20)	0(0.0)	2
Ceftazidime	1(20)	1(20)	0(0.0)	2
Imipenem	1(20)	1(20)	0(0.0)	2
Piperacillin/Tazobactam	0(0.0)	1(20)	1(20)	2
Tetracycline	1(20)	1(20)	0(0.0)	2
Cefoxitin	1(20)	1(20)	0(0.0)	2

Table 5: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa*

Antibiotic	**Sensitive**		**intermediate**		**Resistant**		**Total**
	No	**%**	**No**	**%**	**No**	**%**	**No**
Azitreonam	5	(62.5)	2	(25)	1	(12.5)	8
Piperacillin	4	(50)	2	(25)	2	(25)	8
Ciproflaxicin	6	(75)	1	(12.5)	1	(12.5)	8
Gentamicin	5	(62.5)	2	(64)	1	(12.5)	8
Piperacillin/Tazobactam	7	(87.5)	1	(12.5)	0	(0.0)	8
Colistin sulphate	1	(12.5)	2	(25)	5	(62.5)	8
Ceftazidime	5	(62.5)	2	(25)	1	(12.5)	8
Imipenem	4	(50)	2	(25)	2	(25)	8

Table 6: Antibiotic susceptibility pattern of *Klebsiella* spp

Antibiotic	**Sensitive**	**intermediate**	**Resistant**	**Total**
	No %	**No %**	**No %**	**No**
Azitronam	2 (85.7)	0 (0.0)	6 (85.7)	8
Piperacillin	4(57.1)	2(85.7%)	2 (85.7)	8
Ciproflaxicin	5(71.4)	2(85.7%)	1 (85.7)	8
Gentamicin	6(85.7)	1(85.7%)	1 (85.7)	8
Piperacillin/Tazobactam	7(100)	0(0.0%)	1 (85.7)	8
Colistin sulphate	5(71.4)	1(71.4%)	2 (85.7)	8
Ceftazidime	4(57.1)	2(85.7%)	2 (85.7)	8
Imipenem	7(100)	0(0.0%)	1 (85.7)	8

Table 7: Antibiotic susceptibility pattern of *Escherichia coli*

Antibiotic	Sensitive No %	intermediate No %	Resistant No %	Total No
Aztreonam	1(63.6)	0(0.0)	3(63.6)	4
Piperacillin	3(90.9)	0(0.0)	1(90.9)	4
Ciprofloxacin	4 (72.7)	0(0.0)	0(0.0)	4
Gentamicin	2(81.8)	1(63.6)	1 (81.8)	4
Piperacillin/Tazobactam	3(100)	0(0.0)	1(100)	4
Colistin sulphate	3(90.9)	1(63.6)	0(0.0)	4
Ceftazidine	0(0.0)	0(0.0)	4(63.6)	4
Imipenem	2(90.9)	1(63.6)	1(90.9)	4

Table 8: Antibiotic susceptibility pattern of *Proteus spp*

Antibiotic	Sensitive No %	intermediate No %	Resistant No %	Total No
Aztreonam	1(66.7)	0(0.0)	5(66.7)	6
Piperacillin	5 (100)	1(100)	0(0.0)	6
Ciprofloxacin	5 (83.3)	0(0.0)	1(83.3)	6
Gentamicin	4 (100)	1(100)	1(100)	6
Piperacillin/Tazobactam	6 (100)	0(0.0)	0(0.0)	6
Colistin sulphate	2 (66.7)	0(0.0)	4(66.7)	6
Ceftazidine	1 (66.7)	1(66.7)	4(66.7)	6
Imipenem	5 (83.3)	0(0.0)	1(83.3)	6

Table 9: Age distribution of patient with wound infection

Variables			**Culture positive No (%)**		**P – Value**
Age Group (Years)	**≤ 10**	**(25)**	**7**	**(9.7%)**	
	11-20	**(34)**	**12**	**(16.7%)**	
	21-30	**(8)**	**11**	**(15.3%)**	**P > 0.05**
	31-40	**(3)**	**14**	**(19.4%)**	
	41-50	**(2)**	**10**	**(13.9%)**	
Total		**72**	**54**	**(75%)**	

Table 10: Sex distribution of patient with wound infection

Gender	**No of isolates**	**No of culture positive (%)**	**p-value**

Male	**44**	**31**	**(43.05%)**	
Female	**28**	**23**	**(31.9%)**	**p>0.05**
Total	**72**	**54**	**(75%)**	

Discussion

Infected wounds are odorous and painful and present greater discomfort to the patient. Loss of skin integrity or wound formation exposes the subcutaneous tissue that provides a moist nutritious environment to the microbial colonization and proliferation that results in morbidity and mortality [2]. Epidemiological surveillance of infections is indispensable for effective management of disease and the creation and implementation of control measures. This is particularly important in resource constraint settings, where data on disease prevalence is sparsely documented. In this study a total of seventy-two (72) wound swabs were collected out of which 54 (75%), were found to be infected with bacteria, and this is in agreement with findings of Oladeinde *et al*. [18] who reported an average prevalence of 70.1% in a 5 year study in Edo state Nigeria and Akoachere *et al*. [19] who reported a prevalence of 100% in Buea Cameroon. The aetiology of wound infections varies from one hospital to another, region to region and country to country. This in conjunction with differences in nature and site of wound infection in study center may account for this variation.

In the result of this study bacteria isolated were, *Staphylococcus aureus, Pseudomonas, Escherichia coli, Klebsiella, proteus and Coagulase negative staphylococci* in various proportions, this is in agreement with findings of Oladeinde *et al*. who reported same organisms and more in infected wounds in Edo, and Pandukur *et al*. [20] in Jos. *Staphylococcus aureus* was the predominant isolate (35.1%), followed by *streptococcus spp* (32.5%) in the study. This is in agreement in a study conducted in India by Rao *et al.* [21]. *Staphylococcus aureus* was the most common isolate (24.29%) and *streptococcus spp* (21.49%). The high proportion of *staphylococcus aureus* and *P. aeruginosa* in wound infection in this study might be because of endogenous source of infection or contamination from the environment such as contamination of surgical instruments with the disruption of natural skin barrier as these bacteria are common on surfaces, easily finds their way into wounds [18]. However, Mama *et* al (2014) reported *Staphylococcus aureus* was the predominant microorganism (32.4%) followed by *Escherichia coli* (20%). The isolation of these organisms may be attributed to contamination of wounds with soil, medical devices or the existence of locality variability.

In this study, gram positive isolates (*S. aureus* and CoNS) were most susceptible to pefloxacin (96.3%), gentamicin (85.2%) and ciprofloxacin (74.1%), Amoxicillin (63%) respectively. These findings are similar to those reported by Temitope *et al*. [22] that gram positive isolates were highly sensitive to gentamicin, pefloxacin, Amoxicillin ciprofloxacin and tetracycline. These antibiotics are highly active against most clinically important gram positive and gram-negative bacteria. This is in line with the study performed before commercialization of pefloxacin in Brazil which revealed that it was active against 83% of the isolates tested while streptomycin was active against 91%, tetracycline 75% and ciprofloxacin 73% [23]. Cefoxitin is highly resistant to most of the *Staphylococcus aureus* isolated (85.2%) which is an indication of methicillin-resistant *Staphylococcus aureus* (MRSA). Cefoxitin is considered to be a better predictor than other antibiotics like oxacillin for the detection of heteroresistance because it is a stronger inducer of penicillin-binding protein 2a (PBP2a) [24;25] *Pseudomonas aeruginosa* showed high sensitivity to pefloxacin 25(100%), Ciprofloxacin 24(96%) and amoxicillin 22(88%). The least sensitivity was shown to gentamicin 16(64%) and colistin sulphate 16(64%). Which is much higher compared to a Belgian study [26] but lower than the Turkish study where one third of the isolates were multidrug resistant.

Proteus showed most sensitivity to pefloxacin (100%), septrin (100%), Amoxicillin (83.3%), gentamicin (100%) and ciprofloxacin (83.3%). The results are also similar to those by Kamini *et al.* [27] that showed *Proteus* to be sensitive to Pefloxacin (75%), Amoxicillin (100%) and ciprofloxacin (87%). *Klebsiella* showed more than 50% sensitivity only to Amoxicillin (100%), Pefloxacin (100%), gentamicin (85%) and ciprofloxacin (71.4%). This is similar to study by Rao *et al*., (2014) that showed *Klebsiella* to be most sensitive to Amoxicillin (76.92%), ciprofloxacin (76.92%) and gentamicin (76.92%). *Escherichia coli* showed high sensitivity to Pefloxacin (100%), Septrin (90.9%) colistin sulphate (90.9%), imipenem (90.9%), gentamicin (81.8%), and ciprofloxacin (72.7%). The least sensitivity was showed to aztreonam (63.6%) and ceftazidime (63.6%). This result is consistent with the reports of Kolmos *et al.* [28].

Based on the distribution of wound infections by gender, this study demonstrates that infection is more common in males (69.2%) than in females (30.7%). This finding is supported by reports of Pandakur *et al*. and Oladeinde *et al*. who also documented that males are more commonly infected than females Males are involved in occupations such as agriculture, industrial work, and construction work involving professional hazards where wounds are more likely to occur. Based on the distribution of infection by age group, group range of 31-40 has the highest number of infected wounds (34%). This is consistence with the findings of Pondei *et al.* [29] and Aye *et al.* [30] who reported a prevalence of 34.5% a and 30% in the same age group all in Bayelsa and Benin city state respectively. This finding may not be unconnected to the fact that people in their second to fourth decades of life are more prone to infection since this is the most productive age range.

Conclusion

Because of the detrimental effect of wound infection, among the fifty-four (54) bacterial isolates, *Staphylococcus aureus* was the most common isolated organism followed by *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis* and Coagulase negative *Staphylococcus aureus*. In this study, gram positive isolates were most susceptible to gentamicin, Pefloxacin and Septrin while gram negative isolates were most susceptible to pefloxacin, ciprofloxacin, amoxicillin, Sparfloxacin and Streptomycin. These findings should be taken into account in further studies concerning bacteriological study of wound and their susceptibility to antibiotics in wound infection. Hence there is need to create awareness education on personal hygiene to minimize the occurrence of wound infections.

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