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Prevalence and Antibiogram Pattern of Bacteria from Wound Swabs

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Abstract:
Due to economic downturn which affected health budget, inadequate patient care and antibiotic resistance, there was a need to determine the bacterial agents in wound infections and their antibiogram in order to contribute to data which is currently scanty in JUTH Jos Nigeria. A total of 170 wound swabs were aseptically collected from patients in JUTH. The swabs were cultured on to Blood agar, MacConkey agar, Chocolate agar and Sabouraud Dextrose agar (SAB)(Oxoid England) respectively according to the method previously described by Cheesbrough,(2000).Except for the Chocolate agar plates which were incubated in a candle jar, all other plates were incubated aerobically. Culture plates were incubated in an incubator (Gallenkamp, UK) at 370C for 18 – 24 hours, while SAB plates were incubated at room temperature. Additionally, smears were made on clean glass slides and stained by Gram's stain as previously described by Cheesbrough, (2000). Colonies on culture plates were examined and identified according to methods previously described by Cowan and Steel (1993). A total of 8 bacterial species were identified with Staphylococcus aureus having the highest prevalence (49 or 28.2%). Other pathogens were: Klebsiella spp (20 or 12%); Proteus spp (16 or 9.4%) Pseudomonas aeruginosa (12 or 7.1%); Providencia spp (4 or 2.3%); Escherichia coli (4 or 2.3%); Morganella morganii (1 or 0.6%) and Streptococcus agalactiae (1 or 0.6%). Antibiotic sensitivity was carried out on these isolates on Muella- Hinton sensitivity agar according to methods previously described by Baker and Breach (1980). The antibiotic discs tested were ciprofloxacin, gentamicin, chloramphenicol, Ofloxacin, erythromycin, amoxicillin, ceftriazone and augmentin. Most pathogens were sensitive to first line antibiotics with gentamicin showing multisensitivity to Staphylococcus aureus, Proteus spp, Klebsiella spp, Streptococcus agalactiae. In addition, Staphylococcus aureus showed stronger sensitivity to gentamicin, erythromycin and chloramphenicol. It was suggested that antibiotic treatment for wound infections should not be administered without information on the antibiotic sensitivity of a given bacterium.

Keywords: *wound, infection, bacterial pathogens, antibiogram.*

1. Introduction

Wound infection adds to the increased cases of mortality in Africa [xxii]. Wound is a condition in which the intact skin is broken as a result of surgical incision or physical injury [xiv][xviii]. The consequence of this action is the exposure of the subcutaneous tissue, and thus a loss of the integrity of the skin to protect against infection agents such as bacteria, parasites and chemical agents, leading to an open wound or closed wound [xviii]. Opportunistic bacterial pathogens may thrive as a result of the moist, warm and nutritive environment provided [ix]. As these pathogens overwhelm the body's immune system, infection sets in with the development of pus, a yellowish white fluid which can have a foul smell. Depending on some risk factors such as lowered immunity, malnutrition, cancer, diabetes old age, the wound could lead to severe condition such as wound ulcer, septicaemia, shock and possibly death[vi]. [xxii] reported that wound infection accounted for 45.8% of surgical admissions in Nigeria, while in northern France, it accounted for 1.5% of patient death from surgical site infection (SSI) [iv]. In Africa, wound infection had the highest rate of 25% [i]. Factors such as poverty, equipment, dressings, nutritional status, poor environmental hygiene and poor surgical technique may have been responsible for most cases [i]. Since these periods, there has not been much change, especially with the increasing armed conflict, modernisation

and sedentary life styles. In Nigeria, a 5-year surveillance of wound infections at a rural tertiary hospital showed that there was fluctuation in incidence from 71.4% in 2006, 76.2% in 2007 and 67.0% in 2010 [xix].
Bacterial organisms have been reported to be the major cause of infection. The most prevalent being *Staphylococcus aureus (S. aureus); Pseudomonas aeruginosa (P. aeruginosa); klebsiella* spp*; Proteus* spp*; Escherichia coli (E. coli)* and *Enterobacter* spp [xi][xiii][xvi][xxiii]. The data on the causative bacterial organisms and their antimicrobial sensitivity pattern from wound swabs among patients attending Jos University Teaching Hospital (JUTH) in Nigeria is very scanty. In addition, there is increased abuse of antibiotics (topical and oral) leading to prolonged stay for patients on hospital admission. Thus, this study aimed at isolating bacteria from wound swabs and ascertains their antibiotic pattern.

2. Material and Methods

2.1. Study Population
A total of one hundred and seventy (170) wound swabs were collected from patients with trauma, SSI, diabetes, sickle cell anaemia and cellulitis wound at JUTH Nigeria.

2.2. Ethical Consideration
Written ethical approval was obtained from the Ethical Committee of JUTH.

2.2.1. Informed Consent. Written informed consent was obtained from all subjects.

2.3. Specimen Collection and Processing
All wound swabs were aseptically collected, using commercially prepared sterile swab sticks. The swabs were then processed according to the method described by [vii]. The swabs were aseptically streaked on the surface of blood agar (for Gram negative bacteria), Mannitol salt agar (for *S.aureus*), MacConkey agar (for Gram negative bacteria) (Oxoid England) and incubated (Gallenkamp, UK) aerobically at 37^0C for 18 – 24 hours. In addition, the swabs were also aseptically streaked on the surface of Chocolate agar (for anaerobic bacteria) and Sabouraud dextrose agar (for yeast cells) (Oxoid England). The Chocolate agar plates were incubated at 37^0C in a candle jar while the Sabouraud Dextrose agar was incubated at room temperature for 18 – 24 hours. In addition, smears of the swabs were prepared on slides and stained by Gram technique and examined using 40x and 100x objectives for pus cells, bacteria and yeast cells [v]. Colonies from the culture were further identified.

2.3.1. Identification of Isolates
Bacterial cells were identified according to their characteristic colonial appearance on their media as well as by Gram staining. In addition, they were confirmed by their biochemical reactions according to the criteria previously described by [viii]. This included indole production, hydrogen sulphide production, citrate utilization motility, urease and carbohydrate utilization for the enterobacteriaceae while coagulase, DNAse, bacitracin and catalase for Gram positive bacteria.

2.3.2. Antibacterial Susceptibility Test
The antibiotics (Oxoid, England) tested were: Chloramphenicol(30µg), norfloxacin(10µg), erythromycin(15µg), amoxicillin(10µg), ceftriaxone (30µg), gentamicin(10µg), ciprofloxacine(5µg), augmentin(10µg). Antibacterial susceptibility tests were carried out on Muella-Hinton agar (Oxoid, England) by improved Bauer-Kirby technique previously described by [v]. The diameter of the zone of inhibition for each antibiotic was measured with the aid of vernier calipers. The results were determined after reading from a table and were interpreted as resistant, intermediate and sensitive [v][ii]. Reference strains of *E.coli* and *S.aureus* were obtained from National Veterinary Research Institute Vom, Nigeria and were used for quality control.

2.4. Statistical Analysis
Chi-square (X^2) test was used to determine the significance of the data obtained. A p value of < 0.05 was considered significant.

3. Results
Out of a total of 170 wound patients sampled, wounds caused by trauma had the highest frequency of 113 (66.5%) followed by SSI with frequency of 25 (14.7%); diabetes wound had a frequency of 17 (10.0%); wound from sickle cell anaemia 11 (6.5%) and cellulitis 4 (2.35%).
In addition, 106 (62.40%) wound swabs were culture positive and the highest frequencies were from trauma wound, surgical site infection and diabetes wound respectively. However, there was no significant statistical difference between the site of wound and culture positive swabs at X^2: $p>0.05$. There were more patients in the age range ≥51years with wound infection, followed by the age range 31-40 years and 41-50 years respectively. The age range ≥51 years was most frequently culture positive for various species of bacteria while the age range 11-20 years was least (Table 1).Males (89 or 62.9%) were more frequently infected than females (81 or 61.7%).

Age Range (Years)	No. Sampled	No. Culture Positive
0 - 10	18	12
11 - 20	20	10
21 - 30	26	21
31 – 40	35	16
41 - 50	33	21
≥51	38	26
Total	170	106

Table 1: Age Range (years) of Patients and Number of Wound Swabs Culture Positive

A total of 8 species of bacteria was isolated from the various wound swabs with *S. aureus* having the highest prevalence of 49(28.2%). Others were *Klebsiella* species 20(11.8%), *Proteus* species 16(9.4%), *Pseudomonas aeruginosa*12 (7.1%). Details are in Table 2.

Bacterial Spp	Frequency/Percent
Staphylococcus aureus	49(28.2)*
Klebsiella Spp	20(11.8)
Proteus Spp	16(9.4)
Pseudomonas aeruginosa	12(7.1)
Providencia Spp	4(2.35)
Escherichia Coli	4(2.35)
Morganella morganii	1(0.6)
Streptococcus agalactiae	1(0.6)
Total	106(62.40)

Table 2: Bacterial Species and their Frequency and Percent Prevalence
*Key: * Percent*

The antibiotic susceptibility pattern revealed that most of the bacterial species were sensitive to gentamicin with a frequency of 72. This was followed by ciprofloxacin (61), chloramphenicol (52) and ofloxacin (43). Furthermore, *S. aureus* was most frequently susceptible to all the antibiotics with gentamicin having the highest frequency of 34 or 69.39%. This was followed by erythromycin, 63.27% and chloramphenicol 63.27%. Details are in Table 3.

Bacteria	CPX	GEN	CHL	OFL	ERY	AMX	CEF	AUG
S.aureus(n=49)	31*	69.4	63	33	63	18	31	4
P.aeruginosa(n=12)	42	67	25	25	8	8	17	8
Klebsiella spp(n=20)	85	45	35	45	10	20	25	5
Proteus spp (n=16)	88	75	38	50	19	19	13	19
Providencia spp(n=4)	100	100	25	50	50	0	0	25
Escherichia coli(n=4)	100	75	75	75	0	25	0	0**
Morganella								
Morganii(n=1)	100	100	100	100	100	0	0	0
Streptococcus								
agalactiae(n=1)	100	100	0	100	0	100	0	0

Table 3: Bacterial Species and their Antibiogram in Percent.
*Key: * sensitive; **resistant*
CPX-ciprofloxacin; GEN-Gentamicin; CHL-Chloramphenicol; OFL- Ofloxacin
ERY- Erythromycin; AMX- Amoxycillin; CEF- Ceftriazone; AUG- Augmentin

4. Discussion

A total of 8 different bacterial species have been found to be responsible for wound infections at the Jos University teaching Hospital, Nigeria. These organisms were actually isolated from the wound swabs which were aseptically collected. This showed that these organisms were actually incriminated as the cause of these wound infections as they were found in the body of the patients at the site of the clinical lesions and were isolated in pure culture. This agrees with two of Koch's postulate [xxi]. The site of infection showed that trauma cases accounted for 66.5% of wound infection at JUTH. This finding was different from a similar report by [xvi] who reported that surgical site wound was common at Aminu Kano Teaching Hospital Kano, Nigeria. This could be as a result of occupational hazards such as farming and mining activities which is common in Jos.

The number of wound swabs that were culture positive in the age ranges sampled showed no association between wound infection and age (X^2:$p>0.05$) but there was higher prevalence in males than females. This finding agrees with that by [xviii][xxiii], but does not agree with the findings by, [x]. The difference could be due to the rural community these patients could be coming from and perhaps diabetes as reported by [xxiii].

This study isolated 8 species of bacterial pathogens in JUTH. A similar finding was reported by [xvi] at Aminu Kano Teaching Hospital. However, [xii] isolated 5 species in a similar study; three (3) bacterial species in Cameroon [iii]. These showed that there is a variation from one location to the other due to a number of factors such as standard of health care facility, drug abuse and non-adherence to infection prevention procedures by health care providers amongst other factors [i]. Out of the 8 species of bacterial pathogens isolated (Table 2), *Staphylococcus aureus* was the most prevalent pathogen isolated (28.2%). This meant that it was responsible for most wound infections at JUTH. The same organism has been incriminated in most cases of wound infections as reported by [xii][xvii][xiii][xvi][xx]and [xi], to mention a few. However, this result differed from that reported by [xviii] and [xv] in which *Pseudomonas aeruginosa* was the most frequently isolated pathogen followed by *Klebsiella* species. Other pathogens isolated in this study, in order of frequency were *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa, Providencia* species, *Escherichia coli*, *Morganella morganii* and *Streptococcus agalactiae.* There was no significant difference in the distribution of these pathogens in the age ranges sampled (X^2: $p > 0.05$). This agrees also with the finding by [iii].The multidrug sensitivity pattern to *S. aureus* especially to first line drugs for its treatment is very encouraging especially with most of the pathogens showing strong sensitivity to gentamicin and erythromycin. [xx] reported similar pattern. In addition, the sensitivity to Chloramphenicol a drug that is now rarely used due to its suppression of the bone marrow is a welcome development, as it can be used in the treatment of eye infections caused by *S. aureus* [xiii]. The susceptibility pattern generally showed a wider option for the other pathogens, especially common ones such as *E.coli* and *Proteus* species, thus avoiding the use of more expensive drugs and longer stay on hospital admission.

5. Conclusion

Some of the common bacterial pathogens associated with wound infection have been isolated, with *S. aureus* as the main causative pathogen. In addition, their antibiogram have been determined. Fortunately, most of the antibiotics used were also first line antibiotics, which means that they are affordable and so patients would not ignorantly patronise patent medicine dealers, a practice that could contribute to drug resistance. Furthermore, this study has contributed to knowledge on some of the bacterial pathogens as well as their antibiotic sensitivity pattern, which could assist in prompt treatment of wound infections. Consequently, antibiotic treatment for wound infection should be based on the antibiotic sensitivity pattern of the bacterium.

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