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Original article

Current Microbial Isolates from Wound Swabs, Their Culture and Sensitivity Pattern at the Niger Delta University Teaching Hospital, Okolobiri, Nigeria

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Abstract: Background: Wound infections continue to be problematic in clinical practice where empiric treatment of infections is routine. Objectives: A retrospective cross-sectional study to determine the current causative organisms of wound infections and their antibiotic susceptibility patterns in the Niger Delta University Teaching Hospital (NDUTH), Okolobiri, Bayelsa State of Nigeria. Methods: Records of wound swabs collected from 101 patients with high suspicion of wound infection were analysed. Smears from the wound swabs were inoculated on appropriate media and cultured. Bacterial colonies were Gram stained and microscopically examined. Biochemical tests were done to identify pathogen species. The Kirby-Bauer disk diffusion method was used for antibiotic testing. Results: Prevalence of wound infection was 86.13% (CI: 79.41–92.85). Most bacteria were Gram negative bacilli with *Pseudomonas aeruginosa* being the most prevalent pathogen isolated. The bacterial isolates exhibited a high degree of resistance to the antibiotics tested (42.8% to 100% resistance). All isolates were resistant to cloxacillin. Age group and sex did not exert any effect on prevalence, aetiological agent or antimicrobial resistance pattern. Conclusion: We suggest a multidisciplinary approach to wound management, routine microbiological surveillance of wounds, rational drug use and the institution of strong infection control policies.
Key words: wound infection, bacteria, antimicrobial resistance

INTRODUCTION

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue [1]. Wounds can be accidental, pathological or post-operative. An infection of this breach in continuity constitutes wound infection. Wound infection is thus the presence of pus in a lesion as well as the general or local features of sepsis such as pyrexia, pain and induration. Infection is believed to occur when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system [2].

Wound infection is important in the morbidity and mortality of patients irrespective of the cause of the wound. It is also important because it can delay healing and cause wound breakdown [3]. This is also associated with longer hospital stay and increased cost of healthcare [4]. Wound infections are also significant in that they are the most common nosocomial infection [5].

Studies on wound infection have largely focused on surgical site infections [6–8]. This might be because other types of wound infection are not problematic in the developed world where most of these studies have been done. However, in developing and resource-poor countries, other types of wound infection in addition to surgical site infection are still important causes of morbidity and mortality [9–11]. Where studies have been done on wound infections generally, regional and local variations have been observed in terms of the causative micro-organisms [4, 12, 13]. This means that physicians need to know the prevalent organisms and the resistance patterns existing in their localities.

Diagnosis of wound infection can be a daunting task in resource-poor settings. There is often a lack of adequate diagnostic equipment or requisite personnel [14]. Thus, a diagnostic dilemma confronts physicians in the absence of

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local epidemiological data on wound infections which could aid empiric treatment. This dilemma coupled with the fact that there are no established evidence-based clinical practice guidelines for wound infections, makes management of wound infections difficult in resource-poor settings like the Niger Delta region of Nigeria.

Our study was designed to establish baseline indices of wound infection at the Niger Delta University Teaching Hospital, Okolobiri, by looking at the prevalent microorganisms involved in wound infections, associated factors and drug resistance patterns.

MATERIALS AND METHODS

Study design

This was a retrospective cross-sectional study spanning 15 months from October 2010 to January 2011. The records of wound swab samples obtained from patients with different kinds of wounds, receiving treatment at the Niger Delta University Teaching Hospital (NDUTH), Okolobiri, Bayelsa State, Nigeria, were analysed.

Study area

The NDUTH is a tertiary health institution serving about 1.5 million inhabitants of Bayelsa State, Nigeria as well as patients from the neighbouring Delta State and Rivers State. The majority of inhabitants are farmers, fishermen and civil servants.

Exclusion/inclusion criteria

The records were scrutinised in such a way that all patients with suspected wound infections were added to the study. Wound infection was suspected if a wound was not healing well, getting bigger, exuding pus or fluid. Very ill patients and those undergoing antibiotic therapy two weeks prior to the study were excluded. Patients' age, sex and type of wound were noted. Ethical clearance for the study was granted by the Ethics Review Board of NDUTH.

Sample collection

Sample collection was conducted by medical officers in the out-patient clinic and in the wards using commercially available sterile cotton swabs and following existing departmental guidelines. Only one swab per patient was collected after carefully cleaning the wound with sterile water in order to prevent surface contamination. The samples were transported to the microbiology department within one hour of collection to prevent drying of the swabs.

Swabs were immediately inoculated on MacConkey agar, Chocolate agar, Blood agar and Cystine lactose electrolyte-deficient agar (CLED) and incubated at 37°C aerobically for 24 to 48 hours. Anaerobic cultures were not done due to logistic difficulties. Bacterial colonies on the agar plates were then Gram stained. Bacterial isolates were subjected to biochemical tests for identification and classification. Unclassified coliforms were lactose fermenting on MacConkey agar, yielding pink-coloured colonies, and were uniform gram negative rods.

Susceptibility to antibiotic chemotherapy was determined by the Kirby-Bauer disc diffusion method, and interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards [15]. The antibiotics tested were ceftazidime 30 μg, cefotaxime 30 μg, nitrofurantoin 50 μg, cloxacillin 5 μg, amoxicillin-clavulanic acid 30 μg, gentamicin 10 μg, cefuroxime 30 μg and ofloxacin 5 μg.

Statistical analysis

Differences in antibiotic resistance between wound types and between sexes and age groups were analysed using χ^2 test and Wilcoxon signed-rank test. A difference of $p < 0.05$ was considered significant.

RESULTS

Prevalence of wound infection

A total of 101 wound swab samples received at the Microbiology Department, Niger Delta University Teaching Hospital, Okolobiri, over a 15 month period (October 2010 to December 2011) were analysed. There were 48 male subjects and 53 female subjects, the male:female ratio being 0.9:1. Ages ranged from two months to 85 years, with a mean of 34.99 years (Table 1).

A total of 87 samples (86.13%, CI: 79.41–92.85) yielded significant bacterial growth indicative of wound infection, while 14 samples did not yield significant growth. No sample yielded more than one organism.

Table 1. Age distribution of patients with significant bacterial growth

Age group	Total no. of swabs	No. of infected swabs		p value
		Male	Female	
0 to 10	12	7	4	0.23
11 to 20	12	4	7	
21 to 30	33	11	14	
31 to 40	9	4	4	
41 to 50	8	3	3	
51 to 60	12	6	5	
61 to 70	7	2	5	
>70	8	2	6	
Total	101	39	48	

Type of wound

Non-traumatic causes (including diabetic foot ulcers) accounted for more than a third of all infected wounds (Table 2). There was no association between the type of wound and the type of micro-organism isolated ($p = 0.34$). All swabs obtained from patients with traumatic wounds yielded bacterial growth, and the majority of these patients were male (95.45%).

Age

There was greater incidence of wound infection in the 21 to 30 year age group, but there was no significant association between age and the incidence of wound infection ($p = 0.23$).

Sex

There was no significant association between the type of organism isolated and the sex of the subject ($p = 0.66$) or between the wound type and the sex of the subject ($p = 0.7$).

Micro-organisms isolated

Gram-negative bacilli were the most prevalent bacteria isolated from the wound swabs (85.05%). *Pseudomonas aeruginosa* was the most prevalent pathogen detected in the swabs, while *Escherichia coli* was the least detected isolate.

Gram negative bacilli were responsible for 85% of wound infections. *Staphylococcus aureus* was the only gram positive organism isolated. *Pseudomonas aeruginosa* was the predominant micro-organism isolated from the wound swabs, accounting for almost half of the isolates (Table 3). No particular organism was peculiar to any of the wound types (Table 4).

Antibiotic resistance

The bacterial isolates exhibited a high resistance to the antibiotics tested, with most isolates having resistance levels of between 42.8% and 100%. All of the organisms isolated were absolutely resistant (100 %) to cloxacillin (Table 5). About half of the organisms were also absolutely resistant to amoxicillin-clavulanic acid. *Pseudomonas aeruginosa* exhibited a very high resistance to the tested antibiotics, the lowest recorded resistance being 58.9% (Fig. 1). *Klebsiella pneumoniae* was relatively susceptible to nitrofurantoin.

Multiple-antibiotic resistant strains

Twenty-five (25) isolates (28.7% of isolates) were resistant to all of the antibiotics tested. Fourteen of these isolates were *Pseudomonas aeruginosa*, eight were coliform and three were *Staphylococcus aureus*. Also, there were 13 isolates sensitive to only one of the antibiotics tested.

No significant difference in resistance was observed with regard to age, sex or type of wound.

Table 2. Frequency of wound types

Type of wound	no. of swabs	no. of infected swabs	Patients with infected wounds	
			Males	Females
Surgical	31(30.69%)	27 (87%)	6	21
Trauma	22 (21.78%)	22 (100%)	21	1
Non-traumatic	40 (39.6%)	32 (80%)	10	22
Burns	8 (7.92%)	6 (75%)	2	4
Total	101	87	39	48

Table 3. Frequency of micro-organism isolated

Micro-organism isolated	Male	Female	p value
Pseudomonas aeruginosa	11	28	
Staphylococcus aureus	7	6	0.6601
Coliform (except *E.coli*)	8	4	
Proteus mirabilis	6	4	
Klebsiella pneumoniae	5	2	
Escherichia coli	2	4	
Total	39	48	

Table 4. Frequency of micro-organisms according to wound types

Micro-organism	Type of wound				Total
	Surgical	Trauma	Non-traumatic	Burns	
Pseudomonas aeruginosa	7	9	19	4	39
Staphylococcus aureus	2	9	2	0	13
Coliform (except *E.coli*)	6	0	4	2	12
Proteus mirabilis	3	2	5	0	10
Klebsiella pneumoniae	5	2	0	0	7
Escherichia coli	4	0	2	0	6
Total	27	22	32	6	87

Table 5. Degree of susceptibility of micro-organisms isolated to antibiotics expressed in percentage resistance

Organism	CAZ	CTX	NIT	CXC	AMX-CLA	GEN	CRX	OFL
Pseudomonas	66.7	82	94.9	100	94.9	58.9	92.3	58.9
Staphylococcus	84.6	100	100	100	76.9	46.1	46.1	61.5
Coliform (except *E.coli*)	63.6	63.6	63.6	100	100	81.8	63.6	81.8
Proteus	50	50	70	100	80	90	80	80
Klebsiella	42.8	85.7	28.6	100	100	57.1	57.1	100
E.coli	33.3	50	50	100	100	50	83.3	83.3

CAZ—ceftazidime; CTX—cefotaxime; NIT—nitrofurantoin; CXC—cloxacillin;
AMX-CLA—amoxicillin-clavulanic acid; GEN—gentamicin; CRX—cefuroxime; OFL—oflaxacin

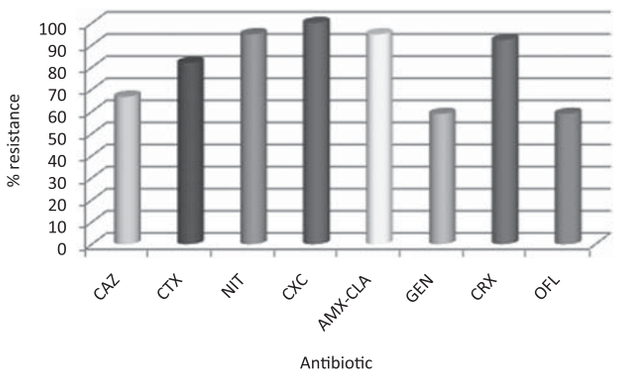

Fig. 1. Susceptibility pattern of *Pseudomonas aeruginosa* isolates to antimicrobial agents.
CAZ—ceftazidime; CTX—cefotaxime; NIT—nitrofurantoin; CXC—cloxacillin; AMX-CLA—amoxicillin-clavulanic acid; GEN—gentamicin; CRX—cefuroxime; OFL—oflaxacin

DISCUSSION

Bacterial contamination of wounds is a serious problem in the hospital, especially in surgical practice where the site of a sterile operation can become contaminated and subsequently infected [16, 17].

Our study demonstrated a high prevalence (86.1%) of pathogenic bacteria in wounds. This high figure is consistent with that obtained in similar studies in Nigeria [12, 18, 19], but different from another study in East Africa reporting a prevalence of 70.5% [20]. These differences may be due to study design. The rates might be equally high if only wounds with a high suspicion of infection are investigated as opposed to all wounds.

Although there was no association between the type of wound and the type of micro-organism isolated, it is important to note that all swabs from traumatic wounds yielded significant bacterial growth and were thus deemed to indicate infection. However, two previous studies also done in Nigeria had associated specific micro-organisms with particular wound types [21, 22]. More studies are required to clarify this observation.

Ideally, the age of a patient seems likely to have a bearing on wound infection and healing, people at the extremes of life being more prone to wound infections. However, we observed no association between age and wound infection, a finding that is inconsistent with the results of a study done in the Niger Delta region [13] and another in Ethiopia [20].

As in previous studies, Gram-negative bacteria were the most commonly isolated pathogens. Our observation of *Pseudomona aeruginosa* as the most common pathogen in wound infections differs from other studies in Nigeria reporting *Staphylococcus aureus* to be predominant [12, 13, 18, 19]. *Klebsiella pneumoniae* was observed as the most common pathogen in wounds in a study in Western Nigeria [4]. This is evidence of the existence of local and regional variability and shows that each health facility has to determine the prevalent micro-organisms and other associated indices.

Most of these studies, including ours, are limited by the fact that anaerobic cultures were not done for a variety of reasons, the main one being a lack of equipment and funds. Thus, anaerobic bacteria, which are also important in wound infections, could not be isolated.

Antibiotic resistance by the isolates to commonly prescribed antibiotics was high. This high level of resistance is a cause for concern. The absolute resistance to cloxacillin was not unexpected considering the fact that cloxacillin is a component of Ampiclox, an antibiotic frequently implicated in self-medication in Nigeria [23]. Cephalosporins, which are among the least prescribed antibiotics in Nigeria [23], are neither widely abused in this country nor easily affordable by the patients in the Niger Delta region. The development of resistance to cephalosporins observed in this study is thus a wake-up call for action on antimicrobial resistance. The poor availability of antibiotics, as well as their unregulated use and misuse, has been shown to contribute to increasing antimicrobial resistance in developing countries [14].

The lack of diagnostic facilities in these developing re-

gions encourages empiric treatment and overtreatment, which contribute to the increased resistance [14].

Multiple antibiotic resistance in bacterial populations is a great challenge in the effective management of wound infections. This calls for monitoring and optimization of antimicrobial use. We suggest a multidisciplinary approach to wound infection management involving both clinicians and microbiologists. Strengthening of laboratory services at local and national levels will ensure effective surveillance of antimicrobial resistance [24]. We also advocate routine microbiological surveillance of wounds and testing for antimicrobial susceptibility before drug use.

CONCLUSIONS

Severe antimicrobial resistance in wound infections was observed among patients in NDUTH, Okolobiri, Bayelsa State of Nigeria. There is a need for serious and urgent intervention to stem the spread and further evolution of this resistance. A rigorous infection control policy combined with rational drug use play an important role in this fight against antimicrobial resistance. We suggest the inclusion of anaerobic culture in routine microbiology culture investigations.

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