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Original Research Article

Bacteriological Pattern of Wound Swab Isolates in Patients with Chronic Leg Ulcer

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Abstract

PURPOSE: To determine the pattern of bacterial pathogens and their antibiotic sensitivity profile in patients with infected chronic leg ulceration.
METHODS: Sixty swab specimens obtained from chronic leg ulcer (CLU) patients were cultured aerobically and the antibiotic sensitivity pattern of the recovered organisms determined by the modified Kirby-Bauer disc-diffusion method.
RESULTS: 47 (78.3%) of the ulcers were infected out of which 39 (83.0%) were culture positive. Most of the culture positive ulcers were on the distal third of the leg. The isolated bacteria from the wounds were *Pseudomonas aeruginosa* (33%), *Staphylococcus aureus* (24%), *Proteus spp* (15%), *Klebsiella spp* (13%), *Citrobacter spp* (13%) and *Escherichia coli* (2%). None of the patient without clinical evidence of wound infection had bacterial positive wound swab culture. All isolates were sensitive to third generation cephalosporin and floroquinolones but majority were resistant to ampicillin.
CONCLUSION: *Pseudomonas aeruginosa, Staphylococcus aureus. Proteus spp*, *Klebsiella spp*, *Citrobacter spp* and *Escherichia coli* sensitive to third generation cephalosporin and floroquinolones have been recovered from 78% of patients with chronic leg ulcers in a tertiary health facility in Nigeria.

Keywords: Bacteriology; Chronic leg ulcer; Wound swab

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Introduction

An ulcer is a type of wound which is described chronic if it does not show any healing tendency within six weeks. Ulcers (wounds) are regularly encountered in surgical practice. They may arise post-operatively, following trauma or burns, or in association with certain medical conditions such as diabetes mellitus, haemoglobino-pathy, lower extremities arterial disease, vasculitis, ulcerative skin diseases and malignancies, etc.

Chronic ulcers are at increased risk of being contaminated with infectious agents[1]. Fifty percent of wounds contaminated with bacteria are estimated to become subsequently infected[2]. Wound infection (WI) in surgical practice is a major contributor to nosocomial infections accounting for between 3 and 11% of hospital acquired infections at various health centres[3]. Besides, WI increases the disease burden by increasing hospital stay, cost of treatment and sometimes may lead to death particularly when complicated with septicaemia and tetanus[4].

The current spread of multi-drug resistant bacterial pathogens has added a new dimension to the problem of WI[5]. This is particularly worse in resource poor countries where sale of antibiotics is under poor control[6]. A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive qualitative health care particularly when blind treatment is a necessity as in underdeveloped and developing nations.

The present study therefore is aimed at establishing the common bacterial agents associated with chronic leg ulceration and their antibiotics sensitivity profile in a tertiary health institution in Nigeria.

METHODS

This is a prospective study carried out at the University of Ilorin Teaching Hospital, Ilorin, Nigeria (UITH) between January 2004 and June 2006. UITH is a tertiary hospital with 450 beds, and an annual average of 10,000 and 120,000 admissions and out-patients hospital visits, respectively, in the last five years.

All patients with chronic leg ulcer (CLU) presenting to the surgery unit of the hospital with or without clinical evidence of WI had their wound swabs taken and sent to the Medical Microbiology laboratory unit of the hospital having obtained their consents. Patients on antimicrobial therapy within 72 hr of presentation were excluded from the study. WI was diagnosed clinically if there was sero-sanguinous or purulent wound discharge and/or evidence of cellulitis. CLU was defined as wound on the leg of 6 or more week's duration.

Wound swabs were taken from each patient and these were transported to the laboratory inside Stuarts transport medium. Swabs were inoculated either unto blood and MacConkey agar plates which were incubated aerobically or chocolate agar which was incubated inside candle extinction jar, all at 37 ^{o}C for 18-24 hr. Growth on culture plates were identified by colony morphology, Gram staining and standard biochemical tests[7].

Antibiotics sensitivity test was performed using the modified Kirby-Bauer disc-diffusion method[8]. Pure colonies of the isolated organisms were suspended in sterile normal saline inside Bijou bottles and the turbidity of the suspension adjusted to 0.5 McFarland's standard. A sterile cotton swab was dipped into the suspension and squeezed against the side of the bottle. Swab was then used to inoculate Mueller Hinton agar before the application of single antibiotic discs and subsequent incubation at 37 ^{o}C aerobically for 24 hr. Zone diameters of inhibition around each disc were measured using a calibrated

ruler and interpreted according to National Committee for Clinical Laboratory Standard (NCCLS)[9]. *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 and P*seudomonas* ATCC 27853 were used as control strains as appropriate.

The antibiotic discs used were cefuroxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) from Oxoid Ltd England, gentamycin (10 µg) from Abteck Biologicalas Ltd UK, ampicillin (25 µg, Abteck Biologicalas Ltd. UK, ciprofloxacin (5 µg) and ofloxacin (5 µg) from Fidson Health Care Ltd, Nigeria.

Results

A total of 60 patients were seen within the study period with CLU. Forty-seven (78.3%) of the patients had clinical WI out of which 39 (82.98%) were culture positive and 8 (17.02%) were culture negative. Out of the 39 culture positive wound swabs, 20 were males and 19 were females. None of the patients without clinical evidence of wound infection had bacterial positive wound swab culture.

Most of the ulcers were on the distal third of the leg (61.70%) and 93.10% of the ulcers on the distal third of the leg were positive for bacterial growth thereby constituting the most infected group of CLU (Table 1).

The bacterial isolate of CLU swab cultures is presented in Table 2 while the distribution of bacterial isolates according to the anatomical site of the wound is presented in Tables 3. *Pseudomonas aeruginosa* and *Staphylococcus aureus* constituted the majority of the isolates recovered from CLUs in this study accounting to (32.61%) and 23.91%, respectively. *Escherichia coli* was the least recorded isolate (2%). Thirty (76.92%) of the culture positive patients had a single bacterial species recovered from their wound swabs while 9 (23.08%) had multiple bacteria isolates. *Pseudomonas aeruginosa* was most commonly found bacteria in association with other isolates and mainly recovered from ulcer on the distal third of the leg.

The antibiotic sensitivity pattern of isolates is presented on Table 4. All isolates were sensitive to the tested fluoroquinolones while most were resistant to the second generation cephalosporin, cefuroxime, with the exception of *Proteus spp* and *Escherichia coli* which were 85.7% and 100% sensitive, respectively. All isolates were resistant to ampicillin (except *Escherichia coli* and *Citrobacter*) but sensitive to gentamicin except *Staphylococcus aureus* which was 36.4% resistant.

Discussion

An ulcer with delayed healing is at increased risk of contamination with microorganisms and subsequent infection. An infected wound, on the other hand, undergoes delay healing and get complicated if not properly handled[4]. In this study, 78.3% of the patients with CLU had clinical evidence of wound infection out of which 82.98% were bacteriologically culture positive. These values were high but similar to the 90% reported by Otokunefor and colleague[10], and

Table 1: Anatomical distribution and bacteriological yield of chronic leg ulcer swab cultures in a tertiary health institution in Nigeria

Anatomical site	Number of ulcers (%)	Number of ulcers with positive culture (%)
Proximal 1/3	8 (17.0)	4 (10.3)
Middle 1/3	10 (21.3)	8 (20,5)
Distal 1/3	29 (61.7)	27 (69.2)
Total	47 (100)	39 (100)

Table 2: Bacterial isolates of chronic leg ulcers wound swab cultures in a tertiary health institution in Nigeria

Organism	No of patients (%)
Pseudomonas	15 (33)
Staphylococcus aureus	11 (24)
Klebsiella	6 (13)
Proteus	7 (15)
Citrobacter	6 (13)
Escherichia coli	1 (2)

83.5% reported by Taiwo *et al*.[2]. This therefore calls for attention.

The fact that all patients without clinical evidence of WI (21.7%) had negative wound swab cultures is in support of the results of previous studies that correlated ulcer clinical status, histology and bacteriology such that any of these parameters can be used to predict WI[11]. However, 17.02% of the subjects with clinical evidence of WI had negative wound culture result. The accurate interpretation of this proportion is limited by the non-determination of anaerobic organisms in the wounds since anaerobic bacteria have been proven responsible for a significant proportion of WIs[10,12].

The preponderance of the two isolates, *Pseudomonas aeruginosa* and *Staphylococcus aureus,* is in line with previous studies[4,10]. Unlike in previous studies however, *Pseudomonas aeruginosa* is more implicated than *Staphylococcus aureus* in this study. This may be related to the greater risk of soil borne *Pseudomonas aeruginosa* contamination of distal leg ulcers which constituted the majority of ulcers seen in this study compared with proximal ulcers. *Pseudomonas aeruginosa* causing wound infection has been proven to be acquired from poor environmental sources[4,13].

Table 3: Distribution of single and mixed bacterial isolates according to the anatomical site of chronic leg ulcers in a tertiary health institution in Nigeria

Isolates	Number of isolates		
	Proximal 1/3	Middle 1/3	Distal 1/3
Single			
Citrobacter spp.	1	0	3
Klebsiella spp.	1	0	2
Proteus spp	0	2	1
Ps. aeruginosa	0	2	7
Escherichia coli	0	0	1
Staph. Aureus	2	2	6
Total	4	6	20
Mixed			
P. aeruginosa, Klebsiella spp. S. aureus and *Proteus spp.*	0	1	0
P. aeruginosa, Proteus spp.	0	1	1
Klebsiella spp, Citrobacter spp	0	0	1
Klebsiella spp. Proteus spp	0	0	1
P. aeruginosa, Citrobacter spp	0	0	1
P. aeruginosa Klebsiella spp.	0	0	1
P. aeruginosa S. aureus	0	0	2
Total	0	2	7

Table 4: Antibiotic sensitivity profile of chronic leg ulcer wound swab isolates in a tertiary health institution in Nigeria

Antibiotics	*Pseudomonas aeruginosa* N=15	*Staphylococcus aureus* N=11	*Klebsiella spp* N=6	*Proteus spp* N=7	*Citrobacter spp* N=6	*Escherichia coli* N=1186
Gentamicin	13 (86.7%)	4 (36.4%)	3 (50.0%)	4 (57.1%)	4 (66.7%)	1 (100%)
Ofloxacin	12 (80.0%)	10 (90.9%)	5 (83.3%)	6 (85.7%)	5 (83.3%)	1 (100%)
Ciprofloxacin	9 (60.0%)	9 (81.8%)	6 (100 %)	7 (100%)	5 (83.3%)	1 (100%)
Ceftriazone	4 (26.7%)	7 (63.6%)	4 (66.7%)	5 (71.4%)	3 (50.0%)	1 (100%)
Ceftazidime	11(73.3%)	5 (45.5%)	5 (83.3%)	4 (57.1%)	3 (50.0%)	1 (100%)
Cefurozime	6 (40.0%)	2 (18.2%)	2 (33.3%)	6 (85.7%)	1 (16.7%)	1 (100%)
Ampicillin	4 (26.7%)	4 (36.4%)	1 (16.7%)	1 (14.3%)	5 (83.3%)	1 (100%)

Most isolates obtained in this study were resistant to cefuroxime with the exception of *Proteus spp* and *Escherichia coli* which were 85.7% and 100% sensitive respectively. The resistance to the second generation cephalosporin, cefuroxime, may be related to the fact that it was the first cephalosporin to be introduced in our hospital and the most prescribed. A similar resistant pattern has been recorded previously in our health facility[14]. Nonetheless, further studies will be required to clarify this observation. Sensitivity of most isolates to gentamicin notwith-standing, its use for treatment of infected CLU cannot be recommended since one of the common agent of CLU infection (*Staphylococcus aureus)* is only 36.4% sensitive to it. It is worthy of note that majority of isolates were resistant to ampicillin except *Citrobacter* and *Escherichia coli*. The resistance to ampicillin may be related to the pressure of prolonged usage and regular abuse in our society. The sensitivity of all isolates to the third generation cephalosporins (ceftaxidime and ceftrizone) and floroquinolones (ofloxacin and ciprofloxacin) tested however makes them the drug of choice in the empiric management of infected CLU at our centre except when contraindicated.

Conclusion

Bacterial pathogens found in infected chronic leg ulceration of patients studied were *Pseudomonas aeruginosa, Staphylococcus aureus. Proteus spp*, *Klebsiella spp*, *Citrobacter spp* and *Escherichia coli*. The isolates were sensitive to third generation cephalosporin and floroquinolones which should be used for first line treatment of patients with infected CLUs except when contraindicated.

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