Antibiotic Susceptibility of Wound Swab Isolates in a Tertiary Hospital in Southwest Nigeria

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Abstract

Background: Wounds are commonly encountered in the clinical practice. Microbacterial colonization and infection negatively affect wound outcomes. With increasing emergence of antibiotic-resistant strains, it is essential to determine local patterns of wound microbiological profile and antibiotic susceptibility to guide rational empirical antibiotic use. Materials and Methods: Consecutive patients who presented to the plastic surgery unit were recruited to the study over a 6-month period. Wound swab cultures were performed at presentation using standard protocols and media. The wound swab was performed by the Levine technique and data were analyzed using a statistical software package. Results: Eighty-five microbial isolates were obtained from the eighty patients (55 males and 25 females) recruited. Gram-positive isolates were 35 (41.2%) and Gram-negative were 50 (58.8%). There was equal distribution of acute and chronic wounds. Pseudomonas aeruginosa was the most common isolate at 30.6%, followed by Staphylococcus aureus (27.1%), Escherichia coli (9.4%), Streptococcus species (8.2%), and Morganella morganii (7.1%). The isolates demonstrated resistance to amoxicillin-clavulenate, ampicillin, cloxacillin, cefuroxime, ceftazidime; low-to-moderate sensitivity to erythromycin, gentamicin, streptomycin, tetracycline, ciprofloxacin, and ofloxacin; and a moderate sensitivity to ceftriaxone and a high sensitivity to imipenem. There was significant difference in antibiotic resistance patterns between Gram-positive isolates from acute and chronic wounds but not for acute and chronic wound Gram-negative isolates. Conclusion: Most of the microbial isolates, particularly the Gram-negative isolates demonstrated moderate low sensitivity to commonly used antibiotics and moderate-to-high sensitivity to less commonly used newer antibiotics.

Keywords: Antibiotic resistance, microbial isolate, swab sample

Résumé

Contexte: Les plaies sont généralement rencontrés dans la pratique clinique. La colonisation et l’infection Microbacterial négatif sur blessure Les résultats. Avec l’augmentation de l’émergence de souches résistantes aux antibiotiques, il est essentiel de déterminer les tendances locales de plaie profil microbiologique Sensibilité aux antibiotiques et de guider l’utilisation d’antibiotiques empiriques rationnelle. Matériel et méthodes: Patients consécutifs qui ont présenté à la Unité de chirurgie en plastique ont été recrutés pour l’étude sur une période de 6 mois. Écouvillon blessure cultures ont été effectuées à la présentation en utilisant des protocols standard et des médias. L’écouvillonnage de la plaie a été effectuée par la technique de Levine et les données ont été analysées à l’aide d’un logiciel statistique. Résultats: Quatre-vingt-cinq isolats microbiens ont été obtenues à partir de 80 patients (55 hommes et 25 femmes) recrutés. Les isolats Gram-positifs ont été 35 (41,2%) et Gram-négatives sont 50 (58,8%). Il y a égalité de répartition des blessures aigus et chroniques. Pseudomonas aeruginosa est le plus fréquent d’isoler à 30,6%, suivi de Staphylococcus aureus (27,1%), Escherichia coli (9,4%), streptocoques (8,2%), et de Morganella morganii (7,1%). Les isolats présentaient une résistance à l’amoxicilline-clavulanate, ampicilline, cloxacillin, céfuroxime, cefazidine, faible à modérée de la sensibilité à l’érythromycine, la gentamicine, la streptomycine, la tetracycline, la ciprofloxacine, l’ofloxacine et; et une sensibilité modérée à la ceftriaxone et une forte sensibilité à l’imipénème. Il y avait une différence dans les profils d’antibi résistance entre les isolats de Gram-positives des infections de plaies aigus et chroniques mais

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Omoyibo, et al.: Antibiotic susceptibility of wound swab isolates

Introduction

Wounds are commonly encountered in plastic surgical practice and constitute a significant source of physical, psychological, and economic burden to patients in particular, and the society at large in terms of the huge resources spent in treating such wounds as well as time/days lost at workplaces. Clinically, wound may be categorized as acute or chronic based on the duration of their existence, 6 weeks usually the dividing line.\(^{1,2}\)

The precise global burden of wounds is not known due to poor data from developing countries. However, in the developed world, the economic burden of chronic wounds is well documented.\(^{[9]}\) In the United States, chronic wounds affect 6.5 million patients and the care costs over 25 billion dollars.\(^{[4,5]}\)

In the United Kingdom, the prevalence of wounds is about 3.55/1000 population and between 2006 and 2007, wound care costs 2.03 million pounds/100,000 of the population amounting to 1.44% of the health-care budget.\(^{[6]}\) Wound prevalence in India was 15.03/1000 of the population (acute wound was 10.55/1000 and chronic wound 4.48/1000).\(^{[7]}\) The point prevalence of chronic wounds at a tertiary hospital in Nigeria was found to be 11%.\(^{[8]}\)

Microbial colonization or infection of wounds is an important factor in poor wound healing.\(^{[9]}\)

The patterns and antibiotic susceptibility of these organisms in particular environment vary with the continuing evolution of microbes and the pattern of antibiotic use or abuse. This makes such a study imperative from time to time. The gold standard for determining wound bacterial bio-burden is tissue biopsy,\(^{[9]}\) but other techniques such as needle aspirate of wound fluid and wound swab are easier performed in the clinical setting, probably to avoid invasiveness with damage to healing tissue, significant pain in sensate soft tissue, and increased cost.\(^{[9,10]}\)

The swab techniques most commonly used are swabs of wound exudate, swabs using a broad Z-stroke over the entire wound bed, and swabs using the Levine technique.\(^{[10]}\) Swabs of wound exudates are usually taken before cleansing the wound and therefore specimens obtained in this manner sample microorganisms on the wound surface. Wound cleansing is advocated before obtaining swabs using either the Z-stroke or Levine’s technique, so the culture isolates wound tissue microorganisms and not just wound surface microorganisms.

In the broad Z-stroke technique, the wound is swabbed from margin to margin in a 10-point zigzag fashion and because a large portion of the wound surface is sampled, the specimen collected may reflect surface contamination rather than tissue involvement. For the Levine technique, the swab is rotated over a 1-cm² area with sufficient pressure to express fluid from within the wound tissue. This technique is believed to be more reflective of tissue involvement than swabs of exudates or swabs taken with a broad Z-stroke.\(^{[10]}\)

Bacteria causing wound infection could occur as single seeds of a (planktonic) bacterium or as biofilms. In the planktonic form, bacteria are susceptible to antibiotics, some antiseptics and the immune system.

They are also easily identified and cultured. In acute wounds, bacteria tend to exist in the planktonic form, while in chronic wound, the bacterium often takes on a different form usually occurring as biofilms.\(^{[11]}\) Biofilms comprise of aggregates of microcolonies of planktonic bacteria encased in an extracellular polymeric matrix which they manufacture themselves.\(^{[12]}\)

Mixed populations of both aerobic and anaerobic microorganisms are commonly found in both acute and chronic wounds. However, chronic wounds have a higher proportion of anaerobes in comparison with acute wounds.\(^{[13]}\)

The development of resistance to antibiotics by bacteria presents a challenge to the management of wound infection. There has been a rise of antibiotic resistance in hospitals and communities which has been concomitant with the use of antibiotics.\(^{[14]}\) There is a need for a more rational approach to the use of antibiotics based on microbial prevalence and antibiotic susceptibility. Thus, it is necessary to determine the pattern of microbiological profile of wounds and the current sensitivity pattern of the microbiological organisms isolated from wounds in our setting which will serve as a guide for empirical antibiotic use.

Materials and Methods

This is a prospective study of patients with wounds at a plastic surgery unit of a tertiary hospital in Southwest Nigeria. The patients were recruited consecutively as they presented for care from January to June 2014; those with clinical or histopathological evidence of malignancy were excluded from the study. Institutional ethical approval was obtained and informed consent was obtained from all consenting patients.

A wound swab was obtained from each patient by the Levine technique after cleaning the wound surface with saline. The sample was kept in a sterile container and transported at room temperature to the microbiology laboratory.

Culture of samples was done by inoculation onto blood agar, Chocolate agar, Sabouraud dextrose agar, and MacConkey agar (Oxoid, England) at 37°C for 24 h.
The isolates were subjected to Gram staining to differentiate Gram-positive from Gram-negative organisms. Colonies with Gram-positive coccal appearance on Gram staining were tested for the enzyme catalase to distinguish Staphylococci from Streptococci. Staphylococci were tested for coagulase production by the slide tests using citrated human plasma.

Colonies with Gram-negative bacilli appearance on Gram staining were subjected to Oxidase test to differentiate the Enterobacteriaceae (oxidase-negative) from other Gram-negative bacteria (oxidase-positive). Gram-negative bacilli were identified with the use of 24E Gram-negative bacilli Microbact (Oxoid, UK) identification kit.

The sensitivity pattern was assessed using single-disc broad spectrum and multidisc antibiotic discs in a diagnostic sensitivity test agar. The Kirby–Bauer Method was used for the antibiotic susceptibility testing. For Gram-positive bacteria isolates, the antibiotic discs used were imipenem, ceftriaxone, ciprofloxacin, erythromycin, gentamicin, streptomycin, tetracycline, chloramphenicol, cloxacillin, amoxicillin/clavulanate, and co-trimoxazole while for Gram-negative isolates, the antibiotic discs used were imipenem, ceftriaxone, ofloxacin, ciprofloxacin, gentamicin, cefuroxime, cefazidime, ampicillin, amoxicillin/clavulanate, and nitrofurantoin.

The data generated from each patient was entered into the Statistical Package for the Social Science (SPSS) version 17 (Chicago, SPSS Inc) sheet and analyzed with the appropriate test statistics.

**Results**

Forty patients with acute wounds and 40 with chronic wounds were studied. A total of 80 swab samples were collected. There were 55 male and 25 female (ratio 2.2:1). Their age ranged from 1 month to 78 years with a mean age of 35.4 years [Table 1]. Forty-five patients (56.2%) were managed as inpatients and 35 (43.8%) were outpatients. Posttraumatic wounds were the most frequent wounds sampled accounting for 36.25% of all the wounds; other etiologies are depicted in Figure 1.

A total of 85 bacterial isolates were obtained from the samples; 35 (41.2%) Gram-positive organisms and 50 (58.8%) Gram-negative organisms. Sixty-two (77.5%) swab samples yielded growth of microorganisms, of which 39 samples (62.9%) grew single bacterial isolate, while 23 samples (37.1%) yielded polymicrobial growth. Eighteen samples (22.5%) did not yield any growth. Forty-six organisms (54.1%) were obtained from the acute wounds, while 39 (45.9%) isolates were cultured from the chronic wounds.

The most common organism isolated was *Pseudomonas aeruginosa*, which accounted for 30.59% of all the bacterial isolates; this was followed by *Staphylococcus aureus* (27.06%). Figure 2 shows the various isolates obtained from the wound swabs.

The most common microorganism from acute wounds was *P. aeruginosa* (25.5%), while *P. aeruginosa* and *S. aureus* were the most common isolates from the chronic wounds, each accounting for 36.8% of the isolates from the chronic wounds [Figure 3 for the comparison of microbial isolates from acute and chronic wounds].

Gram-positive isolates were more prevalent in outpatient wounds accounting for 56.8% of outpatient wound infections, while Gram-negative isolates were more prevalent in inpatient wounds.

### Table 1: Age distribution of patients

| Age group of patients (years) | Chronic (n=40), n (%) | Acute (n=40), n (%) | Overall (n=80), n (%) |
|-----------------------------|----------------------|--------------------|----------------------|
| 0-10                        | 0 (0.0)              | 7 (17.5)           | 7 (8.7)              |
| 11-20                       | 4 (10.0)             | 5 (12.5)           | 9 (11.3)             |
| 21-30                       | 11 (27.5)            | 8 (20.0)           | 19 (23.7)            |
| 31-40                       | 9 (22.5)             | 6 (15.0)           | 15 (18.7)            |
| 41-50                       | 7 (17.5)             | 6 (15.0)           | 13 (16.3)            |
| 51-60                       | 4 (10.0)             | 4 (10.0)           | 8 (10.0)             |
| 61-70                       | 3 (7.5)              | 3 (7.5)            | 6 (7.5)              |
| 71-80                       | 2 (5.0)              | 1 (2.5)            | 3 (3.8)              |
| Total                       | 40 (100.0)           | 40 (100.0)         | 80 (100.0)           |

Figure 1: The distribution of wounds sampled
wounds accounting for 68.7% of inpatient wound infections. Figure 4 compares isolates from inpatient and outpatient samples.

The highest sensitivity of the bacterial isolates was to imipenem. Other patterns of sensitivity are shown in Table 2. The Gram-negative bacilli were resistant to ceftazidime, cefuroxime, and gentamicin while demonstrating a low-to-moderate sensitivity to ciprofloxacin and ofloxacin, particularly the commonly isolated Gram-negative microorganisms. However, the less commonly isolated organisms showed significant resistance [Table 3].

The Gram-positive cocci were least resistant to imipenem and ceftriaxone, with the isolates from acute wounds demonstrating a slightly higher resistance. There was a significant difference in antibiotic resistance pattern between Gram-positive isolates from acute and chronic wound infections ($P = 0.018$) [Table 4].

In general, there was a higher occurrence of resistant Gram-negative strains observed in acute wounds than in chronic wounds, though it was not statistically significant ($P = 0.140$) [Table 5].

**DISCUSSION**

Wound infection interferes with the process of wound healing, and this is increasingly important as emerging strains of antibiotic-resistant microbes occur. In this present study, the most common bacterial isolates were *P. aeruginosa*, *S. aureus*, and *Escherichia coli*, which is similar to the findings in previous studies in Nigeria\(^{[15-18]}\) and in a study among Malaysian participants by Wong *et al.*\(^{[19]}\) The bacterial isolates from these previous studies generally showed resistance to commonly used antibiotics – co-trimoxazole, amoxicillin, tetracycline, ampiclox, cloxacillin, chloramphenicol, and erythromycin. Gram-positive isolates were sensitive to ofloxacin, ciprofloxacin, amoxicillin-clavulanate, ceftriaxone, and
imipenem, while the Gram-negative isolates were sensitive to ofloxacin, ciprofloxacin, gentamicin, amoxicillin-clavulanate, ceftazidime, cefuroxime, ceftriaxone, and imipenem.\cite{16,17,20} Ogunshe \textit{et al}.\cite{21} also noted a high resistance and multiple antibiotic resistance among wound swab bacterial isolates in four health-care institutions in Southwest Nigeria.

![Figure 4: Comparison of bacterial isolates from inpatient and outpatient samples](image-url)
Table 4: Antibiotic-resistant pattern of Gram-positive cocci in acute and chronic wounds

| Antibiotics     | Acute wound resistance (%) | Chronic wound resistance (%) | $\chi^2$ | $P$   |
|-----------------|-----------------------------|------------------------------|---------|-------|
| Imipenem       | 26.3                        | 18.8                         | 21.409  | 0.018 |
| Ceftriaxone     | 47.4                        | 37.5                         |         |       |
| Ciprofloxacin   | 68.4                        | 75.0                         |         |       |
| Gentamicin      | 73.7                        | 68.8                         |         |       |
| Erythromycin    | 63.2                        | 93.8                         |         |       |
| Cloxacillin     | 100                         | 100                          |         |       |
| Co-trimoxazole  | 94.7                        | 100                          |         |       |
| Chloramphenicol | 63.2                        | 31.3                         |         |       |
| Tetracycline    | 84.2                        | 93.8                         |         |       |
| Streptomycin    | 57.9                        | 68.8                         |         |       |
| Amoxicillin/clavulanate | 100 | 100 |         |       |

There was a significant difference in antibiotic resistance pattern between Gram-positive isolates from acute and chronic wound infections.

Table 5: Antibiotic-resistant pattern of Gram-negative bacilli in acute and chronic wounds

| Antibiotics     | Acute wound resistance (%) | Chronic wound resistance (%) | $\chi^2$ | $P$   |
|-----------------|-----------------------------|------------------------------|---------|-------|
| Imipenem       | 29.6                        | 13.0                         | 13.534  | 0.140 |
| Ceftriaxone     | 74.1                        | 43.5                         |         |       |
| Ofloxacin       | 77.8                        | 56.5                         |         |       |
| Ciprofloxacin   | 77.8                        | 56.5                         |         |       |
| Gentamicin      | 92.6                        | 91.3                         |         |       |
| Cefuroxime      | 100                         | 95.7                         |         |       |
| Cefazidime      | 100                         | 95.7                         |         |       |
| Ampicillin      | 100                         | 100                          |         |       |
| Nitrofurantoin  | 85.2                        | 82.6                         |         |       |
| Amoxicillin/clavulanate | 100 | 100 |         |       |

There was no significant difference in antibiotic-resistance pattern between Gram-negative isolates from acute and chronic wound infections.

Gram-negative microorganisms predominated the microbial isolates accounting for 58.8%. A similar finding of 55% prevalence of Gram-negative microorganisms in the wounds of patients attending a tertiary hospital in Nigeria was reported by Garba et al.[17]

The Gram-positive microbial isolates in this present study showed complete resistance to amoxicillin-clavulanate, cloxacillin, and co-trimoxazole. In addition, S. aureus showed poor sensitivity to erythromycin, tetracycline, and gentamicin. This is similar to the findings by Sani et al.[20] and Ogunshe et al.[21] The Gram-positive isolates also demonstrated a low-to-moderate sensitivity to gentamicin (20.0%–57.1%), streptomycin (21.7%–57.1%), and ciprofloxacin (20.0%–42.9%); a moderate sensitivity to ceftriaxone (57.1%–60.9%); and a high sensitivity to imipenem (85.7%–87.0%) except for Staphylococcus epidermidis which showed a low sensitivity (20.0%) to imipenem. The Gram-positive cocci were least resistant to imipenem and ceftriaxone with the isolates from acute wounds demonstrating slightly higher resistance.

The Gram-negative isolates from this study demonstrated resistance to gentamicin, cefuroxime, ceftazidime, amoxicillin/clavulanate, and ampicillin. However, they showed moderate sensitivity to ciprofloxacin; low-to-moderate sensitivity to ofloxacin (12.5%–100.0%); moderate-to-high sensitivity to ceftriaxone (33.3%–100.0%); and a high sensitivity to imipenem (50.0%–100.0%).

In a study by Tamaraiselvi et al.[22] there was no significant difference between the antibiotic resistance pattern between Gram-positive cocci from acute and chronic wound infections, while there was a significant difference in antibiotic resistance pattern between Gram-negative isolates from acute and chronic wound infections. In this present study, there was a significant difference in antibiotic resistance pattern between Gram-positive isolates from acute and chronic wound infections ($P = 0.018$), but there was no significant difference in antibiotic resistance pattern between Gram-negative isolates from acute and chronic wound infections ($P = 0.140$). This difference is probably due to the fact that in the study by Tamaraiselvi et al.[22] Gram-negative bacilli predominated chronic wound infections accounting for 63.93% of chronic wound isolates which were more associated with biofilm production, while in the present study, Gram-positive isolates predominated outpatient wound infection 21/37 (56.8%) and chronic wounds accounted for 27/35 (77.1%) of outpatient wounds. Wong et al.[23] observed a higher occurrence of resistant bacterial strains, particularly Staphylococcus spp. and P. aeruginosa, in chronic wounds than in acute wounds, while in this present study, a higher occurrence of resistant strains were generally observed in acute wounds than in chronic wounds. The possible explanation for the findings in this present study is that acute wounds had more inpatient wounds than outpatient wounds (80% versus 20%), while chronic wounds had more outpatient wounds than inpatient wounds (67.5% versus 32.5%) and organisms causing inpatient wound infections tend to be multidrug resistant due to regular antibiotic use in the hospital setting.[24,25]

One limitation of this study was that anaerobic culture was not done.

**Conclusion**

The bacterial isolates in the study generally showed significant resistance to commonly used antibiotics such as amoxicillin-clavulanate, ampicillin, cloxacillin, gentamicin, cefuroxime, ceftazidime, and co-trimoxazole; low-to-moderate sensitivity to ciprofloxacin and ofloxacin; moderate-to-high sensitivity to ceftriaxone, and a generally high sensitivity to the newer antibiotic imipenem.

In general, isolates from acute wounds, comprising more of wounds managed as inpatient wounds, demonstrated a higher
antibiotic resistance compared with the isolates from chronic wounds. This shows the challenge of managing wounds colonized and infected by microorganisms in the hospital setting, thus necessitating the need for a rational approach to antibiotic use.

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**Conflicts of interest**

There are no conflicts of interest.

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