**ABSTRACT**

To determine the distribution of various bacterial pathogens associated with chronic wounds. A total of thirty wound swabs collected from the patients admitted in surgical ward were included in the study. The swabs were processed according to the standard microbiological methods. The swabs were inoculated onto Nutrient agar, Blood agar, MacConkey agar, Mannitol salt agar, Cetrimide agar and incubated at 37°C for 48 hrs. Bacteria were identified by standard Biochemical reactions and other identification tests. Result of the thirty samples, 10 (33.3%) revealed the growth of Staphylococcus aureus; 7 (23.3%) Pseudomonas aeruginosa; 4 (13.3%) revealed both Staphylococcus aureus and Pseudomonas aeruginosa. Bacterial aetiology was not detected in 9 (30%) samples. S. aureus isolates were sensitive to Methicillin. Resistance to Ampicillin was observed in 3 (30%); Ciprofloxacin, Erythromycin, Clindamycin in 2 % (20%) and Azithromycin resistance in 1% (10%) of S. aureus isolate. Six (8.5%) P.aeruginosa were resistant to Ciprofloxacin; 5(71%) resistant to Amikacin 3(42%) resistant to Cefazolin and 2 (28%) resistant to Ceftazidine and 1 (14%) resistant to Cotrimoxazole and Tetracycline. Staphylococcus aureus is the predominant bacterial isolate recovered from chronic wound swabs. The realization that notorious opportunistic pathogens, such as Staphylococcus aureus and P.aeruginosa are growing as biofilms in chronic wounds urges a search for novel diagnostic and treatment strategies.

**Key words:** Chronic wounds, Staphylococcus aureus, Pseudomonas aeruginosa, Ciprofloxacin

**Introduction**

The number of patients developing chronic wounds is increasing with worldwide increase in lifestyle diseases such as obesity, diabetes, CVDs. In developed countries it has been estimated that 1–2% of the population will experience chronic wound like diabetic foot ulcer, pressure ulcers and venous leg ulcers [1].

The normal wound healing process, involves four main phases: 1) Coagulation, 2) Inflammation, 3) Cell Proliferation and repair of the matrix, 4) Epithelialization and remodeling of the scar tissue.

Chronic wound seems to be arrested in a stage dominated by inflammatory process in which there is continuing influx of Polymorphonuclear Leukocytes (PMNLs). Activated PMNLs release cytolytic enzymes, free oxygen radicals
and inflammatory mediators that cause extensive collateral damage to the host tissues. The presence of bacteria is most likely to influence this imbalance. In bacterial profiles in chronic wounds which have been published for example, chronic venous leg ulcer harboured S. aureus (93.5% of the investigated ulcers) Enterococcus faecalis (71.7%) Pseudomonas aeruginosa (52.2%) Coagulase Negative Staphylococcus (45.7%) Proteus species (41.3%) Anaerobic bacteria (39.1%) [2]. S. aureus and P. aeruginosa are opportunistic pathogenic bacteria and are widely known to cause chronic biofilm-based infections in their hosts. Biofilm are bacterial aggregates enclosed in a self produced extracellular polymeric matrix [3,4,5]. Common characteristics of bacterial biofilms are their resistance against the activities of host immune system and tolerance of antibiotic intervention regimens.

S. aureus is most commonly isolated from chronic wounds [6,7]. It can express no of potential virulence factors and surface proteins which promote its adherence to the damaged tissues to the host [8,9]. P. aeruginosa often causes biofilm based chronic infections and express the virulence factors in particular rhamno lipid that can be eliminated by the activity of PMNLS [1,10]. P. aeruginosa infected wounds appeared significantly larger in terms of area and delay or even prevent the healing process [11,12, 13, 14].

Clinical laboratory investigations of chronic wound infections commonly rely upon bacterial isolation by culture, which most efficiently detects numerically dominant organisms amenable to growth on laboratory media.

This is a useful and well-established approach for the detection of many common pathogenic bacteria associated with wound infections [15]. Here, we report on the distribution of bacteria located on the surface and within the chronic wounds.

## Materials and Methods

A total of 30 wound swabs were obtained from patients attending the surgical unit of Sri Lakshmi Narayana Institute of Medical Sciences. All the samples were analysed in the Central Laboratory facility following the standard microbiological procedures.

### Standard culturing

Routine swabs were taken intra operatively for 30 patients (18 males and 12 females aged between 26-75 years). The swabs were collected in Amie’s transport medium and cultured on aerobic standard agar plates. The aerobic cultures were performed on Enriched media (Blood agar), Differential media (MacConkey) and Selective media. All plates were incubated at 37°C. Plates were examined everyday and differentiation and identification of bacteria were carried out using standard methods. Bacterial species isolated from the Mother inoculum were reported as scanty (<10 colonies), light (first quadrant), moderate (second quadrant), heavy (3rd and 4th quadrant) growth. Bacterial identification based on colony morphology, Grams staining, catalase reaction, coagulase test, oxidase test, Denitrification of nitrites and nitrates and alkalinisation of acetamide and subculture on selective medium were performed. The culture conditions and the medium used for the bacterial growth are shown in Table 1. The bacterial isolates were subjected to antimicrobial susceptibility testing using standard Kirby Bauer disc diffusion method.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Incubation condition</th>
<th>Target bacterial growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient agar</td>
<td>at 37°C for growth</td>
<td>Pigment production</td>
</tr>
<tr>
<td>Blood agar</td>
<td>at 37°C for growth</td>
<td>Haemolysis</td>
</tr>
<tr>
<td>Mac Conkey agar</td>
<td>at 37°C for growth</td>
<td>Lactose fermenting and non lactose fermenting bacteria.</td>
</tr>
<tr>
<td>Mannitol salt agar</td>
<td>at 37°C for growth</td>
<td>Selective growth of S. aureus</td>
</tr>
<tr>
<td>Cetrimide agar</td>
<td>at 42°C for growth</td>
<td>Selective growth of P. aeruginosa</td>
</tr>
</tbody>
</table>

### Table 1: Culture medium and incubation conditions for growth of bacterial pathogens recovered from chronic wound swabs
Results

To investigate the bacterial prevalence in the wounds using standard culturing techniques, out of 30 samples, 10 (33.3%) samples showed growth of S. aureus, 7 (23.3%) samples showed growth of P. aeruginosa and 4 (13.3%) samples showed the growth of both the organisms. Nine (30%) of the samples revealed no bacterial growth. All the bacterial isolates were recovered from both blood agar and Mac Conkey agar. The antibiogram performed on the bacterial isolates and resistance to antibiotics is represented in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>TET Tetracycline</th>
<th>AMP Ampicillin</th>
<th>P Penicillin</th>
<th>AK Amikacin</th>
<th>CZ Ceftazidime</th>
<th>CAC Clavulanic Acid</th>
<th>CIP Ciprofloxacin</th>
<th>AC Amoxycillin</th>
<th>CR Ceftriaxone</th>
<th>OX Oxacillin</th>
<th>COT Cotrimoxazole</th>
<th>G Gentamycin</th>
<th>E Erythromycin</th>
<th>CD Clindamycin</th>
<th>AZ Azithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. aureus + P.</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Antibiogram profile of bacteria recovered from chronic wounds.

Discussion

Although the microflora of chronic wounds is polymicrobial and heterogenous, S. aureus and P. aeruginosa are among the bacteria that are most frequently isolated from the wounds[16,17]. There are reports on the predominant distribution of S. aureus followed by P. aeruginosa chronic wounds. These observation may explain the over representation of S. aureus found in most wounds than P. aeruginosa. S. aureus isolates were sensitive to Methicillin, resistance to Ampicillin was observed in 3 (30%); Ciprofloxacin, Erythromycin, Clindamycin in 2% (20%) and Azithromycin resistance in 1% (10%) S. aureus isolate. Six (85%) P. aeruginosa were resistant to Ciprofloxacin; 5 (71%) resistant to Amikacin 3 (42%) resistant to Cefazolin and 2 (28%) resistant to Ceftazidime and 1 (14%) resistant to Cotrimoxazole and Tetracycline.

S. aureus mainly seen in the upper region of the wounds whereas P. aeruginosa is located in the deeper region of the wound bed. So in our present finding, we found more number of S. aureus as we collected the wound swab from the upper region of the wounds. However more research is required before specific bacterial species in specific modes of growth can be identified as the causative agents in chronic wounds.

It is generally accepted that when bacteria assume the biofilm phenotype, it offers increased protection against antibiotics and the activities of the host defense[2,5,9,11,12]. Accordingly, our investigations suggest that the bacteria embedded in the deeper regions of wounds reside in biofilms[17-22]. It is well known that bacteria such as P. aeruginosa are almost impossible to eradicate from chronic wounds by the use of antibiotics.

In the evaluation of a wound swab or biopsy, it is of paramount importance to consider
the discrepancy between an organism being culturable and non-culturable. Also, culturing from a biopsy does not reveal the presence of a bacterial biofilm. It is well known that bacteria embedded in biofilms are difficult to recover and culture, a fact that may also contribute to “under detection” [23].

For a good healing response, the bacterial load of chronic wounds needs to be optimally managed. Topical antimicrobials can be in some cases effectively control superficial bacterial burdens if the infection is localized but may not be appropriate for highly infected wounds. Systemic antibiotics may be effective in some cases of severe infection with tissue invasion[24]. The use of a nanocrystalline silver dressing was shown to decrease the superficial bacterial burden, as assessed by surface swab investigation, but had no effect on the bacterial burden of the deep wound compartment, as measured by tissue biopsy[25]. Thus, it is of great importance to define the spatial organization of the bacterial species within a chronic wound for the most effective management of the infection.

**Conclusion**

Staphylococcus aureus is the predominant bacteria isolate recovered from chronic wound swabs. The realization that notorious opportunistic pathogens, such as Staphylococcus aureus and P. aeruginosa are growing as biofilms in chronic wounds urges a search for novel diagnostic and treatment strategies

**References**


