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# ANALYSIS OF SKIN GRAFT FOR BACTERIAL INFECTION IN A TERTIARY HEALTH CARE CENTRE

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## ABSTRACT

An intact human skin surface is vital to the preservation of bodily fluid homeostasis, thermoregulation, and the host's protection against infection. Any breach in the skin will lead to compromise in health and well-being of the patient, successful skin grafting is the key to patient survival. But most of the times transplant recipients are predisposed to wide variety of bacterial, viral, fungal and parasitic infections because of immunosuppressant's administration there by prolonging the duration of wound healing. Our objective is to analyze the periodic bacterial infection that occurs in skin graft patients and to evaluate anti-microbial susceptibility testing on the bacteria isolated from the skin graft infection. 300 swabs from 50 skin graft cases were collected from the patients admitted in plastic surgery unit of S.S. Institute of Medical Sciences and Research Centre, Davangere. Periodic swabs were taken on Day 0, week 1, week 2, week 3, week 4 and week 5. The isolates were identified by standard microbiological techniques and antimicrobial susceptibility testing was done as per CLSI guidelines. Detection of Extended-spectrum beta-lactamase (ESβL), Metallo-β-lactamase (MβL) and Amp C-type β-lactamase were done according to standard guidelines. The tissue defects were grouped according to the cause as follows: vascular ulcers (9.2%), burns (14.5%), traumatic tissue defects (36.6%), and flap donor-site defects (39.7%). *Pseudomonas aeruginosa* was isolated in 38.9% of the cases followed by *Klebsiella pneumoniae* in 25.9% cases, *Staphylococcus aureus* in 18.5% of cases. Other organisms isolated are *E.coli, Streptococcus pyogenes,* CoNS*, Acinetobacter baumannii.* In all the cases, the preoperative evaluation indicated an adequate wound-bed preparation. However, graft loss secondary to infection was recorded in 12 patients (24%) and more common in grafts applied to the lower extremities ulcers in case of diabetic foot or when performed at multiple sites in case of burn infections. All the isolates were multi drug resistant. Among Gram negative bacteria, 17.9% were ESβL, 7.7% were AmpC and 21.6% were MβL producers. *Pseudomonas aeruginosa* was the predominant ESβL and MβL producer. 40% of the isolates were MRSA producers as skin transplant infection being a major problem, it becomes important to detect the specific pattern of graft microbial colonization and the antimicrobial sensitivity profiles so to reduce graft rejection.

**KEYWORDS: Skin transplant, bacterial infection, multidrug resistant bacteria**

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# 1. INTRODUCTION

An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection. With such important roles, the skin's ability to regenerate and heal is crucial. Any breach in the skin will lead to compromise in health and well-being of the patient, successful skin grafting is the key to patient survival.[1] Skin grafts have been used for over a century to resurface superficial defects of many kinds. Whether intended for temporary or permanent cover, the transplanted skin does not only protect the host bed from further trauma, but also provides an important barrier to infection.[2] The practice of organ transplantation is associated with two cross-linked and often-interdependent clinical outcomes; rejection and infection.[1] The immunosuppressive drugs used to prevent and treat rejection, predispose the transplant recipient to a wide variety of infections. Interestingly, certain infections are believed to influence occurrence of acute and chronic graft rejection. Therefore the question "Which comes first,infection or graft rejection?" can be answered as "yes" or "either". Indeed, the bidirectional interplay between these two clinical events could lead into a vicious cycle that presents a conundrum in the transplantation field.[3] Infections occurring in skin graft patients may be classified as bacterial viral and fungal based on its causative agent. Common bacteria that cause infection are, *Staphylococcus aureus, Klebsiella sp*, *Pseudomonas sp*. Common virus known to cause infection in skin graft patients is cytomegalovirus. Fungal infections are very rare, it may occur in immune-compromised patients.[4] Hence, the study is undertaken to detect and observe different infections that occur in skin graft patients, which would help the healthcare professionals or workers to take precautionary measures to the infections that occur in such patients and improve the rate of wound healing.

***Objectives***

1. To analyze the bacterial infection that occur in skin graft patients.
2. To do anti-microbial susceptibility testing on the bacteria isolated from the skin graft infection.

# MATERIALS AND METHODS

***Institutional ethical clearance***

The study protocol was approved by Institutional ethical review board, S. S. Institute of Medical Sciences and Research Centre, NH-4, Davangere, Karnataka

***Ethical issues***

Written or verbal consent of patient or legal guardian and permission of the respective authority of burn unit were taken.

***Inclusion criteria***

Patient who has undergone auto graft was included in the study.

***Study type***

Cohort study

This prospective study was carried out on 50 patients who have undergone auto-grafting. 300 swabs from 50 skin graft cases were collected from the patients admitted to plastic surgery unit of S.S. Institute of Medical Sciences and Research Centre, Davangere. Periodic swabs were taken on Day 0, week 1, week 2, week 3, week 4 and week 5. On admission the sampling procedure included collection of swab from clinically deep area of burn wound site prior to any cleansing.[5,6] Later swabs were taken on occasions of surgical debridement or surgical excision and grafting. All specimens were inoculated on 5% blood agar, Mac-Conkey agar and chocolate agar plates and incubated over night at $37^0$C.The aerobic and anaerobic bacteria were identified by standard microbiological techniques.[7] The antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method according to the criteria put forward by the

Clinical Laboratory Standards Institute (CLSI).[8] The test organisms were subjected to screening for the production of ESBL by double disk approximation test as described by Brun Bruisson*et al.*[9] The organisms which were screened and found positive for ESBL production were subjected to confirmatory test by NCCLS phenotypic confirmatory test.[9,10] AmpC disk test was also done for the meropenem resistant strains for detection of AmpC β-lactamases as described by Singhal S *et al*.[5,9] All carbapenem-resistant isolates were subjected to detection of Metallo β-lactamase (MBL) production by Modified Hodge's test and EDTA disk synergy test as described by Black J A *et al*.[6] Methicillin resistance was detected by disc diffusion technique using 30µg cefoxitin disc. The diameter of the zone around the disc was measured and the results were interpreted.[8]

## RESULTS

Fifty patients who received either full- or split-thickness skin grafts to reconstruct soft-tissue defects were included in the study. Among fifty patients studied 38 were males and 12 female. The average age was 48 years ranging from 18 to 81 years. Insulin dependent diabetes was present in 12 patients (24%). The average interval between grafting and follow-up was 10.4 weeks ranging from 8 to 12 weeks. The average age of the ulcers before surgery was 2.8 months and the approximated average size of a single (if solitary) or concomitant ulcers on one extremity were 98.3cm$^2$ ranges (14-156cm$^2$). The tissue defects were grouped according to the cause as follows: vascular ulcers (9.2%), burns (14.5%), traumatic tissue defects (36.6%), and flap donor-site defects (39.7%). Graft loss secondary to infection was recorded in 12 patients (24%) especially in case of ulcers of diabetic foot in lower extremities or when performed at multiple sites in case of burn infections. No obligate anaerobic bacteria were isolated from the swabs collected. Among the aerobic bacteria *Pseudomonas aeruginosa* was isolated in highest among Gram negative bacilli and *Staphylococcus aureus* was isolated in more number among Gram positive cocci. No organisms were isolated on before the graft (0 day). Gram negative organisms were initially prevalent then were gradually superseded by Gram positive organisms on week-2 after the grafting (Table- 1). Mixed cultures were not seen in the study. Isolation of *Staphylococcus aureus* was 40% after second weeks of grafting and was gradually decreased to 30% on third and fourth week and absent on fifth week after the skin grafting. On the other hand single isolation of *Pseudomonas aeruginosa* 38.1% on second week of grafting and 33.3% on 3rd week and 19% on fourth week after the grafting and 9.5% of *Klebsiella pneumoniae* was isolated on fifth week. *Acinetobacter baumannii* was isolated in one case during the first week of grafting. *Streptococcus pyogenes* was isolated in 3 cases after first week of grafting and in subsequent culture no *Streptococcus pyogenes* was isolated. The microbial profile of the skin graft is depicted in the Table 2. Antimicrobial sensitivity pattern of Gram positive organisms isolated from skin graft is shown in Table 3. *Staphylococcus aureus*, CoNS and *Streptococcus pyogenes* were 100% sensitive to vancomycin. Among *Staphylococcus aureus* 50% of the isolates were resistant to Cephoxitin (Methicillin resistant *Staphylococcus aureus*) and 70% sensitive to Clindamycin, 50% to Imipenem and 70% Linezolid. Majority of CoNS were resistant to Gentamycin, Erythromycin and Ampicillin. All the *Streptococcus pyogenes* were sensitive to majority of the drugs. The antibiogram of Gram negative organisms isolated from the burn wound is shown in Table 4. *Acinetobacter baumannii* was found to be 100% sensitive to piperacillin + tazobactam and imipenem but resistance to ceftriaxone and ciprofloxacin. *Pseudomonas aeruginosa* was highly sensitive to piperacillin + tazobactam (61%), and imipenem (61%) but resistance to ceftriaxone (66.7%) and ciprofloxacin (61%). Similarly *Klebsiella species, E.coli* and *Proteus species* were resistant to many drugs screened for sensitivity. All the bacteria isolated were resistant to two or more antibiotics, hence all the isolates in the present study were multi drug resistant. Among gram negative bacteria, 24%

were extended spectrum beta lactamases producers, 8% were AmpC producers and 22% were MβL producers (Table 5). *Pseudomonas aeruginosa* was the predominant ESβL and MβL producer.

**Table1**
***Sequential culture form the skin graft till fifth week***

| Organisms | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 |
|---|---|---|---|---|---|
| Number of culture | 50 | 50 | 50 | 50 | 50 |
| Culture positive | Nil | 29 | 19 | 04 | 02 |
| Culture negative | Nil | 21 | 31 | 46 | 48 |
| *Pseudomonas aeruginosa* | NIL | 08 | 07 | 04 | 02 |
| *Escherichia coli* | NIL | 02 | NIL | NIL | NIL |
| *Klebsiella pneumoniae* | NIL | 09 | 05 | NIL | NIL |
| *Proteus mirabilis* | NIL | 01 | NIL | NIL | NIL |
| *Acinetobacter baumannii* | NIL | 01 | NIL | NIL | NIL |
| *Staphylococcus aureus* | NIL | 04 | 06 | NIL | NIL |
| *Streptococcus pyogenes* | Nil | 02 | 01 | Nil | Nil |
| CoNS | Nil | 02 | Nil | Nil | Nil |

**Table 2**
***Bacteria isolated from skin graft***

| Bacteria isolated | Number |
|---|---|
| *Pseudomonas aeruginosa* | 21 |
| *Klebsiella pneumonia* | 14 |
| *Staphylococcus aureus* | 10 |
| *Escherichia coli* | 02 |
| *Streptococcus pyogenes* | 03 |
| *CoNS* | 02 |
| *Proteus mirabilis* | 01 |
| *Acinetobacter baumannii* | 01 |
| Total | 54 |

**Table 3**
***Antibiotic susceptibility pattern for Gram positive cocci isolated from skin graft***

| Antibiotics | | *Staphylococcus aureus* | | *Streptococcus pyogenes* | | CoNS | |
|---|---|---|---|---|---|---|---|
| amikacin | S | 4 | 40.0 | 2 | 66.6 | 1 | 50.0 |
| | R | 6 | 60.0 | 1 | 33.3 | 1 | 50.0 |
| clindamycin | S | 7 | 70.0 | 3 | 100 | 2 | 100 |
| | R | 3 | 30.0 | 0 | 0 | 0 | 0 |
| ciprofloxacin | S | 1 | 10.0 | 1 | 33.3 | 1 | 50.0 |
| | R | 9 | 90.0 | 2 | 66.6 | 1 | 50.0 |
| cefotaxime | S | 2 | 20.0 | 0 | 0 | 1 | 50.0 |
| | R | 8 | 80.0 | 3 | 100 | 1 | 50.0 |
| cephoxitin | S | 5 | 50.0 | 1 | 33.3 | 2 | 100 |
| | R | 5 | 50.0 | 2 | 66.6 | 0 | 0 |
| cotrimoxazole | S | 3 | 30.0 | 3 | 100 | 0 | 0 |
| | R | 7 | 70.0 | 0 | 0 | 2 | 100 |
| ceftriaxone | S | 4 | 40.0 | 2 | 66.6 | 1 | 50.0 |
| | R | 6 | 60.0 | 1 | 33.3 | 1 | 50.0 |
| doxycycline | S | 5 | 50.0 | 3 | 100 | 2 | 100 |
| | R | 5 | 50.0 | 0 | 0 | 0 | 0 |
| erythromycin | S | 3 | 30.0 | 3 | 100 | 0 | 0 |
| | R | 7 | 70.0 | 0 | 0 | 2 | 100 |
| gentamicin | S | 4 | 40.0 | 2 | 66.6 | 0 | 0 |
| | R | 6 | 60.0 | 1 | 33.3 | 2 | 100 |
| imipenem | S | 5 | 50.0 | 1 | 33.3 | 2 | 100 |
| | R | 5 | 50.0 | 2 | 66.6 | 0 | 0 |
| moxifloxacin | S | 5 | 50.0 | 0 | 0 | 1 | 50.0 |
| | R | 5 | 50.0 | 3 | 100 | 1 | 50.0 |
| piperacillin+ tazobactum | S | 5 | 50.0 | 2 | 66.6 | 2 | 100 |
| | R | 5 | 50.0 | 1 | 33.3 | 0 | 0 |
| penicillin | S | 0 | 0 | 3 | 100 | 1 | 50.0 |
| | R | 10 | 100 | 0 | 0 | 1 | 50.0 |
| linezolid | S | 7 | 70.0 | 1 | 33.3 | 2 | 100 |
| | R | 3 | 30.0 | 2 | 66.6 | 0 | 0 |
| vancomycin | S | 10 | 100 | 3 | 100 | 1 | 50.0 |
| | R | 0 | 0 | 0 | 0 | 1 | 50.0 |

**Table 4**

***Antibiotic susceptibility pattern for Gram negative bacilli isolated from skin graft***

| BACTERIA | | *Pseudomonas aeruginosa* | | *E coli* | | *Klebsiella Pneumoniae* | | *Proteus mirabili* | | *Acinetobacter Baumannii* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| amikacin | S | 9 | 43.0 | 2 | 100 | 9 | 65.0 | 1 | 100 | 0 | 0 |
| | R | 12 | 67.0 | 0 | 0 | 5 | 35.0 | 0 | 0 | 1 | 100 |
| azithromycin | S | 8 | 39.0 | 2 | 100 | 6 | 43.0 | 1 | 100 | 0 | 0 |
| | R | 13 | 61.0 | 0 | 0 | 8 | 57.0 | 0 | 0 | 1 | 100 |
| ciprofloxacin | S | 8 | 39.0 | 2 | 100 | 5 | 35.0 | 1 | 100 | 0 | 0 |
| | R | 13 | 61.0 | 0 | 0 | 9 | 65.0 | 0 | 0 | 1 | 100 |
| chlorampheni col | S | 4 | 19.0 | 2 | 100 | 6 | 43.0 | 1 | 100 | 1 | 100 |
| | R | 17 | 81.0 | 0 | 0 | 8 | 57.0 | 0 | 0 | 0 | 0 |
| cefotaxime | S | 7 | 33.3 | 2 | 100 | 6 | 43.0 | 1 | 100 | 1 | 100 |
| | R | 14 | 66.7 | 0 | 0 | 8 | 57.0 | 0 | 0 | 0 | 0 |
| ceftriaxone | S | 7 | 33.3 | 2 | 100 | 4 | 29.0 | 1 | 100 | 1 | 100 |
| | R | 14 | 66.7 | 0 | 0 | 10 | 71.0 | 0 | 0 | 0 | 0 |
| ceftazidime | S | 15 | 72.0 | 2 | 100 | 10 | 71.0 | 1 | 100 | 1 | 100 |
| | R | 6 | 18.0 | 0 | 0 | 4 | 29.0 | 0 | 0 | 0 | 0 |
| gentamicin | S | 6 | 18.0 | 2 | 100 | 4 | 29.0 | 1 | 100 | 0 | 0 |
| | R | 15 | 72.0 | 0 | 0 | 10 | 71.0 | 0 | 0 | 1 | 100 |
| imipenem | S | 13 | 61.0 | NT | NT | 7 | 50.0 | 1 | 100 | 1 | 100 |
| | R | 9 | 19.0 | NT | NT | 7 | 50.0 | 0 | 0 | 0 | 0 |
| Piperacillin+ tazobactum | S | 13 | 61.0 | 2 | 100 | 8 | 57.0 | 1 | 100 | 1 | 100 |
| | R | 9 | 19.0 | 0 | 0 | 6 | 43.0 | 0 | 0 | 0 | 0 |
| Sparfloxacin | S | 7 | 33.3 | 2 | 100 | 7 | 50.0 | 1 | 100 | 0 | 0 |
| | R | 14 | 66.7 | 0 | 0 | 7 | 50.0 | 0 | 0 | 1 | 100 |

**Table 5**

***Distribution of beta lactamases among different gram negative bacteria***

| Organism | ESβL | AmpC | MβL |
|---|---|---|---|
| *Pseudomonas aeruginosa* | 4 | 2 | 6 |
| *Klebsiella pneumoniae* | 4 | 0 | 2 |
| *E.coli* | 1 | 1 | 0 |

## DISCUSSION

The main advantage of a skin graft is that it is relatively simple procedure and acts as barrier for infections. Sometimes there may be complications such as bleeding, infection, partial or complete loss of skin graft, raised scars and poor cosmetic appearance.[11,13] As per the results of our study, bacteria were not cultured before skin graft and all the patients were on antibiotic treatment. Interestingly, there was no sign of any infection till 7th day of grafting. After 7$^{th}$ day of grafting, the most common infection that occurred in skin graft

patients was by *Pseudomonas aeruginosa*. Skin graft loss due to infection accounted for only minor part in the literature. Unal *et al* found that *Pseudomonas aeruginosa* was an equally prominent danger as *Streptococcus pyogenes* in skin graft survival in routing plastic surgery practice.[13] McGregor in contrast claims that infection with *Pseudomonas aeruginosa* reduces graft take but not to an extent comparable with *Streptococcus pyogenes* and it may reduce graft up take by 5-10%.[14] It is remarkable that even with aggressive treatment of *P. aeruginosa* the results evidently demonstrate a connection between *P. aeruginosa* and deterioration of skin grafts. We believe that the explanation lies in insufficient eradication of *P. aeruginosa* and that it is the persistence of *P. aeruginosa* that deteriorates the clinical outcome. This could be probably explained by ability of colonizing bacteria to establish themselves and proliferate in a biofilm. The clinical implications of bacterial biofilms are particularly pronounced in chronic infections.[15] In addition to being highly tolerant to antibiotics, biofilms are also impervious to the body's natural immune defense system.[16] *P. aeruginosa* and *S. aureus* are well recognized for forming chronic biofilm-based infections in their hosts. Normally radical debridement of the infected area is the treatment of choice in case of biofilm infections. In this study a thorough debridement was performed down to viable and visually non-infected tissue. Despite this, detection of *P. aeruginosa* and *Staphylococcus aureus* prior to surgery, reduced graft takes significantly. This indicates that *P. aeruginosa* and *Staphylococcus aureus* resides deep down in the tissue, and is probably protected from antibiotics and the immune system due to biofilm formation. This is in accordance with a study by Fazli *et al.*[17] showing a non-random distribution of *P. aeruginosa* and *S. aureus* where *P. aeruginosa* is found deeper into the tissue than *S. aureus.* We conclude that bacteria might be involved in and contribute to the lack of healing of chronic wounds. Especially it seems that *Pseudomonas aeruginosa* and *S. aureus* play a crucial role in the healing and we suspect that once chronic ulcers are colonized (weeks or months preoperatively) by *P. aeruginosa*, *S. aureus* the success rate of skin grafting deteriorated despite aggressive treatment.

## CONCLUSION

It is crucial to determine the specific pattern of graft microbial colonization, the time-related changes in dominant flora and the antimicrobial sensitivity profiles so that hospital stay can be shortened thereby improving overall infection related graft rejection and also to reduce morbidity and mortality.

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