Prevalence of the aerobic infections among cases of skin grafting in a tertiary health care centre

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Abstract
Introduction: Skin grafting is done to restore the skin integrity for large surface wounds. Grafts are susceptible to a variety of infections leading to graft failure. The present study was undertaken to analyse the causative agents of skin graft infection and to determine the antibiotic susceptibility of the isolates.

Materials and Methods: This study was conducted on a 60 patients who were undergoing skin grafting at a plastic surgery unit in a tertiary health care centre for 9 months duration. A total of 180 swabs were collected, 3 from each patient. Organisms were identified by standard conventional methods. The antibiotic sensitivity testing of the isolates was done by Kirby Bauer's Disk Diffusion method according to CLSI guidelines.

Results: All the samples collected at the time of admission showed the growth. *Pseudomonas species* (50%) followed by *Staphylococcus epidermidis* (16.7%), *Staphylococcus aureus* (16.7%) are the predominate organisms isolated. Most of the samples collected preoperatively showed no growth (about 73.3%), the rest showed growth of *Staphylococcus aureus* (10%) followed by *Staphylococcus epidermidis* (6.7%) and others. About 16.7% samples collected postoperatively showed no growth and remaining samples showed predominately growth of *Pseudomonas spp.* (40%) followed by *Staphylococcus aureus* (23.3%) and others.

Conclusion: The study revealed that most of the samples showed bacterial growth which can potentially result in graft rejection. Most of these bacteria were resistant to many antibiotics. So, it is crucial to determine causative bacteria and their antibiotic sensitivity profiles, which helps in preventing over all infection related graft rejection.

Keywords: Antibiotic sensitivity, Graft rejection, P*seudomonas spp*, Skin grafting, *Staphylococcus aureus.*

Introduction
Skin graft is one of the most indispensable techniques in plastic surgery and dermatology. Since Reverdin first performed skin auto transplantation in 1869, many pioneers have tried to improve the results of grafting.[1] Skin grafts are used in a many clinical situations, such as traumatic wounds, defects after oncologic resection, burn reconstruction, scar contracture release, congenital skin deficiencies, hair restoration, vitiligo, and nipple-areola reconstruction.[1] Prerequisites for successful skin graft are good graft, adequately vascularised recipient bed, accurate approximation and immobilisation of the graft in relation to the ulcer, avoiding fluid collections beneath the graft, and good nursing care. Even though these prerequisites are met, the graft may fail due to bacterial infcction.[2]

Infection is a major cause for loss of skin grafts leading to increased morbidity and mortality.[3] Microbial growth reduces the chance of the skin graft healing. The surface of the chronic wound is likely to host commensal flora, and it is more likely that an in-depth residing bacterium is more pathogenic than a superficial one.[4] Bacterial load, virulence, host immune response, age of patient, extent of injury, depth of wound are the factors influencing skin graft infection. Both facultative and aerobic gram negative bacilli and aerobic gram-positive cocci can be isolated from wound cultures. Many of these microorganisms are hospital-acquired agents that are resistant to antibiotics to varying degrees. Graft failure results in prolonged hospital stay, increase in the cost of treatment and long term disability. Appropriate antibiotic administration before, during and after surgery, adherence to infection control measures are important in preventing infections.[5] Therefore, bacteriological culture of wounds that are prepared for skin grafting and also post operatively should be performed.[6]

Hence the present study was undertaken to analyse the bacterial infections of skin graft and their antibiotic sensitivity profiles thereby reducing overall infection related graft rejection and also to reduce morbidity and mortality.

Aims and Objectives
1. To isolate aerobic bacteria from the ulcers subjected for skin grafting.
2. To isolate aerobic bacteria from skin graft site postoperatively.
3. To determine the antibiotic susceptibility of all the isolates.

Materials and Methods
Present study was done at Shimoga Institute of Medical Sciences, Shivamogga, for nine month duration from July 2017 to March 2018, after obtaining institutional ethical committee clearance. The study was carried out on 60 patients who were undergoing skin grafting. A total of 180 swabs were collected, 3 from each patient from McGann teaching hospital, attached to Shimoga institute of medical sciences.

3 samples were collected from ulcer using a sterile cotton swab as follows:
1. First sample was collected from the ulcer at the time of admission.

2. Second sample was collected from the ulcer 5 hours before surgery.
3. Third sample was collected 72 hours after skin grafting.

All the samples collected were sent to microbiological laboratory for culture. These samples were processed on blood agar and MacConkey agar media and incubated at 37°C under aerobic conditions. The organisms were identified as per standard conventional methods.[7] Antimicrobial susceptibility testing of isolates were done on Muller-Hinton agar by Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines.[7] Susceptibility testing was carried out using the following antibiotics: Penicillin (P), Clindamycin (CD), Azithromycin (AZM), Gentamicin (G), Ciprofloxacin (CIP), Doxycycline (DO), Vancomycin (VA), Linezolid (LZ), Cefoxitin (CX), Ceftazidime(CAZ), Co-trimoxazole (COT), Gentamicin(G), High level gentamicin(HLG), Tetracycline (TE), Chloramphenicol (C), Ciprofloxacin (CIP), Imipenem (IMP), Meropenem (MRP), Piperacilllin-tazobactam (PT), Amikacin (AK), Aztreonam (AT).[8]

Results

The present study was carried out in the Shimoga Institute of Medical Sciences, Shivamogga. This study included 60 patients who received skin grafting to reconstruct soft tissue defects. A total of 180 swabs were collected, 3 swabs from each patient. Among 60 patients 50(83.3%) were males and 10(16.7%) were females. Highest incidence was 30% between the age group of 31-40 years, followed by 13.3% between the age group of 11-20 and 51-60, 10% between age 1-10, 21-30 and 41-50. The observations made from the study are shown in following tables

Table 1: Demographic characteristics

Sex	**N=60n (%)**
Male	50(83.3)
Female	10(16.7)
Age (in years)	**N=60n (%)**
1-10	6(10.0)
11-20	8(13.3)
21-30	6(10.0)
31-40	18(30.0)
41-50	6 (10.0)
51-60	8 (13.3)
61-70	6 (10.0)
81-90	2 (3.3)
Diagnosis	N= 60n (%)
Healing ulcer	26 (43.3)
Non healing ulcer	18(30.0)
Diabetic ulcer	6(10.0)
Burns	10(16.7)

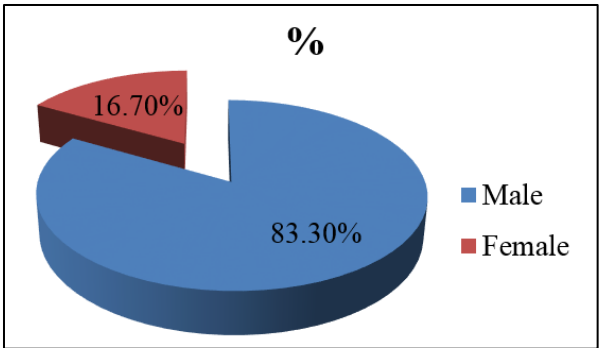

Fig. 1: Sex distribution

Table 2: The various aerobic bacteria isolated from sample 1

Organisms isolated	**N - 60(%)**
Staphylococcus epidermidis	10 (16.7)
CONS	8(13.3)
Pseudomonas spp.	30(50.0)
Staphylococcus aureus	10 (16.7)
Enterococcus spp.	2 (3.3)

CONS- Coagulase negative staphylococcus

All the samples collected at the time of admission showed the bacterial growth. The organisms isolated are *Pseudomonas species* (50%), *Staphylococcus epidermidis* (16.7%), *Staphylococcus aureus* (16.7%), *and Coagulase negative staphylococcus* (13.3%), *and Enterococcus species* (3.3%).

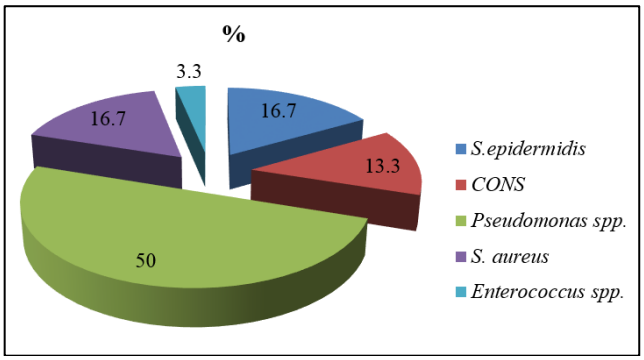

Fig. 2: The various aerobic bacteria isolated from sample 1

Table 3: The various aerobic bacteria isolated from sample 2

Organisms isolated	**N=60(%)**	
No growth	44	(73.3)
Staphylococcus epidermidis	4	(6.7)
CONS	2	(3.3)
Pseudomonas spp	2	(3.3)
Staphylococcus aureus	6 (10.0)	
Enterococcus spp.	2	(3.3)

Most of the samples collected preoperatively showed no growth (about 73.3%), the rest showed growth of *Staphylococcus aureus* (10%), *Staphylococcus epidermidis*

(6.7%), *Pseudomonas sp* (3.3%), *Enterococcus sp* (3.3%), CONS (3.3%).

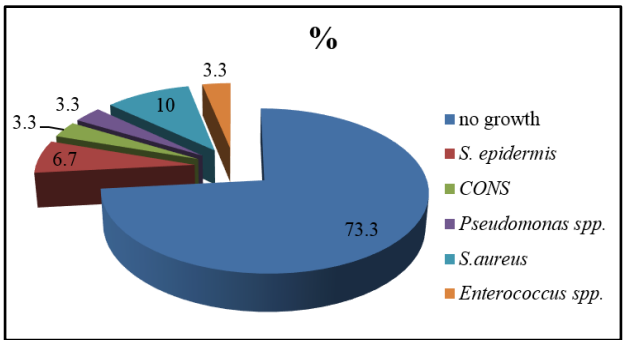

Fig. 3: The various aerobic bacteria isolated from sample 2

Table 4: Percentage of various organisms isolated from sample 3

Organism isolated	**N- 60 (%)**	
No growth	10 (16.7)	
Staphylococcus epidermidis	4	(6.7)
CONS	4	(6.7)
Pseudomonas spp.	24	(40.0)
Staphylococcus aureus	14 (23.3)	
Enterococcus spp.	4	(6.7)

From sample collected postoperatively, Most common organism isolated are the *Pseudomonas spp.* (40%), followed by *Staphylococcus aureus* (23.3%), *Staphylococcus epidermidis* (6.7%), *Enterococcus spp.* (6.7%), *Coagulase negative staphylococcus* (6.7%) and about (16.7%) showed no growth.

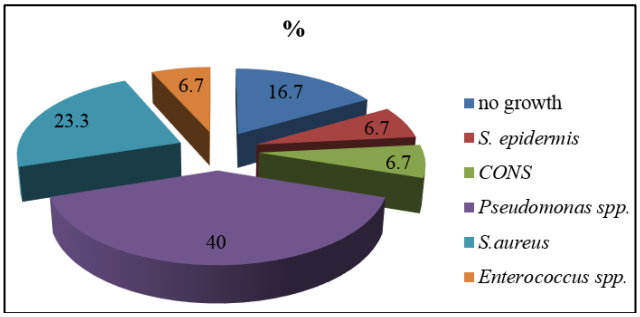

Fig. 4: The various aerobic bacteria isolated from sample 3

Staphylococcus aureus is highly sensitive to linezolid (100%), vancomycin (100%), moderately sensitive to chloramphenicol (60%), doxycycline (53.33) less sensitive to azithromycin (26.7%), clindamycin (33.3%), Co-trimoxazole (33.3%) and were totally resistant to penicillin (100%). Among *Staphylococcus aureus* 6(20%) were MRSA (Methicillin resistant *staphylococcus aureus*), Among CONS 18(56.25%) were MRCONS (Methicillin resistant *coagulase negative staphylococcus*). CONS and *enterococcus spp.* were highly sensitive to linezolid (100%), vancomycin (100%).

Table 5: Antibiotic sensitivity pattern of Gram positive isolates

	Organisms isolated		
Antibiotics tested	***Staphylococcus aureus*, N -30 (%)**	***CONS* N -32 (%)**	***Enerococcus spp.* N- 8 (%)**
Penicillin	0(0)	0	0
Co-trimoxazole	10(33.3)	12(37.5)	-
Clindamycin	10(33.3)	10(31.25)	-
Ciprofloxacin	14(46.66)	14(43.75)	4(50)
Cefoxitin	24(80)	14(43.75)	-
Chloramphenicol	18(60)	18(56.25)	-
Doxycycline	16(53.33)	14(43.75)	-
Azithromycin	8(26.7)	12(37.5)	-
Gentamicin	12(40)	10(31.25)	-
High level gentamicin	-	-	4(50)
Tetracycline	-	-	2(25)
Linezolid	30(100)	32(100)	8(100)
Vancomycin	30(100)	32(100)	8(100)

Table 6: Antibiotic sensitivity pattern of *Pseudomonas spp*

Antibiotics tested	***Pseudomonas spp* n- 56(%)**
Gntamicin	8(14.2)
Amikacin	52(92.9)
Ciprofloxacin	18(32.1)
Imipenem	50(89.3)
Meropenem	48(85.8)
Aztreonam	44(78.6)
Ceftazidime	4(7.1)
Piperacillin- tazobactam	6(10.7)

Pseudomonas species have high susceptibility to amikacin (92.9%), imipenem (89.3%), meropenem (85.8%), and aztreonam (78.6%) and were resistive to ceftazidime (92.9%), piperacillin-tazobactum (89.3%), gentamycin (85.8%), ciprofloxacin (67.9%).

Discussion

Skin grafts are susceptible to a variety of complications leading to graft failure. Most commonly, these include hematoma or shearing movements, inadequate compliance, deficient blood supply, presence of microtrombi in the dermal blood vessels, local fibrin deficiency in the wound, infection, skin pigmentation and skin graft contraction.[9]

In our study out of 60 patients, 50(83.33%) were males and 10 (16.7%) were females. A study by Unal et al.[10] also showed 75% were males and 25% were females. In both the studies male patients were more than females. Study done by E Leslie Gilliland el al showed among 88 patients, 67(76.13%) females and 21(23.86%) males.[2]

In our study, all the samples collected at the time of admission showed the bacterial growth and organisms isolated are predominately *Pseudomonas species* (50%) followed by *Staphylococcus epidermidis* (16.7%), *Staphylococcus aureus* (16.7%), *Coagulase negative staphylococcus* (13.3%) *and Enterococcus species* (3.3%). The reason for above infection by bacteria might be due to

poor hygiene among the patients, diabetes. Most of the samples collected preoperatively showed no growth (about 73.3%), the rest showed predominately growth of *Staphylococcus aureus* (10%), *followed by Staphylococcus epidermidis* (6.7%), *Pseudomonas sp* (3.3%), *Enterococcus sp* (3.3%), *CONS* (3.3%). Most of the samples showed no growth probably due to prior administration of antibiotics and proper dressing of wound with silver sulfadiazine carried out in alternate days. Preoperative wound swabs are routinely performed to identify subclinical wound bed colonization, as well as specific strains of bacteria, such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*, which can have detrimental effects on graft take.[11]

From sample collected post operatively most common organism isolated is the *Pseudomonas spp.* (40%), followed by *Staphylococcus aureus* (23.3%), *Staphylococcus epidermidis* (6.7%), *Enterococcus spp.* (6.7%), *Coagulase negative staphylococcus* (6.7%) and about 16.7% showed no growth. Infection with these aerobic bacteria may be due to hospital acquired or may be due to unhygienic practice followed during dressing. 16.7% samples showed no growth probably due to antibiotic therapy.

Study by E Leslie Gilliland el al. showed out of 88 samples collected at the time of admission 11(12.5%) samples showed no growth, 53(60.23%) samples showed *Staphylococcus aureus* along with other bacteria, 8(9.09%) samples showed *pseudomonas spp.* along with other bacteria, 7(7.96%) samples showed *pseudomonas spp* and *Staphylococcus aureus* and 9 (10.23%) were other bacteria. Preoperatively among 88 samples, 34(38.64%) samples showed no growth, 33(37.50%) samples showed *Staphylococcus aureus* along with other bacteria, 7 (7.96%) samples showed *pseudomonas spp.* along with other bacteria, 2(2.28%) samples showed pseudomonas and *Staphylococcus aureus* and 12(13.64%) were other bacteria. Post operatively among 88 samples, 28(31.82%) samples showed no growth, 24(27.28%) samples showed *Staphylococcus aureus* along with other bacteria, 9 (10.23%) samples showed *pseudomonas spp* along with other bacteria, 5(5.69%) samples showed *pseudomonas spp.* and *Staphylococcus aureus* and 22(25%) were other bacteria.[2]

In a study by S.Geethabanu et al., reported most common pathogens in preoperative quantitative culture were *Staphylococcus aureus* (26.4%) and *Pseudomonas aeruginosa* (26.4%) followed by *klebsiella pneumonia* (8.9%) and others.[6] Prospective study was performed to analyze the causes of infection-related skin-graft loss in a general population of plastic and reconstructive surgery patients by Unal S et al showed among 132 patients who received skin grafts to reconstruct soft-tissue defects, graft loss secondary to infection was recorded in 31 patients (23.5%). The microbiological cultures revealed *Pseudomonas aeruginosa* in 58.1% of the cases, followed by *Staphylococcus aureus*, *Enterobacter*, *enterococci*, and *Acinetobacter spp*.[10] A study conducted by Trine Høgsberg et al. isolated, *Pseudomonas aeruginosa*, *Pseudomonas species*, *Staphylococcus aureus*, haemolytic *Streptococci (*group A, B, C, G), *Proteus*, gram-negative bacilli and anaerobic bacteria from samples collected 12 weeks prior to surgery and preoperatively.[9]

In our study *Staphylococcus aureus* is highly sensitive to linezolid (100%), vancomycin (100%), moderately sensitive to chloramphenicol (60%), doxycycline (53.33) less sensitive to azithromycin (26.7%), clindamycin (33.3%), Co-trimoxazole (33.3%) and were totally resistant to penicillin (100%). *CONS* and *enterococci spp*. were highly sensitive to linezolid (100%), vancomycin (100%). Among *Staphylococcus aureus* 6(20%) were MRSA (Methicillin resistant *staphylococcus aureus*), Among *CONS* 18(56.25%) were *MRCONS* (*Methicillin resistant coagulase negative staphylococcus*). Among 56 *pseudomonas spp.* 92.9% sensitive to amikacin, 89.3% to imipenem, 85.8% to meropenem, 78.6% to aztreonam 32.1% to ciprofloxacin, 14.2% to gentamycin, 10.7% to piperacillin- tazobactam, 7.1% ceftazidime. Study by Saaiq M et al. reported *methicillin-resistant Staphylococcus aureus (*MRSA) constituted an alarmingly high percentage (68.62%) of the *Staphylococcal* isolates and *Pseudomonas spp* showed 80.55% sensitive to piperacillin- tazobactam, 63.88% to imipenem, 44.44% to ciprofloxacin, 8.33% to amikacin 11.11% to ceftazidime.[12]

In our study the predominate organisms isolated were *Pseudomonas species* and *Staphylococcus aureus*. *Pseudomonas aeruginosa and Staphylococcus.aureus* are well known for forming chronic biofilm-based infections in their hosts. Infection with *Pseudomonas aeruginosa* prior to surgery, reduces graft take significantly. This indicates that *Pseudomonas* aeruginosa resides deep down in the tissue, and is probably protected from antibiotics and the immune system due to biofilm formation. *Staphylococci* secrete a large number of toxins and enzymes which include hyaluronidase, fibrinolysins and proteases, that have been suggested to impair the ingrowth of capillaries through the fibrin layer that is laid down between the granulation tissue and the graft.[9,2] It is known that effective surveillance and infection control may reduce infection, mortality rates, length of hospitalization and associated costs.

Conclusion

The study revealed that most of the samples were infected with bacteria which can potentially result in graft rejection. Most of these bacteria were resistant to many antibiotics. So, it is crucial to identify the specific pattern of graft microbial infection, time related changes that occur in the colonized bacteria and the antimicrobial sensitivity profiles, which helps to reduce the overall infection related graft rejection. Thus proper dressing, proper cleaning pattern of the wound, adherence to infection control measures and prior treatment with specific antimicrobial agents can help to reduce the burden of these infections, reducing failure of skin graft and hence reducing morbidity and mortality.

Conflict of Interest: None.

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