ISSN: 2091-0657 (Print); 2091-0673 (Online) Open Access DOI:10.3126/jcmsn.v15i3.24363

Bacteriological Profile of Burn Patients and Antimicrobial Susceptibility Pattern of their Wound Isolates at Nepal Cleft and Burn Center

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ABSTRACT

Background: Burn infections are the major causes of morbidity and mortality in burn patients. The infectious agent could be both Gram positive and Gram negative bacteria. The aim of this research was to study the bacteriological profile of burn patients and anti-microbial susceptibility pattern of their wound isolates. **Methods:** This prospective observational study was conducted in Kirtipur Hospital, Nepal from January 1st to March 31st 2019. A total of 109 burn patients were included and their wound swabs were collected at the time of admission. All isolates were identified by standardized microbiological procedures. Anti-microbial susceptibility tests were performed by Kirby Bauer disc diffusion method. **Results:** Of 109 burn wound samples, 56 (59%) yielded culture growth and the Gram negative isolates were more common than Gram positives (77% vs. 23%). The most common organism isolated was *Klebsiella spp.* (25%) followed by *Acinetobacter spp.* (21%) and *Staphylococcus aureus* (18%). Most burn wound isolates were of resistant strain. Particularly, highly resistant strain of *Acinetobacter spp.* were isolates were common in burn wound isolates and the antibiotic susceptibility pattern was different for different organisms. The use of antimicrobial should be judicious to further not escalate the problem of antimicrobial resistance in the healthcare settings.

Keywords: antimicrobial susceptibility; burn infection; bacteriological profile.

INTRODUCTION

Infection is the major cause of morbidity and mortality in burn patients who are hospitalized.¹ There is an estimation that 75% of the mortality associated with burn injuries is related to sepsis in developing countries.² Burn injury leads to a state of immune system dysfunction that predisposes patients to infection which is aided by the loss of the natural skin barrier.³ There is interplay of pro and anti-inflammatory signals that result in dysregulation off the innate and adaptive immune responses.⁴ After a burn injury there is a massive release of humoral factors, including cytokines, prostaglandins, vasoactive prostanoids and leukotrienes.⁵ The neutrophils have a decreased chemotaxis and bactericidal activity.⁶ There is less phagocytic activity and lymphokine production by macrophages. Natural killer cells activity are diminished⁷. It is due to presence of high amount of dead cells.⁸ As foreign bodies and dead cell are abundant in burnt areas the macrophages reach there and pus will develop as an end result.⁸ The reason for this is due to secondary immunodeficiency.⁸ With the decrease in immune system, burns provide a suitable site for bacterial multiplication and are rich sources of infection because the dead cells provide nutrients to extremophiles.⁹

There are various organisms that are isolated from burn wound.¹⁰ Aerobic bacterial isolates from burn wounds include Gram positive organisms like Staphylococcus aureus, coagulase negative Staphylococci and Enterococcus spp; similarly Gram negative organisms like Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Enterobacter spp, Proteus spp and Acinetobacter spp are isolated.¹¹ The incidence of less common microbes is increasing, as multidrug-resistant strains of the more common isolates.⁹ Polyantibiotic resistance has been noted in Gram positive organisms like Staphylococcus methicillin-resistant aureus (MRSA), and also in Gram negative bacilli like P. aeruginosa and Acinetobacter spp.¹² The nature of microbial colonization of the wound, flora changes, and antimicrobial sensitivity profiles should be taken into consideration in using empirical antimicrobial therapy for burn patients.¹³ Periodical culturing and surveillance is very potential microorganisms and their important

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sensitivity/susceptibility pattern will prompt early management and possible decrease in morbidity and mortality of burn patients from sepsis. The present study aims to find out the common microflora in wounds of the burn patients at Kirtipur Hospital, Nepal. This study will help to assess the bio-burden of infections at the center and antimicrobial susceptibility testing will help to formulate antibiotic policy for better management of these patients.

METHODS

This study was prospective observational study done after approval from the Institutional Review Board (IRB) of NAMS (National Academy of Medical Sciences) between January 1st-March 31st; 2018. Patients who did not fall in the exclusion criteria were included in the study. Exclusion criteria being patient or patient party who refuse to enroll in the study. At the time of admission sterile cotton swabs were smoothly rolled over the burn wound area aseptically.¹⁴ The swabs were transported to the bacteriology laboratory in sterile test tubes and inoculated on MacConkey agar media (HiMedia Laboratories Pvt. Ltd., Mumbai) and blood agar media (HiMedia Laboratories Pvt. Ltd., Mumbai). Aerobic incubation was done at 37° C for 24 hours.¹⁵ The media showing no colony, were noted down as no growth. The media, with colonies, were processed following the standard bacteriological procedures Gram-staining was done from the colonies. Identification of bacterial isolates were done by conventional biochemical tests.¹⁵

Antimicrobial susceptibility testing of the bacterial isolates was done by disk diffusion technique (using Kirby Bauer's method)¹⁶ on Mueller-Hinton agar (MHA) (HiMedia Laboratories Pvt. Ltd., Mumbai) as per Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁵ Inoculum for individual isolate was made by direct colony suspension in nutrient broth. The turbidity of the broth was adjusted to a 0.5 McFarland standard suspension.¹⁵ The drugs listed in British pharmacopeia and commonly used in the center were used.¹⁷ The MHA plates were inoculated and then incubated for 24 hours at 37°C. The MHA plates were examined, the zone of inhibition was noted and the sensitivity pattern of the bacterial isolates to various antibiotics were detected as per CLSI guideline.¹⁵ As a control, antibiotic susceptibility test of ATCC (American Type Culture Collection) 25923 (S. aures) and ATCC 25922 (E. coli) were performed.18

Statistical Package For The Social Sciences (SPSS) version 21 was used for data entry and analysis. The sensitivity and resistance of different organism isolated to the drugs were expressed in terms of percentage. p value of less than 0.05 was considered significant. Quality control and verification of data

were done as follows : A sample was triplicated and repeated two times in a interval of two weeks. Purity plating was performed for the media and equipments were calibrated.¹⁹ Open Epi software version-2 was used to calculate the sample size. $(z-Score)^2 \times Standard deviation \times (1-Standard Deviation)$

(z-Score)²×Standard deviation ×(1-Standard Deviation) margin of error²

For our study for confidence Level of 95 % the Z score = 1.9 Standard deviation = 0.5 Margin of Error = 10 %Sample size= $(1.96)^2 \times 0.5 \times (1-0.5)/(0.1)^2 = 96.04$

with 10 percent drop outs for 96 = 10

Total Sample size = Sample size + 10 (10 percent drop outs of 96)=106

RESULTS

Burn wound swabs were collected from 109 patients during admission. Of them 36 (33%) samples were from male and 73 (67%) were from female. Most of the patient were between 21-30 years' age group twenty-three followed by 0-10 years' age group twenty (Figure 1).

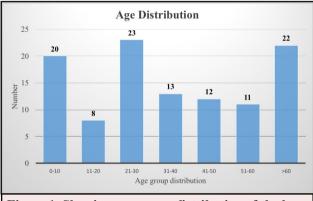
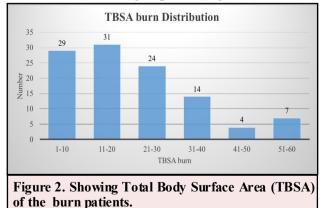


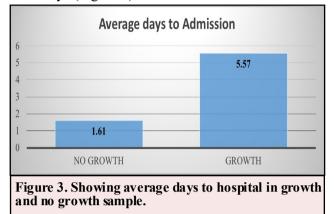
Figure 1. Showing age group distribution of the burn patient.

There was variation in the Total body surface area (TBSA) burn between the groups and most patient were between 11-20 % TBSA group thirty-one.Followed by 1-10 % TBSA group twenty nine and 21-30 % TBSA group twenty-four. The least was 41-50 % TBSA group four (Figure 2).

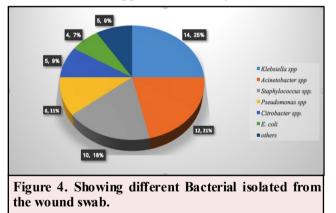


Of the total sample that were collected during the time of admission 53 samples showed no growth

for aerobic bacterial culture and 56 sample showed growth for aerobic bacterial culture. Among the swab sample of the patient who showed no growth to aerobic bacterial culture the average days to admission to our center was 1.61 days and who showed growth to aerobic bacterial culture it was 5.57 days (Figure 3).



Of the total positive samples thirteen (23%) were Gram positive and forty-three (77%) were Gram negative. The most common organism isolated was *Klebsiella spp* fourteen (25%), *Acinetobacter spp* twelve (21%), *Staphylococcus spp*. ten (18%), *Pseudomonas spp*. six (11%), *Citrobacter spp*. five (9%), *E.coli* four (7%), *Enterococcus spp*. three (5%) and *Proteus spp*. two (4%) (Figure 4).



Among the Gram positives, Staphylococcus spp. were sensitive to teicoplanin 90 %, tetracycline hydrochloride 80 %, ceftriaxone sodium 70 % and were less sensitive to cloxacillin sodium 50 %, trihydrate amoxicillin and 30 %. trimethoprim+suphamethoxazole Enterococcus spp. were 100% sensitive to ceftriaxone sodium, trimethoprim+ sulphamethoxazole, cefeperazone+ sulbactam, ciprofloxacin, vancomycin hydrochloride. However, they were less sensitive to gentamicin sulfate 66.66% and tetracycline hydrochloride 50 % (Table 1).

Among the Gram negative, *Klebsiella spp* showed no sensitivity to common drugs like amoxicillin

	Staphylococcus	Enterococcus		
	<i>suphylococcus</i> <i>spp</i> . Sensitivity (%)	<i>spp.</i> Sensitiv ity(%)		
amikacin sulfate	60%	100%		
amoxicillin trihydrate	30%	100%		
cephlexin	50%	ND		
cefepera-				
zone+sulbactam	ND	100%		
ceftriaxone sodium	70%	100%		
ciprofloxacin	ND	100%		
chloramphenicol	60%	ND		
cloxacillin	50%	ND		
trimethoprim+ sulpha-				
methoxazole	30%	100%		
gentamicin sulfate	40%	66.66		
levofloxacin	ND	100%		
teicoplanin	90%	ND		
tetracycline hydrochlo-				
ride	80%	50%		
vancomycin hydro-				
chloride	ND	100%		

trihydrate, ceftazidime, doxycycline and meropenem. They showed very less sensitivity to (35.71%), amikacin cefepime (14.28%),cefeperazone+Sulbactam (44.44%). ceftriaxone (14.28%), trimethoprim+sulfamethoxazole (35.71%), gentamicin (30.76%), levofloxacin They showed good sensitivity to (35.71%). tigecycline (88.88%) and 100 % to colsitin sulphate and polymyxin B. Acinetobacter spp. showed no sensitivity to amoxicillin, amikacin, amoxicillin + clauvanic acid and ceftazidime. They showed very sensitivity cefepime less to (16.67%), cefeperazone+sulbactam (58.33%), ceftriaxone ciprofloxacin (8.33%),(16.67%),trimethoprim+sulfamethoxazole (8.33%),doxycilline (41.66%), gentamicin (25%), imipenem (33.33%), levofloxacin (33.33%), meropenem (33.33%), piperacillin+tazobactam (33.33%). They showed good sensitivity to tigecycline 83.33% and 100 % sensitivity to colsitin sulphate and polymyxin B. Pseudomonas Spp. showed no sensitivity to cefeperazone+sulbactam, doxycycline. They showed very less sensitivity to amikacin (50%), cefepime (33.33%), ciprofloxacin (50%), gentamicin (20%), imipenem (50%), levofloxacin (50%), meropenem (50%), piperacillin+tazobactam (33.33%), teicoplanin (25%) and 100 % sensitivity to colsitin sulphate and polymyxin B. Citrobacter Spp. showed no sensitivity to amoxicillin. They showed less sensitivity to cefepime (60%), cefeperazone+sulbactam (60%), cefotaxim (60%), ceftazidime(40%), ceftriaxone (60%), ciprofloxacin (60%), cotrimoxazole 20%, doxycilline (60%). They showed good sensitivity to amikacin(80%), gentamicin (80%), imipenem (66.66%), meropenem (66.66%) and tazobactam+piperacillin (66.66%) and 100 % sensitivity to colsitin sulphate and polymyxin B. E. coli showed no sensitivity to amoxicillin+clauvanic amoxicillin, acid and

doxycycline. They showed less sensitivity cefotaxime (25%), ceftazidime (25%), ceftriaxone (25%), gentamicin (50%), and more sensitivity to amikacin (75%), cefeperazone+sulbactam (75%), (75%), imipenem (75%), ciprofloxacin levofloxacin (75%) and meropenem (75%) and showed 100 % sensitivity to colistin Sulphate, polymyxin B and tigecycline. Proteus spp. showed no sensitivity to amoxicillin and trimethoprim+sulphamethoxazole. They showed less sensitivity to amoxicillin+clauvanic acid cefeperazone+sulbactam (50%), (50%). ciprofloxacin (50%), gentamicin (50%), levofloxacin (50%) and showed 100 % sensitivity to amikacin, ceftazidime, imipenem, meropenem, piperacillin+tazobactam (Table 2). DISCUSSIONS

is similar to our previous published data by Karki et al $(2018)^{23}$ who found that 0-20% group had the highest number of patient and by Rai et al $(2014)^{24}$ who also found 0-20% group had the highest number of patient.

In our study we found out that 53 (49%) wound swab culture samples showed no aerobic bacterial growth and 56 (51%) showed growth of aerobic bacteria. This was obvious because this was the analysis of the swab that were taken during the time of admission and most of the time it will show no growth until the organism has colonized and the average day to admission of those patient whose swab showed no growth was 1.61 days and whose showed growth was 5.57 days. Which was statistically significant p value

Table 2. Antimicrobial susceptibility pattern of Gram negative isolates in burn patient.								
	<i>Klebsiella</i> <i>spp</i> . Sensi- tivity (%)	<i>Acinetobac- terspp.</i> Sensi- tivity (%)	Pseudomonas spp. Sensitivity(%)	<i>Citrobacter</i> <i>spp</i> . Sensitivity(%)	<i>E. coli</i> Sensitiv- ity(%)	Proteus spp. Sensitivity (%)		
amikacin sulfate	35.71%	0%	50%	80%	75%	100%		
amoxicillin trihydrate	0%	0%	ND	0%	0%	0%		
amoxicillin+ clauvanic acid	ND	0%	ND	ND	0%	50%		
Cefepime	14.28%	16.67%	33.33%	60%	ND	ND		
cefeperazone+sulbactam	44.44%	58.33%	0%	60%	75%	50%		
Cefotaxime	ND	8.33%	ND	60%	25%	ND		
Ceftazidime	0%	0%	80%	40%	25%	100%		
ceftriaxone sodium	14.28%	8.33%	ND	60%	25%	50%		
Ciprofloxacin	50%	16.67%	50%	60%	75%	50%		
colistin sulphate	100%	100%	100%	100%	100%	ND		
trimethoprim+sulphamethoxazole	35.71%	8.33%	ND	20%	25%	0%		
doxycycline hyclate	0%	41.66%	0%	60%	0%	ND		
gentamicin sulfate	30.76%	25%	20%	80%	50%	50%		
imipenem	10%	33.33%	50%	66.66%	75%	100%		
levofloxacin	35.71%	33.33%	50%	80%	75%	50%		
Meropenem	0%	33.33%)	50%	66.66%	75%	100%		
piperacillin+tazobactam	10%	33.33%	33.33%	66.66%	ND	100%		
polymxin-B	100%	100%	100%	100%	100%	ND		
Teicoplanin	ND	ND	25%	ND	ND	ND		
Tigecycline	88.88%	83.33%	ND	100%	100%	ND		

ND: Not done

In our study we found out that the incidence of burn injury was more in females. This finding is in accordance to a study done by Paudel and Dahal $(2010)^{20}$ previously at Bir Hospital in Nepal females (55%) were more affected than males (45%). According to WHO (World Health Organization)²¹ fact sheets the higher risk for females is associated with open fire cooking, or inherently unsafe cook stoves, which can ignite loose clothing. Open flames used for heating and lighting also pose risks, and self-directed or interpersonal violence are also factors. But this data is true only for low and low middle income country for high income country it is the males who are more affected. A systematic review done by Smolle et al $(2017)^{22}$ which included the data mostly of high income countries found the mean male:female ratio of all studies together was 1.92:1. The TBSA(Total Body Surface Area) burn group were mostly between 0 and 20%. This result

less than 0.05.

In our study we found Gram positive isolates from the wound swab were less than Gram negative isolates. The possible explanation could the average day to hospital admission in be positive culture was 5.57 days and by the end of the week Gram negative organism are more common than Gram positive. This is consistent with the previous study done by Mundhada et al $(2015)^{25}$ who found that Gram negative isolates were 3 times more common than Gram positive. Similarly, Aljanaby et al (2018)²⁶ found 34.7 % of the isolates to be Gram positive and 66.3% of the isolates to be Gram negative. Chamania et al $(2012)^{27}$ found Gram positive sample to be 25% and Gram negative to be 75% in burn wound culture.

In our study the most common aerobic bacterial

organism isolated from the wound swab was Klebsiella spp. (25%) followed by Acinetobacter (21%),Staphylococcus spp.(18%), spp. Pseudomonas Spp. (11%), Citrobacter Spp. (9%), E.coli (7%), Enterococcus Spp. (5%), Proteus Spp. (4%). A Similarly study by Al-Aali $(2016)^{28}$ revealed Staphylococcus aureus, Klebsiella spp. and coagulase negative Staphylococci as the most frequently isolated organisms, each representing 20.2%, followed by Pseudomonas aeruginosa 14.6% and E. coli 10.1%. However, Similar study done in Nepal by Rajbahak et al (2014)²⁹ found mostly Pseudomonas aeruginosa (45.6%) followed by Staphylococcus aureus (19.1%), Acinetobacter spp. (17.7%) and coagulase negative Staphylococci (CONS) (5.6%). Sharma et al (2017) ³⁰ in their study found the most common isolate was Pseudomonas aeruginosa (38%), followed by Staphylococcus aureus (35%), Klebsiella spp.(8%), Acinetobacter species (5%), Staphylococcus epidermidis (5%), Proteus species. (3%) and E. coli (1%). Sewunet et al $(2013)^{31}$ found *S. aureus* (34.04%), and *P.aeruginosa* (31.8%), were $al(2016)^{32}$ predominant. Datta et found Pseudomonas aeruginosa (30%) and Staphylococcus aureus (30%) as the most common isolate from burn wounds followed by Klebsiella spp. (20%). In a study done by Ramakrishna et al $(2006)^{33}$ the commonest isolate was *Pseudomonas* aeruginosa in 41% followed by Staphylococcus aureus (37%), Escherichia coli (10%), Klebsiella spp.(10%) and anaerobes in (2%). Bhat et al (2010) ³⁴ in their study found the commonest organism was S. aureus (27.7%), followed by K. pneumoniae (13.4%), Proteus mirabilis (12.4%), Group D streptococcus (9.4%), P. aeruginosa (8.9%) and E. *coli* (6.2%). Lakshmi et al $(2015)^{35}$ the predominant isolate was Pseudomonas (33.6%) followed by E.coli (20.9%), Klebsiella spp. (18.5%), Proteus *spp.* (17.3%), *S. aureus* (5.7%) and *Acinetobacter* spp (3.9%). Mundhada et al $(2015)^{25}$ in their study found the most common isolate was Klebsiella pneumoniae (34.40%) followed by Pseudomonas aeruginosa (23.94%), Staphylococcus aureus (22.94%), Escherichia coli (7.34%), Acinetobacter species (2.75%), Proteus mirabilis (2.75%), and Citrobacter species. Chamania et al $(2012)^{27}$ in their study found the highest incidence was of Pseudomonas aeruginosa (43%). As we can see in all the studies Gram negatives were more than Gram positives isolates in burn wound infection. However, the Gram negative organisms were different in different center. From this we can infer that different organism may be common in different centers depending upon this we can know which organism may be suspected if we do not have culture reports.

In our study *Acinetobacter spp.* was as high as 21% which is alarming as we can see that none of the

centers mentioned above had such high rates of Acinetobacter infection. Acinetobacter is increasingly recognized as a significant healthcareassociated, opportunistic and multidrug-resistant pathogen.³⁶Acquiring multidrug resistant (MDR) Acinetobacter spp infection is associated with an increased risk of patient mortality, and outbreaks have led to the closure of wards.³⁷ The appearance of MDR strains of Acinetobacter spp. continues to rise and persists as a complication of burns worldwide.³⁸ Currently, carbapenem resistance is one of the leading challenges in Acinetobacter healthcare-associated managing infections. In addition, there are recent reports of outbreaks with pan-drug resistant Acinetobacter baumannii (additional resistance to polymyxin and colistin).³⁹ In our study Acinetobacter spp. showed only sensitivity to colistin sulphate (100%) and polymyxin B (100%) and tigecycline(83.33%). With meropenem and imipenem it had just 33.33% sensitivity which is similar to other study by Chandrasekaran et al (2017).⁴⁰ They also did not find Pan resistance to Acinetobacter spp.

In our Study, Pseudomonas spp. showed no sensitivity to cefeperazone+sulbactam and doxycycline. They showed very less sensitivity to amikacin (50%), cefepime (33.33%), ciprofloxacin (50%), gentamicin (20%), imipenem (50%), (50%), levofloxacin meropenem (50%), piperacillin+tazobactam (33.33%), teicoplanin (25%) and 100% sensitivity to colsitin sulphate and polymyxin B. In the study done by Ramakrishna et al (2006)³³ Pseudomonas were found to be highly sensitive to carbapenams followed bv aminoglycosides and quinolones. In another study done in Nepal by Rajbahak et al (2014)²⁹ they found that antimicrobial sensitivity of P. aeruginosa recovered from patient's samples was lower than other isolates. P. aeruginosa was found to be resistant to most of antimicrobials used.

In our study E. coli showed no sensitivity to amoxicillin+clauvanic amoxicillin, acid and They doxycycline. showed less sensitivity cefotaxime (25%), ceftazidime (25%), ceftriaxone (25%), gentamicin (50%), showed more sensitivity to amikacin (75%), cefeperazone+sulbactam (75%), ciprofloxacin (75%), imipenem (75%), levofloxacin (75%) and meropenem (75%) and showed 100 % sensitivity to colistin Sulphate, polymyxin B and tigecycline. Similarly results by Al-Ali (2016)²⁸ showed E. coli was least resistant to cefepime (1.1%), imipenem 1.1%, piperacillin+tazobactam 1.1%, trimethoprim+sulphamethoxazole 22.4%. ceftazidime 15%, ceftriaxone 19% and amikacin 17 %.

In our study *S. aureus* were Sensitive to teicloplanin 90 %, tetracycline 80 %, ceftriaxone 70

% and were less sensitive to cloxacillin 50 %, amoxicillin and cotrimoxazole 30 %. In another study by Bhat and Vasaikar $(2010)^{34}$ most of the *S*. *aureus* isolates (99%) were resistant to penicillin However, all were sensitive to vancomycin and most (90%) to fusidic acid. In a similar study done in India by Meheta et al $(2007)^{41}$ *S. aureus* were highly resistant to amoxicillin(69.04%), erythromycin (75.27%), and netilmicin (77.75%).

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CONCLUSIONS

Gram negative were more common isolates from the swab of burn wound. The main organism isolated was *Klebsiella spp. Acinetobacter spp* were also significantly high and there were many MDR strains hence judicious use of antibiotic for further development of resistant strain in Nepal Cleft and Burn Center, Kirtipur hospital is recommended.

problems in Italy. Annals of Burns and Fire Disasters. 2003;16(4):182-7.

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Citation: Lamichhane A, Nakarmi KK, Dahal P, Basnet SJ, Pokharel PB, Bhattarai S, Rai SM. Bacteriological Profile of Burn Patients and Antimicrobial Susceptibility Pattern of their Wound Isolates at Nepal Cleft and Burn Center. JCMS Nepal. 2019; 15(3):160-6.