

**ABSTRACT** Introduction: Burn is a devastating trauma with multiple acute as well as chronic complications. Infection is the most common complication affecting every patient and responsible for 70% of deaths. Burn wounds are an excellent culture medium for the growth of organisms and cause sepsis.

**Method and materials:** Study was conducted for 1 year (March 2019-2020) in SMS medical college and Hospital, Jaipur (Rajasthan, India). Total of 400 wound swabs was cultured with positive culture in 357 isolates. Wound swabs were inoculated and incubated. Later microbiological tests were applied to identify the organisms. The modified Kirby-Bauer disc diffusion method was used for antibiotic sensitivity.

**Results and discussion:** Almost all the isolates were Gram-negative bacilli, Pseudomonas being the most common organism (58%) followed by Enterobacter sp (38.1%). Acinetobacter baumanni and Staphylococcus aureus were found in wound swabs. Coagulase Negative Staphylococcus was the only gram-positive organism. Pseudomonas was sensitive only to Colistin while Enterobacter sp was sensitive only to Tigecyclline and Polymyxin B. E. coli and Coagulase-negative staphylococcus were the least resistant organisms.

**Conclusion:** The golden era of antibiotics is now coming to an end. The increased incidence of resistance towards broad-spectrum antibiotics are alarming. Prevention of infection by hand washing and sterile dressings is required. This data will help the treating doctor to choose the antibiotic wisely and prevent further increases in resistance. Such studies need to be conducted at frequent intervals to look for changing trends in antibiotic sensitivity pattern.

## **KEYWORDS** : Antibiotic sensitivity, Burn, Bacteria

## **INTRODUCTION:**

India ranks second in terms of population in the world and has an annual incidence of 6-7 million new cases of burn every year. Out of these, 10% of patients require admission and inpatient care (1). World Health organization estimated 265,000 annual deaths worldwide and mostly from the South East Asia region (2,3). Burns is a devastating trauma (4). These patients require the utmost prevention and care to protect them from infections. These patients are relatively immune-compromised with exposed body surface area and hence highly susceptible to hospital-acquired infections. Burn wounds are initially sterile but eventually get colonized. Infections are the most common complication of burn wounds. Infections and subsequent sepsis are also responsible for most burn-related deaths (5,6). Burn wounds provide an excellent culture medium for organism growth. These infections are mostly spread by either commensals or secondary contamination from dressings or other contaminants of the hospital.

Burn infections cause a delay in wound healing and deep scar formation. Most common pathogens infecting burn wounds are Pseudomonas aeruginosa, Acinetobacter baumanni, Staphylococcus aureus, Klebsiella pneumonia, E.coli, Proteus mirabilis, Citrobacter sp., Enterobacter sp, etc (2,4,7). Antibiotic susceptibility patterns isolated from hospitalized patients are continuously evolving and changing. These patterns guide the management protocols followed by treating physicians. This study was conducted to determine organism growth and their antibiotic susceptibility pattern at SMS medical college and hospital, Jaipur (Rajasthan). This susceptibility pattern of antibiotics against specific organisms will help the physicians and surgeons in adequately managing treatment protocols.

### MATERIALAND METHODS:

This study was performed on 400 patients in Burns ward in the department of burn and reconstructive surgery, SMS medical college and hospital, Jaipur (Rajasthan). This study was conducted during the 1 year from March 2019 to March 2020. All patients with acute burns were included in the study while old burn wounds or wounds due to other causes were excluded from the study population. Patients of all age and sex groups were included in the study. Wound swab samples were collected under suitable circumstances and sent with proper measures to the microbiology lab for testing and culture. Wound samples were collected on Day 3 of burn. Inoculation of the culture plate was done within one hour of sample collection. The culture was done on Blood and Mac Conkey agar. These culture plates were incubated at 37°C for 24 hours. If no growth is observed then repeat

incubation is done for the next 24 hours. A sample was rendered sterile if no growth is obtained after 48 hours of incubation. After incubation, the identification of micro-organisms was done using gram staining and standard microbiology techniques. Standard biochemical tests were used to specify the type of organisms. The antibiotic susceptibility testing was done using the Kirby-Bauer disc diffusion method. Antibiotic susceptibility testing was done for the following antibiotics, the antibiotics with specified concentration used are shown in table 1. All procedures were performed in compliance with relevant laws and institutional guidelines and after the appropriate institutional committee approval.

Table I. Antibiotics for sensitibilit	v testing (mcg-micrograms)

Amikacin (30mcg)	Ciprofloxacin (5mcg)
Ampicillin (10mcg)	Cotrimoxazole (1.25/23.75mcg)
Aztreonam (30mcg)	Erythromycin (15mcg)
Tobramycin (10mcg)	Imipenem (10mcg)
Gentamicin (10mcg)	Tazobactum+piperacillin (100/10mcg)
Cefepime (30mcg)	Teicoplanin (30mcg)
Cefoxitin (30mcg)	Doxycyclline (10mcg)
Cefosulbactum (75/10mcg)	Linezolid (30mcg)
Cefotaxime (30mcg)	Tigecyclline (15mcg)
Ceftazidime (30mcg)	Polymyxinn B (300U)
Vancomycin (30mcg)	Colistin (10mcg)

### Statistical analysis:

All data was stored and managed in Microsoft Excel. Descriptive statistics were applied to find frequencies, percentages, and means. Quantitative data such as age was expressed in mean and standard deviation. Qualitative data like type of bacteria or their sensitivity to antibiotics were expressed as percentage and frequency.

## **RESULTS:**

A total of 400 wound swabs were sent for culture sensitivity collected from 328 patients. Out of 400 wound swabs 357 swabs revealed growth (89.2%) while remaining swabs were sterile. Out of 328 patients, 225 (68.6%) were males and 103 (31.4%) were females. The mean age of patients was  $35\pm10.2$  years. 131 of 357 swabs revealed poly-microbial (two or more organisms) growth (36.7%) and rest were mono-microbial. A total of 7 microorganisms were cultured from wound swabs i.e. Pseudomonas aeruginosa (58.0%), Enterobacter sp. (38.1%), Proteus mirabilis (8.10%), Citrobacter (7.3%), E. coli (5.61%), Klebsiella pneumoniae (5.04%), Coagulase Negative Staphylococcus (3.07%) (Table II) (Figure. I)

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Table II. Relative frequency of cultured organisms								
	Organism	Relative Frequency (n=357)	Percentage (%)	Gram Staining				
1.	Pseudomonas aeruginosa	207	58.0%	Gram Negative				
2.	Enterobacter sp.	136	38.1%	Gram Negative				
3.	Proteus mirabilis	29	8.1%	Gram Negative				
4.	Citrobacter	26	7.3%	Gram Negative				
5.	E. coli	20	5.61%	Gram Negative				
6.	Klebsiella pneumoniae	18	5.04%	Gram Negative				
7.	Coag. Neg. Staphylococcus	11	3.07%	Gram Positive				

Relative frequency of sensitivity to antibiotics in different organisms were studied and results shown below Table III.

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Test	Pseudomanas	Enterobacter	Proteus	Citrobacter	E. coli	Klebsiella	Coag. Neg. Staph.
Amikacin	11.8%	7.5%	13.80%	-	85.0%	-	-
Ampicillin	-	-	-	-	50.0%	-	81.8%
Aztreonam	16.7%	-	-	-	-	-	-
Cefepime	10.8%	4.5%	6.7%	-	90.0%	-	54.5%
Ceftazidime	9.8%	7.5%	13.8%	-	95.0%	-	-
Cefosulbactum	12.7%	-	-	-	-	-	-
Ciprofloxacin	10.8%	4.5%	-	-	50.0%	-	90.9%
Cotrimoxazole	-	-	-	38.4%	50.0%	-	81.8%
Erythromycin	-	-	-	-	-	-	54.5%
Gentamycin	5.9%	6.0%	6.7%	-	90.0%	-	100.0%
Imipenem	20.6%	19.4%	-	-	45.0%	-	-
piperacillin-tazobactum	13.7%	19.4%	48.3%	-	40.0%	-	90.9%
Teicoplanin	-	-	-	-	-	-	54.5%
Vancomycin	-	-	-	-	-	-	90.9%
Cefoxitin	-	-	-	-	-	-	81.8%
Doxycycline	-	-	-	-	-	-	54.5%
Linezolid	-	-	-	-	-	-	100.0%
Tigecyclline	-	82.1%	79.3%	80.8%	95.0%	88.8%	-
Polymyxin B	6.9%	92.5%	6.7%	80.8%	100.0%	88.8%	-
Colistin	87.2%	4.5%	-	-	-	-	-
Tobramycin	-	-	-	-	-	-	-
Cefoxatime	-	-	6.7%	-	50.0%	-	-

**96**.93% of organisms were Gram-Negative bacilli while 3.07% were Gram-positive cocci. Pseudomonas was the most prevalent organism, cultured in more than half wound swabs followed by Enterobacter sp. Antibiotic sensitivity pattern of these organisms show that Pseudomonas was sensitive to Colistin in about 90% cases and Imipenem in 20% cases while Enterobacter sp., Klebsiella and Citrobacter were sensitive to Tigecyclline and Polymyxin B in more than 80% wound swabs. Proteus mirabilis was sensitive to Piperacillin + Tazobactum in about half of the growth plates while sensitive to Tigecycline in about 80% wound swabs.



# Figure II. Relative sensitivity pattern of Pseudomonas and Enterobacter to different antibiotics.

Relative sensitivity pattern of Pseudomonas and Enterobacter sp. to different antibiotics is shown in Figure II. E. coli and Coagulase Negative Staphylococcus was the most sensitive organisms showing sensitivity for more than 5 antibiotics. We did not find Acinetobacter baumanni and Staphylococcus aureus in any of the wound swabs.

#### **DISCUSSION:**

Infection is the most prevalent complication of burn wounds and responsible for about 75% burn-related deaths (8). It is possible to challenge this infection and prevent death amongst the affected population by having an antibiogram against the specific organism. We collected 400 wound swabs with positive growth obtained in 357 swabs (89.2%). Similar positive results were found in other studies conducted by Kaushik et al (9), Arabiyat et al (10) and Badea et al (11). Kaushik et al found 87.2% wound swabs to be positive for growth while AL- Bdour et al reported 84.6% and Badea et al reported 86.2% growth (11). In other studies, high culture positivity was reported by Agnihotri et al (96%)(12). On the other hand, low culture positivity

was reported by Vaez et al (31%)(13) and Mohamed et al (60%)(14). Growth plates showed growth of multiple organisms in 36.7% samples while remaining plates documented mono-microbial growth. In a comparable study, similar results with 37.5% poly-microbial growth samples were reported by Rajput et al in 2008(15). However, a study conducted by Badea et al revealed a higher number of samples with poly-microbial growth i.e. 53% (2).

Burn wounds are primarily infected by Gram-positive organisms in initial weeks and gram-negative organisms in subsequent weeks. However, our study revealed a gram-negative organism in 96.93% cases and gram-positive organisms in only 3.07%. The gram-positive organism in our study was coagulase-negative staphylococcus in contrast to staphylococcus aureus in most of the studies conducted worldwide. Similarly, Minakshi et al also found Staphylococcus aureus in only 1.62% samples (5). However, Badea et al reported Staphylococcus aureus to be the most common culture organism from burn wound swabs (11). This difference is attributed likely to the use of antibiotics immediately on admission and increased nosocomial gram-negative infection. We also did not find Acinetobacter baumanni in our culture swabs in contrast to many other studies.

Pseudomonas is the most prevalent organism growth worldwide. Our study documents Pseudomonas in 58% wound swabs and was the most common organism. Pseudomonas infection can be suspected clinically by signs of gram-negative sepsis and the bluish-green color of dressings produced by pyocyanin. Another study from Iran province by Khorasani et al reported 57.3% incidence of Pseudomonas in their study (16). Similarly, Agnihotri et al also reported a prevalence of 59% Pseudomonas in their study (12).

All Gram-negative organisms were highly resistant to antibiotics except E. coli. Pseudomonas aeruginosa was sensitive to Colistin (87.2%) and to some extent Imipenem (20.6%). Similarly, Enterobacter sp was resistant to all other antibiotics except Tigecyclline (82.1%) and Polymyxin B (92.5%). Similarly, Proteus, Klebsiella, and Citrobacter were sensitive only for one or two antibiotics. These findings are consistent with a study conducted by Minakshi et al with 87.05% Pseudomonas isolates sensitive to

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Colistin. In contrast to others, we report a higher resistance of Pseudomonas and other organisms for Carbapenem, Amikacin or Ciprofloxacin (17, 18). Colistin acts by damaging the outer membrane of bacteria and hence does not require a specific target whereas Tigecycline is a recently commercialized broad-spectrum antibiotic with low resistance. The world currently has these two antibiotics as the last available options for Carbapenem-resistant gram-negative infections (5, 19).

These figures are alarming and indicate the possible scarcity of available antibiotic options in the near-immediate future. Hence, we require the introduction of new antibiotics with possibly less resistance to tackle such highly resistant organisms. Injudicious use of antibiotics is possible, the commonest reason for high antibiotic resistance seen in our burn wards.

The limitation of the current study was that we did not study any other sample like tissue biopsy and anaerobic culture/ fungal culture medium.

#### **CONCLUSION:**

Based on the present study, we conclude that in our hospital, Gramnegative bacteria are responsible for most of burn wound infections. Pseudomonas is the commonest gram-negative bacteria. This golden era of antibiotics is coming to a near end. The current trend towards high antibiotic resistance is alarming and dictates the need for new antibiotics against gram-negative organisms. Preventive practices must be practiced to avoid cross infection and reduce the requirement of antibiotics. The data will help the clinicians to prescribe antibiotics judiciously. This also helps in formulating protocols for the rational use of antibiotics and antibiogram for the hospital. Such studies need to be conducted at frequent intervals to look for changing trends in antibiotic sensitivity pattern.

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