

Original Research Article

Bacteriological profile with antibiotic sensitivity pattern of burn wound infections in a peripheral tertiary care hospital

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ABSTRACT

Background: India, has an estimated burn incidence of 6-7 million annually. Nearly 10% of these are life threatening and require hospitalization, and main cause of mortality and morbidity of these burn patients were wound infection and sepsis after 1st 24 hours. The present study was tried to determine specific pattern of burn wound infections, and antibiotic susceptibility of those isolates.

Methods: After matching inclusion and exclusion criteria, total 55 patients were taken for this institution based, prospective observational study. Wound swabs were collected on day 7 and cultured aerobically in MacConkey agar and 5% blood agar and antibiotic susceptibility testing was done on Muller Hinton agar using Kirby-Bauer disc diffusion method.

Results: Among study population 69.1% patients were female and majority (29.1%) of the patients belongs to age group from 21 to 30. It was found that 48 wound swabs were positive for microorganisms, of which *Pseudomonas aeruginosa* was most common isolated organism (23.6%), followed by *Klebsiella pneumoniae* (16.4%) and *Staphylococcus aureus* (14.5%). The most effective antibiotic found in this study was piperacillin/tazobactam, followed by imipenem/cilastatin.

Conclusions: It was seen that gram-negative organisms were more prevalent. *Pseudomonas aeruginosa* was the most common microorganism and piperacillin/tazobactam was most effective antibiotic.

Keywords: Burn, Wound infection, Bacteriological profile, Antibiotic susceptibility

INTRODUCTION

Burns, is caused by application of heat or chemical substances to the external or internal surface of the body which causes destruction of tissues.¹ As per the World Health Organization (WHO) globally, there is around 300,000 annual death due to burn, of which >95% occur in developing countries, with the Southeast Asia region contributing to 57% of the deaths.²

India records 16,00,000 cases of fire and 27,027 death in 2017 according to 195 nation analysis by global diseases burden published in the BMJ injury prevention journal.

It has been estimated that about 75% of the mortality associated with burn injuries is related to sepsis especially in developing countries.³ The most commonly recovered pathogens depend on the site of burn wounds and reflect the hospital's nosocomial pathogens.

Thermal burn causes destruction of the skin barrier with concomitant depressions of both local and systemic host immune responses, leading to infectious complications in patients with severe burn. The protein rich avascular necrotic tissue of burn wound surface provides a favourable niche for microbial colonization and proliferation. Migration of host immune cells and delivery of systemic antimicrobial agents to burn site is impaired

due to toxic substance released by microorganisms and avascularity of eschar.

Burn wound infections are sterile immediately following injury but the normal bacteria present on the skin rapidly colonize open skin wounds within 72 hours. The causative organisms of burn wound and their characters change with time. In the early post burn period gram-positive organisms predominate and are replaced by gram negative organisms by the second week. The density of bacteria grows progressively if topical antimicrobial agents are not applied and the microorganisms penetrate the eschar by migration along sweat glands and hair follicles until they reach the eschar/nonviable tissue interface.

Additional microbial proliferation occurs in the sub-eschar space, enhancing the lysis of denatured collagen and sloughing of the eschar. Proliferating organisms in the sub-eschar space can invade the underlying viable tissue, when the density and invasiveness of the microorganism exceed the host's defence responses, leading to invasive wound infection and even systemic spread to remote tissues and organs and sepsis.

Certain strain-specific factors appear to be important in the pathogenesis of invasive burn wound infection such as the production of enzymes collagenase, elastase, protease and lipase can enhance the organism's ability to penetrate the eschar. Moreover, bacterial motility appears to be important in development of invasive infection. For instance, proteinases, collagenases and hyaluronidase produced by *Staphylococcus aureus*, digest the extracellular matrix and delayed wound healing. They also can excrete exotoxins, such as toxic shock syndrome toxin-1 and enterotoxins A, B and C which are responsible for development of toxic shock syndrome in a susceptible patient. Whereas, *Pseudomonas aeruginosa* produces a characteristic pigment (pyocyanin) which is toxic and exotoxin A produced by some proportion of strains, causes inhibition of protein synthesis and cell death, leading to local necrosis and septicaemia.

Many advancements have reduced the incidence of burn wound infections over the last few decades and these include, antimicrobial therapies both topical and systemic, early excision and closure of burn wound and the introduction of infection control measures in modern burn units such as isolation facilities. Deaths due to hypovolemia and hyperosmolar shock are uncommon now due to the advancement of resuscitation methods in burn patients. Meanwhile, sepsis is now the commonest cause of death following burn injury and contributes to almost 75-85% of all burn victim's deaths.^{6,7}

First 24 hours all burn wounds are sterile so there is no need for antibiotic therapy in first 24 hours. Normally antibiotics prophylaxis should be avoided as prophylaxis does not reduce chances of infection. Sterility should be maintained during all procedures like catheterization, IV Line and Ryle's tube. Tetanus toxoid vaccine and tetanus

immunoglobulin injection should be given for passive immunity. All burn units must have their own antibiogram whenever antibiotics are to be started for prevention or control of invasive sepsis. Entire bacterial flora found on patient's wound surface, should be covered by antibiotics. In case culture and sensitivity reports are not available, antibiotics are required to cover both gram negative and gram-positive bacteria. Fungal cover may also be required in some long-standing cases.

The objective of this study is to find out the microbiological profile of wounds of burn patients admitted this peripheral tertiary care hospital with antibiotics sensitivity pattern of these organisms. This will help us to determine proper empirical systemic antibiotic therapy for early management of septic episodes before the results of microbiologic cultures become available, thus reduce the morbidity and mortality of burn patients by controlling septic episodes as much as possible.

METHODS

This is an institution based, prospective, observational study has been carried out in General Surgery Department of BSMCH from March 2019 to August 2020. The study population comprised of total 55 patients, satisfying below mentioned inclusion and exclusion criteria. The primary data for this study were patient's details and investigation reports, collected in predesigned case record form.

Sample size

Sample size is determined using following formula with adding 10% extra.

$$n = Z^2 P(1 - P) / d^2$$

Where, n=sample size; Z=confidence level=1.96 (for confidence interval 95%); P=expected prevalence=15%; d=precision=0.01

Inclusion criteria

Patients of any genders, having burn injury more than 20% of TBSA.

Exclusion criteria

Patients having burn injuries other than thermal burn i.e., electric burn, chemical burn and patients who have diabetes mellitus, HIV infection or any immunosuppressive disease or receiving immunosuppressive therapy in the preceding 6 months.

Study technique

This study has been conducted after getting ethical approval from institutional ethical committee and proper written informed consent from each patient or legally

acceptable representative of patient after explaining the study procedure to them in their own vernacular language.

Wound swabs were collected from the depth of the wound using sterile cotton swab, then samples were transported to the Institutional microbiology laboratory for culture and sensitivity. Swabs were inoculated evenly over two agar plates aerobically; MacConkey agar and 5% blood agar at 37 °C for 24 to 48 hours. After that the plates were examined for bacterial colony and growth was identified. Plates with no growth were discarded.

Antibiotic susceptibility testing of isolate was done on Muller Hinton agar using Kirby-Bauer disc diffusion method. Antibiotic susceptibility or resistance has been decided according to CLSI (formerly NCCLS) guideline by measuring zone of inhibition.

RESULTS

Distribution of age and sex

Out of 55 patients 69.1% patients were female and 30.1% were male patients as shown in the Figure 1, and majority (29.1%) of the patients belongs to age group between 21 to 30 years as shown in Table 1.

Table 1: Distribution of age of study population.

Age group (years)	No. of patients
<10	5
10-20	3
21-30	16
31-40	14
41-50	6
51-60	5
>60	6
Total	55

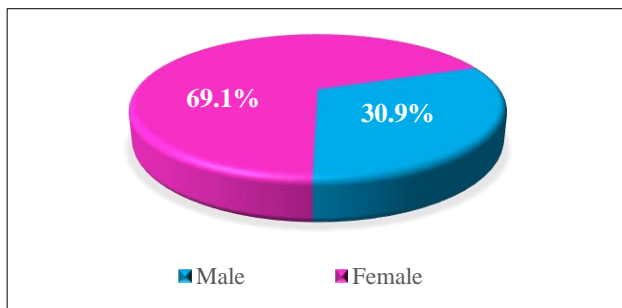


Figure 1: Distribution of gender.

Distribution of isolates

It was found that 48 wound swabs were positive for organisms (Figure 2), of which *Pseudomonas aeruginosa* was most common isolated organism, it is found in 23.6%

of study population, followed by *Klebsiella pneumoniae* (16.4%) and *Staphylococcus aureus* (14.5%). Other bacteria were *Escherichia coli*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Proteus mirabilis*, *Citrobacter koseri*, and *MRSA* (Table 2).

Table 2: Distribution of isolates.

Isolated bacteria	Number
<i>Citrobacter koseri</i>	2
<i>Escherichia coli</i>	5
<i>Klebsiella oxytoca</i>	4
<i>Klebsiella pneumoniae</i>	9
<i>MRSA</i>	1
<i>Proteus mirabilis</i>	3
<i>Proteus vulgaris</i>	3
<i>Pseudomonas aeruginosa</i>	13
<i>Staphylococcus aureus</i>	8
Total	48

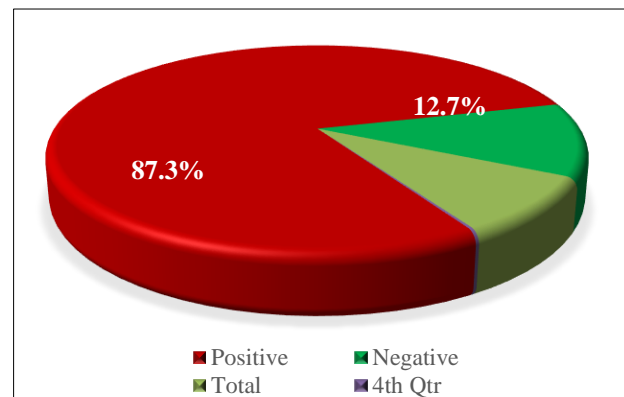


Figure 2: Distribution of swab culture report.

Antibiotic susceptibility of isolates

The most effective antibiotic found in this study was piperacillin/tazobactam, followed by imipenem/cilastatin. Piperacillin/tazobactam was sensitive against 81.3% of isolated bacteria found in swab culture. The 2nd most effective antibiotic imipenem/cilastatin had sensitivity of 56.3%.

The most resistant antibiotic found in this study was ceftriaxone, it had resistance of 43.8% against isolated bacteria in this study. Ciprofloxacin was the 2nd most resistant antibiotic with 41.7% resistance. Distribution of antibiotic susceptibility shown in Table 3 and Figure 3.

The drug most effective against *Pseudomonas aeruginosa*, the most common isolate, were piperacillin/tazobactam followed by imipenem/cilastatin (Figure 4). Ceftriaxone, followed by ciprofloxacin were most resistant tested antibiotic to *Pseudomonas aeruginosa* with 46% and 38% resistance accordingly.

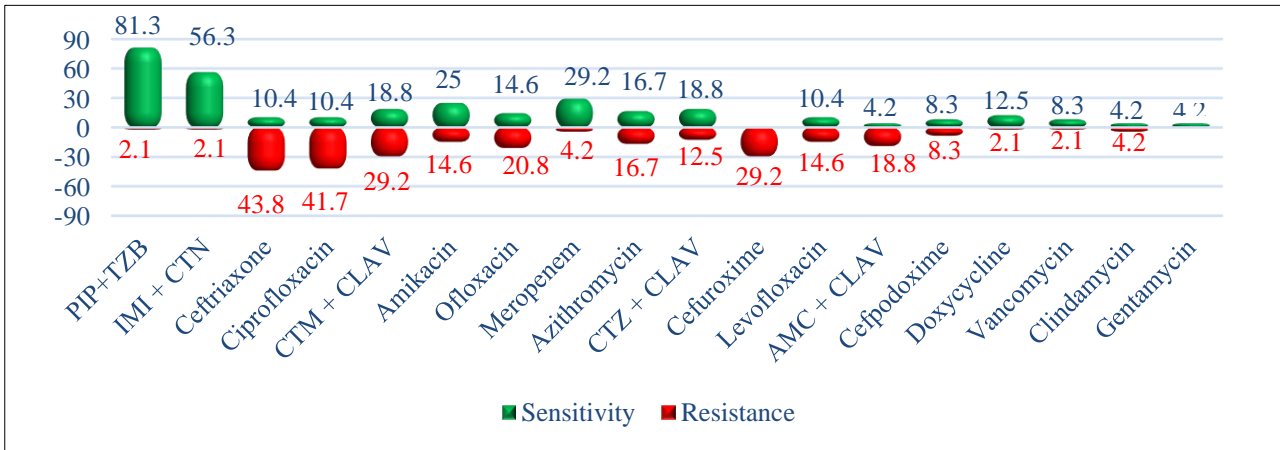


Figure 3: Distribution of sensitivity and resistance pattern of antibiotics.

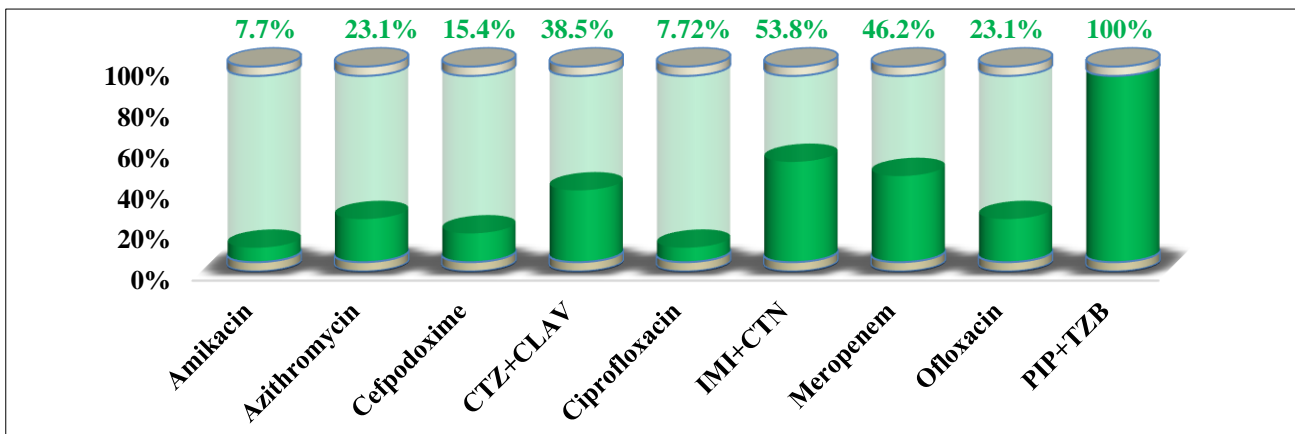


Figure 4: Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*.

DISCUSSION

Distribution of age and sex

In this study majority of the patients belongs to age group between 21 to 40 years (54.5 %) and the youngest patient was 9 months of age and the oldest patient was 77 years of age. The mean age of the patients was 34.8 years. Similar results were seen by Chakraborty et al who reported that 56.6% of the cases were of 20-39 years age.⁸

Likewise, Jaiswal et al stated that most of the cases were between 21–30 years of age.⁹

Incidence was more in females than males. The incidence in female was 69.1% and in male it was 30.9%. This is similar to findings by Kaur et al, Rajput et al¹¹ and Ganesamoni et al.^{10,11} In contrast, Ramakrishnan et al and Ekrami et al reported that the incidence was higher in males in their studies.^{12,13}

High incidence of burns in females is probably due to occupational hazards of working in the kitchen as the kitchen is the most common place to receive a burn injury.

Pattern of burn wound microbial colonization

In the present study, the overall 87.3% wound sample found positive for microorganism and 12.7% sample was negative. This was comparable with findings of Srinivasan et al (86.3%).¹⁴ Others have reported higher isolation rates such as 93% by Ramakrishnan et al and 95% by Kaur et al.^{10,12}

A total 48 (87.3%) wound swab were positive for organisms, of which *Pseudomonas aeruginosa* was most common isolated organism, it is found in 23.6% of study population. *Pseudomonas aeruginosa* was most common isolated organism in a study conducted by Nagesha et al and the incidence was 40%.⁴ Similar results were also found in other studies conducted by Bairy et al, Nagoba et al and Lari et al.¹⁵⁻¹⁷ In contrast, study reports of Ozumba et al indicated a decrease in burn wound colonization with *Pseudomonas aeruginosa*.¹⁸

It has been opined that with the advent of antibiotics against Gram positive organisms a significant rise in *Pseudomonas* infection of burned patients had occurred.⁴ Prevalence of *Pseudomonas species* in the burn wards

maybe due to the fact that the organism thrives in a moist environment.¹¹

The second most common isolate was *Klebsiella pneumoniae*. It was positive in 16.4% of burn patients of study population. Nasser et al in 2003 evaluated the pattern of burn wound colonization and found a high frequency of *Pseudomonas aeruginosa* (21.6%), followed by *Klebsiella pneumoniae* (15.2%).¹⁹ Ozumba et al and Kehinde et al observed that *Klebsiella pneumoniae* was the most common organisms isolated from their burn patients.¹⁸⁻²⁰

As for *Staphylococcus aureus*, they accounted for 14.5% of all the organisms isolated in our study. The incidence of *Proteus* species is reported at frequencies as high as 11% to no incidence at all.²¹ In the present study, we found *Proteus mirabilis* and *Proteus vulgaris* had total incidence of 11% (5.5% each). In this present study we also found some other bacteria, these were *Klebsiella oxytoca*, *Citrobacter koseri* and *MRSA*.

Contrary to the findings in the pre-antibiotic era, the isolation of beta haemolytic streptococci from burn wounds has now become rare.^{11,22} This was also confirmed in this study where we did not find any isolates of beta haemolytic streptococci.

Acinetobacter baumannii has also gained importance as an emerging nosocomial pathogen of burn wounds because of rapid increase in its resistance to a variety of antimicrobial agents.²³ Fortunately, in the present study we did not isolate this organism.

Pattern of antimicrobial sensitivity

The antimicrobial sensitivity pattern of the isolate, to different antimicrobials agents varied depending on the isolated bacteria.

The most effective antibiotics found in this study was piperacillin/tazobactam (PIP+TZB), followed by Imipenem/cilastatin (IMI+CTN). Piperacillin/tazobactam was sensitive against 81.3% of isolated bacteria found after swab culture. The 2nd most effective antibiotics imipenem/cilastatin had sensitivity of 56.3%. The other antibiotics i.e. meropenem and amikacin had sensitivity of 29.2% and 25% accordingly. Rest tested antibiotics had sensitivity below 20%. On the other hand, amoxicillin/clavulanic acid, doxycycline, clindamycin was least sensitive against isolated bacteria with 4.2% sensitivity among the tested antibiotics. No isolated bacteria found sensitive to cefuroxime.

The most resistant antibiotics found in this study was ceftriaxone, the most commonly used antibiotics in our hospital. It had resistance of 43.8% against isolated bacteria in this study. Ciprofloxacin was the 2nd most resistant antibiotics found in this study. It was resistant against 41.7% of isolated bacteria. No isolated bacteria found resistant to gentamicin.

The drugs most effective against *Pseudomonas aeruginosa*, the most common isolate, were piperacillin/tazobactam (sensitivity 100%) followed by imipenem/cilastatin (sensitivity 53.8%). Ceftriaxone, followed by ciprofloxacin were most resistant tested antibiotics to *Pseudomonas aeruginosa* with 46% and 38% resistance accordingly.

Piperacillin/tazobactam (81.3%) was effective against all the isolates, followed by second most effective drug was imipenem showing 56.3% sensitivity. This is in accordance with a study by Guggenheim et al.²⁴ *Klebsiella pneumoniae* accounted for 16.4% of all the isolates. It found highly sensitive against piperacillin/tazobactam, meropenem and imipenem/cilastatin. The other gram-negative isolates, namely *Escherichia coli*, *Proteus mirabilis* and *Citrobacter koseri* showed good sensitivity to imipenem/cilastatin and piperacillin/tazobactam.

Mehta et al saw a significantly high percentage of resistance among gram negative bacilli to aminoglycosides, ciprofloxacin, carbenicillin, tobramycin and ceftriaxone.²⁵ But in comparison, the combination of imipenem with cefoperazone/sulbactam were found to be effective. Macedo et al and Lari et al also reported a high degree of resistance to antimicrobial agents.^{26,27}

The gram-positive isolates showed 100% sensitivity to vancomycin and linezolid, followed by 94.29% sensitivity to piperacillin/tazobactam. Only 24.29% of the isolates were sensitive to penicillin. We found that 10.25% of the isolates of *Staphylococcus aureus* were methicillin resistant. This had less incidence with other studies on *MRSA* in burn patients by Rajput et al and Oncul et al.^{11,28}

Resistance to antibiotics in burn isolates reported previously has shown a gradual increase over time.²¹ Many studies have shown that most of the organisms causing infection in burn patients are highly resistant to routinely used antibiotics.²⁹

The resulting antibiograms were concerning because the predominant bacterial isolates were relatively resistant to antibiotics available commonly, more economical antimicrobials. However, this was not entirely un-expected as hospitals are an important breeding ground for the development and spread of antibiotic resistance. This is the consequence of exposing to heavy antibiotic use, a high density of patient population in frequent contact with health care staff and patient attendant increases the risk of cross infection.

Limitations

This study has several limitation as single wound swab was taken from every patient on day 5 changes of microorganisms during hospital stay cannot be assessed. Furthermore, antibiotics susceptibility pattern of individual microorganism is not shown in this study. In this study small number of samples is taken for limited

duration of time, so further long duration study with bigger sample size is needed for accurate determination of microorganism and antibiotics susceptibility pattern for empirical antibiotics therapy. In our institution Sterility of overcrowded female burn ward is poor than male burn ward, but this factor is not count in this study.

CONCLUSION

It was seen that gram-negative organisms were more prevalent. *Pseudomonas aeruginosa* was the most common microorganism and piperacillin/tazobactam was most effective, so piperacillin/tazobactam can be given as empirical systemic antibiotic therapy for early management of septic episodes before the results of microbiologic cultures become available.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee, BSMCH, Bankura, West Bengal, India

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