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Original Research Article

Emergence of multi-drug resistant strains among bacterial isolates in burn wound swabs in a tertiary care centre, Nanded, Maharashtra, India

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ABSTRACT

Background: Infection is a major cause of morbidity and mortality among burn patients. The worldwide emergence of antimicrobial resistance among a wide variety of burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections. The study was conducted in the department of Microbiology, Dr. S.C.G.M.C, Nanded, Maharashtra, India to determine aerobic bacterial isolates from burn wound swabs and describe their antibiogram.
Methods: Two wound swabs were taken from 570 patients, cultured aerobically. The isolates were identified by standard microbiological methods and antibiotic sensitivity pattern was determined.
Results: Among 570 patients, 434 (76.14%) were female and 136 (23.85%) were male. Out of the total swabs collected, 548 (96.14%) were culture positive and 36 (6.56%) were having 2 isolates. *Pseudomonas aeruginosa* (34.93%) was the commonest isolate followed by *Staphylococcus aureus* (22.77%), *Klebsiella pneumoniae* (13.87%), *Escherichia coli* (13.01%) and *Coagulase negative staphylococcus* (11.31%). Incidence of MRSA was 59.39% and ESBL producers were 61.46 %. Gram positive isolates were 100% sensitive to Vancomycin, Linezolid and Gram negative organisms to Imipenem.
Conclusions: Routine periodic sampling of burn wounds would facilitate the selection of appropriate empirical therapy and reduce the incidence of multidrug resistant infections among burn patients.

Keywords: Burn wound infections, ESBL, MRSA, Multidrug resistant, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

INTRODUCTION

Burns are one of the most common and devastating forms of trauma and a major public health concern in all around the world. Globally an estimated 195000 deaths occur annually. In India, over 1000000 people are moderately or severely burnt every year.[1]

Infection is a major cause of morbidity and mortality among burn patients. 75% of all deaths following burns are related to infection. There is a higher rate of nosocomial infections in burn wards.[2]

Open and large wounds, make burn patients more susceptible to infection.[3] There is disruption of the normal skin barrier at the site, as well as a large amount of necrotic tissue and protein-rich wound exudate at the burn surface, provide a rich growth medium for colonisation and growth of microorganisms, which is poorly controlled due to depressed immune responses.[4]

The worldwide emergence of antimicrobial resistance among a wide variety of burn wound pathogens, particularly nosocomial isolates, limits the available therapeutic options for effective treatment of burn wound infections. Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, extended spectrum beta- lactamases (ESBL) and metallo beta-lactamases (MBL) producing Gram-negative bacteria have been emerging as serious challenges in hospitalized patients. These organisms can be transmitted easily from one patient to another. Thus, burn units are common places where explosive and prolonged outbreaks of infections caused by resistant organisms occur.[5]

Therefore, it is just not sufficient to be aware of the microorganisms that pose a problem for burn patients. To have an in-depth knowledge of the organisms that are predominant in that treatment facility during the period along with their sensitivity pattern is vital as many septic burn patients need to be treated with antibiotics before the results of microbiological cultures are available. This would be crucial to reduce the overall infection related morbidity and mortality.[6]

Aim and objectives

The present study was conducted in the Department of Microbiology, Dr. Shankarrao Chavan Government Medical College, Nanded, Maharashtra, India in order to determine aerobic bacterial isolates from burn wound swabs in our hospital setting and describe their resistance patterns, which would enable the determination of empiric antimicrobial strategies for the early treatment of imminent septic events.

METHODS

A total of 570 patients admitted to burns ward from July 2014 to April 2015 were studied. Two wound swabs were collected and transported to the laboratory.[7] The swabs were cultured aerobically using blood agar and MacConkey agar. Bacterial isolates were identified by conventional methods.[8] Antibiotic susceptibility tests were performed using Kirby Bauer's disc diffusion method per Clinical and Laboratory Standards Institute (CLSI) guidelines 2014.

Drugs used for Gram positive isolates were Erythromycin (15µg), Clindamycin (2µg), Penicillin (10 Units), Linezolid (30µg), Cefoxitin (30µg), Cotrimoxazole (1.25/23.75µg), Vancomycin (30µg). MRSA were screened using Cefoxitin (30µg) disc by disc-diffusion technique.[9] *Staphylococcus aureus* ATCC 25923 was taken as the positive control strain. The antibiotics included for Gram negative isolates were Amikacin (30µg), Ampicillin (10µg), Piperacillin (100 µg), Piperacillin-Tazobactum (100 /10 µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Amoxyclav (20/10µg), Ciprofloxacin (5µg), Imipenem (10µg), Gentamicin (10µg), Tobramycin (10µg) and Ceftazidime-Clavulanic acid (30/10µg). *Pseudomonas aeruginosa* resistant to three of the four 'in use' drugs i.e. Piperacillin+Tazobactam, Imipenem, Ceftazidime, and Gentamicin, was taken as MDR.[10] *Pseudomonas aeruginosa* ATCC 27853 was taken as control strain for culture and antibiotic sensitivity test for each batch. All Gram-negative bacteria belonging to *Enterobacteriaceae* which were resistant to Cefotaxime and/or Ceftazidime by Kirby Bauer disc diffusion test were screened and confirmed by double disc synergy test (DDST).

Double disc synergy test (DDST)

Discs of Ceftazidime (30µg) and Ceftazidime-Clavulanic acid (30 µg /10 µg) were placed at 30 mm from center to center in a straight line on a plate of Mueller Hinton Agar (MHA) being inoculated with the test strain. The plates were incubated at 37ºC aerobically overnight. An increase in zone size ≥5 mm with the combination of the antibiotic tested confirmed ESBL producer.[9] A non ESBL producing organism (*Escherichia coli* ATCC 25922) was used as negative control and an ESBL producing organism (*Klebsiella pneumoniae* ATCC 700603) was used as positive control.

RESULTS

Among 570 patients, 434 (76.14%) were female and 136 (23.85%) were male (Figure 1). Out of the total swabs collected, 548 (96.14%) were culture positive and 36 (6.56%) were having 2 isolates.

Figure 1: Sex-wise distribution of the study population.

Table 1: Various bacterial isolates found in burn wound swabs.

Bacterial isolate	Number	Percentage
Pseudomonas aeruginosa	204	34.93 %
Staphylococcus aureus	133	22.77 %
Klebsiella pneumoniae	81	13.87 %
Escherichia coli	76	13.01 %
Coagulase negative staphylococcus	66	11.31 %
Klebsiella oxytoca	11	1.88 %
Citrobacter freundii	7	1.19 %
Proteus mirabilis	6	1.02 %
Total	584	100 %

Pseudomonas aeruginosa was the commonest isolate followed by *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and *Coagulase negative staphylococcus* (Table 1).

Table 2: Resistance pattern of Gram positive isolates.

Antibiotics	MSSA (54)	MRSA (79)	CONS (66)
Vancomycin (30µg)	00	00	00
Linezolid (30µg)	00	00	00
Clindamycin (2µg)	35.19 % (19)	35.45 % (28)	31.82 % (21)
Cefoxitin (30µg)	00	100 % (79)	62.13 % (41)
Erythromycin (15µg)	53.71 % (29)	53.17 % (42)	56.07 % (37)
Cotrimoxazole (1.25/23.75µg)	64.82 % (35)	68.36 % (54)	68.19 % (45)
Penicillin (10 Units)	83.34 % (45)	84.82 % (67)	89.39 % (59)

(MSSA- Methicillin Sensitive *Staphylococcus aureus*, MRSA- Methicillin Resistant *Staphylococcus aureus*, CONS-*Coagulase Negative Staphylococcus*).

Out of the total 133 isolated *Staphylococcus aureus*, 79 were found to be methicillin resistant *Staphylococcus aureus* (MRSA). So incidence of MRSA was found to be 59.39%. MRSA showed maximum sensitivity to Vancomycin (100%) and Linezolid (100%) followed by Clindamycin (64.55%). Similar pattern of sensitivity was observed in Methicillin Sensitive *Staphylococcus aureus* and *Coagulase negative staphylococcus*.

Table 3: Resistance pattern of Gram negative isolates.

Antibiotics	*P. aeruginosa* (204)	*K. pneumoniae* (81)	*E. coli* (76)
Imipenem (10µg)	00	00	00
Amikacin (30µg)	5.39 % (11)	3.71 % (3)	5.27 % (4)
Ciprofloxacin (5µg)	42.65 % (87)	45.68 % (37)	44.74 % (34)
Gentamicin (10µg)	44.12 % (90)	37.04 % (30)	35.53 % (27)
Tobramycin (10µg)	38.73 % (79)	39.51 % (32)	40.79 % (31)
Ampicillin (10µg)	--	77.78 % (63)	76.32 % (58)
Piperacillin (100µg)	46.08 % (94)	--	--
Piperacillin/ Tazobactam (100 /10µg)	9.88 % (20)	--	--
Amoxyclavuclanic acid (20/10µg)	--	75.31 % (61)	72.37 % (55)
Ceftriaxone (30µg)	71.57 % (146)	82.72 % (67)	80.27 % (61)
Ceftazidime (30µg)	75.98 % (155)	80.25 % (65)	82.89 % (63)
Cefotaxime (30µg)	73.53 % (150)	81.49 % (66)	78.95 % (60)

Pseudomonas aeruginosa was the commonest isolate showing 75% resistance to commonly used Ceftazidime. 9.8% of *Pseudomonas aeruginosa* isolates were resistant to all the three drugs- Piperacillin + Tazobactam, Ceftazidime, and Gentamicin and were multi drug resistant. However, the isolates were 100% sensitive to Imipenem.

Amongst 81 *Klebsiella pneumoniae* species isolated, 64.12% were found to be ESBL producers. Also, 57.89% *Escherichia coli* species were ESBL producers.

So, incidence of ESBL producers in our study was 61.46%. These organisms showed maximum sensitivity to Imipenem followed by Amikacin.

DISCUSSION

In this study, female predominance (76.14%) was higher than male. It is in accordance to other studies done by Pragathi et al and Dash et al.[3,11] In India higher incidence of burn injuries among females may be related to inadequate precautions during cooking, unawareness about fire safety measures in illiterate people, unsafe measures used for cooking like kerosene pressure stoves, traditional chulhas as observed by Domple et al.[12]

Throughout the period of our study, Gram negative organisms predominated over Gram positive organisms. *Pseudomonas aeruginosa* was the commonest isolate. This finding conforms to many published studies such as

Kumar et al, Vinitha CT et al, Mehta et al and Saaiq et al.[13-16]

36.2% multi drug resistant *Pseudomonas aeruginosa* was reported by Biswal et al.[10] 9.8% *Pseudomonas aeruginosa* isolates were multi drug resistant in our study. This may be due to restricted use and higher cost of the antibiotics.

In the present study, prevalence of MRSA amongst burn wound patients was 59% which resembles with the reports of Kohle et al and Pragathi et al.[3,17] MRSA reservoirs are found not only in hospital but also exist outside health care facilities. Both the health care associated MRSA (HAMRSA) infections as well as the community associated MRSA (CAMRSA) infections, pose a threat to burn injury patients who are managed on outdoor basis with the traditional conservative dressings instead of proper surgical excision and grafting.[16] Present study proved that Linezolid and Vancomycin were the agents that could be confidently employed on empirical basis to combat life threatening infections caused by multi drug resistant strains of MRSA.

In present study rate of ESBL producing organism was 61.14% which correlated with the study reports of Pragathi et al.[3]

The high percentage of multidrug resistant isolates is probably due to empirical use of broad-spectrum antibiotics prior to development of infection, extended duration or previous hospitalization and non-adherence to hospital antibiotic policy. Strict infection control practices i.e., physical isolation in a private room, use of gowns and gloves during patient contact, hand washing before and after each patient visit and appropriate antibiotic therapy are essential tools to reduce the incidence of infections due to these multidrug resistant organisms.

Therefore, routine institutional laboratory surveillance system involving periodic sampling of burn wounds would facilitate the selection and administration of appropriate empirical systemic antibiotic agents prior to the availability of microbiological culture and susceptibility test results.

CONCLUSION

To conclude, burn patients were most commonly infected with *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Majority of these isolates were multidrug resistant. For Gram-negative isolates, Imipenem or Piperacillin/Tazobactam and for Gram-positive, Vancomycin or Linezolid were found to be most effective.

Therefore, burn unit-specific nosocomial infection surveillance system may be introduced to reduce the incidence of multidrug resistant infections among burn patients, and for selecting appropriate antimicrobial agents.

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