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Bacteriological Profile and Antibiotic Resistance Pattern and Prevalence of MRSA in Isolates from Burns Patients

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Abstract: Burn wound colonization and infection is not only associated with delayed wound healing and scar formation, but may also lead to sepsis related mortality. A wide variety of microorganism like, *Staphylococcus aureus* , pseudomonas aerugiuosa, klebsiella pneumouiae and Escherichia coli are involved. And Methicillin resistant *Staphylococcus aureus* (MRSA) poses a great risk to burn patient with potential to cause significant morbidity and mortality. According to an Indian study, the prevalence of infections caused by MRSA has increased from 12 percent in 1992 to 80.3 percent in 1999. This study was conducted to determine the common aerobic bacterial isolates, there antimicrobial resistance patterns and prevalence of MRSA in isolates from 150 burn patients admitted in Osmania general hospital Hyderabad. All samples were processed according to standard laboratory protocols .Among 150 isolates, culture positives were 143 and 7 were culture negative and among which *Staphylococcus aureus* was the commonest organism isolated (51.3%) the MRSA accounts for 57.1%. Regular surveillance of burn wound organism and their antimicrobial resistance patterns will help in determining empirical antibiotic therapy for subsequent related septic events.
Keywords: Burn wounds, Bacterial colonisation, Antimicrobial resistance, sepsis, severity of burns, MRSA.

INTRODUCTION

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality [1]. Burn wounds are highly vulnerable to colonization and infection by microorganism and this is a major problem in management of burn victims. Immediately following thermal injury, burn wounds are sterile, but later they eventually get colonized with microorganisms [2]. As a result of thermal injury to skin, there is disruption of normal skin barrier at the site and there is large scale release of various cytokines, prostaglandins, leukotrienes which leads to a significant alteration of immune function [3].

It has been estimated that 75% of the deaths following thermal injuries are related to infections[4].The pattern of infection differs from hospital to hospital[2], *Staphylococcus aureus* is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital infection all over the world. Of these Methicillin became the standard treatment for *Staphylococcus aureus* . In 1961, the first methicillin resistant strains of *Staphylococcus aureus* (MRSA) were isolated in Europe [5]. Methicillin resistant *Staphylococcus aureus* is now endemic in India. The incidence of MRSA varies from 25 percent in western part of India to 50 percent in south India [6].

Despite various advances in infection control measures like early detection of microorganisms and use of newer broad spectrum antibiotics, management of burn septicemia still remain a big challenge and it continues to be the leading cause of death in burns patient [2].

In view of the above facts, the present study was carried out to determine the bacteriological profile, antimicrobial resistance and prevalence of MRSA among the patients admitted in burns unit.

METHODS

A total of 150 samples were taken for the study. The area around the burn wound was cleaned

with 70% ethyl alcohol and the sample was collected from the depth of the wound using two sterile cotton swabs. The samples were transported immediately to the laboratory for further processing. Then the samples were processed by direct microscopic examination using the first swab a smear was made on a clean glass slide. Smear of positive and negative controls were made with *Staphylococcus aureus* ATCC 25923 and Escherichia coli ATCC 25922 respectively. After fixation with heat, it was stained with a Gram stain. The stained smear was screened carefully for the presence or absence of pus cells, bacterial morphology, arrangement and their Gram reaction.

Then the second swab was inoculated onto preincubated plates of Mac.conkey agar and 5% sheep blood agar by rolling the swab over the agar to make a primary well and then streaking from the primary inoculums using a sterile bacteriological loop to form secondary, tertiary and quaternary streak lines then these plates were incubated at 37degrees for 24 hours. Plates showing no growth were incubated further aerobically at 37 degrees for next 24 hrs. Plates not showing any growth after 48 hrs of aerobic incubation were discarded. The plates which were showing growth were processed further after 24 hr. First by doing a Gram stain with an isolated colony and observed under oil immersion lens for Gram reaction, morphology,and arrangement of the organisms. Then biochemical reactions were put up like the 1. Coagulase test both slide and tube, 2. Catlase test for gram positive. And for Gram negative organisms, 1) Oxidase test 2)Motility test 3)Indole test 4) Methyl red test, 5) Voges praskauer test, 6) Citrate test 7) Urease test, 8)Triple sugar iron test 9)Nitrate reduction test, 10)Sugar fermentation test 11)Decarboxylase test .

Followed by Antibiotic sensitivity testing of isolates was done on Muller-hinton agar using Kirby bauer disc diffusion methods. Bacterial suspension was prepared by inoculating few isolated colonies of similar morphology into 4-5 ml of peptone water and incubated for 2-4 hr, the turbidity of the broth was adjusted to 0.5 Mc Farland turbidity standards and lawn culture was made on the surface of the medium using sterile cotton swabs. Antimicrobial discs were applied with the help of sterile forceps and the plates were incubated at 35 degrees for 24hrs. The antimicrobial discs were obtained from Hi media laboratories private limited, Hyderabad.

Table-1: Antibiotics used in the testing

GPCS	GNBS
1)Vancomycin(VA) 30 micro gm	1)Imipenem (IPM) 10 micro gm
2)Cefoxitin(CX) 30 micro gm	2)Ciprofloxacin (CIP) 5micro gm
3)Amikacin(AK) 30 micro gm	3)Piperacllin and tazobatum (PIT) 30micro gm
4)Amoxyclav (AMC)	4) Amikacin (AK) 30 micro gm
5)Ofloxacin (O) 5 micro gm	5) Ceaperazone and Sulbactum (CFS)
6)Tetreacyclin (TE) 30 micro gm	6) Cetazidime (CAZ) 30 micro gm
7)Penicillin (P) 10 units	7) Ceftrioxone (CTR)
8)Cotrimoxazole (COT) 25 micro gm	8)C otrimoxazole (COT) 25 micro gm

Detection of Methicillin resistance

- Methicillin resistance was detected using 30 micro gm of Cefoxitin disc in susceptibility testing and the interpretation was done as per CLSI guidelines.
- Confirmation of methicillin resistance is done by inoculating on a chrome agar MRSA plate and the results were read interpreted after 24hr and 48hr of incubation at 35 degree pink or mauve coloured colonies indicate MRSA.

Fig-1: AST SHOWING MRSA

Fig-2: MRSA CHROMAGAR

RESULTS

Out of 150 isolates, 143 were culture positive and 7 were culture negative, and among the culture positive isolates, 131 were monomicrobial and 12 were polymicrobial in nature. Among the isolated organisms, *Staphylococcus aureus* was the most common, accounting for 51.3% (77 isolates) followed by Klebsiella species 16%, Pseudomonas aeruginosa 9.3%, Cons 2.6%, Proteus species and Escherichia coli each 2.6%, Citrobacter species 2%. Out of total 143 cultures positive, 56.6% were gram positive and 43.6% were Gram negative organisms.

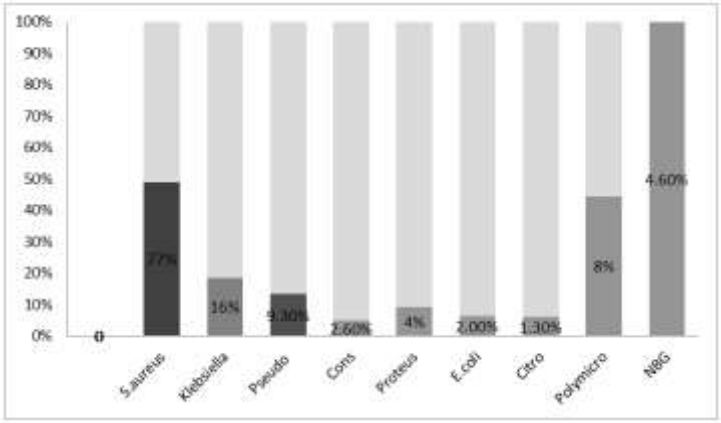

Fig-3: Graph showing different organisms

And among the total 77 *Staphylococcus aureus* , 44 (57.1%) were MRSA and 33 (42.9%) were MSSA.

DISCUSSION

Bacterial infections of the burn wound still remains a major cause of morbidity and mortality in thermally injured patients [7]. The burned patient is prey for a wide variety of microorganisms [8] as burns present an extensive surface with large mass of dead tissue and free exudation of serum which is favorable for bacterial growth. The burn site initially becomes colonized with microorganism which if uncontrolled progresses to invasion and gives rise to bacteremia and sepsis, which is a major cause of mortality in burn patient [7].

In the present study, the overall isolation rate was found to be 95.4%. This was comparable with finding of isolation rates such as 93% by Ramakrishnan MK *et al*. 95% by Kaur H *et al*. and 97.1% by Mehta M *et al*. In contrast with the lower isolation rates findings of Srinivasan S *et al*. (86.3%) [9].

And the most common isolate is the *Staphylococcus aureus* ; this is similar to some studies especially from developed countries which report *Staphylococcus aureus* as the most important organism in burn patients and also in comparison with study of VG Bhat *et al*. Alghalibi *et al*. and Naveen saxena *et al*. [2].

Staphylococcus aureus causes variety of infection ranging from relatively benign skin infections to life threatening systemic illness. MRSA became a major problem for health care providers because it is hard to treat and is called as Super bug. Early and accurate detection of MRSA is essential for the treatment of overt infections and for the implementation of infection control practices.

CONCLUSION

Burn wound infections are showing changing trends in the relative importance and bacterial colonization pattern as well as their antimicrobial sensitivities.

To ensure early and appropriate therapy, routine microbiological surveillance and a regular updates of their antimicrobial susceptibility pattern could help in prevention and development of multidrug resistance, and help in formation of effective guidelines for therapy, thus improving overall infection related morbidity and mortality.

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