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Methicillin Resistant *Staphylococcus aureus* Amongst the Patients in Burns Unit

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Infection is the most important problem in the treatment of burns. The pathogens that infect the burn wound are primarily gram-positive bacteria such as Staphylococcus aureus and gram-negative bacteria such as *Acinetobacter baumanii, Pseudomonas aeruginosa* and *Klebsiella* species. MRSA is an important causative agent of burns infections in India. Many of these MRSA isolates are becoming multidrug-resistant, and are susceptible only to glycopeptides. Our study is aimed to know the prevalence of MRSA strains in JJM Medical college hospital burns unit, its minimum inhibitory concentration and phage type. Total of 120 burns cases were studied over a period of March 2010 to December 2010. Pus samples were collected aseptically for bacteriological examination from burnt sites. All the *Staphylococcus aureus* isolates were tested for methicillin susceptibility. All Methicillin Resistant strains were confirmed by Oxacillin screening using 6μg/ml of Oxacillin in Muller Hinton agar with 4% NaCl plates and were incubated at 30°C. Phage typing was performed at National phage typing centre, Maulana Azad Medical College, New Delhi. All the MRSA strains were typed using a set of 8 MRSA phages.Out of the 120 samples, *Staphylococcus aureus* were isolated from 100 samples and from other 20 samples *Klebsiella* species, *Pseudomonas* species and *Proteus* species were isolated. Our study showed 43 isolates as MRSA and 57 isolates as Methicillin Sensitive *Staphylococcus aureus*. MRSA phage typing was done by using new set of phages. In this maximum strains belonged to group III (70.7%), group II (9.27%), group IV(3.08%). It is essential to control introduction and spread of MRSA infection. This can be achieved by observing universal precautions and conducting regular epidemiological studies to know the changing trends.

Key words: *Staphylococcus aureus,* MRSA, Phage typing.

Infection is the most important problem in the treatment of burns. Skin acts as a physical barrier against infection. When this barrier is damaged, pathogens have a direct route to infiltrate the body, possibly resulting in infection. In addition to the nature and extent of the burns influencing infections, the type and quantity of microorganisms that colonize the burn wound appear to influence the future risk of invasive wound infection. The pathogens that infect the burn wound are primarily gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *Acinetobacter baumanii, Pseudomonas aeruginosa* and *Klebsiella* species[1, 2].

Some strains of *Staphylococcus aureus* developed resistance to penicillin by producing an enzyme (ß-lactamase) which could break down

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the penicillin molecule. A number of synthetic derivatives of penicillin, resistant to the ß-lactamase enzyme, were developed. 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. They were first reported in Australia in 1966 in the eastern states and in the United States in 1968. Other strains were identified that had a broad pattern of resistance, not only to methicillin, but also to the aminoglycosides and cephalosporins [3,4].

Burns patients have been shown the potential to become colonized and infected more readily than other patients due to the deprivation of mechanical barrier provided by the skin and mucous membrane as well as the depression of immunological response. MRSA is an important causative agent of burns infections in India. Many of these MRSA isolates are becoming multidrug-resistant, and are susceptible only to glycopeptides [5,6].

MATERIAL AND METHODS

The present study was prospectively carried out at the Department of Microbiology, JJM Medical College Davangere, India. Total of 120 burns cases were studied over a period of March 2010 to December 2010. Pus samples were collected aseptically for bacteriological examination from burnt sites after 3 days of admission. Burns patients with more than 10 days of admission were excluded from the study.

A preliminary Gram stain was performed to determine the likely organism present. The samples were aerobically incubated on 5% sheep blood agar and MacConkey agar. All isolates were identified by standard conventional bacteriological and biochemical methods.

All the *Staphylococcus aureus* isolates were tested for methicillin susceptibility. All Methicillin Resistant strains were confirmed by Oxacillin screening using 6µg/ml of Oxacillin in Muller Hinton agar with 4% NaCl plates and were incubated at 30°c .Strains showing the growth on this medium were taken as MRSA strains. Simultaneously MRSA were also confirmed by using cefoxitin disc (30µg). All the strains of MRSA were subjected to minimum inhibitory concentration estimation against Oxacillin using agar dilution method as per standard recommendations.

All the MRSA isolates were preserved in 0.5% nutrient agar media in screw capped bottles and were sent to National phage typing centre, Maulana Azad Medical college, New Delhi as per standard guidelines for mailing biohazardous materials. All the MRSA strains were typed using a set of 8 MRSA phages. The phages were M3, M5, M12, MR8, MR25, C30, C33, and C38.

RESULTS

A total of 120 patients were included in the present study. The patient's age ranged from 10 years to 50 years with female predisposition. Out of the 120 samples, *Staphylococcus aureus* were isolated from 100 samples and from other 20 samples Klebsiella species, Pseudomonas species and Proteus species were isolated. (Fig. 1)

All the 100 *Staphylococcus aureus* isolates were tested for methicillin susceptibility. Our study showed 43 isolates as MRSA and 57 isolates as Methicillin Sensitive *Staphylococcus aureus* (MSSA). All the MRSA isolates were further tested for Minimum inhibitory concentration of Oxacillin. All isolates had MIC value above 4µg/ml (Table 4).

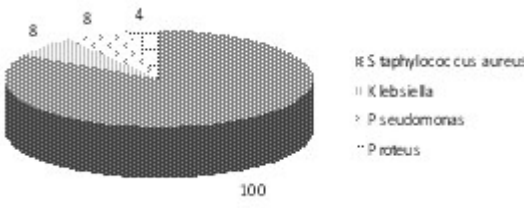

Fig. 1. Bacteria isolated

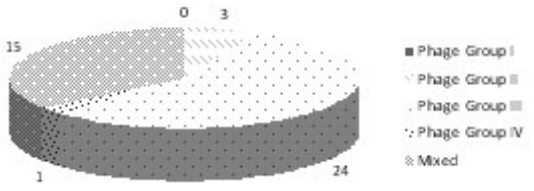

Fig. 2. Phage Types

Table 3. Antibiotic sensitivities of MRSA and MSSA (%)

Antibiotics	MRSA (n=43)		MSSA (n=57)	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin/Amoxycillin	0	43(100)	21(37)	36(63)
Cloxacillin	8(19)	35(81)	53(93)	4(7)
Chloramphenicol	41(95)	2(5)	54(95)	3(5)
Cotrimoxazole	4(9)	39(91)	43(75)	14(25)
Ciprofloxacin	15(35)	28(65)	52(91)	5(9)
Erythromycin	3(7)	40(93)	49(86)	8(14)
Roxithromycin	6(14)	37(86)	52(91)	5(9)
Gentamycin	8(19)	35(81)	56(98)	1(2)
Cephalexin	1(2)	42(98)	57(100)	0
Cephotaxime	11(26)	32(74)	57(100)	0
Amikacin	20(47)	23(53)	54(95)	3(5)
Vancomycin	43(100)	0	57(100)	0

Table 4. Table MIC of Oxacillin for MRSA isolates

MIC value	No. of isolates	Percentage of isolates
0.25-2	0	0
4.0	10	23.2
8.0	8	18.6
16.0	2	4.6
32.0	2	4.6
64.0	6	13.9
128.0	12	27.9
256.0	6	13.9
512.0	1	2.3
1024.0	0	0

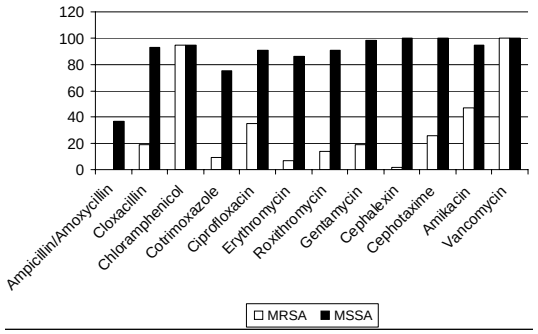

Fig. 3.

All the MRSA isolated were sensitive for Vancomycin (100%). MSSA were sensitive to Vancomycin, Erythromycin and Cephotaxime. (Table 3)

All the *Staphylococcus aureus* strains were subjected to phage typing using routine set of phages. MRSA phage typing was done by using new set of phages. In this maximum strains belonged to group III (55.8%) group II(6.9%) group IV (2.3%). (Fig. 2)

DISCUSSION

Infection is the most important problem in the treatment of burns. As the infection of burns wound is common after 3 days of admission all the samples were collected between 3 to 10 days of admission [4,5]. The bacteriology of burns wounds is often polymicrobial in nature and the presence of multidrug resistant organism is often associated with more severe clinical manifestations, poor response to antimicrobial therapy and mortality. The epidemiology of MRSA has continued to evolve since its appearance 30 years ago initially there were sporadic reports of Methicillin Resistance amongst *Staphylococcus aureus* isolates in burns unit and later MRSA became a well established pathogen[6,7].

Our study indicates that the epidemiology of MRSA in country is changing over the past few decades. We reported 43% of MRSA from the burns wound while in previous studies the incidence was found to be 32.8% in1994, 24% in1996, and 32% in 1997. This shows that the incidence of infection by MRSA keeps changing every year and is on a rise since four years[8-10].

Antibiogram showed that all MRSA isolates were significantly resistant to antibiotics as compared to MSSA isolates ($p<0.05$). The resistance of MRSA to ß lactam antibiotics like Ampicillin and Amoxicillin was 100% and 81.3% isolates were resistant to Cloxacillin. Erythromycin and Cephalexin were resistant to 93.0% isolates and 97.6% isolates. This was much higher than the resistance obtained by Vidhani *et al*. This has serious implications as for the treatment of MRSA infection is considered. All MRSA isolates were sensitive to Vancomycin. So there is a need to test new group of antibiotics like Vancomycin and Teicoplanin in a regular routine manner[11]. Broad spectrum antibiotic Chloramphenicol was sensitive to 95.3% MRSA isolates. Further studies regarding the minimum inhibitory concentrations of Chloramphenicol is needed to supplement our data in this regard (Fig. 3). Our study also showed high MIC values of Oxacillin for MRSA isolates. MIC_{50} was found to be 64µg/ml and MIC_{90} was 256 µg/ml. These values were higher than previous studies[11].

Bacteriological typing is an important method of epidemiological typing for *Staphylococcus aureus* but due to the emergence of methicllin resistance, typeability has decreased substantially[12]. Typeability of MRSA by routine set of phages was found to be low when compared to typeability in previous years. This signifies the need for development of new set of phages for MRSA typing. In the present study majority of MRSA isolates belonged to group III phage group, which was comparable to a study by Vidhani *et al*.

In conclusion, the study showed higher prevalence of MRSA in burn infections and higher degree of antibiotic resistance. The potential risk factors for acquisition of MRSA may be due to indiscriminate use of antibiotics, over the counter availability of antibiotics, self medication[13, 14]. Thus the control of MRSA is essential to curtail the introduction and spread of infection. This can be achieved by observing universal precautions and conducting regular epidemiological studies to know the changing trends.

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