EXCELLENT PUBLISHERS

Original Research Article

Risk Factors and Antibiotic Sensitivity Pattern of *Staphylococcus aureus* Isolates from Suppurative Lesions at a Tertiary Care Hospital

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A B S T R A C T

The early detection of Methicillin resistance along with its risk factors is of prime importance in the prognosis of *S. aureus* infections. The marked difference between the antibiogram of MRSA and MSSA isolates creates a difficult scenario in initiating treatment for *S. aureus* infections. The study aims to identify the risk factors and antibiotic sensitivity pattern of MRSA from pus samples. The present study was conducted at the Department of Microbiology, Kanyakumari Medical College from September 2015 to August 2016 from 100 non-duplicate *S. aureus* isolates from pus samples. The *S. aureus* isolates were confirmed by standard methods and methicillin resistance detected by Cefoxitin (30µg) disc. The antibiotic sensitivity pattern was interpreted as per CLSI guidelines. Among the 34 MRSA isolates, 25 were from males and 9 from females of which majority 9(36%) were from boys of ≤15 years of age. Among the risk factors associated with MRSA, surgery had accounted for 26.5% and diabetes 11.8%. Resistance to Erythromycin, Gentamicin and Ciprofloxacin was increasingly noted among MRSA isolates and all of them were sensitive to Vancomycin, Teicoplanin, Tigecycline and Linezolid. The early detection of Methicillin resistance among *S. aureus* together with timely and ideal antibiotic initiation helped by antibiotic sensitivity testing is the need of the hour, which limits the spread of MRSA infections in hospital setting. The role played by a clinical microbiologist in early detection of MRSA is very critical which helps to avoid multidrug resistance, in an era of very limited antibiotics.

Introduction

The cluster-forming micro-organism, *Staphylococcus aureus* (*S. aureus*) is usually associated with a number of human diseases, which involves intense suppuration and necrosis of tissue. The high prevalence of *S. aureus*, together with its propensity to infiltrate tissues, colonize foreign body material, form abscesses and toxins, makes it by far the most feared micro-organism in healthcare associated infections. In recent times, the early detection of Methicillin resistance is of prime importance in the

prognosis of *S. aureus* infections, since a steady rise of them is being noted.

Emergence of Methicillin Resistance *S. aureus* (MRSA) is attributed to multiple factors like longer duration of stay in hospital, admission in an intensive care unit, prolonged antimicrobial therapy, surgical procedures, device exposure (urinary catheter, central venous catheter) and even close proximity to a patient in the hospital who is infected or colonized with MRSA[1]. Infections by MRSA are more difficult to be dealt than those caused by Methicillin-Sensitive *S. aureus* (MSSA) isolates [2]. The marked difference between the antibiogram of MRSA and MSSA isolates creates a difficult scenario in initiating treatment for *S. aureus* infections. MRSA easily spreads among patients in hospital adding to the problem.

The multidrug resistance of MRSA isolates is of alarming problem paving way for costlier treatment regimens.[3] Effort for manufacturing newer antibiotic for Gram positive infections remains uncertain and only fewer antimicrobials remains as alternatives for them. MRSA strains are resistant to all beta-lactam agents, and often to other drugs such as aminoglycosides and fluoroquinolones[2]. At present, Glycopeptides and Linezolid are the only available agents of choice in the treatment of systemic infections with MRSA. The study aims to assess the risk factors for MRSA and their drug susceptibility pattern from pus specimens at a tertiary care teaching hospital in South Tamil Nadu.

Materials and Methods

The present study was conducted at the Department of Microbiology, Kanyakumari Government Medical College from September 2015 to August 2016 from 100 non-duplicate *S. aureus* isolates from pus samples. Informed written consent and a filled in proforma were obtained from the participants involved in the study. The *S. aureus* isolates were confirmed by standard methods and methicillin resistance detected by disc diffusion on Mueller-Hinton agar by Cefoxitin (30µg) disc.

The following antibiotic discs were used to determine the sensitivity of *S. aureus* isolates-Penicillin (10 IU), Erythromycin (15µg), Clindamycin (2µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Clotrimoxazole (1.25/23.75µg), Vancomycin (30µg), Teicoplanin (30µg), Tigecycline (15µg) and Linezolid (30µg) (HiMedia Laboratories, Mumbai, India). The drug sensitivity for the isolates was interpreted as per CLSI guidelines[4]. ATCC 25923 *S. aureus* strain was included for each and every procedure as quality control.

Data analysis

Data regarding the subjects were described in terms of percentages. The ages of the subjects were compared between the genders by student's unpaired 't' test. The sensitive, resistant and intermediately susceptible were described in terms of percentages. The statistical procedures were performed with the help of the statistical software IBM SPSS statistics 20. The p value less than 0.05 was considered as significant (p <0.05) in two tailed test.

Results and Discussion

During the one year period, 100 *S. aureus* isolates were screened for methicillin resistance by Cefoxitin disc diffusion method from pus samples. A total of 34 isolates showed resistance to methicillin while the remaining 66 of them were methicillin sensitive. Among the MRSA isolates, 25 were from males and 9 from females of which majority 9 (36%) were from boys of ≤15 years of age (Figure 1). The mean age of males was

30.7 years and that of females 39.2 years among MRSA isolates and was not found significant (p>0.05).

A total of 24 (75%) and eight (25%) of the MRSA isolates were from patients with less than two weeks stay in hospital and more than two weeks respectively. Figure 2 depicts the categorization of MRSA isolates on infection basis. Most of the MRSA isolates are from Surgery department 11(32.3%) followed by Pediatrics 7(20.6%), Orthopedics 4(11.8%), Obstetrics and Gynecology 3(8.8%), Dermatology 3(8.8%) ENT 1(2.9), Ophthalmology 1(2.9%) and an isolate (2.9%) from Neurosurgery department.

Table 1 shows the risk factors associated with MRSA where surgery had accounted for 26.5% and diabetes 11.8%. Marked differences in antibiotic sensitivity pattern of the MSSA and MRSA isolates were observed (Table 2). Resistance to Erythromycin, Gentamicin and Ciprofloxacin was increasingly noted among MRSA isolates and all of them were sensitive to Vancomycin, Teicoplanin, Tigecycline and Linezolid.

The increasing prevalence of MRSA among clinical specimens, conditions the patient for prolonged treatment and the associated risk factors adds to the problem. The easily available and standard Cefoxitin disc diffusion method picks up Methicillin resistant isolates from the specimens. Difficulty in deciding the initial antibiotic exists for *S. aureus* and so it is wise to perform antibiotic sensitivity testing for all *S. aureus* isolates before initiating treatment.

In this study, majority of the MRSA isolates were from males (25%) while the remaining were females (9%). This is similar to the study by Thangavel *et al.,*[5], where 30% of the MRSA isolates were from males while 10% from females. On the contrary, the study

from Doon valley hospitals[6], showed that most of the MRSA isolates were from females 60.86% while males were 39.13% only. The study by Buzaid *et al.,*[7], found no significant difference between males 28 (31.8%) and females 34 (30.4%) among MRSA isolates.

Majority of the MRSA isolates were from the Surgery department (32.3%), followed by Paediatrics (20.6%), Orthopaedics (11.8%), and Dermatology (8.8%) in the present study. The study by Arora *et al.,*[8], too had found highest prevalence from surgical units (54.8%). Surgery was definitely an associated factor in MRSA infections as those patients are bound to take antimicrobials for long duration. Sarma *et al.,*[9], had found that MRSA was associated with 34% of infections in Orthopaedics and 18% in surgical units but only 1% in medical units. The highest percentages of isolates were from Intensive Care Units (34%) in the study by Sadaka *et al.,*[10].

In this study, almost most of the MRSA were isolated from wound infection (29.4%) while surgical site infections constituted 26.5%. The remaining isolates were from boil/furuncle (20.6%), abscess (14.7%) and burns (2.9%). This is comparable to the study by Terry Ali *et al.,*[11], where wound infection (21.4%), isolated majority of the MRSA isolates. The study from Pondicherry[12], found a high prevalence from post-operative surgical infections (80%). Frazee *et al.,*[13], found that MRSA, was strongly associated with infection type-furuncle. Most of the MRSA isolates were from patients who had undergone surgery (26.5%), while 11.8% isolates with diabetes, 2.9% with burns, 2.9% with HIV and 2.9% with Job's syndrome in the present study. Surgical site infections, both superficial and deep, could be caused by MRSA. Repeated surgeries and hence prolonged stay, makes way for MRSA infections. Among diabetics,

the decrease in the neutrophil activity plays a predominant role for MRSA infection. But the study from New Delhi[14] found a 51.6% association with high risk patients like burns. This was attributed to the longer stay in hospital and the usage of multiple antibiotics.

In this study, duration of stay at hospital was not a risk factor for MRSA infection. Similarly, the study from Northeastern India[9] had found that duration of hospital stay had no significant association with MRSA infection. But Mehta *et al.,*[15], observed significant association of MRSA and hospital stay of more than 15 days duration. Regarding Macrolides, Erythromycin resistance seems to be on rise among MRSA isolates. In the present study, resistance was noted among 5 (7.6%) of the MSSA isolates while it was 21 (61.8%) among MRSA isolates. Arora *et al.,*[8], found that 61.7% of MRSA isolates were resistant to this drug. Frazee *et al.,*[13], and Rao *et al.,* [16], found 56.8% and 45-48% resistance among MRSA isolates respectively. This is in contrast to the study from Coimbatore[17], where the Erythromycin resistance was 20.5% only.

Table.1 MRSA and risk factors

Risk factors	MRSA	
	No	%
Surgery	9	26.5
Diabetes	4	11.8
Burns	1	2.9
Job's syndrome	1	2.9
HIV	1	2.9
Unidentified	18	52.9
Total	34	100

**Table.2** Antibiotic sensitivity pattern of *S. aureus*

Drug	MSSA				MRSA				
	S	I			R	S	I	R	p value
Penicillin	04	-	62		0	-		34	< 0.05
Erythromycin	21	40	05		02	11		21	< 0.05
Clindamycin	46	17	03		11	05		18	> 0.05
Gentamicin	44	04	18		07	03		24	> 0.05
Amikacin	52	07	07		13	09		12	< 0.05
Ciprofloxacin	21	16	29		04	08		22	> 0.05
Cotrimoxazole	28	21	17		10	08		16	>0.05
Vancomycin	66	-	0		34	-		0	> 0.05
Teicoplanin	43	23	0		20	14		00	> 0.05
Tigecycline	66	-	0		34	-		0	>0.05
Linezolid	66	-	0		34	-		0	> 0.05

S= Sensitive, I= Intermediate, R= Resistant

Fig.1 Distribution of MRSA isolates by age and gender
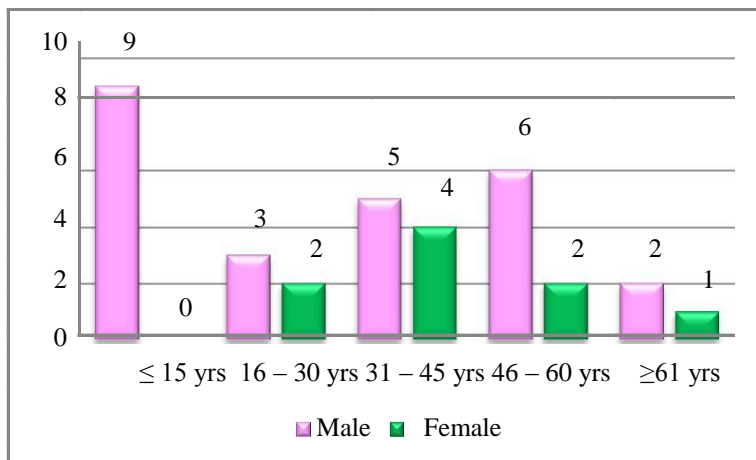
Fig.2 MRSA categorization on infection basis
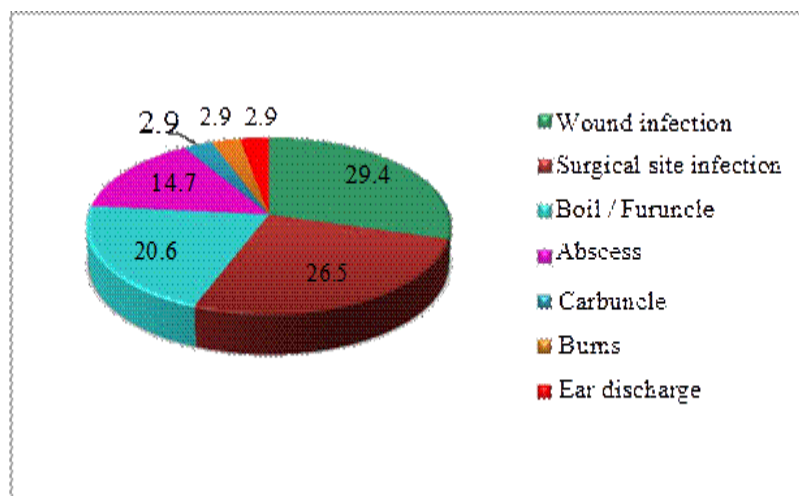
The role of Erythromycin as an alternative among MRSA infections is uncertain because of its higher resistance. Gentamicin resistance was 18 (27.3%) among MSSA isolates and 24 (70.6%) among MRSA isolates. Terry Ali *et al.,*[11] had found 56.5% resistance and the study from Manglore[16] found 40-50% of the MRSA isolates were resistant to Gentamicin. A 100% resistance was observed among the MRSA isolates in the study from Assam[18]. But the study by Rajaduraipandi *et al.,*[17] had reported 20.5% to Gentamicin among MRSA isolates. Among aminoglycosides, the role of

Gentamicin for MRSA infections can be ruled out, as it is frequently administered for Gram negative infections.

In this study, 7 (10.6%) resistance among MSSA isolates and 12 (35.3%) among MRSA isolates were noted for Amikacin. This is similar to the study from Amristar[8], where the resistance to Amikacin was 12 (8.9%) among MSSA isolates and 43 (37.4%) among MRSA isolates. This is in opposition to the study by Mullah *et al.,*[19] where the Amikacin resistance was found to be 52.6%. But a study

from Iran[20], had showed only 13.8% resistance. Amikacin still remains effective for MRSA infections. Resistance to Ciprofloxacin is considered to be surrogate marker for MRSA infections. In this study, Ciprofloxacin resistance was found to be 64.7% among MRSA isolates. The study by Rao *et al.,*[16] found 53-56% resistance for Ciprofloxacin among MRSA isolates. The study by Pai *et al.,*[21] found only 31.8% resistance among MRSA isolates. Quershi *et al.,*[22], found higher resistance among MRSA isolates (90%) to Ciprofloxacin.

In the present study, multidrug resistance was observed more among MRSA isolates like Erythromycin, Clindamycin, Gentamicin, Amikacin, Ciprofloxacin and Co-trimoxazole. These are considered as the first line treatment for MRSA infections and their resistance is of major concern as treatment goes in favour of glycopeptides, which are the reserve drugs and are also expensive. This can give rise to unexpected outbreaks in hospital.

In conclusion, emergence of antibiotic resistance contributes to significant mortality and morbidity and continues to remain as main hindrance in the control of infectious diseases. The early detection of Methicillin resistance among *S. aureus* together with timely and ideal antibiotic initiation helped by antibiotic sensitivity testing is the need of the hour, which limits the spread of MRSA infections in hospital setting. The role played by a clinical microbiologist in early detection of MRSA is very critical which helps to avoid multidrug resistance, in an era of very limited antibiotics.

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