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Increasing trend of methicillin-resistant *Staphylococcus aureus* in Jaipur, Rajasthan, India

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Multidrug resistant strains of *Staphylococcus aureus* have become a serious threat to hospitalized patients and health workers. Recently, this bacterium has emerged as methicillin-resistant *S. aureus* (MRSA) and this bacterial strain has turned into a superbug in the health domain. The main objective of the present study was to find out the prevalence of MRSA in different clinical samples. A total number of 155 clinical samples were collected from Sawai Maan Singh hospital in Jaipur from June, 2014 to January, 2015 and subjected to MRSA screening using biochemical and microbiological methods. An antibiotic sensitivity test was performed for the confirmation of MRSA. Out of 147 strains of Gram positive cocci isolated from clinical samples, 79 (53.74%) were found to be methicillin-resistant. Moreover, this study revealed that the major MRSA isolates were from pus swabs (37.70%) followed by wound swabs (30.40%), hand swabs (8.90%), surgical wound swab (7.60%), axilla swabs (6.32%) and nasal swabs (10.12%).

Key words: Methicillin, *Staphylococcus aureus*, penicillin, oxacillin, cefoxitin.

INTRODUCTION

MRSA stands for methicillin-resistant *Staphylococcus aureus*, which is a general skin bacterium that is resistant to a series of antibiotics such as methicillin, cefoxitin, oxacillin, amoxicillin and penicillin. MRSA strains were primarily described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens which were reported a year after the launch of methicillin (Maple et al., 1989). *S. aureus* has been long recognized as the most important pathogen of hospital acquired infections. Over the past decade, methicillin-resistant *S. aureus* (MRSA) strains have become very common in hospitals worldwide. In accumulation, it is now a developing community pathogen in many geographical regions (Lowy, 1998). MRSA, in addition to being methicillin-resistant, is also resistant to other β-lactam antibiotics, with the exclusion of glycopeptides antibiotics (Chambers, 1997; Brumfitt and Hamilton, 1989). MRSA is associated with high morbidity and mortality rates because of the development of multidrug antibiotic resistance. Rapid and accurate detection of

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Table 1. Isolation of MRSA in different clinical samples from SMS Hospital, Jaipur, Rajasthan, India.

Clinical sample	Sample quantity	Gram positive cocci seen	*Staphylococcus* species	*S. aureus*	MRSA	MRSA (%)
Pus swabs	57	57	20	37	29	37.70
Wound swabs	44	40	10	30	24	30.40
Hand swabs	17	16	6	10	7	8.90
Nasal swabs	16	15	5	10	8	10.12
Surgical wound swabs	12	12	3	09	6	7.60
Axilla swabs	09	07	01	06	5	6.32
Total	155	147	45	102	79	53.74

MRSA is an important role of clinical microbiology laboratories to avoid treatment failure. Prolonged hospital stay, indiscriminate and irregular use of antibiotics, lack of awareness, treating with antibiotics before coming to the hospital, etc., are the factors of MRSA infections appearance (Anupurba et al., 2003). Serious endemic and epidemic MRSA infections occur worldwide as infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staffs promote transmission.

Currently, the treatment options for MRSA infections are limited to very few and costly drugs like teicoplanin, vancomycin, linezolid and Daptomicin. Thus, control of MRSA is essential to curtail the introduction and spread of infection (Siddiqui et al., 2002). The main aim of the study was to find out the occurrence of MRSA in different clinical samples.

MATERIALS AND METHODS

Study design

A total of 155 clinical samples such as pus swabs, axilla swabs, wound swabs, surgical wound swabs, hand swabs and nasal swabs were collected over a period of eight months from June 2014 to January 2015 for research purpose from Sawai Maan Singh (SMS) Hospital in Jaipur district (Rajasthan, India). Sterile dry screw cap cotton swabs (Hi media) were used for the collection of sample. For a collection of axilla swab, nasal swab, hand swab, and the swabs were rubbed, very well by rotating 5 to 7 times over the surface and inner wall of ala and nasal septum. Before sample collection for the isolation of *S. aureus* proper explanation about this study was given to all these patients and consent was taken from them. Detailed history, including age, gender, profession, site of lesion, periods of illness and associated symptoms were also recorded from the patients.

Isolation and biochemical identification of *S. aureus*

A preliminary gram staining was performed to find out the likely organism present. The samples were inoculated on 5% blood agar plates and incubated at 37°C for 18 to 24 h. Morphological examinations were carried out to differentiate *S. aureus* from the other related organisms. Nonetheless, the specimens collected from the patients were subjected to culture on blood agar medium to observe β-haemolysis a defining feature of *S. aureus*. Moreover, biochemical tests, for example catalase and coagulase were performed to confirm the bacteria (Dubey and Padhy, 2012).

Antibiotic sensitivity test

A suspension of each *S. aureus* isolate was prepared to a 0.5 McFarland standard and swabbed on Mueller Hinton Agar (MHA) by lawn streaking. Antibiotic discs were then placed on the streaked agar surface and the plates were incubated overnight at 37°C. Antibiotic sensitivity testing was performed for the following antibiotics: penicillin (10 units/disc), cefoxitin (30 µg/disc), oxacillin (1 µg/disc). These antibiotic discs were obtained from commercial sources (Hi- Media, Mumbai). *S. aureus* ATCC 25923 was used as control strain. The results were interpreted according to the guidelines of the Clinical Laboratory Standards (CLSI, 2009).

RESULTS

A total number of 147 (94.83%) Gram positive cocci reported out of 155 clinical samples. Out of 147 Gram positive bacteria, 57 were from pus swab, 40 from wound swab, 16 from hand swab, 15 from nasal swab, 12 from surgical wound swab, and 7 from axilla swab. Out of a total of 147 Gram positive cocci, 102 (69.39%) were coagulase positive and 45 (30.61%) coagulase negative. Out of 102 *S. aureus*, 37 were from pus swabs, 30 from wound swabs, 10 from hand swabs, 10 from nasal swabs, 09 from surgical wound swabs, and 06 from axilla swabs (Table 1 and Figure 1).

By using Kirby-Bauer's disc diffusion method out of 102 isolates, 79 (53.74%) isolates were MRSA. The MRSA was the most common in pus swabs (37.70%), followed by wound swab (30.40%), hand swabs (8.90%), nasal swabs (10.12%), surgical wound swabs (7.60%), and axilla swabs (6.32%) (Table 1 and Figure 1). All these three antibiotics, that is, penicillin (10 units/disc), cefoxitin (30 µg/disc) and oxacillin (1 µg/disc) are 100% resistant

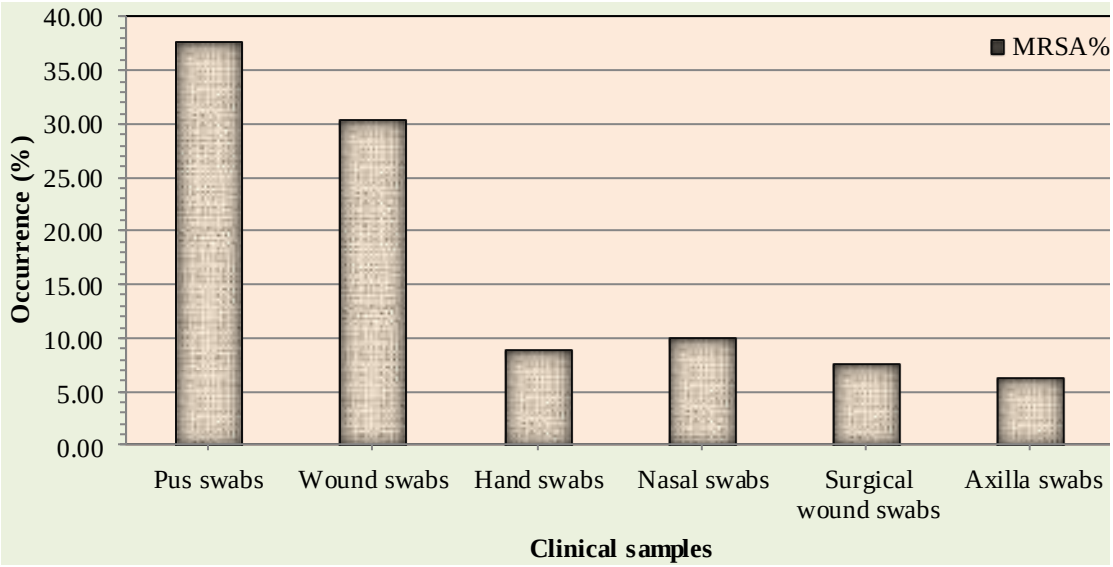

Figure 1. The prevalence of MRSA in different clinical samples.

against 79 (53.74%) caogulase positive isolates.

DISCUSSION

S. aureus is a major human pathogen that is very general and highly virulent. Increased antimicrobial resistance for such an organism is, therefore a cause of concern. In recent years, there has been an alarming increase in the *S. aureus* strains showing resistance to methicillin and reduced susceptibility to vancomycin. The potential reservoirs of MRSA include infectious patients, hospital personnel and hospital environment.

During the earlier period of 15 years, the emergence and world-wide spread of many of such clones have caused major therapeutic problems in many hospitals (Rajaduraipandi et al., 2006). The prevalence of MRSA varies from hospital to hospital in various countries and is constantly high in many countries. In many American and European hospitals, the percentage of MRSA ranged from 29 to 35% (Tahnkiwale et al., 2002; Chaudhary et al., 2009). The incidence of MRSA in India ranges from 30 to 70% (Rajaduraipandi et al., 2006; Verma et al., 2000). In the present study, we have isolated 79 (53.74%) MRSA among 102 *S. aureus* strains from clinical samples. This is an agreement with the study of Assadullah et al. (2003) who also reported prevalence of methicillin-resistant *S. aureus* (52.90%) in Assam. The prevalence of MRSA was reported by the other authors in India. Bandaru et al. (2012) also reported prevalence of MRSA (52%) in Andhra Pradesh. Anupurba et al. (2003) conducted a study of prevalence of MRSA in tertiary care referral hospital in Eastern Uttar Pradesh reported 54.8% occurrence of MRSA. Sasirekha et al. (2012) conduct a study in Bangalore reported out of 198 clinical samples, 153 *S. aureus* were isolated. MRSA screening by phenotypic methods using E-test MIC as standard. Subsequently, biotyping and biofilm production was performed for confirming MRSA isolates. Antibiotic susceptibility test by disc diffusion was also performed for all *S. aureus* isolates. Out of 153 *S. aureus* isolates, 42 (57.7%) were found to be methicillin-resistant. Pandya et al. (2014) worked on characterization of MRSA from various clinical samples at a tertiary care hospital of rural Gujarat, reported among 200 isolates of *S. aureus,* 117 (58.5%) were methicillin-resistant. Dar et al. (2006) studied the molecular epidemiology of clinical and carrier strains of MRSA in the hospital settings of North India reported 54.85% of MRSA from Aligarh Muslim University, Aligarh, India. Bala et al. (2014) conducted a study of prevalence of MRSA and its antibiotic susceptibility pattern in a tertiary health care reported 69.2% prevalence of MRSA from PGI Rohtak. 41% prevalence of MRSA was reported by INSAR in 2013. Rajduraipandi et al. (2006) reported 37.9% of MRSA from Tamilnadu, India. Shinde et al. (2016) also reported 34.61% prevalence of methicillin-resistant from South India.

The prevalence of MRSA was reported by other authors in abroad. Susethira et al. (2015) conducted a study on *S. aureus* nasal carriage among health care workers in a Nepal Hospital reported 46.2% prevalence of MRSA from Nepal. Okon et al. (2013) conducted a study on epidemiology and antibiotic susceptibility pattern

of MRSA recovered from tertiary hospitals reported 12.5% occurrence of MRSA in Nigeria. Hafiz et al. (2002) reported 42% occurrence of MRSA in Pakistan.

In the present study, the high occurrence of MRSA was observed in pus Swab samples (37.70%). Similar prevalence rate of MRSA in pus samples was observed by Pandya et al. (2014) in Gujarat. On the other hand, the prevalence of MRSA in Nasal swabs was found to be 10.12%, followed by surgical wound infection (7.60%). Nasal carriage of *S. aureus* is an important risk factor for developing a surgical site infection as it is a normal flora in the nostrils and the skin. Risk factors for surgical site infection are divided into patient related (preoperative), procedure related (pre-operative), and postoperative categories. Patient related factors again categorized into modifiable (diabetes mellitus, obesity, immuno suppressive drugs, prolonged pre-operative stay) and unmodifiable (age) factors. The preoperative, procedure related factors are class of wound type (clean, clean-contaminated, contaminated, dirty and infected), length of surgery, hair removal, hypoxia and hypothermia. In the immediate postoperative period, glucose control, oxygenation, hypothermia and wound care are the major risk factors (Vidhani et al., 1998).

Conclusion

The present study highlights the prevalence of MRSA and this is a problem for the healthcare sector in India. A high number of MRSA isolates were from pus swabs. So, there is a need to make a strict antibiotic policy and maintaining strict hand hygiene practices in medical staff to avoid cross contamination among patients and to prevent MRSA spread. The regular surveillance of hospital related infections, including monitoring antibiotic sensitivity patterns of MRSA and formulation of definite antibiotic course of action may be helpful in reducing the incidence of MRSA infections.

Conflict of Interests

The authors have not declared any conflict of interests.

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