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Research Article

Hospital-based study of methicillin-resistant *Staphylococcus aureus* in surgical site infections with special reference to determination of environmental and human sources

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ABSTRACT

Background: Surgical site infection (SSI) due to methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with increased morbidity and mortality. Carriage of MRSA by healthcare personnel is a potential source for SSI. The present study was carried out to study the frequency of MRSA in SSI; to identify the most suitable identification test for routine use; and to determine the environmental and human source of MRSA.
Methods: In this prospective study, 195 SSI-pus samples were processed for primary staining, culture and biochemical tests. Cefoxitin and oxacillin disc diffusion test and oxacillin broth dilution test were used to detect MRSA and to determine the minimum inhibitory concentration (MIC) among the identified *S. aureus*. Environment was sampled periodically using air settle plates and swabs from various sites. Healthcare personnel were screened for nasal and hand carriage of MRSA.
Results: Of the 205 isolates, 46 were *S. aureus*, and among these, 18 strains were MRSA. There was no discrepency in the result by any of the three methods used. MRSA carriage, found on the hands of three healthcare personnel, had same anti-biogram as those strains simultaneously obtained from the patients. All three personnel responded to Mupirocin treatment. No MRSA was obtained from the environment.
Conclusions: MRSA is an important source of SSI. Cefoxitin disc diffusion method seems suitable technique for routine use. Periodic screening of healthcare workers for carriage of MRSA will prevent outbreaks of nosocomial infections.

Keywords: MRSA, Oxacillin MIC, Cefoxitin, Surgical site infections

INTRODUCTION

Staphylococcus aureus, a Gram positive coccus, is a major human pathogen and a predominant cause of surgical site infections (SSIs) worldwide with a prevalence rate ranging from 4.6% to 54%.[1] Special interest in *S. aureus* SSI is mainly due to its predominant role in hospital associated infections and emergence of methicillin-resistant *Staphylococcus aureus* (MRSA).[2] SSI due to MRSA are responsible for a seven-fold increased risk of death, 35-fold increased risk of hospital re-admission, more than 3 weeks of additional hospitalization, and additional costs, when compared to that of uninfected controls.[3] The emergence of multidrug resistance MRSA has posed serious therapeutic challenges, leaving glycopeptides as the drug of choice.[4]

Most MRSA infections occur in health care settings and are called health care-associated MRSA (HA-MRSA). Such infections are generally associated with invasive

procedures, intravenous catheters, surgical wounds and open wounds.[5]

Frequently, *S. aureus* is found to colonize the mucosa of the anterior nares.[6] MRSA infection occurring in the community among healthy people is called community-associated MRSA (CA-MRSA) and the vulnerable groups include prisoners and other people who live in crowded conditions. While healthy individuals may harbour MRSA asymptomatically for varying duration, patients with deficient immune systems are at risk for symptomatic secondary infection.

The present study was undertaken to determine the frequency of MRSA in SSIs; to compare oxacillin disk diffusion, cefoxitin disk diffusion and oxacillin Minimum Inhibitory Concentration (MIC) for MRSA; to ascertain the source of MRSA in order to prevent outbreak of MRSA.

METHODS

The present cross-sectional, prospective study was conducted over a period of one year in the department of Microbiology in a teaching hospital in Maharashtra, Western India after obtaining permission of the Institutional Ethics Committee.

A total of 195 pus samples, obtained from patients of surgical site infections, were processed for primary staining and culture on Blood Agar and MacConkey Agar. The cultures were incubated aerobically at 37^0C for 18-24 hour. Strains of *Staphylococcus aureus* were identified by Gram staining, colony morphology, catalase production test, slide and tube coagulase tests, and mannitol fermentation test.[7]

Antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines, by Kirby-Bauer Disk Diffusion Technique on Muller Hinton agar. The plates were incubated at 37^0C for 18-24 hour.[7] Detection of MRSA was done by cefoxitin (30 µg) disk diffusion testing, oxacillin (1µg) disk diffusion testing with 4% NaCl in Muller Hinton agar and incubation temperature of 35ºC for 24-48 hour and MIC of oxacillin by Broth dilution method using Muller-Hinton broth. MIC ≥4µg/ml indicated methicillin resistance.

Detection of organisms in the hospital environment

Air Settle plate study was done every Monday morning from 07.30 to 08.00 hours. Fifty such plates (Nutrient agar and MacConkey agar) were studied in Operation theaters.

Once every month, sterile swabs moistened in nutrient broth were used to collect the samples from operation table, suction machine, Boyle's apparatus, drapes, gloves, dressing trolleys in wards, mattresses, and blankets. These swabs were inoculated on blood agar and MacConkey agar. The aerobic organisms were identified from isolated colonies and subjected to antimicrobial susceptibility tests.

Study of carriers

Sterile swabs, moistened in nutrient broth, were used to swab the anterior nares of nurses in the operation theatre and resident doctors in three surgical departments. Nasal swabs were inoculated on blood agar, MacConkey agar and mannitol salt oxacillin screen agar.

Similarly, finger impressions of both hands of the same persons were taken directly on blood agar, MacConkey agar and mannitol salt oxacillin screen agar. The plates were incubated at 37ºC for 24 hour. The bacteriological study of isolates was done to identify the organism and to study the antibiotic sensitivity pattern.[8]

RESULTS

A total of 208 aerobic bacterial isolates were obtained from 195 pus samples. Of these, 46 (22.1%) were *Staphylococcus aureus.* Eighteen out of 46 (39.13%) were MRSA by all three methods, signifying no discrepancy in the results. The antimicrobial susceptibility of MRSA is given in Table 1.

Table 1: Antibiotic susceptibility of MRSA.

Organism	P	Am	Ox	Va	E	Co	Ak	Cf
MRSA	0 (0%)	0 (0%)	0 (0%)	18 (100%)	4 (22.2%)	10 (55.5%)	12 (66.6%)	6 (33.3%)

P = Penicillin; Am = Ampicillin; Ox = Oxacillin; Va = Vancomycin; E = Erythromycin; Co = Cotrimoxazole; Ak = Amikacin; Cf = Ciprofloxacin.

Broth dilution method for MRSA using Oxacillin: Out of 18 strains, seven had an MIC of 16µg/ml, indicating a very high oxacillin Resistance. Six strains had an MIC of 8µg/ml, whereas five had 4µg/ml (Table 2). Since MIC detection has definitive therapeutic implications and MIC of oxacillin could be judged in broth dilution method, which, though time consuming and labour intensive, seems the best method to detect MRSA.

Air settles plates

The frequency of organisms obtained from 50 air settle plates exposed in the operation theatre was-Coagulase Negative Staphylococci (CoNS) 14 (28%), Methicillin Susceptible *Staphylococcus aureus* 5 (10%), *E.coli* 2 (4%). None of the plates showed presence of MRSA.

Table 2: MIC and size of inhibition zone of MRSA isolates.

MRSA strain No	Oxacillin MIC (µg/ml)	Zone of inhibition (mm)	
		Oxacillin	Cefoxitin
1	16	7	7
2	4	9	14
3	8	8	12
4	16	6	6
5	8	7	8
6	16	6	9
7	8	8	7
8	4	8	9
9	8	8	9
10	16	7	9
11	8	8	11
12	8	7	7
13	16	6	6
14	4	8	12
15	4	9	13
16	4	8	10
17	16	6	6
18	16	6	8

Carriers

From nurses in the operation theatre and doctors in three surgical departments, 35 nasal swabs and 70 fingerprints were obtained on blood agar, MacConkey agar and mannitol oxacillin salt agar. None of the nasal swabs or fingerprints was found sterile (Table 3).

The details of antimicrobial susceptibility of isolates from carriers are given in Table 4.

Three strains of MRSA were obtained from two staff nurses and one junior resident. During the same period, 5 cases of surgical site infection by MRSA had occurred in a single unit of surgical ward. The nasal swabs from all of the 5 patients were negative for MRSA. The antibiogram of the isolate from nasal swab of surgical resident was identical with that of the isolated from patients.

Table 3: Isolates from nasal swabs (n=35) and fingers (n=70) of operation theatre staff.

Organism	Isolates from nasal swabs (n = 35)	Isolates from fingers (n = 70)
CoNS	11 (31.4%)	46 (65.7%)
MSSA	7 (20%)	14 (20%)
MRSA	3 (8.5%)	0 (0%)
Strepto. spp	17 (48.5%)	10 (14.2%)
E.coli	2 (5.7%)	8 (11.4%)

CoNS=Coagulase Negative Staphylococci; MSSA=Methicillin Susceptible *Staphylococcus aureus*; MRSA=Methicillin Resistant *Staphylococcus aureus*

Table 4: Antibiotic susceptibility of isolates from carriers.

Organism	P	Am	Ox	Va	E	Co	Ak	Cf
MRSA	0 (0%)	0 (0%)	0 (0%)	3 (100%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)

P = Penicillin; Am = Ampicillin; Ox = Oxacillin; Va = Vancomycin; E = Erythromycin; Co = Cotrimoxazole; Ak = Amikacin; Cf = Ciprofloxacin

One week after mupirocin treatment, swabs of resident doctor and staff nurses were found negative for MRSA. This study helped prevent similar future incidences.

DISCUSSION

Staphylococcus aureus has developed resistance to most classes of antimicrobial agents. Penicillin was the first choice of antibiotics to treat Staphylococcal infections.[1] In 1944, by destroying the penicillin by penicillinase, *S. aureus* became resistant to it.[9]

More than 90% strains of *S. aureus* are resistant to penicillin. Outbreaks of multi-resiastant *S. aureus* were overcome with penicillinase-stable penicillin.

Methicillin, semi-synthetic penicillin, was used to treat penicillin resistant *Staphylococcus aureus* but MRSA strains emerged within a year of drug's launch. Some early MRSA strains were colonist rather than invaders but by 1998-1999, its proportion rose to 34-37%. This was because of dissemination of two new epidemic strains, EMRSA 15 and 16. These might be more virulent than earlier MRSA or this dissemination reflected changing hospital practices.[10]

MRSA SSI are significantly associated with discharge to long term care facility, duration of post-operative antibiotic therapy of more than 24 hour, use of surgical drains for more than 24 hour. None of the other pre-operative patient attributes like, age, diabetes, lack of

vancomycin as surgical prophylaxis, and intra-operative factors, such as, surgical site, class, in patient status, duration of surgery, surgeon appeared to be independent predictors of MRSA SSI among infected patients, which have been reported risk factors in SSI caused by organisms other than MRSA.[11]

The frequency of MRSA in the current study is 39.13% of *S. aureus*. This is slightly higher than that mentioned in other studies which range from 15.4% to 28.6%.[12-14]

Detection of MRSA by different methods

The results of oxacillin disk diffusion and cefoxitin disk diffusion tests were comparable in this study. But oxacillin disk diffusion test makes use of special medium (Muller-Hinton Agar with 4% NaCl supplement) and optimum temperature required for incubation also differs slightly (35ºC) than others (37ºC).

This calls for an extra efforts and investment of material. Also, the time required for incubation to obtain reliable results is not less than 24 hour. As compared to this, cefoxitin disk can be placed on the routine media. Plate can be incubated at 37ºC for 18 hour. Hence it can be used along with other antibiotic discs. Hence according to this study, cefoxitin disk diffusion test is most suitable as a routine laboratory method. Also, cefoxitin is a surrogate marker and detects all staphylococci that are *mec A* positive, *S. aureus* and CoNS.

The discs are stable and give accurate results allowing the discrimination between MRSA and borderline resistant *S. aureus*.[15,16] Cefoxitin disc testing has been shown to detect MRSA with 100% specificity and 100 % sensitivity, while oxacillin disc testing had a specificity of 100% and sensitivity of 95.2-96.4%, depending on the inoculum used.[17] The oxacillin disk diffusion method was found least reliable, with a specificity of 89.8% and a sensitivity of 96.5%.[18]

The conventional MRSA detection assays are simple and relatively cheap for detecting methicillin resistance. The sensitivity and specificity of conventional methods were found to be 97% and 100%, respectively. However difficulties occur when organisms have their MICs near the break point.[19]

Heterogenous nature of resistance in many strains to methicillin and oxacillin makes its detection difficult. Even automated susceptibility testing systems misclassify susceptible *S. aureus* strains as MRSA.[18] In such instances, molecular techniques are useful for the detection of mec A gene.[19]

The MIC testing has a definite clinical implication. ampicillin-sulbactam therapy is likely to be effective for the strains having low levels of resistance to oxacillin (<16 µg/ml).[18] Thus we can prevent overuse and abuse of vancomycin and other drugs in low-level oxacillin resistance.

Study of carriers

A highly variable MRSA carriage rate ranging from 0-22.2% in healthcare workers has been reported.[20,21] The results of the current study are comparable. In a study from south India, a very high rate of nasal carriage (57.9%) followed by conjunctival carriage (40%) has been reported.[21] In the current study, 14.2% penicillin resistance is observed in MSSA, while another study has reported 100% resistance.[21]

Study of environmental samples

Bacteriological study of operation theatre, various articles, surgical ward was done once a month. It did not demonstrate any growth from operation table, floor and walls of operation theater, suction machine, Boyle's apparatus, drapes and gloves.

However, from dressing trolley, positive cultures were obtained 5 times, showing growth of CoNS (4), *B. subtilis* (1). Bacterial growth was obtained from mattresses and blankets 13 out of 24 times, demonstrating CoNS (7), *S. aureus* (2), *K. pneumonia* (2), *B. subtilis* (2). No MRSA was isolated from operation theatre.

Limitations

The limitations of the present study are that phage typing and PCR studies for detection of *mecA* gene of the MRSA isolates could not be done due to resource constraints.

CONCLUSION

MRSA is an important cause of surgical site infections. Among the three tests available for detection, cefoxitin disc diffusion test is simple, easy to perform as routine antimicrobial susceptibility testing and gives satisfactory results. MRSA carriage in healthcare personnel is potential focus for spread of hospital acquired infections. Simple periodic screening will prevent such outbreaks and the further consequences.

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