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MRSA Threat in a Tertiary Care Hospital in North India

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Staphylococcus aureus **is known to cause both hospital and community acquired infections. Hospital acquired strains are usually multidrug resistant. Treatment of infection due to these organisms and their eradication is very difficult. Monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community. The present study was conducted in the Department of Microbiology, JNMC, India for a period of two years. All subjects in the study were divided into two groups: GROUP 1- consisted of patients admitted in various wards. GROUP 2: Consisted of the health care workers. Detection of methicillin susceptibility was done by both phenotypic and by genotypic methods. Antibiotic susceptibility testing of all the MRSA strains was done. A total of 412 *S. aureus* were isolated from the clinical samples. 138(33.49%) of the clinical isolates were found to be methicillin resistant by oxacillin disc diffusion method. However 134(32.44%) isolates were confirmed to be methicillin resistant genotypically. Prevalence of MRSA was highest amongst the orthopaedics 56(46.67%) and the surgery 34(35.42%) wards. 109(81.34%) were MDR-MRSA (resistant to >3 antibiotics). 34% of the health care workers were MRSA carriers. Phage typing showed that among the clinical MRSA strains group I was the predominant phage type. The resistance of MRSA towards commonly used antibiotics is alarmingly high. Health care workers are an important source of transmission of infection between the hospitalised patients and they should acclimatize to proper hand washing and other simple infection control practices to inhibit such transmission.**

Key words: Antimicrobial resistance, MRSA, Transmission.

Staphylococcus aureus is a frequent and important human pathogen which is also found as non-pathogenic microorganism in human samples[1-5] . Around a third of humans are colonised with *S.aureus*[6] .It is known to cause both hospital and community acquired infections[4,7-9]. Since methicillin resistant *S.aureus* (MRSA) was first described in 1961 in England[10] it has become an important problem around the world . Hospital acquired strains are usually multidrug resistant and pose a serious threat for the patients who are already in a state of immunological challenge because of the diseased state. One study demonstrated that a patients normal colonising flora changes within 24-48 hours under selective antibiotic pressures[11] . Treatment of infection due to these organisms and their eradication is very difficult. Monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community.

The present study was undertaken with the aim of determining the phenotypic and genotypic epidemiology of MRSA from clinical isolates and from healthy hospital personnels in a tertiary care hospital in northern India.

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MATERIAL AND METHODS

The present study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh, India for a period of two years from August 2005 to July 2007.

All subjects in the study were divided into two groups

Group 1

Consisted of patients admitted in variuos wards of JNMCH for various surgical and nonsurgical treatment (duration of stay more than 48 hours).These patients were considered to have hospital acquired infection. Various samples were collected from different sites from these patients as indicated by the illness.

Group 2

Consisted of the health care workers including the doctors and the nursing staff in the wards of JNMCH. These were taken as healthy carriers. Screening of the health care workers was done by the following method: a moistened sterile swab was rotated in each nostril 5 times and then inoculated on Mueller Hinton agar with 4% NaCl[12].

Only those samples from which *Staphylococcus aureus* was isolated were included in the study.

Criteria for exclusion: Indoor patients with isolation of MRSA within 48 hours of hospital admission and health care workers having history of MRSA infection in the last one year.

The samples were inoculated on 5-10% sheep blood agar, MacConkey agar, Mannitol salt agar and Robertson's cooked meat broth. All the isolates suggestive of *S.aureus* were identified by the standard biochemical procedures[13]. The methicillin susceptible strain ATCC 25923 was used as a control for the diagnostic procedures. All isolates were maintained in 0.5%-1% semisolid nutrient agar stabs and sealed with cork stoppers soaked with hot sterile paraffin until analysed[13].

Oxacillin disc diffusion test: All the isolates were subjected to oxacillin disc diffusion test using oxacillin 1µg disc.A 0.5 McFarland turbidity standard suspension of the isolate was made and lawn culture was done on Mueller-Hinton agar (MHA) plates containing 4% NaCl. Plates were incubated at 35°C for 18 hours and zone diameters were measured. An inhibition zone diameter of ≤10mm was reported as methicillin resistant and ≥13mm was taken as methicillin sensitive .

MIC determination: MIC was determined by agar dilution test. 10 different dilutions of oxacillin were selected such that the concentrations that allowed determination of MIC breakpoints defining susceptible (≤2µg/ml)[14] and resistant (≥4µg/ml)[14] values were included. Lowest concentration at which the growth was inhibited by 80% or more was recorded as MIC.

PCR amplification for mec A and fem B genes: Multiplex PCR[15] was carried out on all the *S.aureus* strains found methicillin resistant on MIC determination. All the MRSA strains were analysed for the *mec A* and *femB* genes using the following oligonucleotides sequence.*mec* A1-5' GTA GAA ATG ACT GAA CGT CCG ATA A-3', *mec* A2-5' CCA ATT CCA CAT TGT TTC CGT CTA A-3', *fem* B1-5' TTA CAG AGT TAA CTG TTA CC-3', *fem* B2-5' ATA CAA ATC CAG CAC GCT CT-3'. A 50 µl PCR reaction mixture consisted of 45 µl of mastermix containing PCR buffer (1X), d NTP mix(0.2mM of each), primer(0.5µM), Taq DNA polymerase(0.25U), and $MgCl_2$(1.5mM) with 5 µl of template DNA. Cycling parameters were set to- hot start 94°C for 4 minutes followed by 35 cycles of melting at 94°C for 45 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 1 minute. Analysis of amplified products was done by gel electrophoresis. Amplicons of 310bp were consistent with *mec* A and of 651bp with *fem* B gene amplification.

Antibiotic susceptibility testing of all the MRSA strains was done using Kirby Bauer's disk diffusion method (as per CSLI guidelines) for the following antimicrobial agents. amikacin 30 µg, ciprofloxacin 5 µg, clindamycin 2 µg, cotrimoxazole 25 µg, erythromycin 15 µg, gatifloxacin 5 µg, gentamycin 10 µg, levofloxacin 5 µg , linezolid 30 µg, ofloxacin 5 µg, sparfloxacin 5 µg, vancomycin 30 µg.

Phage typing of the MRSA strains isolated from the indoor patients and the health care workers was carried out by the standard method described by Blairs & Williams (1961) at the National Staphylococcal Phage Centre, Department of Microbiology, Maulana Azad Medical College, New Delhi.

RESULTS

A total of 412 *S. aureus* were isolated from the clinical samples. Around 50% of the *S.aureus* isolated were from the orthopaedics 120(28.85%) and the surgery 96(23.08%) wards. Out of 412 *S. aureus* strains 138(33.49%) were found to be methicillin resistant on phenotypic detection by oxacillin disc diffusion test. However, on genotypic detection with the help of multiplex PCR 134(32.44%) strains had both *mec* A(310bp) and *fem* B(651bp) gene and were confirmed to be methicillin resistant. MIC of all the MRSA isolates was more than 4µg/ml but none was greater than 256µg/ml (Table 1). Prevalence of MRSA was highest amongst the orthopaedics 56(46.67%) and the surgery 34(35.42%) wards (Table 2). Out of 134 MRSA strains 109(81.34%) were MDR-MRSA(resistant to >3 antibiotics) and only 25(18.66%) strains were nonMDR-MRSA (Table 3). 100(74.63%) strains were found to be resistant to five or more antibiotics. A significant number of strains were resistant to eight or more antibiotics (Table 3). Maximum resistance was shown to cotrimoxazole (89.41%) followed by clindamycin (83.53%) ciprofloxacin (81.17%), gentamycin (69.41%), erythromycin (62.35%) and amikacin (60.00%). Moderate resistance was shown to chloramphenicol(54.12%), gatifloxacin (45.88), sparfloxacin(38.88%) and low level resistance was shown to ofloxacin(23.53%),and levofloxacin(16.47%). All the MRSA isolates were uniformly sensitive to vancomycin and linezolid.

Out of the 100 nasal swabs collected from the health care workers, *S.aureus* was isolated in 38%. Among these 34 isolates were methicillin resistant i.e. 34% of the health care workers were MRSA carriers.

Phage typing showed that among the clinical MRSA strains group I was the predominant phage type. However majority of the strains (71.11%) remained nontypeable by the conventional set of phages . These strains when typed by the MRSA set of phages 6.25% became typable by MR25 and 6.25% by M5/C33. 87.5% nontypable strains still remained nontypeable by the MRSA phages. Among the carrier MRSA strains, phage group I of the conventional set and

Table 1. Isolation rate of s.aureus from various wards in hospital (n= 412)

Hospital wards	Non- MRSA N (%)	MRSA N (%)	*S. aureus* N
Orthopaedics	64(53.33)	56(46.67)	120
Surgery	62(64.58)	34(35.42)	96
Paediatrics	26(72.22)	10(27.78)	36
Gynaecology	28(75.68)	9(24.32)	37
Medicine	14(73.68)	5(26.32)	19
ICU	6(60)	4(40)	10
ENT	11(68.75)	5(31.25)	16
TB & CHEST	8(88.89)	1(11.11)	9
Ophtahlmology	8(88.89)	1(11.11)	9
Skin	19(95)	1(5)	20
Plastic Surgery (Burn)	13(65)	7(35)	20
Nursery	19(95)	1(5)	20
Total	278(67.47)	134(32.53)	412

Table 2. Minimum inhibitory concentration (MIC) of oxacillin by agar dilution method (n = 134)

Test method	No. of strains tested	MIC ((µg/mL) 0.5	1	2	4	8	16	32	64	128	256	>256
Agar Dilution method	85	0	0	0	0	28	59	18	14	11	3	0

Table 3. Pattern of resistance of MRSA isolates to other drugs (n = 134)

No. of other drugs		No. of MRSA isolates (%)
n MDR	0	0(0.00)
	1	6(4.71)
	2	8(5.88)
	3	11(8.24)
MDR	4	9(7.06)
	5	20(15.29)
	6	16(11.76)
	7	9(7.06)
	8	19(14.12)
	9	16(11.76)
	10	13(9.41)
	11	5(3.53)
	12	2(1.18)
Total		134(100.00)

MR25 of the MRSA phages was the commonest phage pattern found similar to the clinical strains.

DISCUSSION

The epidemiology of MRSA has continued to evolve since it was first reported in 1961 by Jevons. Initially there were sporadic reports of methicillin resistance amongst nosocomial *Staphylococcus aureus* isolates but later MRSA became a well established hospital acquired pathogen and represents a serious threat to the health of the hospitalized patients. The important reservoirs of MRSA in hospitalized patients are infected or colonised patients and transient hand carriage on the hands of the health care workers is the predominant mode of patient to patient transmission[16].

In this study, the prevalence and antimicrobial susceptibility pattern of MRSA isolates from two different groups was evaluated : indoor patients as group I and group II comprised of the hospital staff as carriers. Our study indicates that 32.44% of the clinical *S.aureus* isolates were methicillin resistant. Similar findings have been reported from other parts of India[17-20]. Amongst the clinical isolates methicillin resistance was highest among the staphylococci isolated from the orthopaedics 56(46.67%) and the surgery 34(35.42%) wards. This might be because orthopaedics and surgery provide fertile environment for the MRSA to flourish. The open wounds and the frequent dressing changes often necessitates a dressing team or multiple persons plus the prolonged stay of patients in these wards might help in MRSA colonisation. Although the number of staphylococci isolated was less among the ICU 10(2.4%) and the plastic surgery ward 20(4.8%) but most of these were methicillin resistant- 40% in ICU and 35% in the plastic surgery wards. Prolonged stay and the inherent immunosuppression of the ICU and the burn patients might lead to MRSA colonisation. The antimicrobial sensitivity results showed around 75% of the MRSA were multidrug resistant. It was found that highest resistance of around 90% was shown to cotrimoxazole followed by clindamycin (83.53%), ciprofloxacin(81.17%), gentamycin (69,41%), erythromycin(62.35%) and amikacin (60.00%). Sensitivity was better for ofloxacin (23.53%) and levofloxacin (16.47%). Amongst all the antimicrobials tested vancomycin and linezolid were the only drugs to which all the MRSA strains were uniformly sensitive. Most of the studies quote similar antimicrobial sensitivity pattern with nil glycopeptides resistance[17,18,20], although decreased sensitivity as well as resistance to vancomycin has been reported recently[21-23].

A significant observation in this study was the increased isolation of MRSA (34%) from the carrier screening samples which are mainly the health care workers. This is an alarming observation despite the fact that carrier screening samples are less as compared to the clinical samples. Contemporary literature shows highly variable carrier rate ranging from 0-29%[12,24,25]. These carriers might be an important source of the MRSA acquired by the hospitalised patients, as colonisation with *Staphylococcus aureus* is a necessary step during pathogenesis of MRSA infection and it is a source of cross transmission between humans[26,27]. On phage typing these isolates were found to be similar to the clinical isolates. Group I of the conventional set of phages and MR25 amongst the MRSA phages were the commonest.

In conclusion, the resistance of MRSA towards commonly used antibiotics is alarmingly high and vancomycin and linezolid are the only antibiotics that are uniformly sensitive and can be used as the drug of choice for MRSA infections.

However, these drugs should not be used blindly and antimicrobial susceptibility testing of all the clinical isolates should be done before prescribing treatment since reports of glycopeptides resistance are coming up.

Health care workers are an important source of transmission of infection between the hospitalised patients and they should acclimatize to proper hand washing and other simple infection control practices to inhibit such transmission. We suggest the use of 2% chlorhexidine gel and/or mupirocin for the treatment of MRSA amongst the carriers.

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