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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN A RURAL TERTIARY CARE HOSPITAL IN NORTH INDIA.

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ABSTRACT : The emergence of *Methicillin-resistant Staphylococcus aureus* (MRSA) has posed a serious therapeutic challenge. We report the prevalence and antibiotic susceptibility pattern of MRSA in the hospitalized patients in a rural tertiary care hospital in India. The study comprised of 97 *Staphylococcus aureus* isolated from a total of 400 clinical samples obtained from hospitalized patients. Antibiotic susceptibility testing was performed and interpreted as per standard guidelines. Methicillin resistance was detected using oxacillin and cefoxitin disc diffusion method, oxacillin screen agar and Chrom agar method, minimum inhibitory concentration using E test, and Latex agglutination method for PBP2a detection.Methicillin resistance with cefoxitin disc diffusion was 47.42%, with oxacillin disc diffusion 54.64%, Oxacillin screen agar 46.34% and Latex agglutination 45.36%. Chrom agar showed low sensitivity (77.27%) as well as specificity (79.25%) in detecting MRSA. MIC detection with E test resulted in 42.27% strains giving MIC between 8-16µg/ml. Multidrug resistance was observed in majority of MRSA strains. However, no strain was resistant to Vancomycin, Linezolid or Teicoplanin. To reduce the prevalence of MRSA, regular surveillance of hospital acquired infection and monitoring of antibiotic susceptibility pattern is the need of the hour. Proper detection of all MRSA with rapid and accurate methods must be done as a routine laboratory procedure.

Key words: Antimicrobial Methicillin-resistant *Staphylococcus Aureus*

INTRODUCTION

Hospital acquired infections represent a significant epidemiological threat these days, in both developed and developing countries. *Staphylococcus aureus* is one of the principal human pathogens that colonizes healthy individuals as well as causes severe infections in hospitalized patients, especially in high risk areas like ICUs, burn wards, surgical post operative wards and skin wards. They have a differential ability to spread and cause outbreaks in hospitals.[1] The treatment of infections caused by these organisms has become problematic due to the development of methicillin resistance. MRSA strains harbour the mecA gene which encodes a modified penicillin binding protein (PBP2a) having low affinity for methicillin and all β-lactam antibiotics, and resistance to this antibiotic implies resistance to all β-lactam antibiotics.[2] MRSA infections may contribute to longer hospital stays, significantly increase the cost of medical care and are likely to have an important role in the development of antimicrobial resistance. Hence, rapid and accurate identification of MRSA is required for therapeutic and epidemiological reasons; to immediately start the appropriate antimicrobial therapy and to avoid the spread of these strains.[3]

Conventional phenotypic methods to detect methicillin resistance have many discrepancies because expression of resistance is subject to environmental & conditional variations. Errors in determining oxacillin resistance may have serious adverse clinical consequences. False negative susceptibility results may lead to treatment failure and the spread of MRSA, especially if appropriate infection control measures are not applied. Conversely, improper detection of resistance may increase health care cost following unnecessary isolation precautions and may lead to overuse of glycopeptides such as vancomycin. So, accurate detection of MRSA is the need of the hour. The knowledge of prevalence of MRSA and their antimicrobial susceptibility pattern is a must for appropriate treatment of these infections. So, the present study was attempted to know the prevalence of MRSA & their antimicrobial profile in hospitalized patients of our rural tertiary care hospital.

MATERIAL AND METHODS

The study was conducted in the department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, a rural tertiary hospital of Amritsar. Four hundred samples from patients admitted in various departments, presenting with postoperative wound infections and skin lesions, were collected using aseptic precautions. A total of 97 *Staphylococcus aureus* strains isolated from these 400 samples were identified and characterized as per standard recommendations.[4] The isolates were then subjected to antimicrobial susceptibility tests by Kirby Bauer Disc Diffusion Method.[5] Antibiotics tested were penicillin (10 units), ampicillin (10 µg), cephalexin (30 µg), oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), ciprofloxacin (5 µg), ofloxacin, gentamicin (10 µg), amikacin (30 µg), netilmicin (30 µg), linezolid (30 µg), vancomycin (30 µg), teicoplanin (30 µg), chloramphenicol (30 µg).(Hi Media Mumbai). Zone diameters were measured following CLSI criteria.[6] ATCC 29213 strain was used as a control strain.

All the isolates were tested for methicillin resistance by disc diffusion method using oxacillin (1 µg), cefoxitin (30 µg), oxacillin screen agar (OSA) method, chrom agar method, E test to know minimum inhibitory concentration (MIC)) and latex agglutination (Slidex MRSA). . Known positive control MRSA (ATCC 43300) was included in each set.

Oxacillin screen agar [7] – Mueller Hinton Agar (MHA) with 4% NaCl and Oxacillin 6µg/ml, was prepared. The 0.5 mcFarland suspension of the test strains was inoculated as spots over the plates, which were incubated at 35ºC for 24hours. The strains which were able to grow on this medium were designated as MRSA

CHROM agar (HIMEDIA)[8] – M1674 HiChrome MeReSa Agar, Base (HiChrome Methicillin Resistant *Staphylococcus aureus* Agar Base: MRSA Chromogenic Agar) from (HiMedia Laboratory Pvt. Ltd, Mumbai, India) was also used to screen MRSA. The test was performed as per instructions by the manufacturer. After incubation at 37ºC for 18-24 hours, bluish green colored colonies of the isolate were reported as MRSA.

Oxacillin MIC [9] – MIC determination by E Test (AB Biodisk, bioMerieux) using Oxacillin strips was done. The test was performed as per instructions by the manufacturer. The strains for which MIC was > 4µg/ml were considered resistant.

Latex agglutination method (Slidex MRSA)[10]: All the 97 *Staphylococcus aureus* strains were tested for presence of mecA gene product PBP2a by Latex agglutination method (Slidex MRSA from Biomerieux). The test was performed as per instructions by the manufacturer. Agglutination with the sensitized latex particles was taken as positive.

RESULTS

Out of the 400 clinical samples, growth was obtained in 364 isolates, the isolation rate being 91%. Out of these, 97 (26.64%) were Staphylococcus aureus. The maximum isolation of Staphylococcus aureus was from Orthopaedics ward (28.86%), followed by surgery (21.65%) and medicine (16.49%). Table 1.

Table 1: Distribution pattern of 97 *staphylococcus aureus* isolates in various wards

NAME OF DEPARTMENT	NUMBER OF SAMPLES	%AGE OF STAPHYLOCOCCUS AUREUS ISOLATED
SURGERY	21	21.65%
ORTHOPAEDICS	**28**	**28.86%**
MEDICINE	16	16.49%
PAEDIATRICS	13	13.40%
GYNAECOLOGY	05	5.51%
SKIN	03	3.09%
EMERGENCY	05	5.51%
CANCER	02	2.06%
ICU	04	4.12%
TOTAL	97	100%

All these staphylococcus aureus isolates were subjected to MRSA detection by six phenotypic methods, which resulted in detection of 46 isolates as MRSA (47.42%) by Cefoxitin disc diffusion method, while 53 MRSA (56.64%) were identified by Oxacillin disc diffusion method, including 03 showing intermediate zones. Oxacillin screen agar gave comparable results with that of Cefoxitin disc diffusion, detecting 45 (46.39%) strains. Chrom agar detected 45 MRSA including 11 false positives, which were identified as MSSA by other methods. MIC detection by E test resulted in 41 isolates with MIC between 8-16 µg/ml, while the rest 05 strains which were resistant with Cefoxitin disc diffusion, had an MIC between 2-4µg/ml. Out of 46 MRSA strains detected by cefoxitin DD method, 44 strains showed positive reaction for the presence of PBP2a by Latex agglutination (slidex MRSA).{Table 2}
All MRSA strains were highly resistant to penicillin (100%), Cephalexin (88.63%), Ampicillin (77.27%), Ciprofloxacin (75%), Gentamicin (75%) and Ofloxacin (68.18%) while moderately resistant to Clindamycin (38.60%) and Erythromycin (34.09%).

Table 2: Detection of MRSA by different methods:

BACTERIAL ISOLATE	CEFOXITIN DD	OXACILLIN DD	OXACILLIN SCREEN AGAR	CHROM AGAR	E TEST & BROTH DILUTION	LATEX AGGLUTINATION
MRSA	46 (47.42%)	53 (54.64%)	45 (46.39%)	45 (46.39%)	41 (42.27%)	44 (45.36%)
MSSA	51(52.58%)	44(45.36%)	52 (53.61%)	52 (53.61%)	56(57.73%)	53 (54.64%)
TOTAL	97	97	97	97	97	97

MRSA were found to be more multidrug resistant as compared to MSSA. MSSA strains were moderately resistant to Ampicillin (56.60%), Ciprofloxacin (54.71%), Cephalexin (45.28%), Ofloxacin (39.62%), Gentamicin (32.07%), Clindamycin (28.30%) and Erythromycin (26.41%).Low level resistance was observed to Amikacin (11.32%), Chloramphenicol (9.43%) and Netilmicin (6.82%). All (100%) the strains were sensitive to Vancomycin, Linezolid and Teicoplanin, irrespective of their methicillin status.

DISCUSSION

MRSA are being recognized as highly virulent and important human pathogens causing significant morbidity & mortality in hospitals and community and are difficult to eradicate because they are multidrug resistant.The prevalence of MRSA in our hospital was found to be 45.36%. Similar isolation rate was also found in studies from different parts of India, ranging from 40.6% to 54.85% to 59.3%.[11,12,13] however, 26.4% and 19.5% prevalence has also been reported in some studies.[14,15] This variation might be because of variation in antibiotic usage and infection control practices in different hospitals. In the present study, maximum isolation of *Staphylococcus aureus* was from Orthopaedics ward (28.86%), followed by Surgery (21.65%) and Medicine (16.49%). Similarly, in a study by Sanjana RK et al (2008), the majority of the samples were obtained from Surgery (24%) and Orthopaedics units (16%). [16]
We attempted to evaluate six phenotypic methods for the detection of MRSA. Latex agglutination (Slidex MRSA kit, Biomerieux) method was used for the detection of PBP2a protein, which is the protein product of mecA gene. This method detected 44 *Staph aureus* strains as MRSA (45.36%) and 53 as MSSA (54.64%). The results of our study are coherent with the other studies like, David velasco et al (2005), [17] who used two kits for PBP2a detection, MRSA-screen latex agglutination test kit and Slidex MRSA Detection kit. Both kits tested in this study showed high sensitivity (100%) and specificity (96%) and concluded that the Slidex MRSA Detection kit is a reliable method of detecting methicillin resistance.
Cefoxitin disc diffusion was found to be highly sensitive (100%) and specific (96.23%) while sensitivity of Oxacillin disc diffusion was 93.18% and specificity 77.36% when compared with latex agglutination test. The results of disc diffusion methods showed that cefoxitin disc diffusion is a better method of MRSA detection than Oxacillin disc diffusion.

Similar results were quoted by several other studies.[17,18,19] Seven strains of *S. aureus* that were resistant to oxacillin but sensitive to cefoxitin ,had MIC values <2mcg/ml.These strains probably are BORSA(borderline resistant strains) that hyper produce beta lactamase and while they appear oxacillin resistant, do not possess the usual genetic mechanism for such resistance. This was corroborated by the fact that all the isolates that were resistant to oxacillin but sensitive to cefoxitin were negative for PBP 2a detection by Latex agglutination (Slidex MRSA kit, bioMerieux). Sensitivity & specificity of Oxacillin screen agar were 97.73% and 96.23% respectively. The results of OSA were comparable with that of cefoxitin DD method.Similar findings were reported by a study done by HK Tiwari et al.[13] Chrom agar was found to be less sensitive (77.27%) & less specific(79.25%) .Results of E test for MIC (oxacillin) were not so accurate detecting only 41 MRSA (42.27%) giving a sensitivity of 90.91 % and specificity 98.11 %. Strains possessing mecA gene are either heterogenous or homogenous in their expression. Heteroresistant strains may show lower expression resulting in MICs that appear susceptible. Results of cefoxitin DD method were in concordance with Latex agglutination test. The findings of our study are coherent with the other studies.[17]

In the present study MRSA strains were found more multidrug resistant as compared to MSSA strains. Table 3. All Staph aureus isolates, irrespective of their methicillin status were, sensitive to Vancomycin, Linezolid and Teicoplanin. High level of sensitivity was also observed to Netilmicin (93.18%), Chloramphenicol (90.91%) & Amikacin (81.82%) in MRSA strains. Both MRSA (100%) and MSSA (97.94%) strains were highly resistant to Penicillin. Among MRSA, high degree of resistance was encountered for Cephalexin (88.63%), Ampicillin (77.27%), Ciprofloxacin (75%), Gentamicin (75%) and Ofloxacin (68.18%)., Other studies have also reported quite high resistance to these antibiotics e.g. 88.7% resistance to Cephalexin reported by Anupurba et al (2003).[12] 70% Ampicillin resistance noted in a study by Shobha KL et al (2005).[20]

Table 3: showing comparison of antimicrobial resistance rates of MRSA & MSSA.

S.NO.	ANTIBIOTIC	MRSA STRAINS RESISTANCE (%) TOTAL 44	MSSA STRAINS RESISTANCE (%) TOTAL 53	Chi square	df	P value
1	P	100% (44/44)	96.22% (51/53)	1.695	1	0.193NS
2	A	77.27%(34/44)	56.60% (30/53)	4.576	1	0.032*
3	Cp	88.63%(39/44)	45.28% (24/53)	19.848	1	<0.001***
4	E	38.60%(17/44)	26.41% (14/53)	1.651	1	0.198NS
5	Ge	75% (33/44)	32.07% (17/53)	17.736	1	<0.001***
6	Ak	18.18%(08/44)	11.32% (6/53)	0.916	1	0.339NS
7	Cd	34.09%(15/44)	28.30% (15/53)	0.377	1	0.539NS
8	Nt	6.82% (03/44)	NIL	3.729	1	0.053NS
9	Cf	75% (33/44)	54.71% (29/53)	4.289	1	0.0383*
10	Of	68.18%(30/44)	39.62% (21/53)	7.864	1	0.005**
11	C	9.09% (04/44)	9.43% (05/53)	0.003	1	0.956NS
12	Va	NIL	NIL			
13	Lz	NIL	NIL			
14	Te	NIL	NIL			

NS: $p > 0.05$; Not Significant; * $p < 0.05$; significant at 5%;
** $p < 0.01$; Significant at 1%; *** $p < 0.001$; Highly significant
P- Penicillin, A- Ampicillin, Cp- Cephalexin, E- Erythromycin, Ge- Gentamicin, Ak- Amikacin, Cd- Clindamycin, Nt- Netilmicin, Cf- Ciprofloxacin, Of- Ofloxacin, C- Chloramphenicol, Va- Vancomycin, Lz- Linezolid, Te- Teicoplanin.

A study by Kumari et al (2008), showed 67.35% resistance to Ciprofloxacin and 62.24% resistance to Ofloxacin.[14] Quite high resistance to Gentamicin have also been seen in earlier studies .[20,21] In our study moderate level of resistance was seen towards Clindamycin (38.60%) and Erythromycin (34.09%). These results were comparable to study by Sanjana et al (2010), who reported 29.03% resistance to Erythromycin. [16]

In MSSA, moderate level of resistance was seen to Ampicillin (56.60%), Ciprofloxacin (54.71%), Cephalexin (45.28%), Ofloxacin (39.62%), Gentamicin (32.07%), Clindamycin (28.30%) and Erythromycin (26.41%).Low level resistance was observed to Amikacin (11.32%), Chloramphenicol (9.43%) and Netilmicin (6.82%).Highly statistical significant difference in resistance patterns between MRSA and MSSA was found for Cephalexin (p value <0.001) & Gentamicin (p value <0.001) and significant difference for Ampicillin (p value 0.032), Ciprofloxacin (p value 0.0383) and Ofloxacin (p value 0.005). While the difference for other antibiotics were not statistically significant.

CONCLUSION

This report showed a high prevalence of MRSA in our hospital. Hence, rapid and accurate identification of MRSA is required for therapeutic and epidemiological reasons; to immediately start the appropriate antimicrobial therapy and to avoid the spread of these strains. Also, there is a need for constant surveillance of MRSA and its antimicrobial profile. The hospital infection control policy and guidelines should be strictly implemented and followed so as to enable the clinicians to deliver better and proper health care to the patients. Rapid and accurate detection of all MRSA strains should be a routine laboratory procedure. Latex agglutination method is rapid though expensive can be the best predictor to detect MRSA in case of nonavailability of molecular methods. Cefoxitin Disc Diffusion with high sensitivity and high negative predictive value, when combined with tests like Latex agglutination having high specificity, can be the best option to detect MRSA in clinical settings with constraint facilities.

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