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Prevalence and antibiotic susceptibility pattern of Methicillin Resistant Staphylococcus *aureus* (MRSA) in clinical isolates from a tertiary care hospital, Jaipur, Rajasthan (India)

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Abstract

Background: MRSA has been a persistent and ever growing problem for healthcare institutions because of its increasing resistance to a multiple number of drugs, thereby making it a challenging task for clinicians. The aim of present study was to determine the prevalence and antimicrobial susceptibility pattern of MRSA in our hospital in Jaipur, Rajasthan.

Materials and methods: A total of 100 clinical specimens were collected and subjected to MRSA screening using Cefoxitin disc diffusion, Oxacillin screen agar, Chromogenic agar & Hicomb MIC test. Subsequently the antibiotic sensitivity test was performed for the PCR (Gold Standard) confirmed MRSA isolates.

Results: Out of 100 strains of S. aureus isolated from clinical samples, 58 (58%) were found to be methicillin resistant respectively. Almost all clinical MRSA strains were resistant to Erythromycin(98.27%) and Oxacillin (96.55%), 80 -88% strains resistant to Ampicillin, Cephalexin, Clindamycin, Ciprofloxacin, 43.10% to Gentamycin & 1.72% to Linezolid. Multidrug resistance was observed among 27.58 % of MRSA isolates. However, all strains of MRSA were sensitive to vancomycin & Teicoplanin.

Conclusion: The regular surveillance & determination of prevalence and antimicrobial sensitivity pattern of MRSA will help the treating clinicians, in preserving antibiotics like vancomycin, only for life-threatening staphylococcal diseases.

Keywords: Antibiotic, MRSA, prevalence

Introduction

In recent years, the increasing number of MRSA has become a serious therapeutic challenge because of the widespread circulation of Methicillin-resistant Staphylococcus aureus strain, which is resistant to several antibiotics making successful treatment increasingly difficult. For these reasons, accuracy and promptness in the detection of methicillin resistance is of key importance in the selection of appropriate empirical treatment of these infections.

Prolonged hospital stay, indiscriminate & irrational use of antibiotics and lack of awareness are some of the predisposing factors of MRSA emergence. Asymptomatic colonization in patients and health care workers serve as a reservoir in the spread of MRSA strains[10]. Many of these MRSA isolates are becoming multidrug resistant, leaving glycopeptide antibiotics such as vancomycin, as the only drug of choice.

The present study provides an initiative to understand the current trends of antimicrobial resistance among clinical isolates of *S. aureus* and also prepares a platform to initiate epidemiological studies for staphylococcal infections. We conducted this study to determine the prevalence and antimicrobial profile of MRSA at a tertiary referral hospital.

Materials and methods

This was a descriptive and observational one year study conducted in the Department of Microbiology, S.M.S. Medical college, Jaipur (Rajasthan) from April 2012 to March 2013.A total of 100 *Staphylococcus aureus* strains were isolated from various clinical specimens such as pus, blood, sputum, throat swab, ear swab, high vaginal swab, CSF, urine, pleural fluid, semen, bile and corneal swab. The *S aureus* strains in these isolates were identified by the conventional morphological & biochemical tests which included the colony characteristics on culture plate, Grams stain, catalase test, coagulase test (slide & tube coagulase) and mannitol salt agar as per the standard routine procedure followed in our department[4].

Coagulase positive *Staphylococcus aureus* isolates were screened for methicillin resistance using 4 phenotypic tests: Cefoxitin disc diffusion, Oxacillin screen agar, Chromogenic agar & Hicomb MIC test with PCR as the gold standard. These isolates were then tested for antibiotic susceptibility by the Kirby Bauer disk diffusion method [2] as per Clinical and Laboratory Standards Institute (CLSI) guidelines[5]. against a panel of antimicrobial agents which included ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), clindamycin (2 µg), oxacillin (1 µg), cefoxitin (30 µg), erythromycin (15 µg), cephalexin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), linezolid (30 µg), vancomycin (30 µg),Teicoplanin(30µg), norfloxacin (10 µg) and nitrofurantoin (300 µg) . Norfloxacin and nitrofurantoin were used only in urine samples**.** The discs used were obtained from the Hi-media Pvt. Ltd. Zone diameters were measured following CLSI criteria[[5]. Isolates resistant to more than 5 drugs were considered as MDR.

Results

Out of 100 Staphylococcus aureus isolates, 58% isolates were found to be methicillin resistant. Isolation rate of MRSA from various clinical samples was high for pus, wound swab and urine (Table 1).

MRSA isolation rate was 82% in the indoor patients & 18% in outdoor patients (Table 2).

Table 1: Isolation rate of MRSA from different clinical specimens.

Sample	MSSA	MRSA
Pus & Wound swab n = 38	14 (36.84%)	24 (63.16%)
Blood n = 23	11 (47.83%)	12 (52.17%)
Urine n = 17	5 (29.41%)	12 (70.59%)
Throat swab/ sputum n = 17	7 (41.17%)	9 (58.83%)
Others n = 5	4 (80%)	1 (20%)
Total n = 100	42 (42%)	58 (58%)

The antimicrobial resistance in MRSA was found to be 98.27% to Erythromycin, 86.21% to Ciprofloxacin, 81.03% to Clindamycin, and 43-46.55% to Aminoglycosides. None of the strains were

found to be resistant to Vancomycin & Teicoplanin (Table 3).

Table 2: Isolation rate of MRSA from Indoor & Outdoor patients.

INDOOR/ OUTDOOR	MSSA	MRSA
IPD n = 82	33 (40.24%)	49 (59.76%)
OPD n = 18	9 (50%)	9 (50%)
Total n = 100	42 (42%)	58 (58%)

The antimicrobial resistance was significantly higher in methicillin resistant *Staphylococcus aureus* than methicillin sensitive Staphylococcus aureus. Multidrug resistance in MRSA was found to be 27.58 % where as in MSSA it was 4.76% (Table 4).

Table 3: Antibiotic resistance pattern of Methicillin resistant and methicillin sensitive *Staphylococcus aureus.*

Antibiotic	MSSA (42 isolates)		MRSA (58 isolates)	
	No. of resistant strains	%	No. of resistant strains	%
Ampicillin 10µg	12	28.57%	51	87.93%
Amoxicillin/Clavulanic acid 20/10µg	11	26.19%	45	77.59%
Clindamycin 2µg	19	45.24%	47	81.03%
Oxacillin 1µg	4	9.52%	56	96.55%
Erythromycin 15µg	12	28.57%	57	98.27%
Cephalexin 30µg	23	54.76%	51	87.93%
Ciprofloxacin 5µg	24	57.14%	50	86.21%
Gentamycin 10µg	03	7.14%	25	43.10%
Amikacin 30µg	11	26.19%	27	46.55%
Linezolid 30µg	00	0.00%	1	1.72%
Vancomycin 30µg	00	0.00%	00	0.00%
Teicoplanin 30µg	00	0.00%	00	0.00%
Norfloxacin 10µg	00	0.00%	5	41.67%
Nitrofurantoin 300µg	00	0.00%	2	16.67%

Table 4: Multidrug resistance in Methicillin resistant Staphylococcus *aureus*& Methicillin sensitive Staphylococcus *aureus*.

S. No.	Resistance to antibiotics	Number (%) of MRSA strain	Number (%) of MSSA strain
1.	≥ 5	27.58% (n=16)	4.76% (n=2)
2.	Less than 5	72.41% (n=42)	95.23% (n=40)

Discussion

In the light of growing concern about the rapid rise in resistance of *Staphylococcus aureus* to antimicrobial agents, our objective of the present study was to determine the prevalence & pattern of antimicrobial profile of MRSA. Our study comprised of 100 *Staphylococcus aureus* isolates from various clinical samples received in our Bacteriology section. 58 isolates showed presence of mecA gene by PCR, the prevalence being 58%, which agrees with Behera B. et al [3] and R. Kaur et al [9]. On the contrary, some studies have reported

alarmingly high 83.54%[6] & low 29%[8] incidence of MRSA infection over different parts of country. This variation can be attributed to differences in infection control policy and treatment practices. For detection of Methicillin resistance combination of cefoxitin disc diffusion n Oxacillin screen agar proved to be comparable to PCR.

Isolates from indoor patients (82%) exceeded those from the outdoor patients (18%) giving a significant percentage of 59.76% MRSA in the present study (Table 2).

Urine samples accounted for the maximum incidence of MRSA in our study (70.59%) followed by wound discharge and abscess aspirate (63.16%, Table 1).

MRSA and its antibiotic sensitivity profile are regarded with all seriousness in clinical practice and clinical epidemiology, not only because these strains are resistant to multiple antibiotics, but also because they act as a reservoir for drug resistance gene [7].

The antibiotic sensitivity pattern of the present study indicated that 54-57% of the MSSA were resistant to Cephalexin & Ciprofloxacin while 100% were sensitive to Linezolid, Vancomycin & Teicoplanin. Highest resistance in MRSA was seen for Ampicillin, Oxacillin, Erythromycin, Cephalexin, Ciprofloxacin (87-99%) while Gentamycin & Amikacin showed resistance in 40-45% strains. All the MRSA strains were sensitive to vancomycin and Teicoplanin while 98.28% MRSA isolates were sensitive to linezolid. Similar findings were echoed in the studies by Anupurba S et al [1].

Resistance to Ciprofloxacin was found to be 86.21 % while Norfloxacin, which was used only for urine samples, showed resistance in 41.67% of MRSA isolates from urine samples. Staphylococcus species may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3-4 days of initiation of therapy. Hence testing of repeat isolates is warranted.

For MRSA, β lactam agents, penicillins, β-lactam/β-lactamase inhibitor combinations, cephems (with the exception of cephalosporins with anti MRSA activity) and carbapenems, may appear active in vitro, but are not effective clinically. Results for these drugs should be reported as resistant or should not be reported.

The percentage of MDR strains among MRSA was found to be 27.58%. as compared to MSSA 4.76% .In the various reports from other parts of the country, the burden of such strains has ranged from 23.2% to 63.6%[11] .Majumder D *et al*. also reported that coexisting resistance to different antibiotics (except penicillin) with methicillin was significantly higher in comparison to methicillin-sensitive strains[7].

Extensive Indiscriminate use of antibiotics arising from self-medication in the developing countries, where there are no regulatory guidelines in this respect, has rendered the routinely used antibiotics completely ineffective in the treatment of *Staphylococcus aureus* infections.

Accounting of high rates of MDR MRSA leads to the probability of misuse of drugs like vancomycin, Linezolid & Teicoplanin by clinicians. Prudent use and continuous monitoring of MIC levels is required for these drugs, so that we may not fall back into pre-antibiotic era.

To conclude, glycopeptides seem to be the only drug of choice to treat MDR MRSA infections. The high prevalence of MRSA associated with indiscriminate use of glycopeptide drugs, both considered to be risk factors for VRSA, make the widespread dissemination of these organisms, a distressing and realistic possibility once it happens to arise.

In addition to promoting rational use of antibiotics, raising alertness about antibiotic misuse, periodic surveillance of AMR pattern taking into consideration the dosage & rhythm of antibiotics will certainly be of great help in formulating appropriate

antibiotic policy. Return of sensitivity in resistant organisms by antibiotic rotation, can also prove to be another route for increasing longevity of antibiotics.

Conclusion

Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in this part of India. With the prevalence of MRSA being 58% in our study, Vancomycin & Teicoplanin seems to be the only antimicrobial agents, which show uniform sensitivity (100%) even with MDR MRSA infections.

The regular surveillance of hospital-acquired infections of MRSA may be helpful in defining and monitoring the antibiotic policy. This may also help in conserving antibiotics like vancomycin & teicoplanin, for life-threatening staphylococcal diseases while drugs like Aminoglycosides, Fluroquinolones & Linezolid can be used routinely.

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