Research Article

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PREVALENCE AND ANTIBIOGRAM OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES AT A TERTIARY CARE HOSPITAL IN BANGALORE, SOUTH INDIA

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ABSTRACT

Background: The emergence of Methicillin-resistant Staphylococcus aureus (MRSA) has posed a serious therapeutic challenge. MRSA is an important cause of nosocomial infections worldwide.

Objectives: The aim of this study was to determine the prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) from clinical samples in a tertiary care hospital and to analyse the antibiotic susceptibility patterns of MRSA isolates.

Materials and Methods: Various clinical specimens were cultured and staphylococcus aureus was identified using standard tests like catalase test, slide and tube coagulase and growth on Mannitol salt Agar. All staph isolates were then tested for routine antibiotic sensitivity by Kirby - Bauer disc – diffusion method following CLSI guidelines. MRSA were then identified from amongst the Staph isolates by using cefoxitin (10 mcg) discs by the disc-diffusion method. The D-test was performed on all isolates of Staphylococcus aureus to identify erythromycin induced clindamycin resistance.

Results: The prevalence of MRSA in our study was found to be 66.84%. Linezolid and Vancomycin proved to be effective against 93.89% and 89.31% of MRSA isolates. There was statistically significant resistance to Penicillin,Cefoxitin and Oxacillin ($p<0.0001$) and significant sensistivity to Linezolid and Vancomycin among MRSA. Also, 35.11% of MRSA isolates and 24.62% of MSSA isolates showed erythromycin induced clindamycin resistance in the study.

Conclusion: MRSA infection in the hospital set up is on the rise and is a cause of worry due to resistance to commonly prescribed drugs. Regular surveillance and antibiotic sensitivity testing in hospitals can help to formulate antibiotic and other strategies for reducing the load and severity of MRSA infections world-over.

Key Words: Staphylococcus aureus, MRSA, Cefoxitin, Clindamycin, Linezolid, Vancomycin.

INTRODUCTION

In the early 1960s, Methicillin-Resistant Staphylococcus aureus (MRSA) emerged as a nosocomial pathogen. Since then, it has been increasingly reported from hospitals in countries around the world. Today, infection with MRSA is a common hospital-acquired as well as community-acquired infection encountered especially in developing nations. MRSA causes UTI, wound infection and even sepsis, endocarditis, osteomyelitis and other life-threatening conditions which are difficult to treat owing to the multi-drug resistance developed by the organism [1].

Prolonged hospital stay and indiscriminate use of antibiotics has been implicated in the rapid emergence and spread of MRSA. Asymptomatic health-care workers are the major sources and carriers of this pathogen in a hospital.

Fearing MRSA, physicians prescribe Vancomycin and other glycopeptide antibiotics in order to treat their patients, without undertaking appropriate antibiotic sensitivity tests. This has deleterious effects in the long-term as more resistance builds up. Currently, there are Vancomycin resistant staphylococcus aureus (VRSA) strains being isolated from some cases and these are highly difficult to treat.

In this study, clinical samples from patients in a tertiary care hospital in South India were processed for the

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growth of Staphylococcus aureus and their antibiotic sensitivity patterns were obtained by Kirby- Bauer's disc diffusion method. MRSA strains were identified using a 30 mcg Cefoxitin disc according to Clinical and Laboratory Standards Institute (CLSI) standards (2). The study also looks for erythromycin induced clindamycin resistance among staphylococcus isolates.

This study was undertaken with the main aim of estimating the prevalence of MRSA and studying the antibiotic-sensitivity pattern of MRSA isolates among clinical samples from patients attending a Tertiary care centre, Bangalore. As a result, appropriate antibiotic schedules, control measures and prophylactic steps can be introduced in order to reduce infection rates and spread of MRSA.

MATERIALS AND METHODS

The present study was conducted for a period of 6 months on patients attending the OPDs and inpatient admissions to Tertiary care hospital attached to a medical college in Bangalore, South India. During this period 2821 various clinical samples like urine, blood, sputum, stool, body fluids and exudates (mainly pus from wounds, middle-ear infections and pyogenic abscesses) were received and processed in the microbiology laboratory. Institutional ethical clearance and informed consent of the patients was obtained for the study.

The specimens were cultured on blood agar and MacConkey agar plates and incubated aerobically at 37°C for 48 hours. Staphylococcus aureus isolates were identified using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar [3]. Following identification of staphylococcus aureus, antibiotic sensitivity testing was performed by Kirby–Bauer disc diffusion method for the following antibiotics:

Amoxicillin + clavulinic acid (50/10μg), cefotaxime(30μg), oxacillin(5μg), linezolid(30μg), tetracycline(30μg), cotrimoxazole(25μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), clindamycin (2 μg), gentamicin (10 μg), erythromycin (15 μg), netilmicin (30 μg), penicillin (10 units), and vancomycin (30 μg). Carbenicillin, nitrofurantoin and nalidixic acid were added if the isolate of Staphylococcus aureus was from a urine sample.

D test: The test was done on a Mueller–Hinton agar plate inoculated with a lawn culture of 0.5 McFarland bacterial suspension. The erythromycin (15 μgm) disc and clindamycin (2 μgm) disc was then placed at a distance of 15 mm (edge-to-edge). After an overnight incubation at 37^{0}C , flattening of the zone in the area between the two discs giving a (D-shaped zone) around clindamycin disc indicated inducible clindamycin resistance.

Test for methicillin resistance was performed by Kirby–Bauer disc diffusion method using cefoxitin (30μgm) disc according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the test includes incubating a lawn culture of the test isolate on Mueller Hinton agar with 2% sodium chloride and cefoxitin disc (30 mcg) at 37^o C for 24 hours, a zone size <22 mm indicated resistance to cefoxitin and reported as MRSA. [2]

The data obtained was analysed using percentages, normal distribution and contingency tests.

RESULTS

During a period of 6 months a total of 2709 clinical samples were processed. Out of all the samples processed, 196 (7. 23%) yielded Staphylococcus aureus, 580 (21.41%) samples yielded Gram-negative bacilli,14(0.51%) samples grew Candida species and 2031 samples had no growth after 2 days of culture. Among the staphylococcal isolates, MRSA accounted for 66.84% and MSSA 33.16%. [table 1]

Out of the various types of clinical samples, pus / wound swabs / aspirates from abscesses yielded maximum load 63.26 % of Staphylococcus aureus followed by blood, urine, sputum and finally body fluids (ascetic, pleural fluid, csf). The MRSA distribution for each type of clinical sample is shown In Table 2.

Antibiotic susceptibility patterns are demonstrated in Figure 1. Among the MSSA isolates 60% and 89.23% were sensitive to penicillin and oxacillin respectively.

All MRSA isolates were found resistant to Cefoxitin, oxacillin and Penicillin respectively. Linezolid and Vancomycin proved to be effective against 93.89% and 89.31% of MRSA isolates. 59.54%, 57.25%, 54.96%, and 48.85% of MRSA isolates were sensitive to chloramphenicol, gentamicin, clindamycin and erythromycin, respectively. All the antibiotics tested were effective against more than half of the MSSA isolates, except for Netilmicin, in the present study.

35.11% of MRSA isolates and 24.62% of MSSA isolates showed Dtest positive - Erythromycin induced Clindamycin resistance (Table 3).

Table 1: showing the percentage of staphylococcus aureus isolated.

Staphylococcus aureus	Number (n= 196)	Percentage
MSSA	65	33.16
MRSA	131	66.84

Table 2: Shows distribution of MRSA from various clinical samples.

Clinical Sample	Total (n=2709)	S. aureus (n=196)	MRSA (n=131)	Percentage of MRSA
Urine	691	25	20	80
PUS/aspirate/swab	626	124	86	69.35
Body fluids	132	1	1	100
Sputum	697	19	10	52.63
Blood	563	27	14	51.85

Table 3: Showing resistance to Erythromycin and Clindamycin by Dtest.

Staphylococcus aureus		Resistant to Cd and E	Percentage
MSSA	n= 65	16	24.62%
MRSA	n=131	46	35.11%

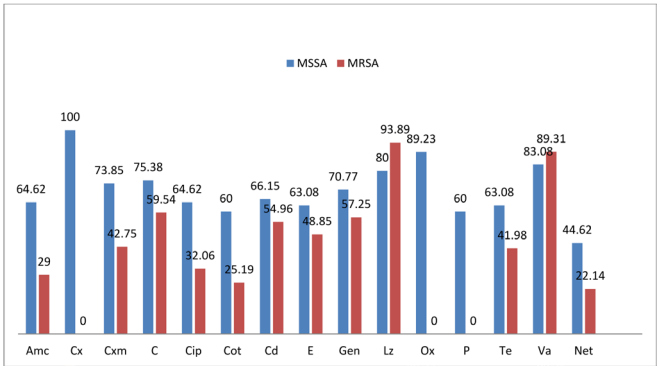

Figure 1: Showing percentage of sensitive patterns of MSSA and MRSA.

DISCUSSION

The prevalence of MRSA in our 6-month-long study was found to be 66.84%. Such alarmingly high percentages have previously been reported in studies from Gulbarga (56.7% MRSA) [4] and Varanasi (59.3% MRSA) [5]. Rates of MRSA vary from place to place and from time to time. For instance, a study in a Delhi hospital showed a prevalence rate of MRSA of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008. [6]

The major load of MRSA isolates in the study was from body fluids, urine and pus/exudates. Majority of the urinary samples that yielded MRSA were found to be from paediatric ICU and are hospital-acquired in nature.

MRSA are difficult to treat owing to the multi-drug resistance shown by them. They produce beta-lactamases and are usually resistant to penicillins and other beta-lactams including methicillin. In the current study as well, 11.45% and 1.5% of MRSA were found to be sensitive to penicillin and oxacillin while 60% and 89.23% of MSSA were sensitive, respectively. Thus, MRSA show a statistically extremely significant ($p<0.0001$) resistance to penicillin and oxacillin. Similar findings were seen in other studies. [7]

93.89% and 89.31% of MRSA isolates were sensitive to Linezolid and Vancomycin, respectively and these drugs are found to be the most effective in treatment of MRSA infections. However, increased use of these high-end drugs is not only expensive but also causes drug resistance to spread further and is worrying as we are rapidly running out of antibiotics.

However, with appropriate antibiotic-sensitivity testing, other suitable antibiotics may be used in treatment of MRSA and use of vancomycin can be reserved for only severe, last-resort cases. In the current study chloramphenicol, gentamicin, clindamycin and erythromycin were found to be useful in treating MRSA with 59.54%, 57.25%, 54.96%, and 48.85% of MRSA isolates being sensitive to them, respectively. In a study from Mangalore[1], Ciprofloxacin was found to be the most effective drug apart from Vancomycin. However, in another study from Maharashtra, no drug other than Vancomycin was found useful in treatment of MRSA infections.[8] This may be due to different drug pressure in different communities leading to varying degrees of resistance to same antibiotics.

Among MRSA isolates 35.11% showed erythromycin induced clindamycin resistance while 24.62% of MSSA isolates showed erythromycin induced clindamycin resistance. Other studies also give similar values and are in concordance with our study. For instance, inducible resistance of 24.4% in MRSA and 14.8% in MSSA was got in one study from Turkey [9]. while in another study from India, inducible resistance was 30% in MRSA and 10% in MSSA. [10]

CONCLUSION

Prevalence of MRSA is alarmingly high and causes a great worry to doctors in management of infections. Higher costs, unavailability and risk of developing resistance are the reasons physicians should choose to treat MRSA infections with alternative antibiotics instead of Vancomycin and Linezolid. Chloramphenicol, Gentamicin, Erythromycin and Clindamycin are some such suitable alternatives. Therefore, routine monitoring of MRSA infections along with their antimicrobial susceptibility pattern can assist

in formulating a suitable antibiotic policy which may be helpful in reducing the burden and spread of MRSA infections in hospitals across India.

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