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Original article

Prevalence and antimicrobial resistance of methicillin resistant *staphylococcus aureus* (MRSA) isolated from blood culture in tertiary care hospital in Haryana

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Abstract

Introduction and Aim: It has been observed that there is rise of *MRSA* related morbidity and mortality worldwide. Due to its antibiotic resistance nature, it is difficult to treat. So, in this study our aim was to detect the prevalence and antibiotic resistance of *MRSA strains*, isolated from blood culture in PT. B.D. Sharma institute of medical science, PGIMS, Haryana.

Materials and Methods: Total 7510 samples were sent to Microbiology Laboratory of between 1st June 2021 to 31st December 2021. All blood culture bottles after an incubation period of 24 hrs were plated onto a blood agar plate and the plates were incubated at 35°C for 24 to 48 h. Staphylococcus spp. was identified by doing gram staining, catalase test, coagulase test and antibiotic susceptibility tests were performed by Kirby-Bauer Disk Diffusion method. Methicillin resistance was detected by cefoxitin disc diffusion test.

Results: Total of 104 *Staphylococcus aureus* positive samples were detected. Interestingly, 40 (38.4%) of total *Staphylococcus aureus* positive samples were *Methicillin-Resistant Staphylococcus aureus* and 64 (61.6%) of them were *Methicillin-Sensitive Staphylococcus aureus*. The prevalence of *MRSA* strains was higher in males 27 (67.5%) than females 13 (32.5%). Maximum numbers of *MRSA* strains isolates were from inpatient 28 (70%) than outpatient 12 (30%). Maximum MRSA strains were isolated from patients in age group of 0-14 yrs, 15 (37.5%) followed by >60 years of age group9, (22.5%). The maximum number of *MRSA* strains were isolated from the patients of intensive care units 14 (35%) followed by surgery 10 (25%). When the antibiotic resistance profiles among *MRSA* strains isolates were checked, it was seen that penicillin resistance seen in all the isolates i.e. 100 %, erythromycin resistance seen in 77.5% isolates, clindamycin resistance in67.5%, gentamicin resistance in 67.5%, ciprofloxacin resistance in 62.5% and cotrimoxazole resistance in 55%. Most *MRSA* strains were sensitive to second line of antibiotics like Vancomycin 100 %, Linezolid 92.5%.

Conclusions: The antibiotic resistance is getting increased by uncontrolled antibiotic usage and wrong choices in empiric therapy day by day. Each hospital/institute has to detect its own antibiotic resistance profiles and apply empiric therapy according to these profiles. Strict infection control policies must be implemented to check its spread in hospital.

Keywords: Antimicrobial resistance, Methicillin resistant *staphylococcus aureus,* Blood culture

Introduction

Healthcare associated blood stream infections (BSIs) are associated with major morbidity and mortality all over the globe. Also, the incidence of healthcare associated BSIs and the prevalence of antibiotic resistance among the micro-organisms are getting increased with time.[1,2] Coagulase negative Staphylococcus (CONS) and *Staphylococcus aureus* (*S.aureus*) are the most commonly isolated gram-positive bacteria from blood culture samples.[3] *S.aureus* is gram positive coccus of size 0.5-1.5 µm in diameter, it is facultative anaerobe which produces catalase and coagulase. This microorganism is commensal organism of skin, skin glands and mucous membranes particularly in the nose of healthy individuals. It is associated with skin and soft-tissue infections, osteomyelitis, pneumonia, infective endocarditis, brain abscesses, and meningitis and bloodstream infection.[4-6] After the discovery of penicillin, the number of infections caused by penicillin-resistant strains of *S.aureus* increased in hospitals in the mid-1940s.[7] On the other hand, Methicillin Resistant Staphylococcus aureus (MRSA) were first reported in the early 1960's in United Kingdom and are now regarded as

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a major hospital acquired pathogen worldwide. As per European Antimicrobial Resistance Surveillance Network report 2018, the prevalence of MRSA ranges from 16% to 44% in various European countries.[8,9] Similarly, prevalence of MRSA was reported to be 65% by Brog, *et al*. in 2006 in Jordan.[10] According to National Nosocomial Infection Surveillance System (NNIS) report, in USA, 50% of hospital acquired infections in ICUs are due to MRSA.[11] Studies conducted across various centers in Korea on CA-MRSA report it to be around 13–16%.[12,13] In India, incidence of MRSA is increasing with time as reported by many studies spread across the country, according to Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India in 2008 and 2009 MRSA prevalence was reported to be 41%.[14]

MRSA strains are a reservoir for multiple drug resistant genes; therefore, the limitation of treatment options is a serious health problem. Due to widespread β-lactam antibiotic resistance in MRSA strains, non-β lactam antimicrobial agents have recently come to the fore in the treatment of infections caused by MRSA strains. So that, it is important to determine their role in treatment of infections and their effectiveness with antimicrobial susceptibility tests.[15,16] Erythromycin and clindamycin belong to the macrolide, lincosamide and streptogramin (MLS) group of antibiotics. Macrolides are major alternative antimicrobial agents for the treatment of infections caused by gram-positive bacteria. Clindamycin can be administered orally, good tissue penetration and is tolerable, therefore it is usually used to treat skin and bone infections. Macrolides and lincosamides resistant *S.aureus* strains are increasingly reported in clinical isolates. Both antimicrobial agents exhibit their action by inhibiting protein synthesis by binding to the 50S ribosomal subunits of bacterial cells.[17-19] Regular monitoring of antimicrobial drug resistance is important for continued updating of hospital antibiogram policies, determination of prescription and choice of drug for empirical therapy. The aim of this study was to find the prevalence and antibiotic resistance of *MRSA* strains, isolated from blood culture in PT. B.D. Sharma institute of medical science, PGIMS, Rohtak, Haryana.

Material and Methods

A total of 7510 samples were sent to Dept. Of Microbiology, PT. B.D. Sharma Institute of Medical Sciences, PGIMS between 1st June 2021 and 31st December 2021. In this study, the prevalence and antibiotic resistance of *MRSA* strains isolated from blood culture were investigated retrospectively. Blood for culture was collected under sterile conditions. A total of 5 mL of blood was added to each of two bottles containing sodium polyanethole sulfonate (SPS) broth and bile broth respectively. Both bottles were incubated aerobically at 35-37˚C for seven days. Subcultures were done on 5% sheep blood agar and MacConkey agar after 24 hours.

The bacterial colonies were identified by conventional methods such as gram staining, catalase test, slide and tube coagulase, mannitol fermentation test and DNase production. Antibiotic susceptibility tests also were performed by using Kirby-Bauer Disk Diffusion method on Muller Hinton Agar (MHA) plate. The antibiotics tested were Cefoxitin (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Co-trimoxazole (1.25/23.75 µg), Clindamycin (2 µg), Erythromycin (15 µg), Linezolid (30 µg), and Vancomycin (30 µg).[20] Methicillin resistance was detected by phenotypic methods, such as cefoxitin disc diffusion test.Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 were used as negative control strain.The results were evaluated according to the standards of Clinical and Laboratory Standards Institute (CLSI) guideline 2021.[20]

Statistical analysis

The statistical analysis was performed using IBM SPSS 21 for Windows Version.

Results

During the six-month period started at 1st June 2021 till 31st December 2021, a total of 7510 samples were sent to Dept. Of Microbiology, Pt. B.D. Sharma Institute of Medical Sciences. During this period, total 104 *S.aureus* positive samples were detected.

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TABLE 1: Show total Staphylococcus aureus strains isolated.

TOTAL STAPHYLOCOCCUS AUREUS STRAINS ISOLATED (N=104)	
Methicillin sensitive Staph aureus (MSSA)	**Methicillin resistant Staph aureus (MRSA)**
64(61.5%)	40 (38.5%)

TABLE 2: Show sex wise distribution of patients with MRSA strain.

TOTAL NUMBER OF PATIENTS WITH MRSA STRAINS (N=40)	
MALE	**FEMALE**
27 (67.5%)	13 (32.5%)

TABLE3: Show age group wise distribution of patients with MRSA strain.

AGE GROUPS	NO. OF MRSA STRAIN ISOLATED (N=40)
>60 YRS	9 (22.5%)
45-59 YRS	7 (17.5%)
30-44 YRS	6 (15%)
15-29 YRS	3 (7.5%)
0-14 YRS	15 (37.5%)

TABLE 4: Show admission wise distribution of patients with MRSA strain.

TOTAL NO. OF PATIENTS WITH MRSA STRAIN (N=40)	
INPATIENT	**OUTPATIENT**
28 (70%)	12 (30%)

TABLE 5: Show department wise distribution of patients with MRSA strain

DEPARTMENT WISE SEGMENTATION OF PATIENTS WITH MRSA STRAIN (N=40)	
ICU	14 (35%)
SURGERY	10 (25%)
PAEDIATRIC	3 (7.5%)
MEDICINE	6 (15%)
ORTHO	4 (10%)
GYNAE	3 (7.5%)

TABLE 6: Show resistance pattern of MRSA strain.

RESISTANCE PATTERN OF MRSA STRAINS (N=40)	
ANTIBIOTIC TESTED	**NO. OF STRAIN RESISTANT**
PENICILLIN	40 (100%)
ERYTHROMYCIN	31 (77.5%)
CLINDAMYCIN	27 (67.5%)
CIPROFLOXACIN	25 (62.5%)
GENTAMICIN	27 (67.5%)
COTRIMOXAZOLE	22 (55%)
LINEZOLID	3 (7.5%)
VANCOMYCIN	0 (0%)

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A total of 104 isolates of *S. aureus* was isolated and out of which 64 (61.5%) isolates were found to be MSSA and 40 (38.4 %) isolates were MRSA (Table 1).The prevalence of MRSA strains was higher in males 27 (67.5%) than females 13 (32.5%) (Table 2). Maximum MRSA strains were isolated from patients in age group of 0-14 yrs 15 (37.5%) followed by>60 years of age group, 9(22.5%) (Table 3). Maximum numbers of MRSA isolates were from inpatient 28 (70%) than outpatient 12 (30%) (Table 4). The maximum number of MRSA was isolated from the patients of intensive care units 14 (35%) followed by surgery 10 (25%) (Table 5). Antibiotic resistance profiles among MRSA isolates were checked, it was seen that penicillin resistance seen in all the isolates i.e. 100 %, erythromycin resistance seen in 77.5% isolates, clindamycin resistance in 67.5%, gentamicin resistance in 67.5%, ciprofloxacin resistance in 62.5% and cotrimoxazole resistance in 55%. Most MRSA were sensitive to second line of antibiotics like Vancomycin 100 %, Linezolid 92.5% (Table 6).

DISCUSSION

In the present study, from1st June 2021 and 31st December 2021 the prevalence of *MRSA* strains was 38.4%. Many studies showed similar trend of *MRSA* from across India ranging from 26.14% to 43%.[21,22] This increased prevalence may be due to multiple risk factors like carriage of *MRSA* by health care workers (HCWs) , laxity in patient isolation protocols, poor compliance to hand hygiene, lack of active surveillance programs for *MRSA*, misuse and abuse of antimicrobials, irrational use of medical devices, presence of open wounds, prolonged hospitalization, no strict visitors policy and lack of bundle care approach.

In the present study while comparing gender distribution of *MRSA* infections, males (67.4%) were more affected than female patient (32.6%). The similar trends were observed by Rao *et al*. in 2012.[23] The more affected age group was of both extremes i.e 0-14 yr age group and >60 years in our study. Although advancing age by itself is not considered a risk factor for *MRSA* infection, age more than 65 years is a significant risk factor for hospitalization. Hence, advancing age is indirectly linked to *MRSA* acquisition.[11] *MRSA* was observed more in admitted patients (70%) as compared to outpatients (30%) cases which could be attributed to presence of *MRSA* strains in various ICU`s and wards.

In the present study, highest number of *MRSA* came from various ICU i.e. 35% followed by surgery dept. 25 %, medicine dept. 15% and other departments. An obvious reason for this observation may be due to prolonged antibiotic usage, colonization of skin by *MRSA* and the chances of invasion increasing with use of invasive approach as with surgical departments, indwelling devices in intensive care units and laxity in infection control practices. Similar trends were observed by Mallick and Basak and Sanjana *et al*.[24,25]

Considering the antibiogram pattern of *MRSA* out of 40 *MRSA* isolates penicillin resistance seen in all the isolates i.e 100 %, erythromycin resistance seen in 77.5% isolates, Clindamycin resistance in 67.5%, gentamicin resistance in 67.5%, ciprofloxacin resistance in 62.5% and cotrimoxazole resistance in 55%. Most *MRSA* isolates were sensitive to second line of antibiotics like Vancomycin 100 %, Linezolid 92.5%. Similar pattern was obtained in other studies.[26-28]

CONCLUSION

In conclusion there is rise of *MRSA* infections associated morbidity and mortality worldwide. *MRSA* with multidrug resistant could lead to treatment failure. Proportion of *MRSA* and antibiotic resistance observed are different in various countries and hospitals. These different results between countries and hospitals show once again the importance of local epidemiological surveillance study. All hospitals should determine their resistance profiles, the appropriate antibiotic policy and current information should be followed for empiric therapy and prophylactic treatment. Furthermore, specific antimicrobial therapy should be initiated according to the culture results. Strict infection control guidelines in hospitals should be implemented.

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