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RESISTANCE PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM SAMPLES OF CRITICAL CARE UNIT PATIENTS WITH SPECIAL REFERENCE TO INDUCIBLE CLINDAMYCIN RESISTANCE

Mohit Kumar MBBS, Postgraduate Student, Department of Microbiology, Shri Ram Murti Samarak Institute of Medical Sciences. Bareilly, Uttar Pradesh, India.

Rahul Kumar Goyal MD, Professor & HOD of Department of Microbiology, Shri Ram Murti Samarak Institute of Medical Sciences. Bareilly, Uttar Pradesh, India.

Vandana Sardana* MD, Professor, Department of Microbiology, Shri Ram Murti Samarak Institute of Medical Sciences. Bareilly, Uttar Pradesh, India. *Corresponding Author

Fauzia Khan MD, Associate Professor, Department of Microbiology, Shri Ram Murti Samarak Institute of Medical Sciences. Bareilly, Uttar Pradesh, India.

ABSTRACT **Background & objectives:** Occurrence of methicillin resistant Staphylococcus aureus (MRSA) is an area of therapeutic concern. MRSA isolates with inducible clindamycin resistance (ICR) are resistant to erythromycin and sensitive to clindamycin on routine testing. This present study was carried out to study the resistance pattern of methicillin resistance Staphylococcus aureus and ICR among isolates from critical care unit. **Methods:** The clinical samples from ICU patients submitted at the Microbiology laboratory were processed and all Staphylococcus aureus isolates were included in this study. All isolates were identified morphologically and biochemically by standard laboratory procedures and subjected to antibiotic sensitivity testing (AST) by Kirby-Bauer's disk diffusion method as per CLSI guideline to estimate the occurrence of MRSA and their resistance pattern. D-test was employed to detect ICR. **Results:** Out of 5986 various samples from ICU, S.aureus were found to be 118. The frequency of MRSA among the isolates of S.aureus was found to be 75.42% (89). Out of 89 MRSA 18 (20.22%) were ICR positive. Resistant to vancomycin and linezolid were found to be 5.6% and 4.5% respectively. **Conclusions:** Continuous surveillance of infection and monitoring of antibiotic sensitivity pattern of S. aureus is required to reduce MRSA burden.

KEYWORDS :

INTRODUCTION

Staphylococcus aureus is a common human pathogens and have the ability to cause a wide range of infection, which may be the skin disease or life threatening infection [(1)]. Among all microorganisms it is the most common causing both nosocomial and community acquired infections [(2)]. MRSA strains were identified in the early 1961 soon after the discovery of methicillin in clinical settings. MRSA infections are associated with increased morbidity and mortality in hospitalized patients and has the potential to cause sudden outbreaks in hospitals [(3)].

MRSA infections have emerged as a worldwide problem of public health importance. Methicillin resistance occurs due to altered penicillin binding protein (PBP-2a) that causes resistance to all β-lactam antimicrobial drugs [(4)]. Clindamycin is not useful in infections which are caused by clindamycin inducible resistant isolate. Antibiotic sensitivity testing (AST) by Kirby-Bauer disc diffusion method of MRSA with ICR isolates show resistant to erythromycin and sensitive to Clindamycin. D-test detects inducible clindamycin resistance if present and help in taking decision to whether clindamycin could be used as a therapeutic option or not [(5)].

The prevalence of MRSA in India has been estimated to be 23.3% to 73% [(2)]. Isolates of MRSA depicting resistance to other antibiotics posses a therapeutic challenge for the clinicians. The wide spread of MRSA in developing countries, like India, calls for the need of implementation of strict infection control & prevention practises.

MATERIAL & METHODS

The present study was conducted from 1st July 2021 to 30th June 2022 at the Microbiology Department of Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly India. Study group includes admitted patients in critical care unit. The clinical samples such as sputum, blood, pus, cvp tip, Foley's tip, tracheal aspirate, umbilical tip, pleural fluid, swab, E.T tip, urine etc. were subjected to culture and sensitivity. All samples were first inoculated on Blood agar and then on MacConkey agar and CLED agar (for urine samples). All Culture plates incubated at 37°C aerobically for 24 hours and if no growth were found then further incubated for 24 hours(total 48 hours). Culture plates showing growth were observed for typical colony characteristics of *S.aureus* on Blood agar (β-hemolysis) and MacConkey agar (lactose fermenting). After getting the colonies on the media the Gram's staining was performed from a single isolated colony and observed for Gram positive cocci in clusters, under 100X oil immersion lens of microscope. Further the bacterial identification was done by standard biochemical tests [(6,7,8)]. AST of all isolates were done using by Kirby-Bauer disc diffusion method on Mueller-Hinton agar plate and evaluated using Clinical and Laboratory Standards Institute (CLSI) guidelines 2021 [(9)]. The antibiotics included in AST were Penicillin (10 units), Nitrofurantoin (300 mcg), Azithromycin (15mcg), Ciprofloxacin (5 mcg), cefoxitine (30 mcg) Levofloxacin (5 mcg), Clindamycin (2 mcg), Gentamycin (10 mcg), Moxifloxacin (5 mcg), Minocycline (30 mcg), Doxycycline (30 mcg), Co-trimoxazole (25 mcg), chloramphenicol (30 mcg), linezolid (30 mcg), vancomycin (30 mcg) each. Detection of inducible clindamycin resistance producer was done by D – Test according to CLSI guidelines 2021 [(9)].

RESULTS

Total 5986 different clinical specimens were collected from the patients admitted in critical care unit and processed during the study period. From all samples 118 isolates were identified as *S.aureus* of which 89 (75.42%) were found to be MRSA as depicted in Figure-1. The prevalence of MRSA was found higher in males (48; 53.93%) than females (41; 46.07%) (Figure-2). Maximum number of MRSA strains were isolated from patients >60 years of age, 37.07% of the total MRSA population followed by 0–15 years age (24.72%), 15–30 year age group (20.23%), 45–60 year of age group (10.12%), and the least was found in 30–45 year age group of patients (7.86%) as depicted in Table-1. The highest number MRSA, was observed in blood samples (19; 21.35%) followed by E.T.aspirate (17; 19.10%), tracheal (13; 14.61%) and least was sputum (1; 1.12%) as depicted in Figure-3. The results of antibiotic susceptibility tests were studied [Table 2 & Figure 4]. In 89 isolates of MRSA, all were resistant to Penicillin, followed by Azithromycin (91%), Nitrofurantoin (89.9%), ciprofloxacin (76.4%), levofloxacin (75.3%), Clidamycin (58.4%), Gentamycin (50.6%), Moxifloxacin (44.9%), Doxycycline (38.2%), Co-trimoxazole (37.1%), Chloramphenicol (25.8%), relatively lower resistance pattern were observed with Minocycline (6.7%), Vancomycin (5.6%), linezolid (4.5%). Eighteen of 89 (20.22%) isolates were found positive for ICR. Seventy one out of 89 (79.78%) showed ICR negative (Table.3 & Figure.5).

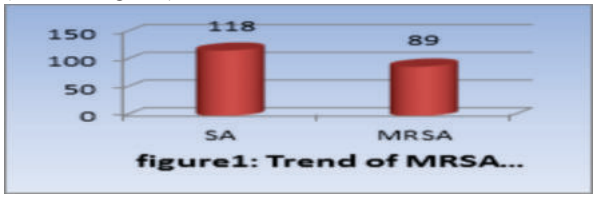


figure1: Trend of MRSA...

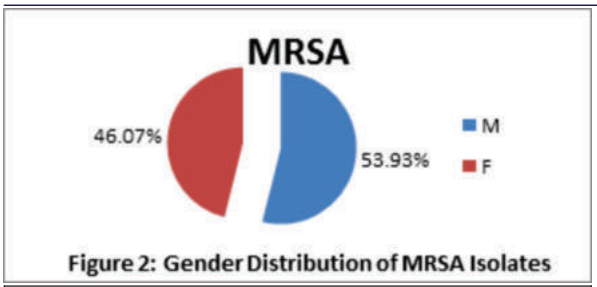


Figure 2: Gender Distribution of MRSA Isolates

Table 1: Distribution of MRSA isolates on the basis of Age

Age group	MRSA (n=89)
0–15	22 (24.72%)
15–30	18 (20.23%)
30–45	07 (7.86%)
45–60	09 (10.12%)
>60	33 (37.07%)

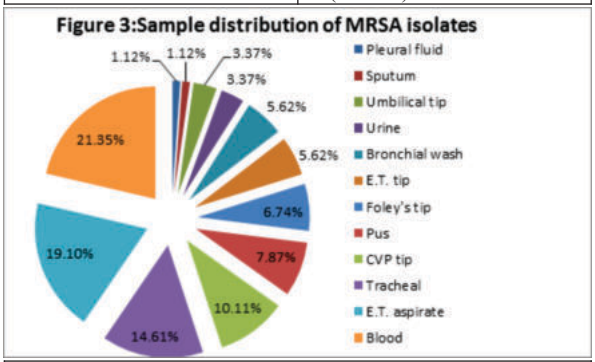


Table 2: Resistance pattern of MRSA isolates by Kirby Bauer disc diffusion method

Antimicrobials Tested	Resistance pattern of MRSA isolates (n=81)	
P	89	100.0%
AZM	81	91.0%
NIT	80	89.9%
CIP	68	76.4%
LE	67	75.3%
CD	52	58.4%
GEN	45	50.6%
MO	40	44.9%
DO	34	38.2%
COT	33	37.1%
C	23	25.8%
MI	6	6.7%
VA	5	5.6%
LZ	4	4.5%

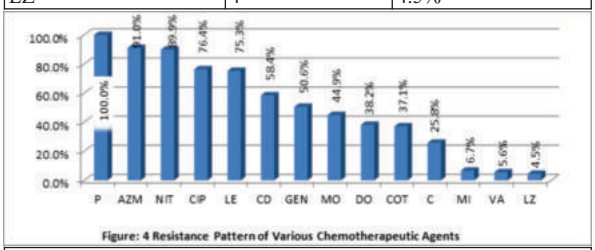
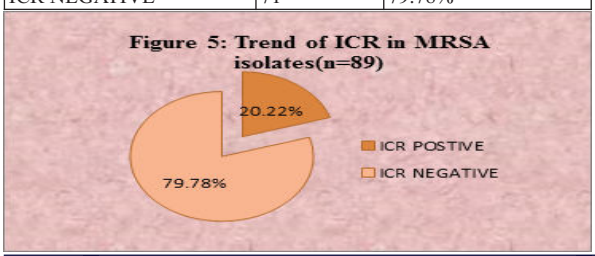


Figure: 4 Resistance Pattern of Various Chemotherapeutic Agents

Table 3: ICR detected by D-Test

ORGANISM	MRSA isolates(n=89)	
ICR POSTIVE	18	20.22%
ICR NEGATIVE	71	79.78%



DISCUSSION

Escalation of antimicrobial drug resistance is a continuous threat for the humanity. It's not the problem of one country rather entire globe is facing the new challenges everyday due to development of antimicrobial resistance in various microbes. It is a matter of concern that the present study reports the MRSA prevalence in ICU is 75.42%. The study done by Kumar Gaurav et al. reported 10.2% MRSA prevalence in ICU in lucknow [10]. Whereas the study of Sharanagouda S. Patil et al. reported 55% prevalence of MRSA in ICU of Jammu and Kashmir and 21% prevalence of MRSA in Maharashtra [11]. The MRSA prevalence rate varies in various countries. The study by Tahir Mehmood Khan et al. reported 55% MRSA prevalence rate in ICU of Malaysia [12]. The study done by Lennox Archibald et al. Reports, 33% MRSA prevalence rate in ICU of US [13]. As per the study conducted by Shania Indah Chineko et al. in 2021 reported that 1178 of 3763 results of total positive culture tests were reported from the ICU consisting of 741 (62.9%) positive culture were MRSA which is close to our study [14]. The factors responsible for variations in the prevalence of different studies may due to difference in geographical area, variation in sample sizes and length of study, specimens, methods used for testing, antibiotic policies, and status of infection control.

On comparing MRSA isolates on basis gender distribution, males (53.93%) were more affected than female patient (46.071%). The similar type of distribution were observed by Rao et al. in 2012 [15]. The most affected age group was found to be elderly patients >60 years in this study. A similar trends were obtained by Sharma S, Mall A et al. in 2011 [16].

MRSA isolates were founded more from blood and e.t.aspirates, 21.35% & 19.10% respectively. In the study done by Dar et al. reported pus sample on higher side in Aligarh (35.5%), Srinivas et al. in Andhra Pradesh (64%), Tiwari et al. in Varanasi (42%), and Rao and Mallick and Basak in Maharashtra (61.4%) all reported pus sample on higher side[15,17,18].

In the AST pattern of MRSA, penicillin was founded to 100% resistant. Out of 89 MRSA isolates, 18 isolates were postivie for ICR (20.22%). More than 50% of MRSA isolates were resistant to azithromycin, nitrofurantoin, ciprofloxacin, levofloxacin, Clindamycin and Gentamicin. Moxifloxacin, doxycyclin, co-trimoxazole, chloramphenicol, minocyclin resistance was seen in 44.9%, 38.2%, 37.1%, 25.8%, 6.7% respectively. Similar pattern was obtained in other studies done by Sanjana RK, Shah R et al., Oberoi L, Kaur R et al., Gadepalli R, Dhawan B et al., Foster TJ et al. [19,20,21,22]. Our study reported maximum sensitivity with vancomycin (94.4%) and linezolid (91%) sensitive which is closely related with the study done by Soumyadeep Ghosh et al. vancomycin and linezolid (100%) sensitive for antibiotic tested against MRSA in 2016 [23].

CONCLUSION

Present study showed need of continuous monitoring of MRSA with detection of ICR. With the spread of ICR producing MRSA strains in hospitals all over the world, it is necessary to know the prevalence of ICR in a hospital so as to use of Clindamycin judicially in area where MRSA is much higher. Equally important is the knowledge of ICR in the MRSA from a patient to avoid misuse of Clindamycin which could lead to treatment failure if not tested for ICR. The burden of MRSA can be reduced by the implementation of appropriate infection control practices and avoiding the injudicious / misuse of broadspectrum antibiotics. Also there is need of regular MRSA surveillance of HCWs, strict compliance to hand hygiene, and formulation of antibiotics policies. These control measures if implemented can control the spread of MRSA in hospitals as well as community.

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