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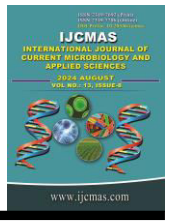

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Phenotypic Detection and Antibiotic Susceptibility of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Clinical Isolates at Maternal & Child Tertiary Care Hospital-Western Rajasthan

Richa Agrawal*, Pinky Bhagat and Rajendra Singh Parihar

Department of Microbiology, Dr. S. N. Medical College, Jodhpur, India

**Corresponding author*

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A B S T R A C T

Methicillin Resistant *Stahylococcus aureus* (MRSA) continue to be a major nosocomial pathogen causing increased morbidity and mortality worldwide. Emergence of hospital acquired MRSA strain posses great challenge for treatment leading to higher morbidity and mortality. This was a retrospective study done in a tertiary care hospital from November 2022 to July 2023. Various clinical samples such as pus, blood, CSF, vaginal swab, pleural aspirate were received & processed and *Staphylococcus aureus* species were identified as per standard laboratory protocol. AST was performed & MRSA was detected by disc diffusion test as per CLSI guideline 2022. Total 214 *Staphylococcus aureus* were isolated. 142 were MRSA, 72 were MSSA. Maximum number of *Staphylococcus aureus* (both MRSA & MSSA) were isolated from blood (48.13%) followed by pus (42.05%). MSSA were found 100% sensitive to Amikacin, & Lenezolid followed by Cotrimoxazol (97.22%), Piperacillin –tazobactam (97.22%), gentamicin (95.83%). MRSA strain 100% sensitive to Linezolid followed by Amikacin (95.77%), Gentamicin (91.54%) and Ciprofloxacin (72.53%). The result showed that *Staphylococcus aureus* isolated were resistant to most of the commonly used antibiotics. MRSA isolates were found sensitive to Linezolid, Amikacin and ciprofloxacin. There is need to establish antimicrobial surveillance system which will help in making antibiotic policy to prevent overuse and misuse of antibiotics.

Introduction

Staphylococcus aureus is a pathogen causing wide range of infections from mild localized skin lesions to severe life threatening systemic infection such as septicaemia, pneumonia, endocarditits etc (Boucher and Corey, 2008; Valle *et al.,* 2016) in both community as well as in hospital settings. Among Gram positive cocci, Methicillin Resistant *Stahylococcus aureus* (MRSA) continue to be a major nosocomial pathogen causing increased morbidity and mortality worldwide.

In 1942 penicillin resistance *Staphlyococcus aureus* strain were reported which led to the introduction of a new drug Methicillin in October 1960 (Jevons, 1961), 2 years later *MRSA* strain were reported widely. *MRSA* is due to presence of mecA gene, resulting in synthesis of an altered penicillin binding protein (PBP-2a) by the

virtue of which the organism have low affinity for β-lactam antibiotics and also to a range of different antibiotics classes such as macrolide, flouroquinolones, tetracyclines (Maple *et al.,* 1989).

The factors responsible for emergence of *MRSA* could be attributed to carriage of *MRSA* in nose, axilla, perineum and hands of patients and healthcare workers (HCW), irrational use of antibiotics, prolonged hospital stay, presence of indwelling catheters and cannula, immunosuppression, elderly age, decubitus ulcer, insulin dependent diabetes (Naves *et al.,* 2012).

Initially *MRSA* were hospital acquired but it is seen that community acquired *MRSA* is being increasingly reported in India (Grema *et al.,* 2015).

It is known that *MRSA* is endemic in India with variation in the antimicrobial susceptibility patterns based on geographical region & patient population (Agarwal *et al.,* 2015).

Emergence of hospital acquired MRSA strain posses great challenge for treatment leading to higher morbidity and mortality (Chatterjee *et al.,* 2018).

According to Indian Council of Medical Research–Antimicrobial Resistance Surveillance Network (ICMR–AMRSN) report, the total *MRSA* prevalence in India escalated from 32.9% in 2017 to 38.6% in 2018, with North India reporting the highest MRSA prevalence of 52.8%, followed by West India (48.1%) (AMRSN Annual Report, 2018).

It is listed as Priority 1 (High) in the 2017 WHO list of bacteria for which new antibiotics are urgently needed (WHO, 2017). The CDC has also classified *MRSA* as a serious threat and therefore listed it in the 2019 Antibiotic Resistance Threat Report (CDC, 2019).

Understanding the prevalence, antibiotic resistance patterns and information on accurate and reliable detection methods of MRSA strains are necessary for appropriate antibiotic treatment and effective infection control.

Materials and Methods

Study setting: Microbiology laboratory of Maternal and Child tertiary care hospital, Western Rajasthan India.

Study duration: November 2022 to July 2023 (Nine months).

Study design: Retrospective observational study.

Sample size and sampling

Various clinical samples such as pus, blood, CSF, vaginal swab, pleural aspirate were received in the department of microbiology for aerobic bacterial culture & sensitivity. Samples were collected with proper aseptic procedure and were processed as per standard microbiological laboratory methods (Garry W. Procop *et al.,* 2016).

Data collection: Demographic details (age /Gender/ IPD/OPD) and clinical data (chief complaints, gynae/ paediatric etc) were noted in the proforma after taking institutional ethical committee permission.

Antimicrobial sensitivity testing

A standardized Kirby–Bauer disk diffusion method in the Muller–Hinton Agar (MHA) plate technique was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2022 (CLSI, 2022). A bacterial suspension equivalent to the 0.5 McFarland turbidity standard was prepared for inoculation. Standard antimicrobial disks representing multiple drug classes were subsequently set and the plates were incubated at 37°C for 24 hours in Mueller–Hinton agar.

An inhibition zone diameter of each antimicrobial was then measured and interpreted as resistant (R), intermediate (I), and sensitive(S). The antibiotics tested (Himedia, India) were Cefoxitin (30 µg), Penicillin (10 µg), Gentamicin (10 µg), Amikacin (30ug), Cotrimoxazole (1.25/23.75 µg), Clindamycin (2 µg), Erythromycin (15 µg), Linezolid (30 µg), Piperacillin-Tazobactam (100/10ug), Amoxicillin clavulanic acid (20/10ug), Ciprofloxacin (5 ug).

Detection of methicillin resistance

Methicillin resistance was detected by phenotypic method of cefoxitin disc diffusion test (CDD), all isolates were tested by CDD test using 30 ug Cefoxitindisc after making a lawn culture of 0.5 McFarland suspension of isolates on Muller Hinton Agar (MHA) plate. Plates were read after incubating at 37°C for 18 h. Any zone diameter

of ≤21 mm was reported as cefoxitin resistant and reported as *Methicillin resistance Staphylococcus Aureus* (MRSA) (CLSI, 2022).

Results and Discussion

Total 4338 clinical samples were received in the study time period. Out of 4338 samples growth were present in 1377 samples and out of these *Staphylococcus aureus* were isolated in 214 (15.54%) samples.

Out of 214 *Staphylococcus aureus* isolates, 142 were *MRSA*, 72 were *MSSA*, 142 were detected in paediatric population & 72 isolated in OBG patients (Table 1 & 2).

Maximum number of *Staphylococcus aureus* (both *MRSA & MSSA*) were isolated from blood (48.13%) followed by pus (42.05%), vaginal swab (6.54%), CSF (1.40%) (as shown in table 3).

Methicillin sensitive staphylococcus aureus strain were found 100% sensitive to Amikacin, & Lenezolid. They were sensitive most of the routinely used antibiotics like Cotrimoxazol (97.22%), Piperacillin –tazobactam (97.22%), gentamicin (95.83%), Ciprofloxacin (88.88%) and clindamycin (75%) (Table.4)

MRSA strain showed maximum sensitivity to Linezolid (100%) followed by Amikacin (95.77%), Gentamicin (91.54%) and Ciprofloxacin (72.53%) (as shown in table 5).

In this study we found increasing trend of *MRSA* isolates (142) as compared to MSSA (72). This can be explained by presence of various risk factors like carriage of *MRSA* by HCWs and patients, poor hand hygiene practices, prolonged hospital stay and lack of any surveillance programs for *MRSA*. One of the major factor contributing to this increasing trend could be attributed to the fact that ours is a tertiary care centre where by the time patient reaches us they have already been on various antimicrobials agents (either by easily available over the counter medication or given by various quacks).

Similar trends were also observed in study by Mallick *et al.,* (2010); Patel *et al.,* (2010); Gopalkrishnan *et al.,* (2010); Sangeeta Joshi *et al.,* (2013); Lohan *et al.,* (2021) in India. Whereas in study by Ramanand kumar *et al.,* (2019) from Bihar reported in their study that out of 200 *Staphylococcus aureus* isolates, 73 (36.6%) were methicillin resistant (*MRSA*) and 127 (63.4%) methicillin sensitive (*MSSA)*. This difference in incidence of MRSA could be due to different geographical region.

In present study maximum number of *Staphylococcus aureus* (both *MRSA & MSSA*) were isolated from blood (48.13%) and pus (42.05%) samples followed by vaginal swab (6.54%), CSF (1.40%). A study by Anurupa *et al.,* (2003) found maximum isolation of *MRSA* from pus and wound swabs (52.5). The article by Sangeeta *et al.,* (2013) published in 2013 said that overall *MRSA* strains was highest for skin and soft tissue infections (64% in 2008 and 61% in 2009) followed by blood (48%) and respiratory samples including bronchial washings, endotracheal secretions and sputum (41%). A study by Purav Patel *et al.,* (2014) in a tertiary care teaching hospital, Western India also found out that skin and soft tissue infection (72.37) accounted for most of the *MRSA* cases. Ramanand kumar *et al.,* (2019) reported in their study that maximum isolates of pus was isolated from pus (41.09%), postoperative wound infection (17.80%), blood samples (21.91%) (Naves *et al.,* 2012), miscellaneous sample (13.69%), urine (5.47%).

A study by Lohan *et al.,* (2021) observed maximum isolates of *MRSA* was from pus (61%) followed by urine (23.4%), blood (12.3%) and least was from catheter tip (1.2%).

Methicillin sensitive staphylococcus aureus strain in this study were found 100% sensitive to Amikacin & Linezolid. They were sensitive to most of the routinely used antibiotics like Cotrimoxazole (97.22%), Piperacillin –tazobactam (97.22%), gentamicin (95.83%), Ciprofloxacin (88.88%) and clindamycin (75%).

Whereas *MRSA* strain in our study showed maximum sensitivity to Linezolid (100%) followed by Amikacin (95.77%), Gentamicin (91.54%) and Ciprofloxacin (72.53%).

Similar findings were reported *by* Ramanand kumar *et al.,* (2019) in which 100% MRSA strain were susceptible to linezolid and vancomycin, moderate susceptibility (71,14%) to gentamicin, cefuroxime and least susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% respectively). All *MRSA* strains (100%) were sensitive to vancomycinin a study from north India Arora *et al.,* (2010) and the *MRSA* isolates were found to be more resistant to other antibiotics than *MSSA*. Sangeeta *et al.,* (2013) reported no resistance documented against vancomycin and linezolid in *MRSA* strain (100%).

Table.1 Distribution of *MRSA* and *MSSA* from total clinical isolates (n=214)

SN	Total *Staphylococcus aureus* N(%)	*MRSA* N(%)	*MSSA* N(%)
1.	214 (100)	142(66.35)	72(33.64)

Table.2 Gender wise distribution of *Staphylococcus aureus* isolates. (n=214)

	Peadiatric (%)	OBG (%)	Total (%)
Male	89 (62.23%)	-	89 (41.58%)
Female	53 (37.32%)	72 (100%)	125 (58.87%)
Total	142 (100%)	72 (100%)	214 (100%)

Table.3 Sample wise distribution of MRSA and MSSA isolates**.**

Sample type	*MRSA* N(%)	*MSSA* N(%)	Total N(%)
Pus	52 (36.61)	38 (52.77)	90 (42.05)
Blood	75 (52.81)	28 (38.88)	103 (48.13)
Vaginal swab	09 (6.33)	05 (6.94)	14 (6.54)
CSF	02 (1.40)	01 (1.38)	03 (1.40)
Pleural fluid	02 (1.40)	00	02 (0.93)
Ascitic fluid	01 (0.70)	00	01 (0.46)
Sputum	01 (0.70)	00	01 (0.46)
Total	142 (100)	72 (100)	214 (100)

Table.4 Antibiotic Sensitivity pattern of Methicillin Sensitive *Staphylococus aureus* (MSSA)

Drugs	Methicillin sensitive *Staphylococcus aureus (MSSA)* N-72(%)
Penicillin (10 µg)	12(16.66%)
Cefoxitin (30 µg)	72 (100%)
Cefazolin (30 µg)	58 (80.55%)
Amoxicillin clavulanic acid (20/10 µg)	58 (80.55%)
Erythromycin (15µg)	36 (50%)
Clindamycin (2 µg)	54 (75%)
Gentamicin (10 µg)	69 (95.83%)
Amikacin (30 µg)	72 (100%)
Piperacillin-tazobactam (100/10µg)	70 (97.22%)
Ciprofloxacin (5µg)	64 (88.88%)
Cotrimoxazol (1.25/23.75 µg)	70 (97.22%)
Linezolid (30µg)	72 (100%)

Table.5 Antibiotic sensitivity pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates**.**

Drugs	Methicillin Resistant *Staphylococcus aureus (MRSA)* N-142(%)
Penicillin (10 µg)	00
Cefoxitin (30 µg)	00
Cefazolin (30 µg)	00
Amoxicillin clavulanic acid (20/10 µg)	33 (23.23%)
Erythromycin (15µg)	12 (8.45%)
Clindamycin (2 µg)	36 (25.35%)
Gentamicin (10 µg)	130 (91.54%)
Amikacin (30 µg)	136 (95.77%)
Piperacillin-tazobactam (100/10µg)	03 (2.11%)
Ciprofloxacin (5µg)	103 (72.53%)
Cotrimoxazol (1.25/23.75 µg)	20 (14.08%)
Linezolid (30µg)	142(100%)

Resistance to antibiotics amongst the MRSA isolates was more than that in methicillin sensitive *S. aureus* (*MSSA*) (P<0.001). Singh *et al.,* (2022) reported that MRSA strains present maximum resistance against Ciprofloxacin (71.8%), Erythromycin (70.6%), Clindamycin (59.3%), Gentamicin (36.8%) and Cotrimoxazole (34.1%) and all *MRSA* isolates were discovered to be sensitive to Linezolid and Vancomycin.

Staphylococcus aureus isolated were resistant to most of the commonly used antibiotics. *MRSA* isolates were found sensitive to Linezolid, Amikacin and ciprofloxacin. There is need to establish antimicrobial surveillance system which will help in making antibiotic policy of hospital to prevent overuse and misuse of antibiotics. Also there should be ongoing and continuous training of hospital infection prevention &control program to prevent spread of drug resistant microorganism.

Limitation of study

Vancomycin susceptibility could not performed due to limited resources for MIC (Minimum inhibitory concentration) detection. As disc diffusion test of Vancomycin for *Staphylococcus aureus* is not recommended as per CLSI guidline 2023.

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Author Contributions

Richa Agrawal: Investigation, formal analysis, writing—original draft. Pinky Bhagat: Validation, methodology, writing—reviewing. Rajendra Singh Parihar:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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