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Antibiotic susceptibility and prevalence of Methicillin-resistant *Staphylococcus aureus* in different clinical isolates in a tertiary care hospital

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ABSTRACT

Background: *Staphylococcus aureus* is a Gram-positive cocci bacterium responsible for variety of infections. Emergence of Methicillin-resistant *S. aureus* (MRSA) strains has led to a greater threat in different clinical settings. Early identification of MRSA in different infections is an effective measure to prevent and treat such systemic infections. **Aims and Objectives:** This study aims to isolate and identify MRSA with their antibiotic sensitivity in different clinical samples. **Materials and Methods:** A total of 2041 clinical samples from January to June 2021were collected and processed at the Microbiology Department of Burdwan Medical College and Hospital. *S. aureus* was identified and their antibiotic susceptibility testing was determined. MRSA/methicillin-sensitive *S. aureus* (MSSA) were identified by cefoxitin disc (30 mcg/disc) and their susceptibility to vancomycin and MIC determined by Etest following British Society for Antimicrobial Chemotherapy (BSAC) guidelines. **Results:** Of 2041 samples, 358 (13.36%) isolates were *S. aureus,* of which 207 (57.82 %) MRSA and 151 (42.18%) MSSA. Among the 207 MRSA isolates, 40% were from patients with sepsis, 18.36% from pyrexia of unknown origin, 12.56% from surgical site infection, and 10.62% from ventilator-associated events. The highest number (55.55%) of MRSA was isolated from blood followed by 27.05% of isolates were from pus. All the isolates were sensitive to vancomycin with MICs of 0.5 µg, 0.75 µg, 1.0 µg, and 1.5 µg according BSAC and also very good sensitivity to levofloxacin and piperacillin/tazobactam. **Conclusion:** The increasing prevalence of MRSA is posing a real threat to the health-care system. Careful detection and judicious use of antibiotics are the only answer.

Key words: MIC; MRSA; MSSA; *Staphylococcus aureus*

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) strains are an important cause of community and healthcare-associated infection worldwide.[1] Humans are natural reservoirs of *Staphylococcus aureus* forming an important part of the normal flora of our body and about 30% of them are persistent colonizers of *S. aureus*.[2,3] *S. aureus* is an important cause of various infections of the skin, joints, urinary and respiratory tract, and septicemia.[4] They may also produce infective endocarditis and device-related infections.[5]

The colonization of MRSA in healthy individuals causes serious infections, spreading through infected hands while performing different procedures or at the time of providing care to the patients, and is responsible for poor prognosis in the hospitalized patients. Factors leading to increased

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transmission are contact through breaks in the skin and mucosa (indwelling catheters or wounds), crowded ICUs, prolonged hospital stay, lowered immune status, and poor personal hygiene.[6]

Previously, all these strains of *S. aureus* were susceptible to the beta-lactam group of antibiotics but with rampant and inappropriate use of these antibiotics, resistant strains have developed around 1960s due to the development of the β-lactamase gene mecA by the bacteria. *S. aureus* acquired the β-lactamase gene mecA by horizontal transfer of a mobile genetic element called staphylococcal cassette chromosome mec.[7] A new penicillin-binding protein 2a (PBP2a) was developed by the bacteria with the help of the mecA gene. PBP2a led to the development of cross-linkages in the peptidoglycan present in the cell wall. The PBP2a also has low affinity for β-lactams, resulting in resistance to this entire class of antibiotics.[8]

MRSA strains have become the most common nosocomial pathogens and infection due to these strains is severe, requiring longer hospital stay, higher cost, and increased mortality and morbidity.[9,10]

These infections are of a serious concern as they are resistant to most of the commonly used antibiotics. However, these strains are susceptible to glycopeptides like vancomycin[11] and early control of these MRSA isolates with effective antibiotic is very much essential.

Aims and objectives

This study aims to find this antibiotic susceptibility in the isolates isolated from the different wards of a tertiary care hospital.

MATERIALS AND METHODS

This is a prospective study, conducted at the Department of Microbiology of Burdwan Medical College and Hospital from January to June 2021. A total of 2041 samples (blood, pus, fluid, aural swab, sputum, wound swab, throat swab, and urine) were collected from clinically suspected patients in the pediatric ward: Shishu Niketan Medicine, neonatal intensive care unit (NICU), special newborn care unit (SNCU), pediatric intensive care unit (PICU), gynecology, general surgery, chest medicine, and critical care unit (CCU) of Burdwan Medical College and Hospital in West Bengal. Information regarding age, sex, onset of the lesion, any history of previous antibiotic intake, and presence of any medical complications in the patients was also collected.

The Institutional Ethics Committee permission was also taken and informed consent was collected from the participants. Informed consent was taken from parents or legal guardians for underage children (<18 years).

Inclusion criteria

All the patients with the diagnosis of

- Septicemia
- Meningitis
- Pneumonia and pneumonia on ventilatory support
- Urinary tract infection(UTI)
- Reproductive tract infection
- Hospital-acquired surgical site infection (SSI)
- Deep-seated wound infection (abscess)
- Superficial infection (cellulitis)
- Burn patients

Exclusion criteria

- Colonizers of coagulase-negative *Staphylococcus* but with no clinical symptoms.
- Improperly collected samples.
- Patients with no informed consent.

Processing of the samples

Samples were processed by the standard conventional methods. Double swabs were taken from each site and inoculated in nutrient agar (NA) and then subcultured in mannitol salt agar (MSA) and incubated aerobically for 24 h at 37°C. Blood samples were taken in trypticase soy broth and inoculated on blood agar (BA) and chocolate agar (CA) and then subcultured in MSA. MSA is the selective media for *S. aureus*.

The colonies showing typical characteristics of *S. aureus* like golden-yellow pigmented colonies on NA and yellow colonies with yellow zones on MSA. Further identification was carried out by Gram staining (Gram-positive cocci in clusters), colony morphology, hemolysis on BA, mannitol fermentation test, catalase test, and coagulase test (both the slide and tube coagulase tests).

Antibiotic susceptibility testing was done by Kirby–Bauer disc diffusion method in Mueller-Hinton agar (MHA) media using commercially available HiMedia discs as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines. A bacterial suspension equivalent to the 0.5 McFarland turbidity standard was prepared for the inoculation. The antibiotics which were tested for the Gram-positive cocci are ampicillin (10 mcg/disc), ceftriaxone (30 mcg/disc), azithromycin (15 mcg/disc), gentamicin (10 mcg/disc), amikacin (30 mcg/disc), netilmicin (30 mcg/disc), cotrimoxazole (25 mcg/disc), vancomycin (30 mcg/disc), linezolid (30 mcg/disc), teicoplanin (30 mcg/disc), piperacillin/tazobactam (100/10 mcg/disc), ciprofloxacin (30 mcg/disc), levofloxacin (5 mcg/disc), and nitrofurantoin

(300 mcg/disc). The MHA plates were incubated at 37°C for 24 h.

Detection of MRSA or MSSA (Cefoxitin disc diffusion test)

MRSA or MSSA was identified using the cefoxitin (30 mcg/disc). A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on MHA plate. The MHA plates were incubated at 37°C for 18–24 h and zone diameters were measured.

An inhibition zone diameter was interpreted as resistant (R), intermediate (I), and sensitive (S) by comparing with the standard ATCC 33591 control. Inhibition zones were interpreted according to CLSI guidelines. An inhibition zone diameter of ≤19 mm was reported as oxacillin resistant and ≥20 mm was considered as oxacillin sensitive.

Detection of multidrug drug resistance (MDR) strain

The isolates which were resistant to at least one antimicrobial drug in three or more antimicrobial categories were considered as MDR strain. The groups of drugs tested are penicillin (ampicillin), cephalosporin (ceftriaxone), aminoglycosides (gentamicin, amikacin, and netilmicin), macrolides (azithromycin), fluoroquinolones (ciprofloxacin and levofloxacin), glycopeptides (vancomycin and teicoplanin), and oxazolidinones (linezolid).

Detection of minimum inhibitory concentration (MIC)

Isolates of MRSA were checked for the susceptibility to vancomycin and the MIC was determined by Etest strips (bioMérieux) following the British Society for Antimicrobial Chemotherapy (BSAC) guidelines Etest on MHA plates. MICs of 0.5 µg, 0.75 µg, 1.0 µg, and 1.5 µg were studied based on BSAC guidelines.

RESULTS

In this study, out of 2041 total samples, 358 (17.54%) isolates were *S. aureus,* among which 207 (57.82 %) were MRSA and rest 151 (42.18%) MSSA. The highest number of MRSA was isolated from blood 115 (55.55%) followed by 27.05% (56) MRSA isolates from pus and 13.53% (28) from urine (Table 1). Among the 207 MRSA isolates, 40.1% were obtained from patients suffering from sepsis, followed by 18.36% from pyrexia of unknown origin, 12.56% from SSI, and 10.62% from ventilator-associated events (VAEs) (Table 2). The number of MRSA isolates obtained from male (56.04%) outnumbered the number of MRSA isolates obtained from female (43.96%). Among the total 178 isolates of MRSA obtained from blood, maximum, that is, 49 (68%) isolates were obtained from NICU, followed by 40 (54.8%) isolates were from SNCU and 16 (45.6%) were from PICU – rest from the other wards. Among the 118 MRSA isolates from pus, 35 (48.6%) isolates were from the surgery ward, 5 (21.7%) from CCU from the endotracheal tube secretions (Table 3), and rest from other wards. All isolates were found to be sensitive to vancomycin with MICs of 0.5 µg, 0.75 µg, 1.0 µg, and 1.5 µg according BSAC as seen by the E strips (Figure 1). Linezolid and teicoplanin also showed 100% sensitivity. *S. aureus* isolates obtained from blood showed 81.46% sensitivity to levofloxacin followed by 76.5% to amikacin and 74.5% to netilmicin (Figure 2). Likewise, *S. aureus* isolates obtained from pus revealed 86.44% sensitivity to levofloxacin followed by 65.71% sensitivity to piperacillin/tazobactam and 65.57% to netilmicin (Figure 3). *S. aureus* isolates obtained from urine, revealed highest sensitivity to nitrofurantoin (82.69%) followed by 77.2% to netilmicin and 72.3% to amikacin (Figure 4). *S. aureus* isolates obtained from ET tube and showed 80% sensitivity to levofloxacin, 60% sensitivity to amikacin, and 40% sensitivity to piperacillin/tazobactam.

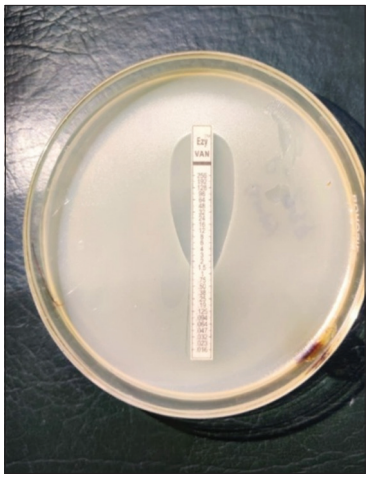

Figure 1: Etest showing vancomycin susceptibility

Table 1: Distribution of MRSA and MSSA among the different types of samples

Types of sample	Total samples, n=2041	*Staphylococcus aureus* isolates, n=358	MRSA, n=207	MSSA, n=151
Blood	419	178 (49.72%)	115 (55.55%)	63 (41.72%)
Urine	840	52 (14.53%)	28 (13.53%)	24 (15.89%)
Pus	548	118 (32.96%)	56 (27.05%)	62 (41.1%)
Sputum	213	5 (1.4%)	3 (1.44%)	2 (1.32%)
Others	21	5 (1.4%)	5 (2.42%)	0

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*

DISCUSSION

MRSA causes a wide variety of infections – boils, carbuncles, impetigo, cellulitis, and wound infections or even the more severe ones like ventilator-associated pneumonia, necrotizing fasciitis, toxic shock syndrome, and sepsis,[12] causing a great burden on all the hospitals and hence the society.

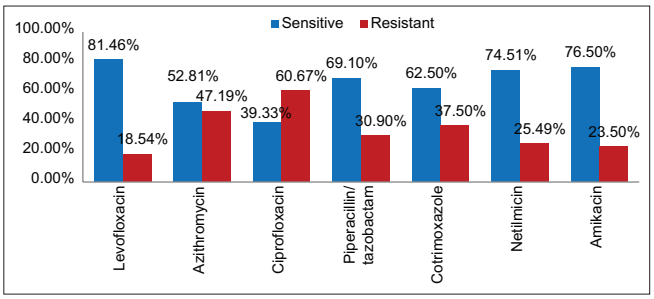

Figure 2: ABST from blood (n=178)

MRSA is a bacterial isolate that shows resistance to multiple drugs and is present in 13–47% infections due to *S. aureus* in India[13] and has emerged as a serious problem worldwide since 1961.[14]

They are responsible for a number of serious infections leading to significant patient morbidity and mortality.[15] Intensive care units are the sites of origin of these multidrug-resistant MRSA and are responsible for their spread within the hospital. Their excessive prevalence in the ICUs is a feature of great anxiety even in countries practicing very good infection control measures. The

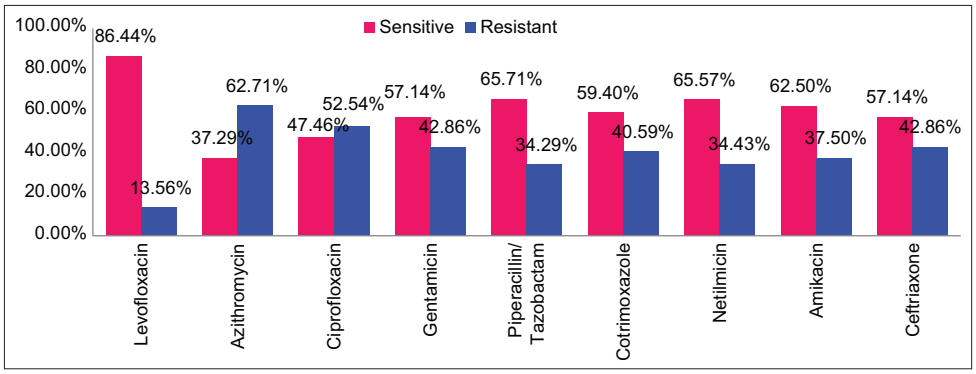

Figure 3: ABST from pus (n=118)

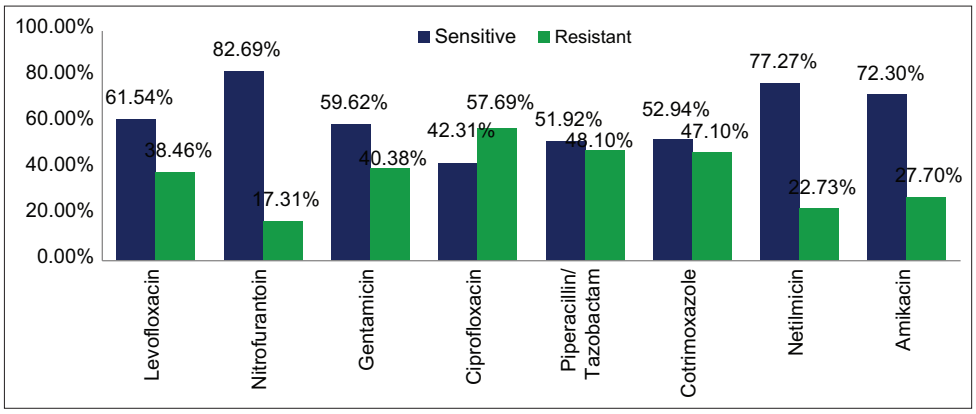

Figure 4: ABST of urine (n=52)

Table 2: Diagnosis wise distribution of MRSA

n=358	MRSA: n=207	%	MSSA: n=151	%
Sepsis (n=110)	83	40.1	27	17.88
Pyrexia of unknown origin (n=65)	38	18.36	27	17.88
Urinary tract infection (n=18)	10	4.8	8	5.29
Surgical site infection (n=45)	26	12.56	19	12.58
Burn (n=20)	16	7.73	4	2.65
Ventilator-associated events (48)	22	10.62	26	17.22
Abscess (n=52)	12	5.8	40	

MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 3: Ward wise distribution

Type of samples	NICU-72		PICU-45		SNCU-73		SNM-45		Surgery 72		Gynae-23		Chest-5		CCU-23	
	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
Blood-178	49 (68%)	23 (32%)	16 (35.6%)	11 (24.4%)	40 (54.8%)	20 (27.4%)	8 (17.8%)	7 (15.6%)	----	----	----	----	----	----	2 (8.7%)	2 (8.7%)
Urine-52	----		5 (11%)	3 (6.7%)	3 (4.1%)	1 (1.4%)	9 (20%)	8 (17.8%)	4 (5.6%)	2 (2.8%)	3 (13%)	8 (34.8%)	----	----	4 (17.4%)	2 (8.7%)
Pus-118	----		6 (13.3%)	4 (8.9%)	3 (4.1%0	6 (8.2%)	6 (13.33%)	7 (15.6%)	35 (48.6%)	31 (43%)	4 (17.4%)	8 (34.8%)	----	----	2 (8.7%)	6 (26.1%)
Sputum-5	----		----	----	----	----	----	----	----	----	----	----	3 (60%)	2 (40%)	----	----
ET tube-5	----		----	----	----	----	----	----	----	----	----	----	----	----	5 (21.7%)	----

NICU: Neonatal intensive care unit, PICU: Pediatric intensive care unit, SNCU: Special newborn care unit, SNM: Shishu Niketan Medicine, CCU: Critical care unit, MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*

World Health Organization revealed that the low- and middle-income countries have 2–3 times more frequency of MRSA than that of high-income countries.[16]

The MRSA prevalence is different in different regions ranging from 4.6% to 54.4% worldwide.[17] In India, it is different in different areas, which is probably because of easy availability of antibiotics, ineffective health-care facilities, and overuse/inappropriate use of different antibiotics in suboptimal doses, leading to resistance to different antibiotics and frequent self-medication by the patients.[18]

In our study, a total number of 358 (17.54%) blood, pus, and urine samples showed the growth of *S. aureus*; of them, 207 samples (57.82 %) showed a prevalence of MRSA. A similar study conducted at AIIMS, New Delhi, by Tyagi et al.,[19] on 2080 pus samples found a prevalence of 44% MRSA among all *S. aureus* isolates. Another study conducted by Patel et al.,[20] found 28 MRSA isolates among the 80 pus samples showing growth of *S. aureus*.

Umadevi reported a sex predilection among MRSA with it being predominantly found in males and in the 15–45 years age group.[17] This fact was also reported by studies conducted by Tsering et al.[21] This sex predilection was also found in our study with males showing MRSA in 56.04% of isolates against 43.96% MRSA in females. This view, however, was not supported by the study conducted by Geyid et al.,[22] who reported no such age/sex preponderance.

In our study, maximum number of MRSA was isolated from blood (178=49.72%,) unlike another study conducted by Suryadevara et al.,[23] who found maximum MRSA from pus followed by urine. This was unlike our study as we found MRSA from pus in 118 isolates (32.96%) and from urine in only 52 (14.53%) isolates.

Furthermore, maximum MRSA was isolated from abscess 15 cases (62.5%), burns 12 cases (60%), diabetes 11 (78.6%), and surgical wounds 21 (87.5%) in a study conducted by Garoy et al.[24] In our study, we found maximum MRSA from patients with sepsis (83/207; 40.1%), followed by SSI, UTI, burns, VAE, and abscess.

In our study, we also found that all these 207 MRSA isolates showed 100% sensitivity to vancomycin, linezolid, and teicoplanin. This finding was corroborated by the study conducted by Umadevi[17] who also found all of the isolates isolated by them to be sensitive to vancomycin, rifampicin, and teicoplanin.

Because of 100% sensitivity to vancomycin was found by us, regular monitoring and strict antibiotic stewardship should

be performed everywhere to prevent the development of vancomycin-resistant MRSA; also, routine testing of other new glycopeptides/drugs should be done if such resistant strains develops.

Limitation of the study

Molecular characterization of isolates and their associated virulence factors were not studied.

CONCLUSION

The incidence of MRSA in healthcare set up is increasing day by day. During this study, we observed that peoples of extreme of ages – aged people and neonates are susceptible to the staphylococcal contamination due to their lower immunity as the maximum MRSA isolates were obtained from NICU in babies with sepsis or bloodstream-related infection. Routine screening for MRSA in clinical laboratories should be done and inappropriate use of these drugs should be checked.

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Authors Contribution:
NC – Concept and design of the study, prepared first draft of manuscript; **TB –** Reviewed the literature and manuscript preparation; **RM –** Statistical analysis and interpretation; **AC –** Laboratory test and interpretation; and **SC –** Concept and design of the study, prepared first draft of manuscript, preparation of manuscript, and revision of manuscript.

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