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ORIGINAL RESEARCH

Phenotypic Determination of Methicillin-Resistant Staphylococcus Aureus Isolated From Tertiary Care Hospitals

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Abstract
Context: Staphylococcus aureus (S. aureus) is an opportunistic pathogen. It can cause various infections in humans and animals. The threat of antibiotic resistance in S. aureus has risen over the years and the treatment costs have also spiralled up. Methicillin-resistant Staphylococcus aureus (MRSA) is resistant to all β-lactam antimicrobials, and these strains contribute to both hospital-acquired and community-acquired infections.
Aim: This study aimed to determine the prevalence rate of methicillin-resistant Staphylococcus aureus in HA-MRSA and CA-MRSA.
Methods: MRSA were subjected to antibiotic susceptibility testing, and oxacillin discs and cefoxitin discs were used to detect them.
Results: Out of 150 Staphylococcus aureus isolates, 45 were MRSA. Of the 45 confirmed MRSA isolates, 18(40%) were HA-MRSA and 27 (60%) were CA-MRSA.
Conclusion: causes of nosocomial pathogens are responsible for causing various human infections that may range from minor skin diseases to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drug-resistant organisms is becoming more common. Therefore, early detection is most important for treating, preventing and controlling such organisms.
Keywords: HA-MRSA, CA-MRSA, Oxacillin & Cefoxitin discs

Introduction

Staphylococcus aureus (S. aureus) is a powerful pathogen that can cause simple skin infections and life-threatening systemic illnesses [1]. This bacterium's ability to quickly adapt to environmental changes and develop antibiotic resistance makes it a major issue for healthcare workers. To address this issue, this study examined methicillin resistance and antibiotic susceptibility in *S. aureus* clinical isolates from a tertiary care hospital in Indore [2,3].

The emergence of antibiotic-resistant strains among these Staphylococci raises concerns and restricts the number of antimicrobials available for the treatment of these infections [4]. MRSA is one of the most common causes of infections acquired in hospitals. Healthcare-associated MRSA (HA-MRSA) infections are a substantial burden on the healthcare system because of the increased morbidity and extra costs associated with extended hospital stays, as well as higher fatalities than those caused by methicillin-susceptible S. aureus (MSSA) [4]

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However, vancomycin-resistant and vancomycin-intermediate S. aureus (VRSA and VISA) has been reported globally, including from different parts of India,[5], and resistance to clindamycin has also been observed.[5] multi-drug-resistant S. aureus poses a challenge to treatment outcomes; therefore, tracking the prevalence and dissemination of such strains is important.[5]

Materials & Methods

The study was conducted in Index Medical College, Hospital & Research Centre, Indore, M.P.A Total of 150 *S. aureus* isolates from different clinical samples were subjected to MRSA screening using conventional microbiological methods. The clinical Specimens included pus, sputum, urine, devices (urinary catheter, cup catheter, etc.), blood, and body fluids.

The standard microbiological methods were followed in this study during culture and antibiotic sensitivity tests following universal precautions All isolates were identified by conventional methods including colony morphology, Gram staining, catalase test, coagulase test (tube & slide), and DNase test.[6]

All the confirmed S. aureus strains were subsequently tested for methicillin resistance based on the Kirby-Bauer disk diffusion method using oxacillin discs (1 µg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered methicillin-resistant if the zone of inhibition was 12 mm or less.

Cefoxitin is reported to be more sensitive in the detection of MRSA strains, therefore all suspected MRSA strains were cross-checked by the Cefoxitin disc diffusion test, using 30 µg disc. An inhibition zone of ≤21 mm was taken as MRSA.[6]. E-test for detection of MIC for oxacillin of MRSA isolates was also performed using Hi comb strips (Himedia, Mumbai).

Further, the antibiotic susceptibility pattern of methicillin-resistant S. aureus strains was determined on the day of their isolation by the modified Kirby Bauer disc diffusion method on Muller Hinton agar, using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials.

The antibiotics used were penicillin-G (10 units); gentamycin (10 µg); amikacin (30 µg);ciprofloxacin (5 µg); co-trimoxazole (25 µg); vancomycin (30 µg); linezolid (30 µg). Finally, the data were recorded and analyzed after the study as per CLSI guidelines.[7].

Results

Of 150 staphylococcus aureus isolates, 45(30%) were MRSA stains. The incidence rate of male MRSA is 30/45 (66.7%), and female MRSA is 15/45 (33.3%). Most MRSA was from the male patient's 31-40 age group (8), and females were from the 31-40 age group (Table 1)

Table 3: Age & sex-wise distribution of different isolates.

Age group (In year)	MRSA		
	Male	Female	Total
10-20	0	0	0
21-30	7	3	10
31-40	8	5	13
41-50	5	3	8
51-60	6	3	9
61-70	3	1	4
71-80	1	0	1
Total	30	15	45

Out of 150 S. aureus isolates, the highest number of S. aureus were isolated from pus samples 92(61.3%), followed by urine 25(16.7%), blood 13(8.7%), ear swab 11(7.3%) and catheter tip

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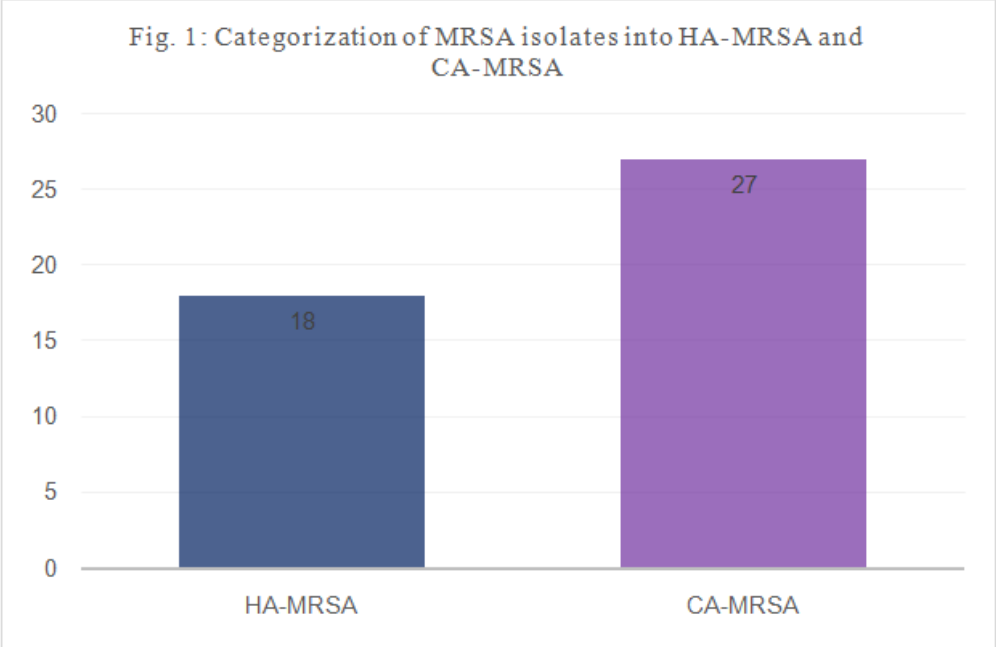
culture 9 (6%) and isolates 45 were MRSA, the maximum isolation of MRSA was from pus 25(27%), followed by Urine 9(36%), Blood 5(38%), ear swab 3(33%), and tip culture 3(33%)—Table 2.

Table 4: Prevalence of MRSA isolates from different clinical samples			
Clinical samples	**Number of S. aureus (n=150)**	**MRSA (n=45)**	**MRSA %**
Pus	92	25	27%
Urine	25	9	36%
Blood	13	5	38%
Ear Swab	11	3	27%
Tip culture	9	3	33%
Total	150	45	30%

Categorization of MRSA isolates into HA-MRSA and CA-MRSA: All MRSA isolates confirmed by phenotypic methods were further categorized into HA-MRSA and CA-MRSA based on the definition by the Centers for Disease Control and Prevention (CDC). Based on the CDC definition, the 45 confirmed MRSA isolates were categorized into 18(40%) HA-MRSA and 27 (60%)CA-MRSA (Figure 1)



Confirmation of MRSA

The overall percentage of MRSA production was 45/150(30%) among the three tests. The Oxacillin was observed in 43 isolates, whereas 45 isolates showed MRSA production by the remaining two tests, including Cefoxitin & E-test.-table 3

Table 3: Comparison of two phenotypic methods with E-test (Hi-comb MIC test) for detection of MRSA			
Isolates	**Oxacillin (1 µg) disc diffusion**	**Cefoxitin (30 µg) disc diffusion**	**E-test (Hi-Comb MIC test) methods**
Staph. aureus (n=150)	43	45	45

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The antibiotic sensitivity pattern amongst the MRSA isolates shows that 100% were sensitive to vancomycin and linezolid, 86.7% were sensitive to Co-trimoxazole, 84.4% were sensitive to clindamycin, 82.2% were sensitive to gentamycin, 75.6% were sensitive to erythromycin, and 64.4% were sensitive to ciprofloxacin. Similarly, no resistance was seen with vancomycin and linezolid.- table 4

Table 4: antibiotics sensitive pattern of MRSA (n=45)

Antibiotics	Sensitive	Percentage	Resistant	Percentage
Clindamycin	38	84.4	7	15.6
Ciprofloxacin	29	64.4	16	35.6
Co-trimoxazole	39	86.7	6	13.3
Erythromycin	34	75.6	11	24.4
Gentamycin	37	82.2	8	17.8
Vancomycin	45	100	0	0
Linezolid	45	100	0	0

Discussion

S. aureus is one of the common causes of nosocomial and community-acquired infections with high mortality and morbidity. Increased methicillin resistance among Staphylococci has posed great difficulty managing such infections. Hence, an accurate and rapid detection of methicillin resistance is essential to choose appropriate antibiotics and control the spread of MRSA. Many phenotypic methods to detect MRSA have been developed but are slow and vary in sensitivity and specificity. Detecting the mec-A gene by PCR is the gold standard for MRSA identification [116]. However, using molecular methods for routine practice is not affordable for many resource-constrained laboratories. Therefore, it is essential to develop a rapid, accurate, and sensitive phenotypic method to detect MRSA [116].

In our study, out of 45 MRSA isolates; the maximum isolation of MRSA was from pus 25(27%), followed by Urine 9(36%), Blood 5(38%), ear swab 3(33%), and tip culture 3(33%). This is consistent with the study done in Yemen [123] and Kerala [124]. In contrast to our study, other studies from Iran and Nigeria reported a high rate of isolation from blood (29%) [125] and urine (76%) [126], respectively. A Kenyatta study observed a high isolation rate from pus (68%) [127]. An increased isolation rate of S. aureus from pus may be due to the exposure of wounds or skin breaches, making them more prone to invasion of S. aureus infections. In many cases, poor hygiene is a predisposing factor.

In our study, the prevalence rate of MRSA was 30%, which was higher than the survey done by Oberoi et al. [122], who reported a prevalence of 28.86% in their study in northern India, which was lower than ours. Silvana et al 109 [128]. Their survey in Brazil documented a prevalence of 37.7% of S. aureus in ICU patients. A study from Southeast Nigeria revealed a prevalence of 60.4% [129].

In another study from Odisha, India, the prevalence rate of MRSA was 31%, slightly higher than our finding [117].

On the contrary, Tebelay et al. [130] observed a 14.3% S. aureus prevalence in their study at Yekatit, Ethiopia, from September 2013 to April 2014. Overall, this was very low compared to our study. Overall, the prevalence varies from place to place and from time to time**.**

In the present study, 45(30%) of 150 staphylococcus aureus isolates were MRSA stains. The incidence rate of male MRSA is 30/45 (66.7%), and female MRSA is 15/45 (33.3%). Most MRSA were from the male patient’s 31-40 age group (8), and females were from the 31-40 age group (5).

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In the present study, comparing two phenotypic methods proved that the cefoxitin (30μg) disc diffusion method is better than the oxacillin (1μg) disc diffusion method in screening MRSA strains.

Another significant finding of this study showed that all MRSA isolates were significantly less sensitive to antibiotics than MSSA. However, all S. aureus isolates were sensitive to vancomycin and linezolid. The sensitivity pattern of MRSA strains with other antibiotics was 86.7% were sensitive to Co-trimoxazole, 84.4% were sensitive to clindamycin, 82.2% were sensitive to gentamycin, 75.6% were sensitive to erythromycin, and 64.4% were sensitive to ciprofloxacin.

Like many other studies from India [120,121] and Iran [131], all the isolates, irrespective of their methicillin sensitivity or resistance status, were sensitive to linezolid (100%), teicoplanin (100%) and vancomycin (100%). Our study adds to the existing facts that glycopeptides (vancomycin and teicoplanin) and linezolid appear to be the most beneficial options available for treating MRSA infections.

In our study, the isolation rate of HA-MRSA and CA-MRSA was 40 % and 60 %, respectively. In discordance with our study, Nagaraju et al. [132] reported a low prevalence of 11.8% CA-MRSA in their study. John et al. [133] documented almost equal prevalence of HA-MRSA (54%) and CA-MRSA (52%) in their study. A study from Raichur, Karnataka, reported 75% and 25% prevalence of HA-MRSA and CA-MRSA, respectively [134]. D'souza et al. reported 54% CA-MRSA [135]. Available reports demonstrated variations in the prevalence of HA and CA MRSA in different places at different times. The prevalence of CA-MRSA infections in males was noticed in many studies [136,137]. Naimi et al. found the prevalence of CA and HA MRSA was 12% and 85%, respectively, in their study in the USA [138].

Conclusion

causes of nosocomial pathogen responsible for causing variety of human infections that may range from minor skin disease to life-threatening infections. In the present era of antibiotic resistance, the emergence of multi-drug resistant organism is becoming more common, therefore early detection is most important for treatment, prevention and control of such organisms.

In this study, cefoxitin was superior to oxacillin for the detection of MRSA by disc diffusion method. Therefore, cefoxitin can be a good surrogate marker for detecting MRSA. However, another high-sensitivity and specificity test, like the E-test, should combine cefoxitin disc diffusion to confirm S. aureus strains showing inhibition zone diameter between 20-22 mm. Linezolid & vancomycin were the highly sensitive drugs against MRSA isolates.

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