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DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND ASSOCIATED RESISTANCE GENES IN HOSPITAL ACQUIRED INFECTIONS

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Abstract:

Background: *The prevalence of multidrug-resistant methicillin-resistant Staphylococcus aureus (MRSA) is one of the primary causes of hospital-acquired infections worldwide and is a significant public health problem, further complicated by resistance genes like mecA, mecC and erm genes. Determine if MRSA is present and investigate the presence of the associated antimicrobial resistance genes in individuals with hospital-acquired infections.*

Methods: *A cross-sectional study was conducted in a laboratory involving 120 clinical samples from patients who were diagnosed with HAI. Staphylococcus aureus was isolated and identified from wound swabs, pus, blood, and urine using standard microbiological methods. Detection of MRSA was done by cefoxitin disc diffusion. The antimicrobial susceptibility testing was performed on commonly used antibiotics according to CLSI guidelines. PCR was used for molecular detection of resistance genes such as mecA, mecC, ermA, ermC, tetK and vanA. SPSS-26.0 software was used to statistically evaluate the data, and a p-value of less than 0.05 was considered statistically significant.*

Results: *Out of 120 samples processed, 74 (61.7%) were positive for Staphylococcus aureus. Among these, 45 (60.8%) were confirmed as MRSA. The highest percentage of MRSA isolates (40.0%) was found in wound swab specimens. MRSA isolates were all resistant to penicillin and oxacillin, and very high rates of resistance to erythromycin, 34 (75.6%) and ciprofloxacin, 29 (64.4%). The mecA gene was present in 41 (91.1%) of MRSA isolates, while ermC was found in 24 (53.3%) and ermA in 19 (42.2%). There was a significant correlation between mecA positivity and multidrug resistance (p=0.012). Vancomycin and linezolid were the most active agents, with most isolates being susceptible.*

Conclusion: *This study demonstrated that MRSA and related resistance genes are prevalent in HAIs, especially in patients in the ICUs and surgical wards.*

Keywords: *Antimicrobial resistance, Hospital acquired infections, mecA, Methicillin resistant, MRSA, Multidrug resistance, PCR, Resistance genes, Staphylococcus aureus*

INTRODUCTION

Hospital acquired infections (HAIs) are a significant public health problem globally as they are linked to increased morbidity, mortality and higher cost of healthcare (1, 2). *Staphylococcus aureus* is one of the most important bacteria that cause HAIs and is associated with bloodstream infection, surgical site infection, wound infection, pneumonia and urinary tract infection (3). It possesses multiple virulence factors and can acquire antimicrobial resistance genes rapidly, enabling survival in hospital environments.

An increasingly problematic issue in healthcare facilities is the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) which is resistant to the β-lactam antibiotics and a number of other antimicrobial agents. MRSA resistance is mainly due to the mecA gene coding for an altered penicillin-binding protein (PBP2a) that has low affinity for β-lactam antibiotics (4-6). Other resistance-associated genes, including mecC, ermA, ermC, tetK and vanA, are also found that confer resistance to macrolides, tetracyclines, and glycopeptide antibiotics, respectively (7, 8).

These resistance determinants are becoming common and are reducing therapeutic options and making

infection control more challenging. Poor infection control, overcrowding, irrational use of antibiotics, prolonged hospitalizations and invasive procedures contribute to the spread of MRSA in the healthcare environment. High risk areas where MRSA colonization or infection is likely include intensive care and surgical wards, because of frequent use of invasive devices and widespread use of broad-spectrum antibiotics (9, 10). Being able to detect MRSA and resistance genes early enough is critical to take the necessary steps to treat infection and prevent its spread.

Hence, the aim of the present study was to identify MRSA and prevalence of MRSA to assess the antimicrobial resistance genes associated with it in the hospital-acquired infections patients. While previous studies have reported MRSA prevalence, none have simultaneously evaluated mecA, mecC, ermA, ermC, tetK, and vanA genes in HAIs using PCR. This study fills that gap. The results obtained from this study can be useful for better surveillance, antimicrobial stewardship and infection control programs in health care facilities. Pakistan.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

A cross-sectional laboratory-based study was conducted in a tertiary care hospital to detect methicillin resistant *Staphylococcus aureus* (MRSA) and associated resistance genes among patients with hospital acquired infections (HAIs). The study was carried out over a defined study period, and all laboratory analyses were performed in the microbiology laboratory of the institution. This study was approved by the Institutional Review Board Committee. Informed consent was obtained from all patients or their legal guardians. Patient data were anonymized before analysis.

SAMPLE COLLECTION

A cross-sectional laboratory-based study included clinical specimens from 120 patients with HAIs were collected. According to standard hospital infection control guidelines, HAIs were defined as infections that appeared more than 48 hours after admission. Specimens included wound blood, swabs, pus, and urine samples.

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS AUREUS

Samples were cultured on standard media including blood agar and mannitol salt agar and incubated at 37°C for 24–48 hours. Colonies suggestive of *Staphylococcus aureus* were identified based on colony morphology, Gram staining, catalase test, and coagulase test (slide and tube methods).

DETECTION OF MRSA

Methicillin resistance was determined using cefoxitin (30 μg) disc diffusion method on Mueller-Hinton agar in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Zone of inhibition was measured and isolates were classified as MRSA or methicillin sensitive S. aureus (MSSA) based on CLSI interpretive criteria.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method against commonly used antibiotics including penicillin, oxacillin, erythromycin, ciprofloxacin, clindamycin, gentamicin, vancomycin, and linezolid. Results were interpreted according to CLSI standards. Isolates exhibiting acquired non-susceptibility to ≥1 agent in ≥3 antimicrobial classes were categorized as multi-drug resistant (MDR) in accordance with international consensus recommendations.

MOLECULAR DETECTION OF RESISTANCE GENES

Genomic DNA was extracted from confirmed MRSA isolates using standard extraction procedures and quantified by Nano spectrophotometry at 260/280 nm. Polymerase chain reaction (PCR) was performed for detection of resistance genes including mecA, mecC, ermA, ermC, tetK, and vanA. It was performed using a master mix containing dNTPs (0.4 mmol each), $MgCl_2$ (3 mmol), and Taq polymerase (0.08 U/μl). The thermal profile included initial denaturation at 94°C for 5 minutes, 37 cycles of denaturation (94°C for 45

s), annealing (58°C for 45 s), and extension (72°C for 45 s), with a final extension at 72°C for 5 minutes. Amplified products were analyzed using 1.5% agarose gel, stained with ethidium bromide, and visualized under UV transillumination.

DATA ANALYSIS

The collected data were entered and analyzed in Ms. Excel and Statistical software (SPSS 26). Frequencies and percentages were computed using descriptive statistics. The association of categorical variables was evaluated with chi-square test with a p value<0.05 considered as statistically significant.

RESULTS

A total of 120 patients were diagnosed with healthcare-associated infections (HAIs). Of these, 56.7% were male and 43.3% were female. The most represented age group was 46–60 years, accounting for 34.2% of cases, followed closely by the 31–45 years age group at 30.8%. Regarding admission type, ICU patients constituted the largest proportion (31.7%). Wound swab specimens were the most frequently collected samples, representing 36.7% of all specimens (Table I).

Table I. Demographic and clinical characteristics of patients with hospital acquired infections (n=120)

Variable	Frequency (n)	Percentage (%)
Gender		
Male	68	56.7
Female	52	43.3
Age group (Years)		
18–30	22	18.3
31–45	37	30.8
46–60	41	34.2
>60	20	16.7
Hospital ward		
ICU	38	31.7
Surgical ward	34	28.3
Medical ward	29	24.2
Orthopedic ward	19	15.8
Type of specimen		
Wound swab	44	36.7
Blood	28	23.3
Pus	31	25.8
Urine	17	14.2

Staphylococcus aureus was isolated from 74 (61.7%) of the 120 processed clinical samples (Table II). The identified Staphylococcus aureus were 45 MRSA and 29 methicillin sensitive Staphylococcus aureus (MSSA). These results have shown that there is a high prevalence of MRSA in hospital related infections.

Table II. Frequency of *Staphylococcus aureus* and MRSA isolates among clinical samples

Parameter	Frequency (n)	Percentage (%)
Total Clinical Samples Processed	120	100
Staphylococcus aureus Isolates	74	61.7
Methicillin Sensitive S. aureus (MSSA)	29	39.2
Methicillin Resistant S. aureus (MRSA)	45	60.8

According to Table III, the majority of MRSA-positive samples were obtained from wound swabs (40.0%), with pus samples accounting for 26.7% and blood samples for 20.0%. The smallest proportion was observed in urine samples, which made up 13.3% of the total.

Table III. Distribution of MRSA isolates according to clinical specimens (n=45)

Specimen Type	MRSA Isolates (n)	Percentage (%)
Wound Swab	18	40.0
Pus	12	26.7
Blood	9	20.0
Urine	6	13.3

All MRSA isolates showed complete resistance to penicillin and oxacillin (100%), while resistance to cefoxitin was observed in 95.6% of isolates. Two isolates were phenotypically cefoxitin-sensitive but carried the mecA gene, confirming them as MRSA by molecular detection. High resistance rates were also detected against erythromycin (75.6%) and ciprofloxacin (64.4%). In contrast, most isolates remained sensitive to vancomycin (93.3%) and linezolid (97.8%), indicating their continued therapeutic effectiveness against MRSA infections (Table IV).

Table IV. Antibiotic resistance pattern of MRSA isolates (n=45)

Antibiotic	Resistant n (%)	Sensitive n (%)
Penicillin	45 (100)	0 (0)
Oxacillin	45 (100)	0 (0)
Cefoxitin	43 (95.6)	2 (4.4)
Erythromycin	34 (75.6)	11 (24.4)
Ciprofloxacin	29 (64.4)	16 (35.6)
Clindamycin	21 (46.7)	24 (53.3)
Gentamicin	18 (40.0)	27 (60.0)
Vancomycin	3 (6.7)	42 (93.3)
Linezolid	1 (2.2)	44 (97.8)

The mecA gene was detected in 91.1% of MRSA isolates, making it the predominant methicillin resistance determinant identified in the study. All PCR runs included positive and negative controls, which performed as expected. The ermC gene was present in 53.3% of isolates, whereas ermA and tetK genes were detected in 42.2% and 35.6% of isolates, respectively. The vanA resistance gene showed the lowest frequency, being identified in only 4.4% of MRSA isolates. The mecC gene was detected in 5 isolates (11.1%) (Table V).

Table V. Detection of resistance genes among MRSA isolates (n=45)

Resistance Gene	Positive n (%)	Negative n (%)
mecA	41 (91.1)	4 (8.9)
mecC	5 (11.1)	40 (88.9)
ermA	19 (42.2)	26 (57.8)
ermC	24 (53.3)	21 (46.7)
tetK	16 (35.6)	29 (64.4)
vanA	2 (4.4)	43 (95.6)

Table VI shows that multidrug resistance was significantly more common among mecA-positive MRSA isolates, where 80.5% demonstrated multidrug resistant characteristics. In comparison, only 25.0% of mecA-negative isolates exhibited multidrug resistance. Statistical analysis indicated a significant association between mecA gene positivity and multidrug resistance pattern (p=0.012).

Table VI. Association between presence of mecA gene and multidrug resistance among MRSA isolates

Variable	MDR present n (%)	MDR absent n (%)	p-Value
mecA Positive (n=41)	33 (80.5)	8 (19.5)	0.012
mecA Negative (n=4)	1 (25.0)	3 (75.0)	

Table VII illustrates that the ICU exhibited the highest prevalence of MRSA, where 75.0% of *Staphylococcus aureus* isolates were methicillin resistant. Surgical wards also demonstrated a high MRSA prevalence of 65.0%, followed by medical wards with 50.0%. The orthopedic ward showed the lowest proportion of MRSA-positive isolates at 41.7%. The difference in MRSA prevalence across hospital wards was statistically significant (p = 0.04).

DISCUSSION

The present work emphasises the huge burden of hospital acquired infections (HAIs) caused by methicillin resistant *Staphylococcus aureus* (MRSA) and the key resistance genes contributing to the antimicrobial resistance profile of MRSA. A significant percentage of the isolates were Staphylococcus

aureus, with a significant number of these being MRSA, highlighting the ongoing presence of MRSA in the hospital environment. This result corroborates earlier reports of S. aureus being a major pathogen associated with HAIs especially in resource-limited and high burden environments.

Table VII. Prevalence of MRSA according to hospital ward

Hospital ward	Total *S. aureus* solates	MRSA Positive n (%)
ICU	24	18 (75.0)
Surgical ward	20	13 (65.0)
Medical ward	18	9 (50.0)
Orthopedic ward	12	5 (41.7)

Surgical site and soft tissue infections are important reservoirs in MRSA epidemiology, as evidenced by the significant finding of MRSA in wound swabs and pus samples (11-13). MRSA was highly prevalent in intensive care units, highlighting the importance of nosocomial transmission in critically ill patients. This risk is probably increased by invasive equipment, extended hospital stays, and heavy antibiotic exposure. These results confirm previous reports that ICUs are colonization and infection hot spots for MRSA (14, 15).

The antibiotic susceptibility patterns of the present study showed that the MRSA isolates were completely resistant to penicillin and oxacillin, which is the classical β-lactam resistant type of MRSA (16, 17). The high resistance levels for erythromycin and ciprofloxacin suggest increased resistance to macrolide and fluoroquinolone drugs that are used as alternatives (18, 19). However, vancomycin and linezolid showed good activity against large majority of isolates, thus there is hope that these drugs can be used as treatment options for severe MRSA infection. Susceptibility patterns are similar throughout the world, and a slow development of resistance to glycopeptides has been increasingly noted (20, 21).

Molecular analysis revealed that the mecA gene was widespread among MRSA isolates, and that it is the key gene responsible for the methicillin resistance in MRSA, which is mediated through change in penicillin-binding protein (PBP2a) (20). The presence of ermC and ermA genes suggest that the macrolide resistance is achieved through ribosomal methylation and the presence of tetK suggests that the tetracycline resistance is mediated through the efflux mechanism (22, 23). VanA was detected only rarely (2/45, 4.4%), indicating that glycopeptide resistance remains uncommon in this setting, though continued surveillance is warranted. The relationship between mecA positivity and multidrug resistance seen in this study was strong, and it indicates the relevance of genetic factors in determining antimicrobial resistance patterns. The low prevalence of mecC (11.1%) is consistent with reports suggesting mecC is more common in livestock-associated MRSA rather than hospital-acquired isolates (24, 25).

This high level of resistance genes and MDR isolates are attributable to antibiotic abuse, poor infection control measures and selective pressure in the hospital setting. The overuse of broad-spectrum antibiotics and the prescription of antibiotics based on clinical judgment without conducting a culture sensitivity test could potentially further drive the development of resistance (26). Cross-transmission of MRSA via healthcare workers and contaminated surfaces is also likely to help maintain MRSA circulation in healthcare settings (26, 27).

The growing burden of antimicrobial resistance in hospital-acquired infections emphasizes the urgent need for molecular surveillance and rational antimicrobial stewardship. Emerging evidence has demonstrated that inappropriate antibiotic exposure and resistant bacterial evolution significantly contribute to poor clinical outcomes, prolonged hospitalization, and increased healthcare burden in critically ill patients. In the present study, wound swabs accounted for the highest proportion of MRSA isolates (40.0%), and recent molecular investigations have highlighted the important role of inflammatory and oxidative stress pathways in chronic wound infections, including those caused by MRSA, further supporting the need for integrated therapeutic and microbiological strategies in managing resistant wound infections (28).

In conclusion, this study highlights the importance of implementing antimicrobial stewardship programs, implementing routine MRSA surveillance and adopting stringent infection control measures. Molecular detection of resistance genes can help direct rational antibiotic treatment and minimize treatment failures in cases of early detection of resistance genes. To prevent the emergence of MRSA and to ensure that present antibiotics continue to be effective, it is essential to improve hospital hygiene, encourage proper

antibiotic prescribing and keep a close watch on antibiotic resistance patterns.

CONCLUSION

The present study confirms that methicillin resistant *Staphylococcus aureus* (MRSA) is a common part of the hospital acquired infection (HAI) landscape with a high proportion of MRSA isolates collected from the surgical wards and the intensive care unit (ICU). The presence of the mecA gene confirms its critical importance in the acquisition of methicillin resistance, and the discovery of other resistance genes like ermA, ermC and tetK has raised the concern of MDR. Vancomycin and linezolid are still mostly effective, but the development of resistant determinants suggests a narrowing therapeutic window for effective treatment.

Resistance genes are closely related to multidrug resistance and continuous molecular surveillance is recommended. These results highlight the need for appropriate infection control measures, and periodic microbiological surveillance. The routine molecular screening is recommended for mecA and erm genes in ICU and surgical ward admissions, and annual antibiogram updates to guide empiric therapy. Improving antimicrobial stewardship programs will help to limit the spread of MRSA and to maintain the utility of existing antibiotic use in practice.

Conflict of interest:

The authors declare no conflict of interest.

Authors' contribution:

MH, MA Conducted the study and collected retrospective data; MB Conceptualized, designed the epidemiological investigation and analyzed seasonal prevalence data; MH Supervised the research and finalized the manuscript; MH, HT Performed statistical analysis of internal and external par asite cases; MH, MA & HT Compiled and organized treatment records from field visits, camps, and hospitals. All agreed to research aspects and approved the final version to be published.

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