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Epidemiological Insights, Mupirocin Resistance, and the Silent Threat of Biofilm Formation

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ABSTRACT

Background

Staphylococcus aureus with methicillin resistance (MRSA) causes life-threatening infections in immune compromised individuals. Categorization of MRSA as Healthcare-associated (HA-MRSA) and Community-associated (CA-MRSA) has an impact on outcome and treatment protocols. Mupirocin helps in the prevention and eradication of nasal MRSA; the detection of mupirocin resistance is crucial.

Aims and Objectives

To estimate the prevalence and risk factors associated with CA-MRSA and HA-MRSA.
To assess the prevalence of mupirocin-resistance strains.
To detect biofilm production and its correlation in Healthcare-associated Infection (HCAI)

Methods

Analysed MRSA prevalence in nasal swabs. Categorisation into CA-MRSA and HA-MRSA by risk factor analysis. Mupirocin resistance as per CLSI guidelines. Biofilm production: 96-well plate method, Tube method, Congo red agar plate method.

Result

Prevalence of MRSA- 3.37 % (74 out of 2190). Number and Prevalence of CA-MRSA – 10 (13.5 %) and HA-MRSA- 64 (86.5 %). Prevalence of mupirocin resistance: 1.56% (1 in 64). All strains of HA-MRSA (100%) were biofilm producers, and one CA-MRSA (10%) showed biofilm production. Risk factor analysis showed HA-MRSA in older populations, prolonged hospitalised patients, ICU admission, indwelling devices, invasive procedures, prior antimicrobial therapy, prior MRSA carrier, and CA-MRSA in young age, crowded environment, sharing of items, breach in skin integrity and those who have tattoos.

Conclusion

MRSA and mupirocin resistance are low in our setting, but most MRSA cases are hospital-acquired. Strict Infection Prevention and Control (IPC) practices are needed to prevent infection spread. Biofilm production in multi-drug resistant HA-MRSA indicates a link with virulence and healthcare-associated infections (HCAI).

Keywords: Biofilm detection, Community-associated (CA-MRSA) and Healthcare-Associated (HA-MRSA), Healthcare-associated Infection (HCAI), Methicillin Resistant Staphylococcus aureus (MRSA), Mupirocin resistance

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INTRODUCTION

Staphylococcus aureus causes life-threatening infections, especially in an immunocompromised individual, through broken skin or mucosa, and oral ingestion of infected food. It is estimated that the methicillin-resistant Staphylococcus aureus (MRSA) causes around 1,71,200 healthcare-related infections every year and 5400 attributable extra deaths. [1]Nasal carriage of MRSA faces a higher infection risk. It is important to categorise as Healthcare-Associated (HA-MRSA) and Community-associated (CA-MRSA), as each has its own impact and outcome when it causes infection. Mupirocin helps in the prevention and eradication of nasal MRSA. However, reports of increasing mupirocin resistance (MR) are of serious concern.

Materials and Methods:

A cross-sectional study was conducted at the Department of Neuromicrobiology, NIMHANS, for one year.

Case definition:

All nasal swabs from hospitalised patients who had isolated MRSA were included in this study.

Categorisation into CA-MRSA and HA-MRSA

Isolation of MRSA strain: For the detection of MRSA, a 30 µg Cefoxitin disc is placed onto Mueller-Hinton agar with a lawn culture of Staphylococcus aureus. After overnight incubation, the zone of inhibition is measured, showing zone size >=22 as sensitive and <=21 as resistant.

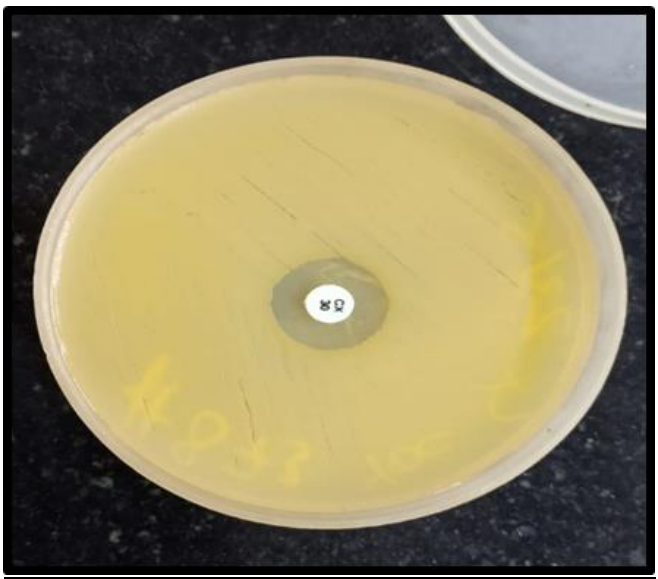

Image 1 showing MRSA-Methicillin-resistant Staphylococcus aureus strain

Minimal inhibition around the Cefoxitin disc (≤21 mm) indicates resistance, signifying Methicillin-resistant Staphylococcus aureus. According to CDC, an MRSA isolate is HA associated if the original entry criteria of hospitalization for >72 hours before culture acquisition was met and if in the year before the present hospitalization, the patient had any 1 of the following: surgery, hospitalization, residency in a long-term care facility, haemodialysis or peritoneal dialysis, or at the present admission had indwelling percutaneous devices or catheters. Most commonly, it causes surgical site infection and is often multidrug-resistant. The definition of CA-MRSA includes cases where MRSA is isolated ≤ 72 h after hospital admission, and it most commonly causes skin infection and is sensitive to Beta-lactams. [2]

Risk factor analysis:

For HA-MRSA infections, the following were addressed

- Age (elderly)
- Prolonged hospitalisation (1year)
- Intensive care unit admission
- Haemodialysis
- Indwelling lines and catheters
- Invasive procedures
- Comorbid medical conditions (heart failure, COPD)

- Institutionalisation (nursing home)
- Presence of surgical site infection
- Sensitivity of the organism
- Persons exposed to frequent antibiotic use
- Prior MRSA carrier
- Prior to other infections (≤ 3 months)

For CA-MRSA infections, the following were addressed

- Age(young)
- Persons in crowded living conditions
- Users of Intravenous drugs
- Athletes in contact sports
- Men who have sex with men
- Persons exposed to MRSA, close contacts (sharing items)
- Breach in skin integrity
- Shave or wax to remove body hair, particularly in the armpits and groin
- Have tattoos or body piercings

Sample collection procedure, Screening, Nasal colonisation treatment, and follow-up screening:

Nasal swabs were taken following ICU entry and prior to surgery. A Sterile normal saline moistened swab was inserted into both nostrils 1-2 cm and was gently rotated on all surfaces for about 3 seconds. Care must be taken not to touch the external surface of the nose. Only non-repetitive nasal swabs were taken into account. For detection of MRSA, a 30 µg Cefoxitin disc is placed onto Mueller-Hinton agar with a lawn culture. After overnight incubation, the zone of inhibition is measured. Zone size of ≥22 was considered sensitive, and ≤ 21 was considered resistant.

For nasal colonisation of MRSA, apply (2%) nasal mupirocin ointment three times a day for 5-7 days. A small amount of ointment (about the size of a matchstick head) should be placed on a cotton bud and applied to the anterior part of the inside of each nostril. The nostrils are closed by gently pressing the sides of the nose together; this will spread the ointment throughout the nares. Mupirocin ointment must be reserved for the treatment of MRSA only, and prolonged course (≥ 7 days) or repeated courses (> two courses per hospital admission) should be avoided to prevent the emergence of mupirocin resistance. If surveillance specimens are taken to confirm clearance of MRSA, they should be taken at least 2– 3 days after completion of decolonisation and/ or antibiotic therapy, as the specimens may show a false negative result due to the presence of antimicrobial agents. Consecutive negative screens from screening sites on two separate occasions are declared MRSA free. [3]

Mupirocin resistance screening

For the detection of High-Level Mupirocin resistance, a 200 µg mupirocin disc is placed onto Mueller-Hinton agar with a lawn culture of MRSA. Reports with no zone are considered as high-level mupirocin resistant, and any zone indicates an absence of resistance. The test was performed based on CLSI guidelines. [4] High-level resistance strains can be treated with(0.5%) neomycin and (0.1%) chlorhexidine nasal ointment four times a day for 10 days. It is useful in reducing the bioload of microorganisms in the nose.

Image 2

Biofilm production:
Three approaches were used to find the production of biofilms.

1) 96-well plate method/tissue culture plate method.
2) Tube method.
3) Congo red agar (CRA) plate method.

96-well plate method (Tissue culture plate)

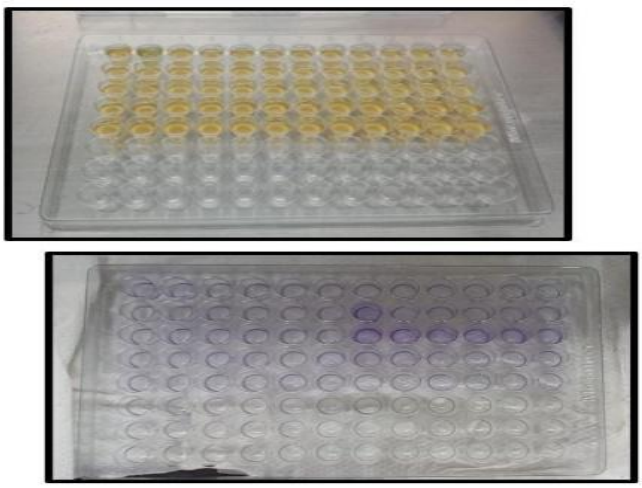

Image 3

A loopful of the test organism from an overnight culture on a Mueller-Hinton agar plate was added to 10 ml of Brain Heart Infusion (BHI) broth tubes, which were then incubated at 37°C for 24 hours. After incubation, 180 µl of the test organism's overnight culture in separate BHI broth tubes was transferred to 96-well plates. Pseudomonas aeruginosa (ATCC27853) served as the positive control.

The 96-well plates containing the cultured organisms were incubated at 37°C for 24 hours. After incubation, the plates were gently tapped, and the wells were washed four times with phosphate-buffered saline (pH 7.2) to remove any non-adherent bacteria. The bottom and walls of the wells were fixed with (2%) sodium acetate and stained with (0.1%) crystal violet. Excess stains were washed off with phosphate-buffered saline, and the plates were dried thoroughly.

The optical density (OD) of the stained adhering biofilm was measured using a micro-ELISA auto reader at a wavelength of 492 nm. The interpretation of the OD values is as follows:
- OD ≥0.12 - <0.2: Weak positive
- OD 0.2 - 0.4: Moderate positive
- OD >0.4: Strong positive

Tube method

Image 4

A loopful of the test organism from an overnight culture on a Mueller-Hinton agar plate was added to 10 ml of Brain Heart Infusion (BHI) broth tubes and incubated at 37°C for 24 hours. Pseudomonas aeruginosa (ATCC27853) served as the positive control. The cultures were decanted, and the tubes were washed with phosphate-buffered saline (pH 7.3). After drying, the tubes were stained with (0.1%) crystal violet. Excess stain was removed with phosphate-buffered saline, and the tubes were dried in an inverted position.

Interpretation:
A clearly stained film coating the tube's wall and bottom indicated successful biofilm production. The results were categorised as absent, weak, moderate, and strong.

Congo Red Agar (CRA) method

Image 5

The medium contained (2%) Congo red dye, 5 g/l sucrose, 15 g/l agar powder, and 15 g/l brain heart infusion broth. A concentrated aqueous solution of Congo red stain was prepared and autoclaved at 121°C for 15 minutes. It was then mixed with sucrose in autoclaved brain heart infusion agar at 55°C. The plates were inoculated with the test organism and incubated aerobically at 37°C for 24 hours.

Interpretation:
Biofilm formation was indicated by black colonies with a dry, crystalline consistency.
Considering the data collection was exclusively for hospital quality improvement purposes and the microbiological aspects were routine laboratory tests, with all patient samples being anonymised, a waiver from ethical approval was sought.

Statistical Analysis:
Data Analysis Approach
Analyses of data were carried out using SPSS for Windows (Version 14) and Microsoft Office Excel 2003. To characterise the number of successes over a series of observations, binomial distribution test, independent t-test and chi-square test were used to compare the relative prevalence of CA-MRSA and HA-MRSA, and the correlation of the different wards, sites, and duration of hospital stay with the two MRSA types. A probability of $P < 0.05$ was

considered statistically significant.

Results

Out of a total of 2190 isolates, 74 MRSA strains were isolated from nasal swabs, contributing to a prevalence of 3.37%.

Table 1: Prevalence and Distribution of MRSA

MRSA Type	Number of Isolates	Prevalence (%)
CA-MRSA	10	13.5
HA-MRSA	64	86.5
Total	74	100

Risk Factor Analysis

Table 2: Risk Factors for HA-MRSA and CA-MRSA

Risk Factors for HA-MRSA	Odds Ratio (OR)	95% Confidence Interval (CI)	P-Value
Older Population (≥ 60 years)	**1.82**	**1.25-2.63**	<0.01
Prolonged Hospitalization	**1.59**	**1.10-2.30**	0.02
ICU Admission	**2.14**	**1.48-3.10**	<0.01
Indwelling Devices	**1.95**	**1.32-2.88**	<0.01
Invasive Procedures	**1.71**	**1.19-2.46**	0.01
Prior Antimicrobial Therapy	**1.63**	**1.13-2.34**	0.01
Prior MRSA Carrier	**2.05**	**1.41-2.97**	<0.01
Risk Factors for CA-MRSA	Odds Ratio (OR)	95% Confidence Interval (CI)	P-Value
Young Age (< 60 years)	1.42	1.05-1.93	0.02
Crowded Environment	1.80	1.27-2.54	<0.01
Sharing of Items	1.68	1.19-2.38	0.01
Breach in Skin Integrity	1.95	1.32-2.88	<0.01
Tattoos	1.56	1.10-2.20	0.01

Prevalence of Mupirocin Resistance
- Mupirocin Resistant: 1 (1.35%)
- Mupirocin Sensitive: 73 (98.65%)

Biofilm Production

Table 3: Biofilm Production (all three methods) by MRSA Type

MRSA Type	Biofilm Producers (n)	Percentage (%)
HA-MRSA	**64**	100
CA-MRSA	**1**	10
Total	**65**	87.8

The binomial test indicates that the difference in prevalence between CA-MRSA and HA-MRSA is statistically significant ($P < 0.05$). The chi-square test for the association between MRSA type and ICU admission is significant ($P = 0.02$), indicating that ICU admission is associated with HA-MRSA.

DISCUSSION

Extensive and inappropriate use of mupirocin has now resulted in an increase in resistance to mupirocin among Staphylococcus aureus strains.

Consecutive negative screens from screening sites on two separate occasions are declared MRSA free, but recolonisation can happen. [3]

In Risk factor stratification analysis for HA-MRSA.

- Older populations are more prone to MRSA carrier state because of comorbid conditions and functional debility. [5]
- Prolonged hospitalised patients are more susceptible to the MRSA carrier state. [6]
- ICU admission serves as a risk factor for infection because of the duration of stay, intravascular devices and the intensity of exposure to infected patients. [7]
- It was stated that patients undergoing dialysis are more prone to MRSA colonisation and carriage, but as our institute's preliminary focus is on neuro cases, we could not find this as a risk factor. [8]
- Indwelling devices play a major role in MRSA infection. [9]
- Invasive procedures help in the systemic spread of MRSA. [10]
- Though many comorbidities are associated with MRSA colonisation (eg, Diabetes mellitus, Antibiotic, Corticosteroid, chemotherapy, various implants, Pressure ulcers, Trauma), our institute has not identified it as a particular risk factor. [11]
- Nursing homes serve as a particular risk factor, but this has not been identified by our institute. [12]
- SSI serves as a risk factor for MRSA, but it is not the same in our study; most of the SSI were caused by gram-negative bacteria. [13]
- Prior antimicrobial therapy is one of the major risks. [14]
- Prior MRSA carrier was identified as a risk factor. [15]
- Prior infection was not identified as a risk factor. [16]

In Risk Factor Stratification Analysis for CA-MRSA

- Young age was a major risk factor for CA-MRSA. [17]
- A crowded environment helps in the easier transmission of infection. [18]
- Though IV drug abusers serve as a risk factor, it is not associated in our study. [19]
- Athletes and men having sex with men have not been associated as risk factors in our study, which is different. [20,21]

- Sharing of items, breach in skin integrity and tattoos were identified as risk factors for acquiring MRSA. [22-24]

Prevalence of MRSA was around 3.37 % (74 in 2190). [25]

Prevalence of CA-MRSA- 13.5 %(10 in 74) and HA-MRSA- 86.5 %(64 in 74). [26]

Prevalence of mupirocin resistance: 1.35 % (1 in 74). [27]

By using the Tissue culture plate, Tube method and Congo red agar method, all HA-MRSA and one CA-MRSA were biofilm producers. [28]

CONCLUSION

Biofilm-producing HA-MRSA showed high resistance to most of the investigated antibiotics, adding to its virulence and pathogenicity. Risk factor analysis for HA-MRSA and CA-MRSA was quite contributory to the study in categorisation of the same. Prevalence of MRSA and mupirocin resistance is low in our settings due to stringent ICU entry and pre-op screening, which is highly recommended. Most of the MRSA isolates were hospital-acquired; hence, strict infection prevention and control practices should be implemented to limit the spread of infection.There are no conflicts of interest. The manuscript has been read and approved by all authors, and the requirements for authorship, as stated earlier in this document, have been met. Each author believes that the manuscript represents honest work.

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