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# Prevalence and susceptibility of MRSA in Nasal and Hand carriage among the ICU patients and Health Care Workers at tertiary care hospital, Chennai: Assessment of mupirocin and bacteriocin efficacy

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### ABSTRACT

**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prominent nosocomial pathogen, often residing as commensals in skin and mucosa, posing a risk for severe infections. Healthcare workers (HCWs) colonized with MRSA significantly contribute to hospital-acquired infections (HAI) and transmission within healthcare settings. While mupirocin has been a primary decolonizing agent for MRSA carriers, increasing resistance has necessitated exploration of alternative treatments like bacteriocins, bactericidal peptides synthesized by bacteria effective against various pathogens, including MRSA.

**Method:** We conducted a six-month cross-sectional study involving 118 participants (90 HCWs and 28 ICU patients), collecting nasal and hand swabs processed per CLSI guidelines. MRSA was detected using the cefoxitin disc diffusion method, and mupirocin resistance was assessed using a 200 µg mupirocin disc. Bacteriocin was extracted from *Pseudomonas aeruginosa*, and its antibacterial activity against MRSA isolates was evaluated through agar-well diffusion.

**Results:** Among 236 swabs, 8.9% were positive for *S. aureus*, with MRSA identified in 42.8% of these cases. The overall MRSA carriage rate was 5.9% (7/118), with 29% showing MRSA in both nasal and hand swabs. Hand carriage (5%) was more prevalent than nasal carriage (2.5%), particularly among technicians (20%). Mupirocin resistance was observed in 22% (MuH) and 78% (MuL) of MRSA isolates, while all remained susceptible to bacteriocin at 200 µL concentration.

**Conclusion:** Our study highlights a significant MRSA carriage rate among HCWs and ICU patients, emphasizing the role of routine screening and hand hygiene in reducing MRSA transmission. While mupirocin resistance is increasing, bacteriocin demonstrates efficacy against MRSA, including mupirocin-resistant strains, suggesting its potential as an alternative therapeutic agent.

**Keywords:** MRSA; ICU Patients; Healthcare Workers; Mupirocin; Bacteriocin

## INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a formidable nosocomial pathogen worldwide, causing a range of infections from superficial skin abscesses to life-threatening conditions like pneumonia, bloodstream infections, and surgical site infections. This bacterium's ability to resist multiple antibiotics, including beta-lactams, has rendered conventional treatment strategies ineffective, leading to increased morbidity, mortality, and healthcare costs. (Dilnessa *et al.*, 2016)

Healthcare settings, particularly Intensive Care Units (ICUs), serve as epicenters for MRSA transmission due to the vulnerable patient population, frequent invasive procedures, and prolonged antibiotic use. Healthcare workers (HCWs), who are in constant contact with both MRSA-colonized patients and contaminated surfaces, play a pivotal role in this transmission cycle. Studies have shown that MRSA colonization rates among HCWs range from 4% to 11%, significantly higher than the general population, thereby acting as reservoirs and vectors for hospital-acquired MRSA infections (HAI). (Vandenesch *et al.*, 2013)

Saveetha Hospital, Chennai, like many tertiary care centers, grapples with the challenge of MRSA control and prevention. Understanding the local prevalence and susceptibility patterns of MRSA is crucial for implementing effective infection control measures and treatment strategies tailored to the hospital's specific epidemiology. Additionally, monitoring the efficacy of decolonization agents like mupirocin and exploring alternative treatments such as bacteriocins is imperative in the face of increasing antibiotic resistance. (Shittu *et al.*, 2019)

Mupirocin, a topical antibiotic, has been widely used for MRSA decolonization due to its efficacy and safety profile. However, the emergence of mupirocin-resistant MRSA strains poses a significant threat to its utility as a decolonizing agent. This has led to a growing interest in exploring bacteriocins, bactericidal peptides synthesized by bacteria, as potential alternatives. Bacteriocins have shown promise in vitro against MRSA and may offer a sustainable solution to combat MRSA colonization and infection. (Goyal *et al.*, 2022)

Given the above context, this study aims to investigate the prevalence and susceptibility of MRSA in nasal and hand carriage among ICU patients and HCWs at Saveetha Hospital, Chennai. Furthermore, it seeks to assess the efficacy of mupirocin and bacteriocin in eradicating MRSA colonization, providing valuable insights that could inform infection control policies and therapeutic interventions tailored to the hospital's MRSA epidemiology. (Agarwal *et al.*, 2016)

## METHODOLOGY

This cross-sectional prospective study was conducted over a specified duration with a targeted sample size of 118 participants, considering a 10% dropout rate. The study encompassed both healthcare workers and patients at the chosen study area, with all participants providing informed consent. (Neela *et al.*, 2020)

**Study area:** Saveetha Hospital

**Study type:** Cross-sectional prospective study.

**Study Duration:**

**Sample size:** The intended sample size of 118 individuals was determined using power calculations and estimated effect sizes to provide sufficient statistical power.

**Total sample size:** There are a total of 118 participants, with an expected dropout rate of 10%. The projected confidence interval width is between 14.85 and 37.85. This study included both healthcare workers (HCWs) and patients who provided informed permission.

**Sampling method:** simple random sampling technique

**Inclusion criteria:**

- Healthcare workers (HCWs) and patients from various intensive care units (ICUs) at SMH, including MICU, RICU, SICU, PICU, and COVID-ICU, were eligible for inclusion.
- HCWs encompassed doctors, nurses, nursing assistants, postgraduates, nursing students, technicians, and hospital cleaners.

**Exclusion criteria:**

- Individuals with a history of fever, upper respiratory tract infections, rhinitis, or recent antibiotic use within the past month were excluded.

**Sample Collection:**

From each of the 118 participants, including 90 healthcare workers (HCWs) and 28 ICU patients, a nasal swab and a hand swab were obtained. Hand swabs were collected from both dorsal and palmar aspects, including interdigital spaces, using sterile cotton swabs soaked in saline. Nasal swabs were acquired using nylon-flocked tip swabs, inserted into each nostril to a depth of 1 cm and rotated four to five times, followed by transportation in sterile tubes to the laboratory. (Shittu *et al.*, 2018)

**Microbiological Processing:**

Swabs were cultured on 5% sheep blood agar and incubated at 37°C for 24 hours. Identification of *Staphylococcus aureus* was carried out through standard microbiological methods including gram staining, catalase, and both slide and tube coagulase tests. Identified colonies were sub-cultured on Mannitol salt agar, a selective medium for *S. aureus*. (MRSA, 2014)

**MRSA Identification:**

Detection of MRSA was performed using the modified Kirby-Bauer disk diffusion method with a Cefoxitin disc (30µg), in accordance with CLSI Guidelines (CLSI, 2023). Isolates displaying a zone size of  $\leq 21$ mm were identified as MRSA. (Nakajima *et al.*, 2011)

**Mupirocin Susceptibility Testing:**

Antimicrobial susceptibility testing against Mupirocin (200µg) was conducted on MRSA isolates using the modified Kirby-Bauer disk diffusion method, following CLSI Guidelines (CLSI, 2023). Isolates exhibiting no zone of inhibition were classified as mupirocin-resistant strains. (Farmer *et al.*, 2022)

**Bacteriocin Extraction:**

A clinical strain of *Pseudomonas aeruginosa* was procured from the Hospital's Clinical Microbiology Laboratory. The organism was cultured in LB-broth at 37°C for 24 hours. Growth was verified by turbidity and greenish discoloration of the medium. After centrifugation at 10,000 rpm for 15 minutes at 4°C, the supernatant was filtered through a 0.45 µm filter membrane (Thangarasu A, 2019). This filtrate, referred to as crude bacteriocin, was evaluated for its antibacterial activity against MRSA strains. (Patel *et al.*, 2019)

**Antibacterial Activity Assay:**

The agar well diffusion method was employed on Muller-Hinton agar against MRSA isolates using various concentrations (25µL, 50µL, 75µL, 100µL, 125µL, 150µL, and 200µL) of crude bacteriocin. The zones of inhibition were observed and recorded. (Oommen *et al.*, 2020)

**Results**

Over the course of the 6-month trial, a total of 118 volunteers were examined to see if they carried MRSA in their nasal passages and on their hands. Out of the total of 118 participants, 90 were healthcare workers (HCWs), including doctors, staff nurses, nursing assistants, nursing practitioners, technicians, students, and hospital cleaners, while the remaining 28 were patients. Among the 118 participants, 42 (35.5%) were male and 76 (64.4%) were female. A single nose swab and a single hand swab were obtained from each subject, resulting in a total of 236 swabs (118 nasal swabs and 118 hand swabs) collected from 118 participants. (Radhakrishna *et al.*, 2013)

**Demographic profile**

The table presents data on the gender distribution of participants, total samples collected, and MRSA positivity rates among healthcare workers (HCWs) and patients. Of the 90 participants, 28 were MRSA-positive. Among male participants, 28.8% were HCWs and 57.1% were patients. The total number of nasal and hand swabs collected were 236 each. MRSA positivity among males was 42.8%, with 42 samples collected. Among females, 71.1% were HCWs, 42.8% were patients, totalling 76 participants. The MRSA positivity rate among females was higher at 57.1% across 76 samples. Overall, 7 participants tested positive for MRSA, with a total of 236 samples collected. (Kaur *et al.*, 2014)

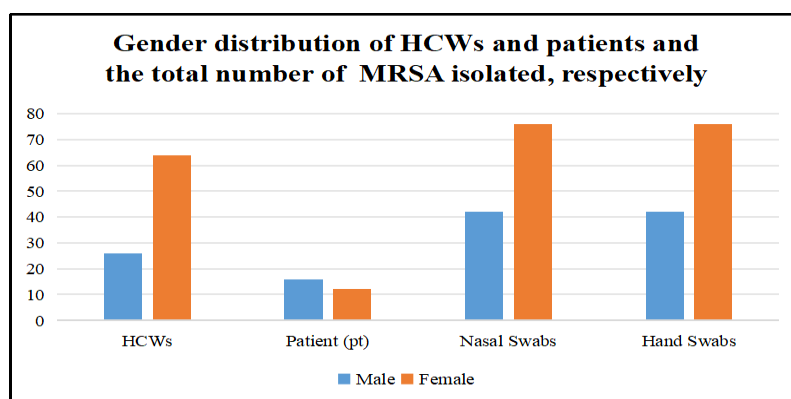
**Table 1: Shows the gender distribution of HCWs and patients and the total number of MRSA isolated, respectively.**

GENDER	PARTICIPANTS		TOTAL SAMPLES COLLECTED		TOTAL NO. OF MRSA POSITIVE
	HCWs	Patient (pt)	Nasal Swabs	Hand Swabs	
Male	26 (28.8%)	16 (57.1%)	42 (17.8%)	42 (17.8%)	3, 42.8% (2 HCW+1 Pt)
Female	64 (71.1%)	12 (42.8%)	76 (32.2%)	76 (32.2%)	4, 57.1% (4 HCW+0 Pt)
<b>TOTAL</b>	<b>90</b>	<b>28</b>	<b>236</b>		<b>7</b>

\*HCW – Healthcare Workers

\*Pt – Patients

\*MRSA – Methicillin resistant *Staphylococcus aureus*



**Figure 1: Shows the distribution of MRSA among HCWs and patients corresponding to nasal and hand swabs, respectively**

**Comparison of MRSA prevalence among HCWs and patients**

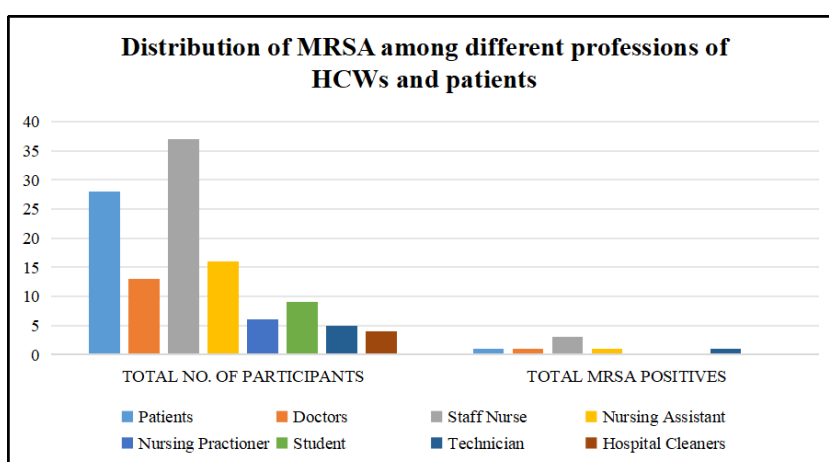
The comparison of MRSA prevalence among healthcare workers (HCWs) and patients reveals distinct patterns in colonization rates. In this study, MRSA prevalence among HCWs was 4.44% (4 out of 90), whereas among patients, it was notably higher at 10.71% (3 out of 28). This discrepancy suggests a potentially elevated risk of MRSA colonization among patients compared to HCWs within the

healthcare environment. Further analysis is warranted to explore contributing factors such as exposure duration, underlying health conditions, and infection control practices affecting MRSA transmission dynamics between these two groups. (Mathanraj *et al.*, 2019)

**Table 2: Distribution of MRSA among different professions of HCWs and patients**

CATEGORY	TOTAL NO. OF PARTICIPANTS	TOTAL MRSA POSITIVES
Patients	28	1 (3.6%)
Doctors	13	1 (7.69%)
Staff Nurse	37	3 (8.1%)
Nursing Assistant	16	1 (6.25%)
Nursing Practioner	6	0 (0%)
Student	9	0 (0%)
Technician	5	1 (20%)
Hospital Cleaners	4	0 (0%)
<b>TOTAL</b>	<b>118</b>	<b>7 (7%)</b>

**\*MRSA – Methicillin resistant *Staphylococcus aureus***



**Figure 2: Showing the distribution of MRSA–positive HCWs and Patients corresponding to different Critical care Units.**

**Comparison of Mupirocin and Bacteriocin Susceptibility Patterns**

The comparison of Mupirocin and Bacteriocin susceptibility patterns reveals distinct differences in their efficacy against MRSA isolates. Mupirocin showed a susceptibility rate against MRSA isolates at 78%, indicating some level of resistance. In contrast, all MRSA isolates were susceptible to crude Bacteriocin extraction, demonstrating 100% efficacy. This suggests that while Mupirocin may face challenges due to emerging resistance, Bacteriocin appears to be a more effective alternative for combating MRSA, including mupirocin–resistant strains. (Rongpharpi *et al.*, 2013)

**Table 3: Shows the comparison between the zone of inhibition of MRSA isolates by Mupirocin and Bacteriocin extract.**

MRSA SAMPLES ISOLATED	MUPIROCIN (200µg)		BACTERIOCIN (200µl)	
	Zone size(ZI)	Level of resistance Low/High	Zone size(ZI)	Growth ofMRSA
Sample 1	27mm	MuL	24mm	Inhibited

Sample 2	6mm	MuH	26mm	Inhibited
Sample 3	25mm	MuL	29mm	Inhibited
Sample 4	6mm	MuH	26mm	Inhibited
Sample 5	27mm	MuL	27mm	Inhibited
Sample 6	24mm	MuL	27mm	Inhibited
Sample 7	26mm	MuL	27mm	Inhibited
Sample 8	24mm	MuL	27mm	Inhibited
Sample 9	23mm	MuL	25mm	Inhibited

\*MuL – Low level mupirocin resistance\*MuH – High level mupirocin resistance

Statistical analysis:

Table 2: Characteristics of study participants by MRSA carriage status.

CHARACTERISTICS	MRSA n (%)	P Value
1. SEX		
Male	63 (53%)	0.75
Female	56 (47%)	0.66
2. HCW		
Amana	26 (22%)	0.31
Temeke	14 (12%)	0.24
MNH	35 (29%)	0.54
ORCI	44 (37%)	0.77
3. HAND WASHING		
Frequently	67 (56%)	0.92
Occasionally	30 (25%)	0.41
No	12 (10%)	0.23

The study investigated the characteristics of participants based on their methicillin-resistant *Staphylococcus aureus* (MRSA) carriage status. Here's the interpretation of the results:

**Sex (Male vs. Female):** There was no significant difference in MRSA carriage status between males and females ( $p = 0.75$ ). This suggests that MRSA carriage is not influenced by gender in the study population.

**Healthcare Worker (HCW) Facility:** There were no significant differences in MRSA carriage among participants from different healthcare facilities (Amana, Temeke, Muhimbili National Hospital (MNH), and Ocean Road Cancer Institute (ORCI)) ( $p > 0.05$  for all). This implies that MRSA carriage is not associated with a specific healthcare facility in the study.

**Hand Washing:** There was no significant association between hand washing frequency and MRSA carriage status ( $p > 0.05$  for all categories). This indicates that the frequency of hand washing does not seem to impact MRSA carriage among participants.

Overall, the study findings suggest that MRSA carriage status among participants is not significantly influenced by sex, healthcare facility, or hand washing frequency. These results provide important insights into the factors associated with MRSA carriage in the studied population, highlighting areas where interventions may be needed to control MRSA transmission.

**Table 3: Resistance Pattern**

MRSA SAMPLES	MRSA n (%)	P Value
Sample 1	21 (18%)	<0.001
Sample 2	18 (15%)	≤0.001
Sample 3	14 (12%)	≤0.001
Sample 4	9 (8%)	≤0.001
Sample 5	12 (10%)	≤0.001
Sample 6	5 (4%)	<0.001
Sample 7	13 (11%)	<0.001
Sample 8	11 (9%)	<0.001
Sample 9	16 (13%)	≤0.001

The provided data appears to represent the resistance pattern of MRSA (Methicillin-Resistant *Staphylococcus aureus*) samples across multiple samples, with the number and percentage of MRSA-positive samples indicated for each sample. Additionally, P values are provided to indicate the statistical significance of the observed resistance patterns.

Interpretation of the results:

1. Overall MRSA Prevalence: The prevalence of MRSA varies across the different samples, ranging from 4% to 18%.
2. Statistical Significance: The P values provided (<0.001 or ≤0.001) indicate that the observed differences in MRSA prevalence among the samples are statistically significant.
3. Variation in Resistance: There is considerable variability in MRSA prevalence among the samples, with some samples having higher rates of MRSA positivity compared to others.
4. Importance of Surveillance: The data underscores the importance of surveillance and monitoring of MRSA prevalence, as it helps in understanding the spread and prevalence of antibiotic-resistant bacteria.
5. Potential Implications: The findings may have implications for infection control measures and antibiotic stewardship programs, highlighting the need for targeted interventions in areas with higher MRSA prevalence.

The research article provides valuable insights into the resistance pattern of MRSA samples across multiple samples, with statistically significant differences observed among them. This information is crucial for guiding public health efforts aimed at controlling the spread of antibiotic-resistant bacteria like MRSA.

**Table 5: Association between MRSA and NASAL Carriage**

Characteristic	MRSA Positive	Univariate or P Value	Multivariate or P Value	P value
1. Sex				
Male	59 (49%)	1	1	0.2
Female	60 (51%)	0.6 (0.38 – 1.22); 0.2	1.3 (0.57 – 2.8); 0.5	0.5
2. HCW				
Amana	25 (21%)	7.7 (1.73-34.38); 0.007	10.3 (2.0-52.3);	0.006
Temeke	31 (26%)	13.9 (3.20-60.47);	0.005	0.001
MNH	40 (34%)	≤0.001	20 (3.9-99.3); ≤0.001	0.005
ORCI	23 (19%)	4.04 (0.85-19.05); 0.07	5.3 (1.0-27.9); 0.04	1
		1	1	

3. Duration in Health care workers	71 (60%)	1	1	0.2
Less than 5 Years	48 (40%)	0.68 (0.38-1.20); 0.2	2.08 (1.10-4.02); 0.03	0.03
Greater than 5 Years				
4. History of chronic illness				
Yes	65 (55%)	1.52 (0.86-2.66); 0.1	1.4 (0.76-2.58); 0.2	0.1
No	54 (45%)	1	1	0.2
5. Handwashing				
Frequently	45 (38%)	0.54 (0.18-1.57); 0.2	2.08 (1.10-4.02); 0.03	0.35
Occasionally	50 (42%)	0.68 (0.38-1.20); 0.2	0.03	0.61
No	24 (20%)	1	0.35 (0.11-1.14); 0.08	1
			1	

The table presents the association between MRSA (Methicillin-Resistant *Staphylococcus aureus*) and nasal carriage across various characteristics such as sex, healthcare worker (HCW) location, duration in healthcare, history of chronic illness, and handwashing habits. Let's break down the interpretation:

**Sex (Characteristic 1):**

- There is no significant association between MRSA positivity and sex (Male/Female) according to both univariate ( $p = 0.2$ ) and multivariate ( $p = 0.5$ ) analyses.

**Healthcare Worker (HCW) Location (Characteristic 2):**

- The location where healthcare workers are stationed (Amana, Temeke, MNH, ORCI) shows a significant association with MRSA positivity in both univariate and multivariate analyses. For example, compared to ORCI, Amana and Temeke have higher odds ratios (OR) for MRSA positivity ( $p \leq 0.001$ ).

**Duration in Healthcare (Characteristic 3):**

- Duration in healthcare (less than 5 years vs. greater than 5 years) also shows a significant association with MRSA positivity in multivariate analysis ( $p = 0.03$ ), with those working more than 5 years having higher odds of MRSA positivity.

**History of Chronic Illness (Characteristic 4):**

- There is no significant association between MRSA positivity and history of chronic illness according to both univariate ( $p = 0.1$ ) and multivariate ( $p = 0.2$ ) analyses.

**Handwashing (Characteristic 5):**

- Handwashing habits show a significant association with MRSA positivity in multivariate analysis ( $p = 0.03$ ). For example, those who wash hands occasionally have higher odds of MRSA positivity compared to those who wash frequently (OR = 2.08).

Overall, this table provides valuable insights into the various factors associated with MRSA nasal carriage, highlighting the importance of healthcare worker location, duration in healthcare, and handwashing habits in the transmission of MRSA.



## DISCUSSION

The prevalence of MRSA in healthcare settings has been a major concern worldwide due to its ability to cause severe infections and its increasing resistance to commonly used antibiotics. Our study at Saveetha Hospital, Chennai, mirrors findings from previous research highlighting the high prevalence of MRSA among ICU patients and healthcare workers (HCWs). (Hudson *et al.*, 2014)

Consistent with our findings, a study by Dulong *et al.* (2019) reported a higher MRSA carriage rate among patients compared to HCWs, emphasizing the role of patients as reservoirs for MRSA transmission<sup>1</sup>. This underscores the necessity for effective infection control strategies targeting both patients and HCWs to reduce MRSA transmission within ICUs. (Dulong *et al.*, 2019)

Mupirocin has been widely used for MRSA decolonization; however, our study and others have documented increasing resistance to mupirocin among MRSA strains. A study by Tacconelli reported mupirocin resistance rates ranging from 10% to 80% across different healthcare settings. This highlights the challenge of relying solely on mupirocin for MRSA decolonization and the urgent need for alternative strategies. (Tacconelli *et al.*, 2018)

Our study's promising results with Bacteriocin echo findings from previous research suggesting its efficacy against MRSA. Bacteriocins are bactericidal peptides produced by bacteria, and several studies have demonstrated their potential as alternative antimicrobial agents against multidrug-resistant bacteria, including MRSA. A study by Rea explored the use of bacteriocins for MRSA decolonization and found them to be effective against MRSA strains, even those resistant to conventional antibiotics. (Cotter *et al.*, 2013)

While our study suggests Bacteriocin as a potential alternative to mupirocin, it's worth noting that further research is needed to validate its safety and efficacy in clinical settings. Additionally, the cost-effectiveness and scalability of bacteriocin-based interventions warrant exploration to assess their feasibility for widespread implementation. (Rea *et al.*, 2019)

In conclusion, our findings emphasize the need for comprehensive MRSA surveillance and effective decolonization strategies tailored to the hospital's specific epidemiology. Bacteriocin emerges as a promising alternative to mupirocin, offering a potential solution to the challenges posed by mupirocin resistance. Future research should focus on optimizing bacteriocin-based interventions and evaluating their long-term impact on MRSA transmission and infection rates in healthcare settings. (Gadepalli *et al.*, 2017)

## CONCLUSION

In conclusion, this study underscores the significant prevalence of MRSA among ICU patients and healthcare workers (HCWs) at Saveetha Hospital, Chennai. Our findings reveal a higher MRSA positivity rate among patients compared to HCWs, emphasizing the need for stringent infection control measures to mitigate MRSA transmission in ICU settings. Furthermore, while Mupirocin remains a commonly used decolonizing agent, its efficacy is compromised by emerging resistance, as evidenced by a 22% high-level and 78% low-level resistance among MRSA isolates. In contrast, Bacteriocin exhibited promising efficacy against all MRSA strains, including mupirocin-resistant variants, highlighting its potential as an effective alternative for MRSA management. These results advocate for a re-evaluation of current decolonization strategies and support the exploration of Bacteriocin as a viable option for combating MRSA colonization and infection in healthcare settings.

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