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Research Article

Mupirocin Resistance in Clinical Isolates

Prevalence of High and Low-Level Mupirocin Resistance in Clinical Isolates of *Staphylococcus Aureus* from Skin and Soft Tissue Infection

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Abstract: The skin has an extremely diverse ecology of organisms that may produce infection. The clinical manifestations of skin and soft tissue infection (SSTIs) culminate in a two-step process involving invasion and the interaction of bacteria with host defenses. Approximately 7% to 10% of hospitalized patients are affected by SSTIs. Mupirocin has been used to treat skin infections and eradicate the nasal carriage of MRSA. Our aim is to estimate the prevalence of low and high-level Mupirocin resistance among the clinical isolates of *Staphylococcus aureus* from skin and soft tissue infection. The study was conducted in the Department of Microbiology, Krishna Institute of Medical Sciences, Krishna Hospital and Medical Research Centre, Karad, District- Satara. Specimen collection and processing of samples were performed as per standards. The Kirby -Bauer disc diffusion method was used for the sensitivity to common antibiotics recommended Clinical and Laboratory Standard Institute (CLSI 2021). Mupirocin resistance was detected using 5µg and 200µg Mupirocin discs to determine Low and High -level resistance. 135 *Staphylococcus aureus* isolates were obtained from patients admitted to various medical, surgical, and intensive care units and patients attending outpatient departments. Out of 415 clinically suspected cases of skin and soft tissue infection, 91.33% were culture positive, and 8.67% were culture negative. Out of 379 microorganisms, 44.06% were Gram-positive cocci, 27.44% were Gram negative bacilli, 24.27% were Gram negative cocci, and 14.22% were Gram-positive bacilli. Out of 167 Gram positive cocci, 135 (80.84%) were *Staphylococcus aureus*, 22 (13.17%) were *Coagulase Negative Staphylococcus* and 10 (5.99%) were *Micrococcus*. We conclude that the prevalence of *Staphylococcus aureus* was 32.53%, among which Mupirocin high and low-level resistance was 5.92% and 7.41%, respectively.

Keywords: Skin and Soft Tissue Infection, *Staphylococcus aureus*, MRSA, High-level Mupirocin resistance, Low-level Mupirocin resistance

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1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) has been recognized as an important pathogen in human diseases.[1] Coinciding emergence of methicillin-resistant *S. aureus* (MRSA) infections is a challenge to clinicians to prevent their spread in hospitals. Close eye monitoring should be kept on the use of antibiotics, duration of hospital stays, and nasal and hand carriage in healthcare staff. Infectious Diseases Society of America Practice Guidelines for skin and soft-tissue infections recommend Mupirocin for treating skin and soft-tissue infections, surgical site infections, and eliminating nasal colonization of MRSA among patients and medical staff. Mupirocin distorts the synthesis of protein in these bacteria. The antibiotic Mupirocin (pseudomonic acid A) is produced by the bacterium *Pseudomonas fluorescens*. Mupirocin calcium ointment was clinically introduced in the late 1980s and has proved to be one of the most successful topical antibiotics for the clearance of nasal *S. aureus*.[2-5] In 1985, Mupirocin was launched in the UK to treat infections caused by *Staphylococcus* and *Streptococcus* and to clear the nasal carriage of MRSA and also used to treat MRSA-associated skin and soft-tissue infections. Mupirocin resistance among MRSA isolates began to emerge in the UK soon after 2 years and was reported after that in Ireland (2%), New Zealand (12.4%), the USA (24%), and in Trinidad and Tobago (44.1%). Two types of Mupirocin resistance have been defined in *Staphylococci*. If minimum inhibitory concentrations (MICs) are in the range of 8–256 µg/ml, it is termed as low-level resistance said to be related to point mutations in the *ileS* gene. In contrast, high-level resistance is considered when MICs, ≥512 µg/ml, are supposed to be plasmid-mediated genes, *mupA* (*ileS2*). The enhanced use of Mupirocin ointment for local applications has increased data on its resistance. Usually, screening for MRSA is done in hospitals to check its spread in people in contact with the hospital environment. Still, unfortunately, Mupirocin antibiotic is not checked for its sensitivity or resistance pattern. Hence, leading to therapy failure and the development of resistance to this drug in MRSA strain.[6] Present study will highlight the current prevalence of mupirocin resistance in this geographical area data generated by the study will help to authority formulation of antibiotic policy for treating *S. aureus* infections and eliminating MRSA carriers working in this healthcare setup.

2. MATERIAL AND METHODS:

After the study protocol presentation and permission of the Institutional ethics committee, all procedures performed in this study involving human participants were by the ethical standard of the institutional ethics committee Krishna Institute Medical Sciences Deemed to be University, Karad (Protocol number 052/ 2021-2022). Written consent was taken from all the participants enrolled in the study. A cross-sectional descriptive study was conducted in the Department of Microbiology Krishna Institute of Medical Sciences, "Deemed to be" University, Karad. The study was conducted from November 2020 to November 2022. A total of 415 participants with skin and soft tissue infections were included in the study. The clinical samples (pus, wound swab) were collected per standard precautions. After the collection of specimens, the sample was transferred into a sterile container and transported under cold conditions to the microbiology laboratory for further processing without delay. After the sample was received in the laboratory, microscopy was done. The aerobic bacterial culture sample was inoculated on plating media, e.g., blood agar, chocolate agar, and MacConkey agar. All the inoculated plates were incubated aerobically for 24 hrs at 37°C. After the growth on culture media, isolates were further subjected to biochemical identification and antibiotic susceptibility as per Clinical and Laboratory Standard Institute (CLSI 2021)[7]. MRSA detection using Cefoxitin (30µg) disc.[7] Mupirocin resistance was detected using 5µg and 200µg Mupirocin discs to determine Low and High -level resistance. ATCC *Staphylococcus aureus* 25923 was used as the control strain of the test. The Inoculum of the test and control organism was prepared and matched turbidity with 0.5 McFarland standard. A lawn culture of the test and control was done on Muller Hinton agar plates, and a Mupirocin High level (200µg) and Low level (5µg) discs were placed. After incubation, the criteria of zone diameter breakpoints for susceptible and resistant isolates were set at >14 and < 13mm, respectively. A zone diameter greater than or equal to 14mm for 5 and 200 µg discs was considered susceptible to Mupirocin. Isolates that showed zone diameters less than 14 mm in the 5µg discs but more than or equal to 14 mm in the 200µg disc were considered Mupirocin low-level (MuL) resistance strains. All isolates with zone diameters less than 14mm for 5 and 200 µg were considered Mupirocin high-level (MuH) resistance strains.[8]

2.1 Inclusion criteria

All age group patients of both gender were clinically diagnosed with skin and soft tissue infections.

2.2 Exclusion criteria

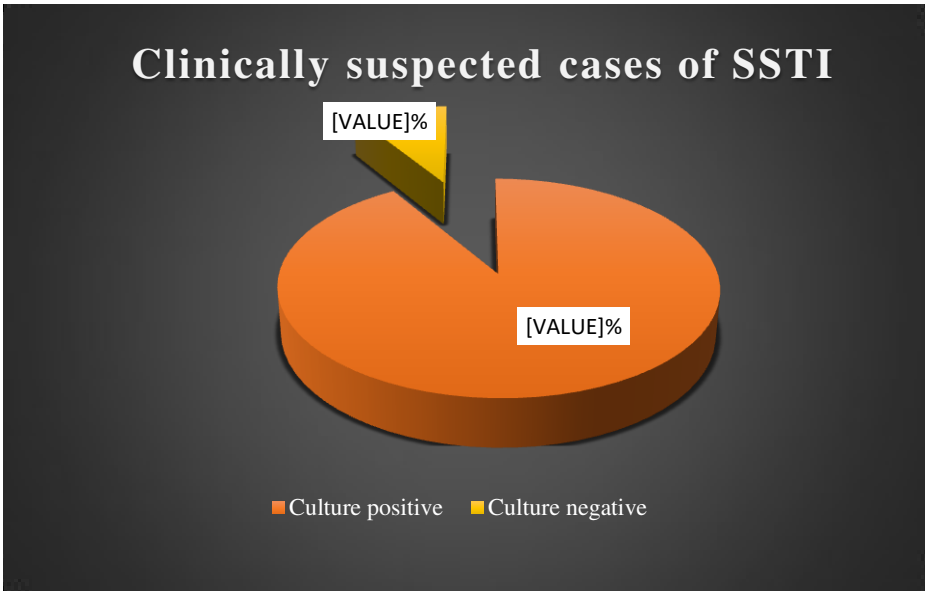
Those patients have other systemic infections like osteomyelitis and receive antibiotics at the time of presentation or within a week.

3. STATISTICAL ANALYSIS

Data were filled in the MS Excel Software. Then, analyzed results were expressed as percentage and p values by Chi-square test using GraphPad Instant software. If the probability is less than 0.05, the association or difference is said to be significant.

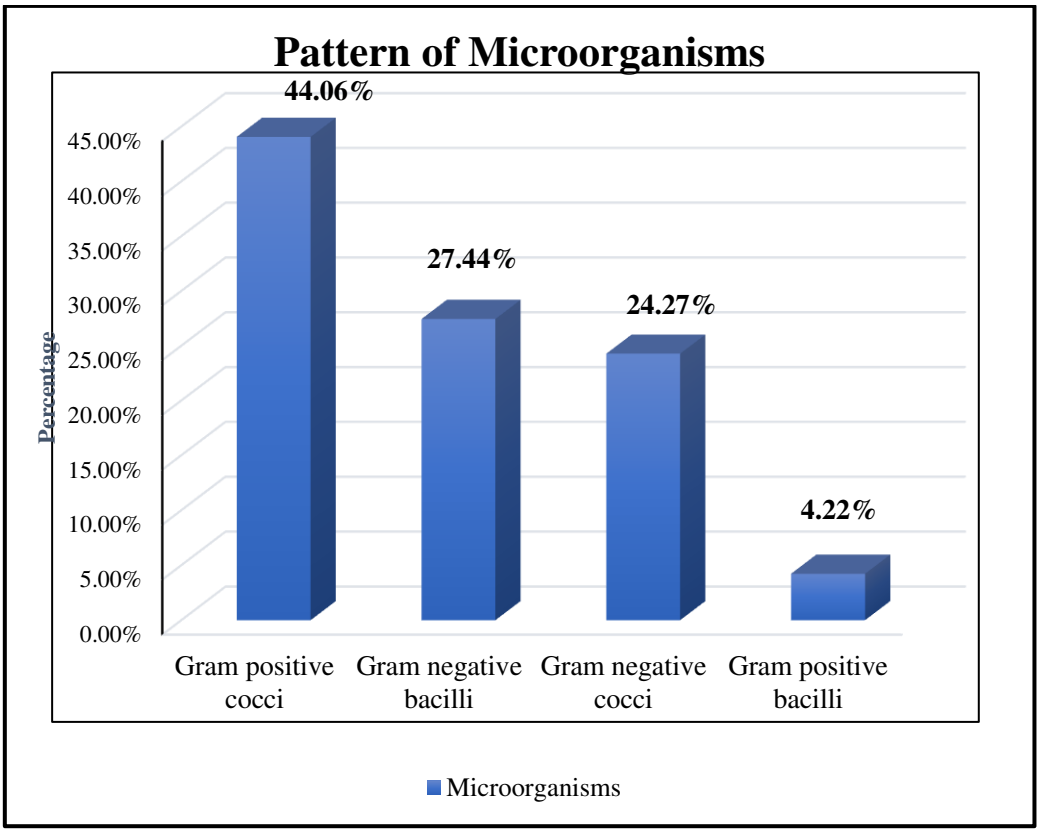
4. OBSERVATIONS AND RESULTS

In the present study, out of 415 clinically suspected cases of SSTI, 379 were culture positive, which accounts for 91.33%. Out of total culture-positive cases of SSTI from all age groups and of both sexes, 135 isolates were *Staphylococcus aureus*, which was further studied to detect Mupirocin resistance.



Graph.1: Clinically suspected cases of SSTI

Out of 415 clinically suspected cases of skin and soft tissue infection,379 (91.33%) were culture positive, and 36(8.67%) were culture negative.



Graph 2: Pattern of Microorganism

Out of 379 microorganisms, 167 (44.06%) were Gram-positive cocci, 104 (27.44%) were Gram-negative bacilli, 92(24.27%) were Gram-negative cocci, and 16 (4.22%) were Gram-positive bacilli.

Table 1 - Distribution of Gram-positive cocci (n=167)

Gram positive cocci	No. of isolates	Percentage
Staphylococcus aureus	135	80.84
Coagulase negative staphylococcus	22	13.17
Micrococcus	10	5.99

Out of 167 Gram positive cocci, 135 (80.84%) were Staphylococcus aureus, 22 (13.17%) were Coagulase Negative Staphylococcus and 10 (5.99%) were Micrococcus.

Table 2-Age and Gender wise distribution of *Staphylococcus aureus*			
Age group	**Male (%)**	**Female (%)**	**Total (%)**
0-10	5(3.70)	4(2.96)	9(6.67)
11-20	5(3.70)	3(2.22)	8(5.93)
21-30	9(6.67)	18(13.33)	27(20)
31-40	11(8.15)	15(11.11)	26(19.26)
41-50	12(8.89)	5(3.70)	17(12.59)
51-60	10(7.41)	2(1.48)	12(8.89)
61-70	14(10.37)	9(6.67)	23(17.03)
≥71	11(8.15)	2(1.48)	13(9.63)
Total	77(57.04)	58(42.96)	135(100)

Maximum isolates were from the 21-30 age group, 20%.

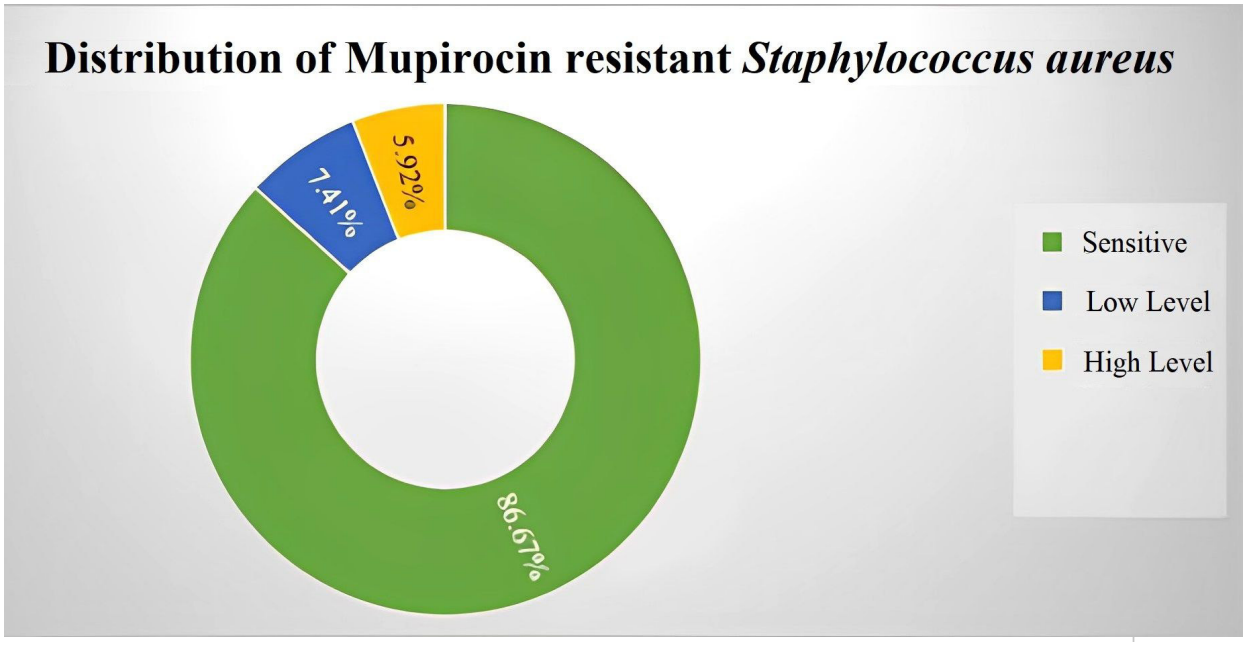
In males, maximum isolates were from the 61-70 age group 10.37% followed by the 41-50 age group 8.89%, 31-40 and ≥ 71 age group 8.15%, 51-60 age group 7.41%, 21-30 age group 6.67%, 0-10 and 11-20 age group 3.70%. On the other hand, in females' maximum isolates were from the 21-30 age group 13.33% followed by the 31-40 age group 11.11%, the 61-70 age group 6.67%, the 41-50 age group 3.70%,0-10 age group 2.96%,11-20 age group 2.22%,51-60 and ≥ 71 age group 1.48%.

Table 3 - Distribution of *Staphylococcus aureus* from clinically diagnosed SSTI samples		
Specimens	**Number**	**Percentage**
Pus	104	77.03
Wound swab	22	16.30
Tissue bit	9	6.67
Total	135	100

Table shows Distribution of *Staphylococcus aureus* from clinically diagnosed SSTI samples obtained from the hospital. The majority of the isolates were from pus 104 (77.04%), followed by wound swab 22(16.30%) and tissue bit 9 (6.67%).

Table 4 - Distribution of Mupirocin resistant *Staphylococcus aureus*		
Mupirocin resistant	***Staphylococcus aureus***	**Percentage**
Sensitive	117	86.67
Low Level	10	7.41
High Level	8	5.92
Total	135	100

Table shows distribution of Mupirocin resistant *Staphylococcus aureus*. Out of 135 isolates, 117(87.67%) were Sensitive, 10(7.41%) were Low level resistant, and 8 (5.92%) were High level resistant.

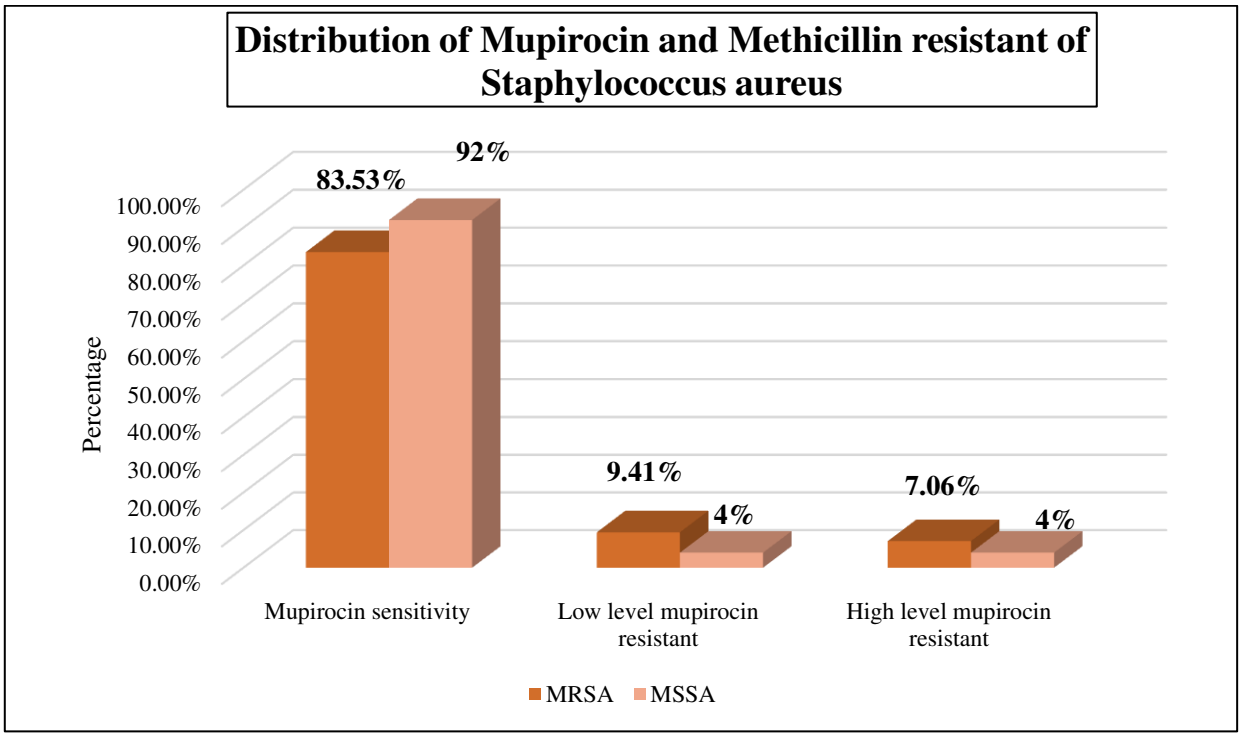


Graph .3: Distribution of Mupirocin resistant *Staphylococcus aureus*

Table .5- Distribution of Mupirocin resistance in MRSA and MSSA				
Methicillin sensitivity	Mupirocin sensitive (%)	Low-level Mupirocin resistant (%)	High-level Mupirocin resistant (%)	Total (%)
MRSA	71(83.53)	8(9.41)	6(7.06)	85(100)
MSSA	46(92)	2(4)	2(4)	50 (100)

χ^2 *-2.002, P- 0.3674, Significant*

Table No.5 shows the distribution of Mupirocin and Methicillin resistance of *Staphylococcus aureus.* Out of 85 MRSA isolates, Mupirocin sensitivity was seen in 71 (83.53%), followed by low-level Mupirocin resistance 8 (9.41%) and high-level Mupirocin resistance 6 (7.06%). Among 50 MSSA isolates, Mupirocin sensitivity was seen in 46 (92%), followed by low-level Mupirocin resistance is 2 (4%) and high-level Mupirocin resistance is 2 (4%).



Graph.4: Distribution of Mupirocin and Methicillin resistant of *Staphylococcus aureus*

Table 6: Comparison of Antibiotic Susceptibility with Mupirocin Susceptibility			
Antibiotic	Mupirocin sensitive (n=117)	Mupirocin low level resistant (n=10)	Mupirocin high level resistant (n=8)
Penicillin	2 (1.71)	0 (0)	1 (12.5)
Oxacillin	24 (20.51)	3 (30)	1 (12.5)
Gentamicin	84 (71.79)	8 (80)	7 (87.5)
Ciprofloxacin	14 (11.97)	2 (20)	0 (0)
Levofloxacin	13 (11.11)	3 (30)	0 (0)
Erythromycin	44 (37.61)	3 (30)	3 (37.5)
Clindamycin	57 (48.72)	5 (50)	4 (50)
Linezolid	117 (100)	10 (100)	8 (100)
Teicoplanin	115 (98.29)	10 (100)	7 (87.5)
Vancomycin	109 (93.16)	10 (100)	7 (87.5)
Tetracycline	104 (88.89)	10 (100)	5 (62.5)
Tigecycline	112 (95.73)	10 (100)	8 (100)
Nitrofurantoin	113 (96.58)	10 (100)	8 (100)
Co-trimoxazole	71 (60.68)	6 (60)	6 (75)

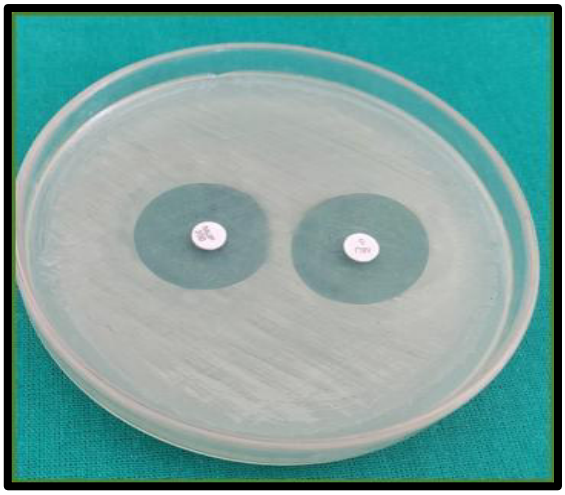


Fig 1: Mupirocin sensitive

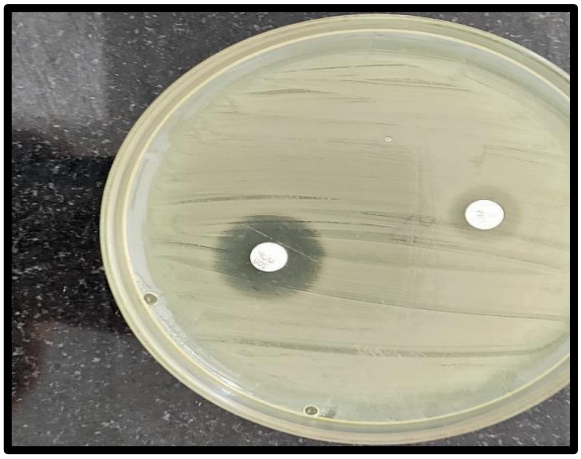


Fig 2: Mupirocin low level resistant

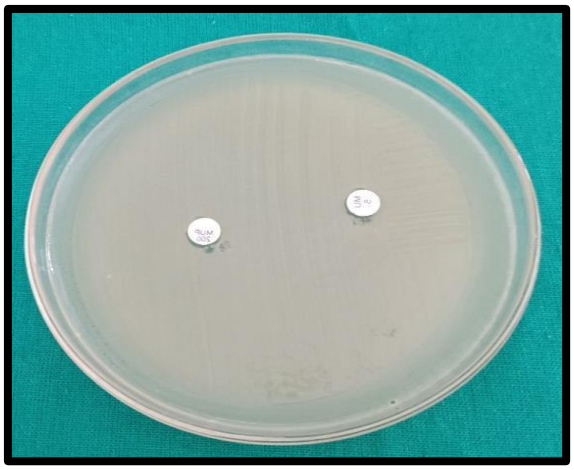


Fig 3: Mupirocin high level resistant

5. DISCUSSION

Mupirocin, a topical antibiotic, treats MRSA-associated skin and soft tissue infections and decolonizes carriers.[9] Globally, Mupirocin resistance was increased in MRSA as irrational, uncontrolled, prolonged, and multiple courses of this drug are the main reasons for the development of resistance. [10] Preventive measures for *Staphylococcus aureus* infections have been widely implemented in health care settings. Specifically, Mupirocin has been prescribed to eradicate *S. aureus* carriage to reduce the risk of nosocomial infections.[11] Outbreaks of MRSA resistant to Mupirocin have accompanied the increased use of this antibiotic, although the frequency of resistance is still low.[12] In the present study, out of 415 clinically suspected cases of SSTI, 379 were culture positive, accounting for 91.33%. Among the 379 positive cultures, 167 (44.06%) were gram-positive cocci, 104 (27.44%) were gram-negative bacilli, 92 (24.27%) were gram-negative cocci, and 16 (4.22%). Were gram-positive bacilli. Among the 167 gram-positive cocci, 135 were *Staphylococcus aureus* isolates which were studied further to know the high and low-level Mupirocin resistance of *Staphylococcus aureus.* Out of 135 isolates in the present study revealed *Staphylococcus aureus* is the most common pathogen causing SSTI and accounts for 57.04% in males and 42.96% in

females. In both sexes, a maximum number of patients belongs to the 21-30 age group of 20%. In the study, among 135 isolates, the majority of the isolates were from Pus 104 (77.03%), followed by wound Swab 22 (16.30%) and Tissue bit 9 (6.67%). This finding can be correlated with the study conducted in 2015 [13] reported that most *Staphylococcus aureus* isolates were from Pus 68.7%. Our study is mostly comparable with the study published in 2019 [14] in which they reported a maximum number of *Staphylococcus aureus* isolates from Pus 70%. The literature from Madhya Pradesh [15] showed 8.2% high-level and 17.3% low-level Mupirocin resistance in *Staphylococcus aureus.* Similarly, Various studies reported 2% high and 9% low level [16], 3.5% high and 2.83% low level [17], 5% high and 1% low level [18], 1.6% high and 2.3% low-level [19] Mupirocin resistance, respectively. These findings are similar to our study, where among 135 *Staphylococcus aureus i*solates, 5.92% were high-level, and 7.41% were low-level Mupirocin resistance. In contrast, compared to our study, another study published in 2020 [20] shows the highest prevalence of high-level resistance, 9% and low-level resistance 4%. Some studies show Mupirocin resistance to only high levels of 11% [21] and 5% [22], respectively.

Table.7 Comparative Study Showing Mupirocin Resistance in MRSA

Author name	Year	Rate of Mupirocin Low-level resistance (MuL)%	Rate of Mupirocin high-level resistance (MuH) %
Nicholoson Am et.al [23]	2010	30	24
B Madhumati et al. [24]	2018	13	11
Khan A et al. [4]	2020	10.6	3.5
Present Study	2022	9.41	7.06

Our MRSA isolates showed 7.06% high-level and 9.41% low-level Mupirocin resistance. It is comparable to the study [10] reported in 2022 showed 9.8% for high-level resistance and 11.8% for low-level resistance. A researcher from Maharashtra [6] observed the prevalence of high-level 5.99 % and low-level 15.35% Mupirocin resistance in MRSA; similarly, another study reported 4% high-level and 8% low-level Mupirocin resistance.[25] The study conducted in 2015 [15] showed a prevalence of high and low-level resistance to Mupirocin in MRSA at 14.7% and 10.5 respectively, which were slightly higher than our research. Similarly, another study noted 25% high and 14.2% low-level resistance Mupirocin.[26]

6. CONCLUSION

High and Low-level Mupirocin resistance in *Staphylococcus aureus* isolates was 5.92% and 7.41%, respectively. As a result, even in hospitals where Mupirocin is not used, routine testing of *Staphylococcus aureus* for Mupirocin resistance is suggested. It will aid in the early detection of resistance and the control and spread of Mupirocin resistance in a healthcare setting.

7. LIMITATIONS OF STUDY

Mupirocin resistance in *Staphylococcus aureus* can be detected by phenotypic and genotypic methods, such as Polymerase Chain Reaction (PCR). Molecular methods are confirmatory for the detection of resistance among the isolates. Still, due to a lack of facilities for genotypic study, we have yet to further study the isolates for molecular characterization. Molecular characterization will help to understand the mechanism of both high and low-level Mupirocin resistance.

8. ETHICAL APPROVAL STATEMENT

Ethical and research clearance was procured from Institutional Ethics Committee, Krishna Institute Medical Sciences Deemed to be University, Karad, for conducting the present research (Protocol number 052/ 2021-2022). All the participants were informed about the research and the study, conducted to provide the required data and informed consent before participating. The authors collected and preserved the patient's written consent per international or university standards.

9. AUTHORS CONTRIBUTION STATEMENT

Dr. Ravindra Shinde done writing – editing, reviewing software, and data curation. Aishwarya Mohite conceptualized and designed the study methodology, curated the data, and prepared the original draft. Dr. Deepak Patil, Dr. Shivaji Mohite, and Dr. Satish Patil discussed the methodology and analyzed the data. Finally, Dr. Anjali Shinde and Dr. Sandeep Shinde provided valuable inputs toward the design of the manuscript.

10. ACKNOWLEDGMENT

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11. CONFLICT OF INTEREST

Conflicts of interest declared none.

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