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Slime-Producing Methicillin-Resistant Staphylococcus Aureus: A Prevalence Study In Hospitalized Patients At A Tertiary Care Hospital.

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Abstract:
Background: *The VITEK 2 Compact system is a reliable, automated tool for the detection of MRSA, utilizing advanced algorithms for precise antimicrobial susceptibility profiling and species identification. In a study of 62 MRSA isolates, the highest antibiotic sensitivities were observed for daptomycin and tigecycline (100%), followed by linezolid (98.38%) and teicoplanin (95.16%). Resistance was particularly high for erythromycin (69.35%) and clindamycin (66.12%).*
Slime-producing Staphylococcus aureus strains, with capsular polysaccharides bound to their cell walls, enhance their colonization and resistance. This slime layer, which promotes bacterial adhesion especially to prosthetic surfaces, reduces antibiotic efficacy, leading to more persistent infections. Detecting oxacillin-resistant strains is crucial to guide treatment and limit vancomycin overuse, which could otherwise drive resistance development.
Material and Methods: *A two-year A laboratory analysis was conducted to evaluate slime production in Methicillin resistant Staphylococcus aureus strains at a tertiary care hospital.*
Results: *A total of 62 slime producing Methicillin resistant Staphylococcus aureus isolates were analyzed. Slime production was detected using the Congo Red Agar method, and in varying degrees using the Standard Tube method (42% weakly positive, 32% moderately positive, 26% strongly positive).*
Conclusion: *Microbial slimes contribute to persistent infections, posing challenges for healthcare and leading to high costs. In this study, identifying slime-producing microbes enables targeted treatments, allowing for early intervention and appropriate antibiotic use. By being proactive, we can improve patient outcomes, cut down on healthcare costs, and lessen the severity of chronic illnesses.*
Key Words: *Methicillin resistant Staphylococcus aureus, Slime, Antibiotic resistance, Congo Red Agar, Standard Tube method, Vitek 2 compact system.*

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I. Introduction

The production of slime is seen as a major A close relationship exists between the bacterial capsule and the bacterial cell wall in certain strains of *Staphylococcus aureus*. Additionally, these strains can contain an additional capsular and labile extra polysaccharidic structure. Previously, the generation of slime by *Staphylococcus aureus* was not thought to constitute a virulence factor. [1] Several researchers recently revealed that slime producing *Staphylococcus aureus* strains had a higher colonization capacity than it's non – slime producing variants did. Therefore, *Staphylococcus aureus* slime may play on role in the establishment of infection.[2]

The importance of the part performed by slime is further increased by its frequent association to reduced antibiotic susceptibility.[3] It has been demonstrated that persistent infections linked to the development of slime are more resistant to antibiotic concentrations than bacteria that do not produce slime.[4] In addition, the identification of staphylococci resistance to oxacillin is crucial for directing treatment and avoiding overuse of vancomycin, an antibiotic that has significant side effects, is expensive, and may select for resistant strains of the bacteria. [5] Many Coagulase Negative Staphylococci and Coagulase Positive Staphylococci strains of coagulase are derived from an adhering bacterial film, or slime, whose production has been linked to bacterial pathogenicity. This is thought to be the method through which the bacteria attach itself to and colonizes specific prosthetic devices. The slime forming Strains adhere better to surface (polystyrene and other materials) and have lower antibiotic susceptibility.[6] Vitek 2 Compact automated microbial identification and sensitivity test system (bioMerieux, France. Software version: 08.01) was used to analyse.

In the tertiary care hospital in Karad, the aim of this study is to assess and report the prevalence of Methicillin resistant *Staphylococcus aureus* that forms slime in hospitalized patients.

II. Material And Methods

The current investigation was conducted in the Microbiology Department of Krishna Institute of Medical Sciences in Karad, with approval from the institutional ethics committee. Specimens were collected from hospitalized patients, including both invasive and non-invasive clinical specimens. Gram staining was done directly on the prepared smears, and the specimens were cultured on Nutrient agar, Blood agar, followed by incubation at 37°C for 24 hours. The identification of bacterial colonies was based on their morphology, Gram staining results, catalase and coagulase tests, and various biochemical reactions, following Koneman's protocols.[7] Slime production of all isolates was evaluated by two different methods, Congo red agar method and standard tube method.

Automated microbial identification and sensitivity (Vitek 2 - compact; Biomerieux) system:

Reference strains of MRSA (ATCC 43300) and MSSA (ATCC 25923) were included as internal quality controls. Cultures incubated for 18 to 24 hours, adjusted to a density of 0.5–0.63 McFarland standard as determined by the system's colorimeter, were inoculated into sterile normal saline (bioMérieux, France). These samples were subsequently processed in the Vitek 2 Compact system, equipped with software version 8.01 (bioMérieux, France), following the manufacturer's protocol. Antimicrobial susceptibility was assessed using AST GP-P628 cards, and bacterial identification was performed using the GP Ref. 21342 ID card, specifically designed for Gram-positive bacteria. [8]

Detection of slime production in methicillin-resistant Staphylococcus aureus (mrsa):

Congo Red Agar (CRA) Method:

Utilizing the Congo Red Agar method described by Freeman et al. [9]:

After the medium had cooled to 55°C, the Congo red stain, which had been separately autoclaved for 15 minutes at 121°C, was added. Following inoculation, the plates were incubated aerobically for 24 hours at 37°C. Figure 2 illustrates that slime-positive isolates were identified by black, crystalline, dry colonies, while pink colonies indicated slime-negative isolates.

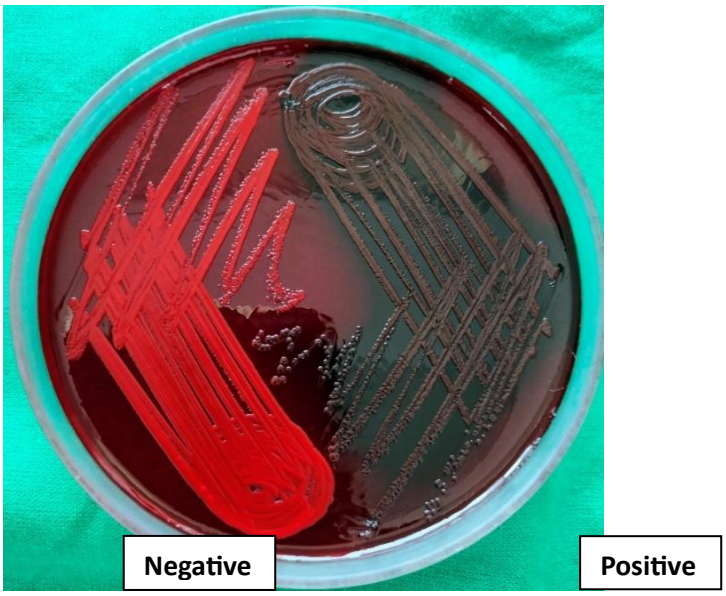


Figure no 1: Congo red agar method

Standard Tube (ST) Method:

The qualitative slime formation assay will follow Christensen et al.'s [10] protocol:

Trypticase Soy Broth (TSB) was inoculated with a loopful of the organism from a single colony on a pure blood agar plate, followed by incubation for 24 hours at 37°C. After the incubation period, the contents were discarded, and the tubes were stained with 1% safranin for seven minutes. A favourable outcome was demonstrated by an adhering layer of stained material on the inner surface of the tube, while stained material restricted to the liquid-air interface was considered unfavourable for slime production.

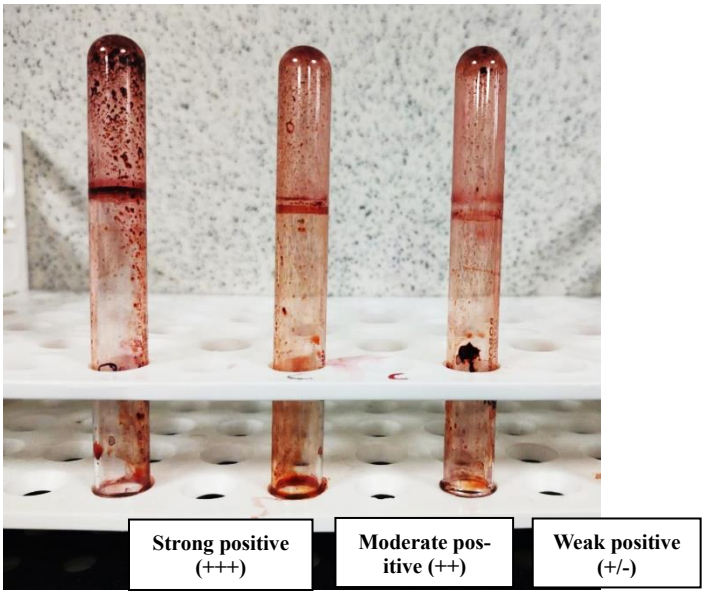


Figure no 2: Standard Tube Method

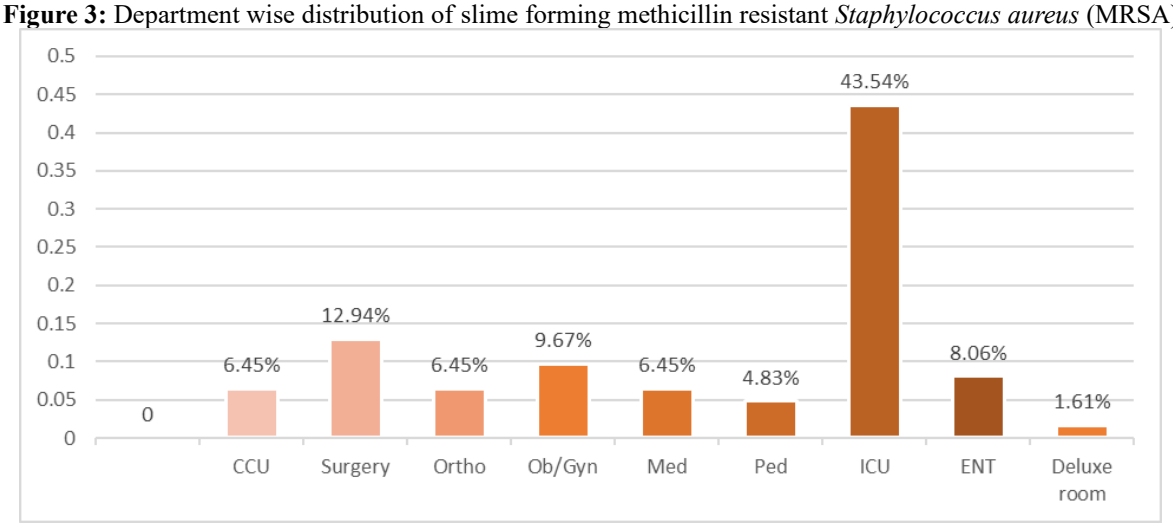
III. Results

Table no. 1: Age sex wise distribution of *Staphylococcus aureus*

Age group	Female patient	(%)	Male patient	(%)	Total (%)
0 – 20	02	8.33%	06	15.78%	07 (24.11%)
21 – 40	10	41.66%	13	34.21%	23(75.87%)
41 – 60	06	25%	10	26.31%	16(51.31%)
61 – 80	06	25%	06	15.78%	12(40.78%)
≥ 81	0	0	03	7.89%	03(7.89%)
Total	**24**	**100%**	**38**	**100%**	**62(100%)**

Table no.1 provides a detailed breakdown of Staphylococcus aureus isolates based on age and sex. The data reveals that the highest number of isolates overall came from the 21 – 40 age group, accounting for 23(75.87%) of the total cases, followed by the 41 – 60 age group with 16(51.31%). The distribution among older age groups decreases, with 12(40.78%) from the 61 – 80 age group, 07 (24.11%) from the 0 – 20 age group, and 03(7.89 %) from the ≥ 81 age group.

Figure 3: Department wise distribution of slime forming methicillin resistant *Staphylococcus aureus* (MRSA)



The distribution of *Staphylococcus aureus* isolates across various departments, as presented in Figure 3, shows the highest prevalence in the ICU, accounting for 43.54% of the total isolates. This is followed by Surgery at 12.94%, Obstetrics/Gynaecology at 9.67%, and Orthopaedics at 6.45%. Other departments had smaller contributions: ENT at 8.06%, Medicine and CCU both at 6.45%, Paediatrics at 4.83%, and the Deluxe room at 1.61%.

Table no. 2: Antibiogram of slime forming methicillin resistant *Staphylococcus aureus* (MRSA)

Antibiotics	Slime Producing Methicillin Resistant *Staphylococcus Aureus*	
	Sensitive N (%)	Resistant N (%)
Cefoxitin Screen	0 (0%)	62 (100%)
Benzylpenecillin	0 (0%)	62 (100%)
Oxacillin	0 (0%)	62 (100%)
Gentamicin	33 (53.22%)	29 (46.77%)
Ciprofloxacin	07 (11.29 %)	55 (88.70 %)
Levofloxacin	07 (11.29 %)	55 (88.70 %)
Inducible Clindamycin Resistance	22 (35.48%)	40 (64.51%)
Erythromycin	19 (30.64%)	43 (69.35%)
Clindamycin	21 (33.87%)	41 (66.12%)
Linezolid	61 (98.38%)	01 (1.61%)
Daptomycin	62 (100%)	0 (0%)
Teicoplanin	59 (95.16%)	03 (4.83%)
Vancomycin	54 (87.09%)	08 (12.90%)
Tetracycline	45 (72.58%)	17 (27.41%)
Tigecycline	62 (100%)	0 (0%)
Nitrofurantoin	59 (95.16%)	03 (4.83%)
Trimethoprim/Sulfamethoxazole	29 (46.77%)	33 (53.22%)

Table 2 presents the antibiotic susceptibility pattern of slime producing Methicillin-Resistant *Staphylococcus aureus* (MRSA).

The antimicrobial susceptibility of 62 MRSA isolates is detailed in Table 2. Daptomycin and tigecycline had the highest sensitivity at 100%, followed by linezolid and nitrofurantoin at 98.38%, and teicoplanin at 95.16%. Vancomycin showed 87.09% sensitivity, with tetracycline at 72.58%. Moderate sensitivities were noted for gentamicin (53.22%) and trimethoprim/sulfamethoxazole (46.77%). Clindamycin and erythromycin exhibited sensitivities of 33.87% and 30.64%, respectively, while levofloxacin and ciprofloxacin were each 11.29% sensitive. Cefoxitin screening was positive in 100% of cases, with full resistance to benzylpenicillin and oxacillin.

Regarding resistance, 69.35% of isolates were erythromycin-resistant, 66.12% clindamycin-resistant, and 53.22% trimethoprim/sulfamethoxazole-resistant. Gentamicin resistance was seen in 46.77% of isolates, tetracycline in 27.41%, and both nitrofurantoin and teicoplanin at 4.83%. Linezolid exhibited the lowest resistance rate, at 1.61%.

Among the MRSA isolates, inducible resistance to clindamycin was present in 35.48%, while the remaining 64.51% showed no inducible resistance to clindamycin.

Figure no 4: Slime production of Methicillin resistant *Staphylococcus aureus* by Congo Red Agar method.

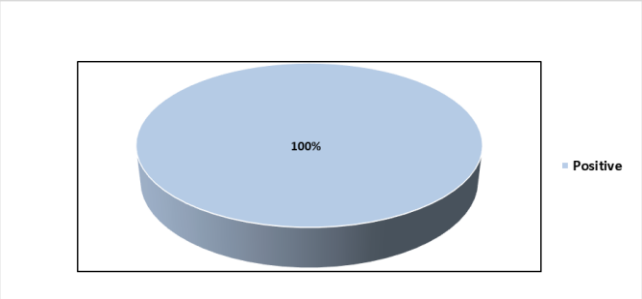


Figure 4 shows that all 62 Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates tested by the Congo Red Agar method demonstrated 100% slime production.

Figure 5: Slime production of Methicillin resistant *Staphylococcus aureus* by Standard Tube method.

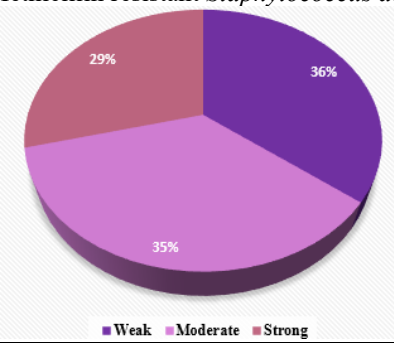


Figure 5 presents the slime formation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates using the Standard Tube method. Among the 62 Methicillin-Resistant *Staphylococcus aureus* strains tested, 22 isolates (35.49%) showed weakly positive slime formation, 22 isolates (35.49%) were moderately positive, and 18 isolates (29%) demonstrated strong slime production.

IV. Discussion

Methicillin-Resistant *Staphylococcus aureus* increases resistance to drugs and immunological reactions in hospitalized patients by forming biofilms, or "bacterial slime," on wounds and medical equipment. As a result, infections become more persistent and challenging to treat.

In this research, 62 Methicillin-Resistant *Staphylococcus aureus* isolates were analysed, revealing that individuals aged 21-40 exhibited the highest frequency, accounting for 23(75.87%) of cases. This is higher compared to the study by Kumar P et al. [11], which reported 46.01% of Methicillin-Resistant *Staphylococcus aureus* cases in individuals aged 20-40 years. Moreover, male accounted for 61.30% of cases, whereas female constituted 38.70% of cases. These findings compared with Siddiqui T et al. [12], who reported a higher prevalence of MRSA among males (70%) compared to females (30%). Among hospital wards, the ICU had the highest proportion of Methicillin-Resistant *Staphylococcus aureus* isolates, with 43.54% of the total, which is comparable to Kumar G et al. [13], who found 43% of Methicillin-Resistant *Staphylococcus aureus* isolates in ICUs, indicating a higher burden of Methicillin-Resistant *Staphylococcus aureus* in critical care units.

Regarding slime production, 62% of Methicillin-Resistant *Staphylococcus aureus* isolates were positive for slime production based on the Congo Red Agar (CRA) method, a result comparable to Bhattacharya S et al. [14], who reported 76.6% slime production. Similarly, Mariana NS et al. [15] found that 78% of MRSA strains were slime producers on congo red agar plates. Using the Standard Tube method, varying degrees of slime production were observed: 42% weakly positive, 32% moderately positive, and 26% strongly positive. These results compared with Manandhar S et al. [16], who reported 53.4%, 16.8%, and 11.2% for weak, moderate, and strong slime production, respectively.

Strengths

1. High Sensitivity Rates: Daptomycin and tigecycline exhibited 100% sensitivity against MRSA isolates, indicating their effectiveness as treatment options.
2. Insight into Resistance Patterns: The study provided critical data on antibiotic resistance, with high resistance rates to erythromycin (69.35%) and clindamycin (66.12%), aiding clinicians in therapy selection.
3. Importance of Early Detection: Emphasizing the need for early identification of slime-producing strains can enhance treatment strategies and improve patient outcomes.

Limitations

1. Biofilm Resilience: The protective nature of biofilms poses significant challenges to treatment efficacy, necessitating advanced management strategies.
2. Challenges in Detection: Traditional culture methods may inadequately identify slime-producing bacteria, leading to misdiagnosis and delayed treatment.
3. Limited Treatment Options: Dependence on certain antibiotics like vancomycin may contribute to resistance, highlighting the need for alternative therapeutic strategies.

V. Conclusion

Microbial slimes play an important part in the development of persistent infections, may present significant obstacles for healthcare professionals and result in considerable economic burdens. These biofilms, formed by bacteria and other microorganisms, create protective layers that shield the pathogens from the host's immune response and reduce the effectiveness of antibiotic treatment. As a result, infections become more difficult to eradicate, often leading to prolonged hospital stays and recurring infections.

Identifying the specific microbes responsible for slime production is crucial for enabling the use of targeted treatment plans. By detecting these biofilm-producing organisms early, healthcare providers can prescribe appropriate and effective antibiotic therapies, tailored to the patient's infection. This not only helps in mitigating the severity and persistence of chronic infections but also reduces the risk of antibiotic overuse, which can contribute to resistance. This proactive approach optimizes healthcare costs by decreasing the need for extended treatments and shortening hospital stays. Targeted therapy and early detection also improve patient outcomes, resulting in faster recovery times and better level of living for individuals with chronic infections.

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