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PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN URINE SAMPLE AND STUDY ON ITS BIOFILM FORMATION AND ANTIBIOTIC SENSITIVITY

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Abstract: The study aimed to assess *Staphylococcus aureus* prevalence in urine samples, its biofilm formation capacity, and antibiotic sensitivity through urine sample isolation, identification, biofilm examination, and antibiotic susceptibility. *Staphylococcus aureus* is known to produce pathogenic factors and poisons, and its development pattern resembles grapes. Drug-resistant bacteria, particularly MRSA, have raised concerns. Symptoms include diarrhoea, vomiting, nausea, and abdominal pain. Prevention involves avoiding contact, wound care, and hygiene.

***Index Terms - Staphylococcus aureus,* Biofilm, Drug-resistant bacteria, Antibiotic sensitivity, MRSA.**

I. INTRODUCTION

Staphylococcus aureus, a gram-positive bacterium, causes various infections due to its growth pattern and toxins. Recently, drug-resistant strains, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA), have emerged, often linked to healthcare-associated and community-associated infections. Prevention and control of *S. aureus* infections involve good hygiene practices, wound care, and avoiding contact with infected individuals or contaminated objects. Timely and appropriate antibiotic use is crucial, especially in the case of drug-resistant strains. Biofilms, complex communities of bacteria embedded within a self-produced matrix of extracellular polymeric substances, provide protection against antimicrobial agents, host immune response, and environmental stressors. (Foster T et al., 1996) (Taylor TA *et al*., 2023). A common bacterial pathogen, *Staphylococcus aureus* (*S. aureus*) can cause invasive or toxin-mediated illnesses with a variety of symptoms. Multidrug resistant bacteria, such as MRSA, which possess a mec gene expressing PBP-2a, make treatment challenging. For precision therapy, collaboration among specialists is essential. (R. *Et al*. 2005). According to a study by Rosa et al., patients with *Staphylococcus aureus* bacteria in a tertiary hospital in Zaragoza had a death rate of 24.2% after 14 days and 40% after 30 days. Patients over 65 and those with comorbidities had a decreased overall survival rate. Sepsis, age above 65, and a higher number of positive blood cultures are all linked to higher fatality rates. (Rosa *et al*., 2022). Cristina et al.'s 2021 study found that daptomycin prevents *Staphylococcus aureus* biofilms from forming due to reactive oxygen species production, while thiourea and catalase reduce ROS levels. (Cristina *et al*., 2021). According to a 2019 study by Zafindrasoa Domonia et al., the coagulase test is a good method to identify Staphylococcus aureus, and factors including age and resuscitation can all result in infection. (Zafindrasoa Domonia *et al*., 2019). Qi Peng et al.'s 2022 study underlines the importance for understanding biofilm production and regulatory mechanisms for efficient treatment of *Staphylococcus aureus* biofilm infections, thereby tackling antibiotic resistance. (Qi Peng *et al*., 2022). Mehdi Goudarzi et al. conducted a study in Tehran, Iran, revealing that penicillin and chloramphenicol were effective against urinary tract infection-causing Staphylococcus aureus, while nitrofurantoin and chloramphenicol were less effective. (Goudarzi Mehdi *et al*., 2019)

According to a 2020 study by Liu et al., environmental factors such as calcium, oxygen regulation, and iron depletion affect the formation of *Staphylococcus aureus* biofilms, which can result in bacterial infections that are resistant to drugs. (Liu *et al*.,2020). Shivani Tyagi et al.'s 2021 study at a tertiary care hospital in North India found that antibiotic resistance and Staphylococcus aureus infections are common, with urine and pyogenic samples being the most common and medications exhibiting 100% susceptibility. (Shivani Tyagi *et al*, 2021). A 2009 study by Franklin D. Lowy on Staphylococcus aureus infections emphasized the pathogen's involvement in serious infections such as pyaemia and septicemia. The difficulties in treating multidrug-resistant bacteria are highlighted by the rising incidence of illnesses. Staphylococcus causes suppurative collections and recurring infections by spreading to particular locations. (Franklin D. Lowy's, 2009). A study by Alshomrani et al. suggests that trimethoprim-sulfamethoxazole could be a useful treatment for Staphylococcus aureus bacteriuria. However, source identification and clinical evaluation are crucial for prevention. (Mohammad K *et al*.., 2014) Pishtiwan Ahmad's study on S. Aureus strains revealed higher antibiotic resistance in MRSA strains, emphasizing the need for efficient biofilm management based on epidemiological and molecular traits. (Pishtiwan Ahmad, 2023).

II. MATERIALS AND METHODS:

The research was conducted from September 2023 to May 2024 at the department of Biotechnology of the institution. The study aims to isolate, identify, and test for antibiotic susceptibility of *S. aureus* from urine samples, focusing on its prevalence and biofilm formation. We used a selective method, the plating method, to isolate *S. aureus* from a urine sample. Mannitol Salt Agar was used as a selective and differential media for the isolation of pathogenic *Staphylococci* isolates. The bacteria isolated from the urine sample were identified, and their morphology was described using Gram staining. The biochemical characteristics of the isolates were investigated using the IMViC, sugar utilization, catalase, and coagulase tests. The tube method was used to examine the formation of biofilms by *S. aureus* isolates from urine samples. *S. aureus* antibiotic susceptibility was investigated using the disc diffusion method.

III. RESULTS AND DISCUSSION:

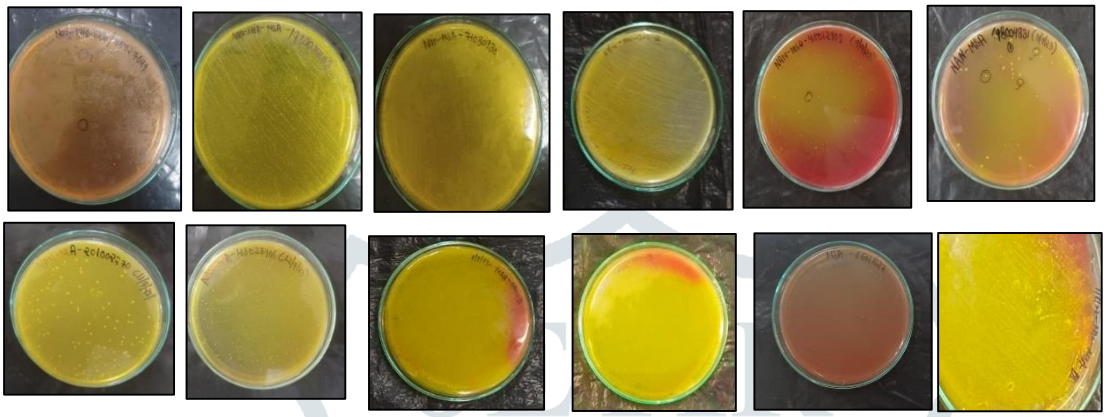


Figure 1: Isolation of *S. Aureus* on selective media MSA.

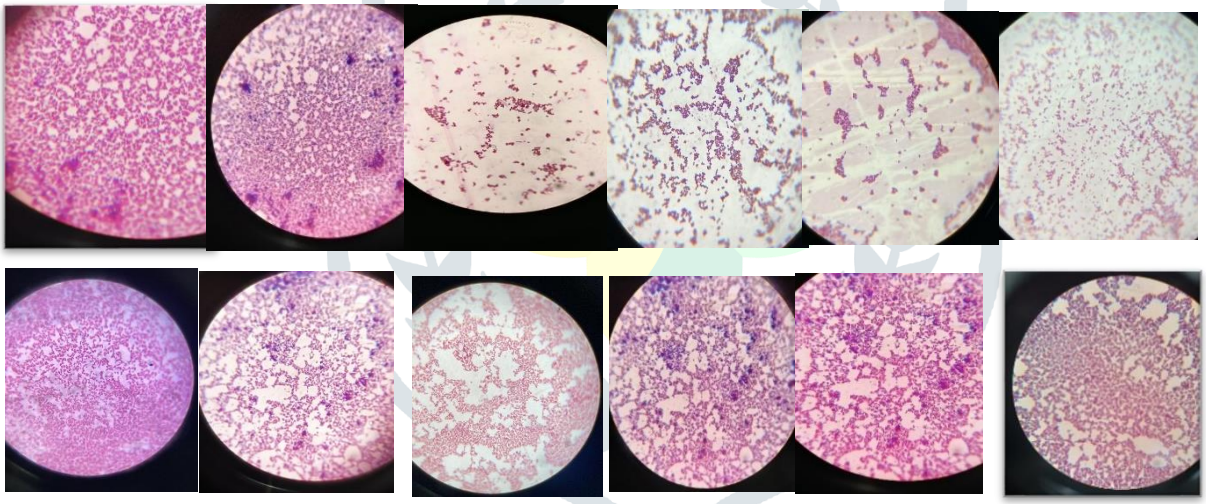


Figure 2: Morphological Characteristic: Gram Staining of isolates

Table 1: IMViC Test results for biochemical Identification

S.no	Isolate number	Indole test	Methyl Red test	Voges-Proskauer test	Citrate test
1	Isolate 1	-	+	+	+
2	Isolate 2	-	+	+	+
3	Isolate3	-	+	+	+
4	Isolate4	-	+	+	+
5	Isolate 5	-	+	+	+
6	Isolate 6	-	+	+	-
7	Isolate 7	-	+	+	+
8	Isolate 8	-	+	+	+
9	Isolate 9	-	+	+	-
10	Isolate 10	-	+	+	-
11	Isolate 11	-	+	+	+
12	Isolate 12	-	+	+	-

13	Isolate 13	-	+	+	-
14	Isolate 14	-	+	+	+
15	Isolate 15	-	+	+	-
16	Isolate 16	-	+	+	-

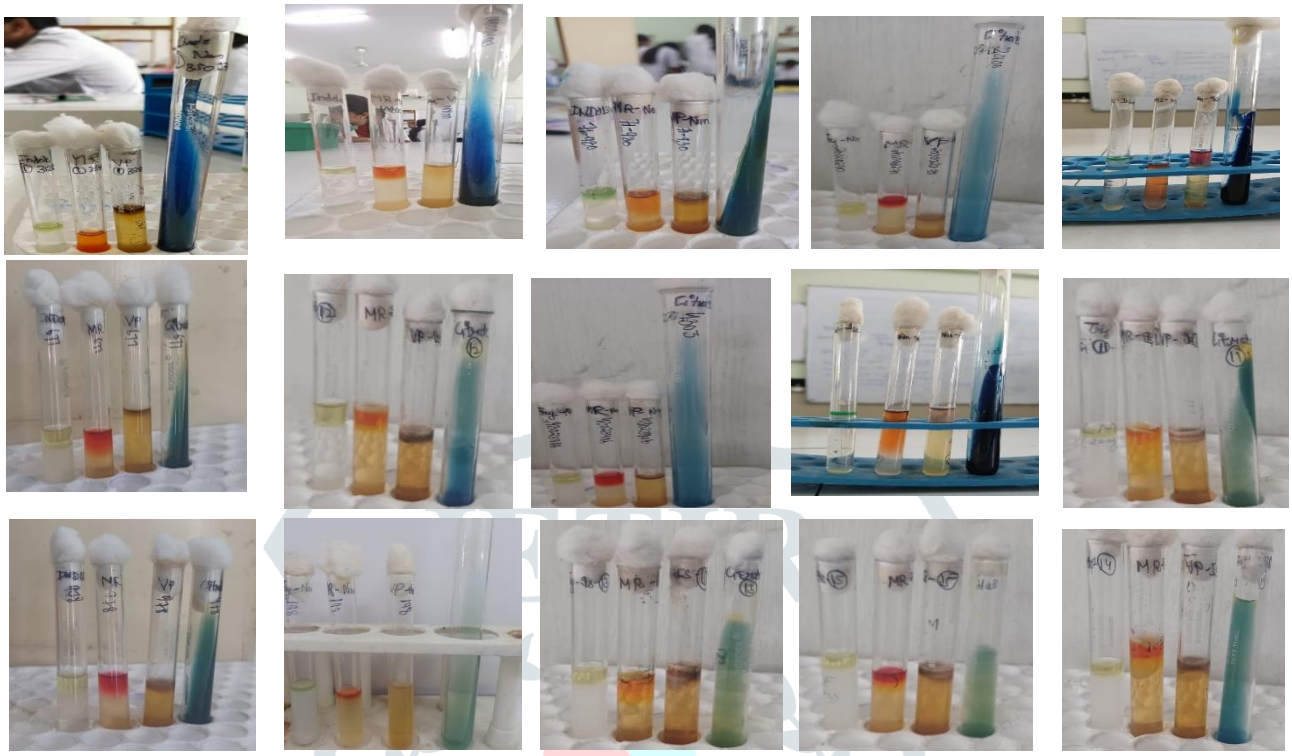


Figure 3: IMViC Test results for biochemical Identification of isolates

Table 2: Sugar Utilization Test

S. No	Isolate Number	Glucose		Sucrose		Mannitol		Mannose	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
1	Isolate 1	+	-	+	-	+	-	+	-
2	Isolate 2	+	-	+	-	+	-	+	-
3	Isolate3	+	+	+	+	+	+	+	+
4	Isolate4	+	+	+	-	+	-	+	-
5	Isolate 5	+	-	+	-	+	-	+	-
6	Isolate 6	+	-	+	-	-	+	+	-
7	Isolate 7	+	-	+	-	+	-	+	-
8	Isolate 8	+	-	+	-	+	-	+	-
9	Isolate 9	-	-	-	-	+	-	-	-
10	Isolate 10	-	-	+	-	+	-	-	-
11	Isolate 11	+	-	+	-	+	-	+	-
12	Isolate 12	-	-	-	-	-	-	-	-
13	Isolate 13	-	-	-	-	-	-	-	-
14	Isolate 14	+	-	+	-	+	-	+	-
15	Isolate 15	+	-	-	-	-	-	+	-
16	Isolate 16	+	-	-	-	-	-	-	-

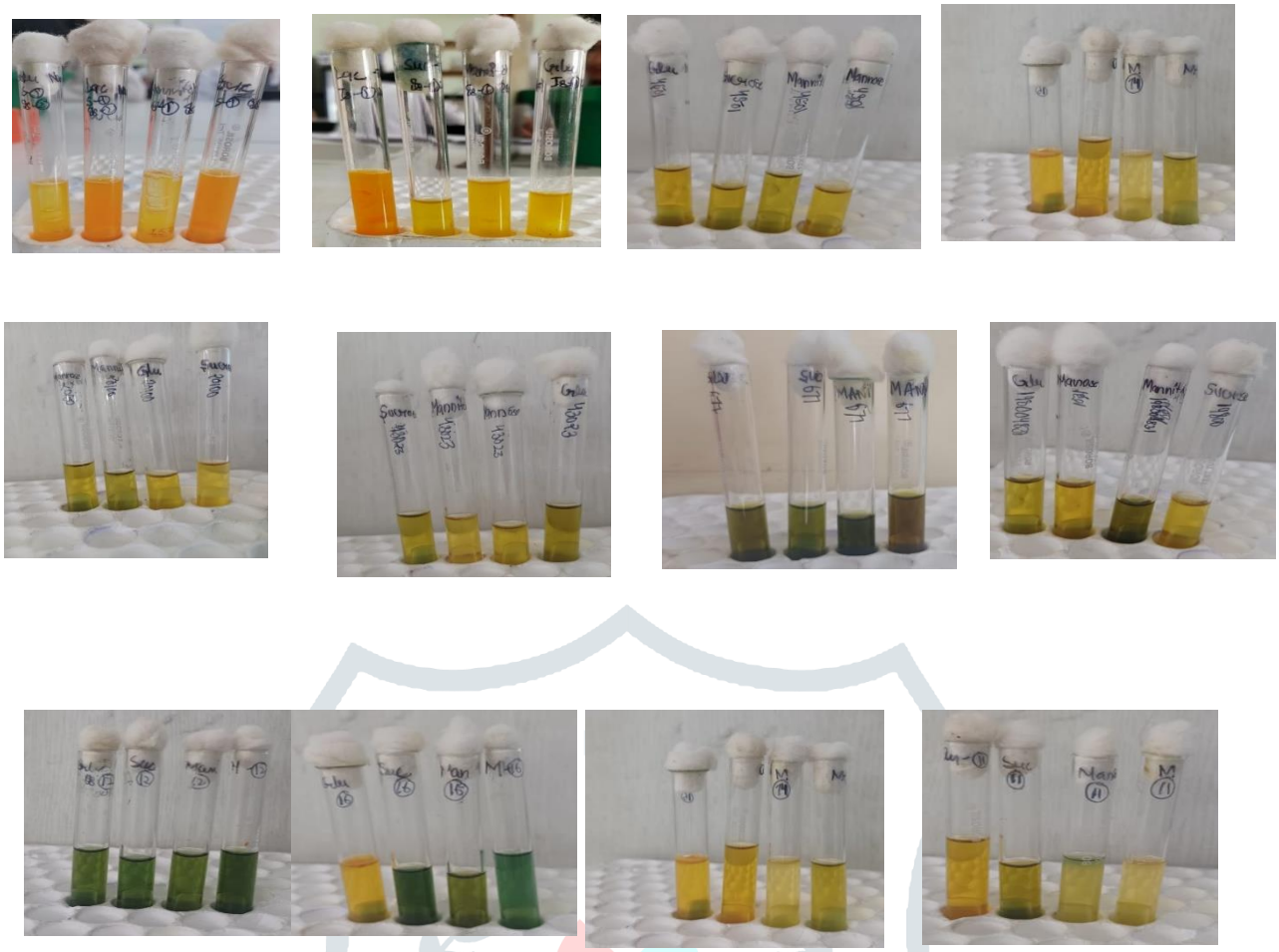


Figure 4: Sugar Utilization Test of isolates

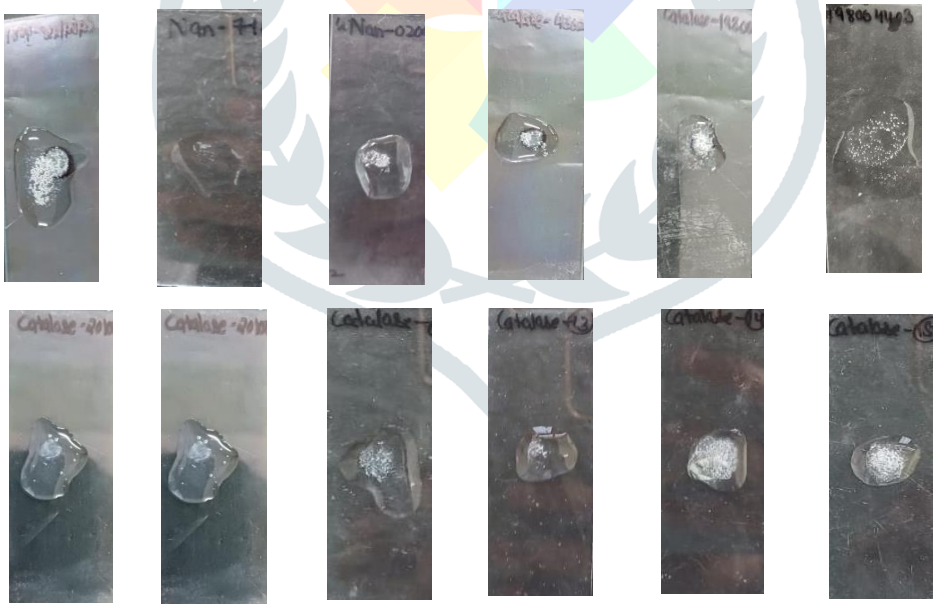


Figure 4: Catalase test results of isolate

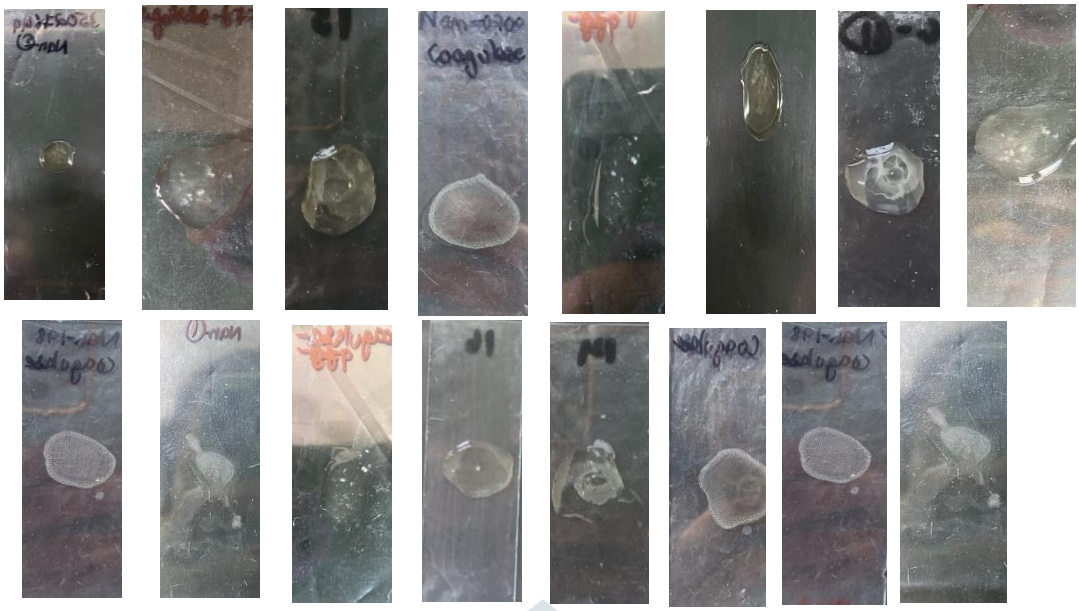


Figure 5: Coagulase test results of isolates

Table 3: Biofilm formation by isolates

S. No	Isolates	Biofilm formation
1	Isolate 1	+
2	Isolate 2	+
3	Isolate3	+
4	Isolate4	+
5	Isolate 5	+
6	Isolate 6	-
7	Isolate 7	+
8	Isolate 8	+
9	Isolate 9	-
10	Isolate 10	-
11	Isolate 11	+
12	Isolate 12	-
13	Isolate 13	-
14	Isolate 14	+
15	Isolate 15	-
16	Isolate 16	-

Figure 6: Biofilm formation by isolates

Table 4: Antibiotic Susceptibility Test for isolates positive to biofilm formation

S. No	Isolates	Ciprofloxacin		Gentamycin		Doxycycline		Penicillin	
1	Isolate 1	10mm	Resistant	16mm	Susceptible	28mm	Susceptible	No zone of Inhibition	Resistant
2	Isolate 2	16mm	Resistant	17mm	Susceptible	No zone of Inhibition	Resistant	15mm	Resistant
3	Isolate3	14mm	Resistant	20mm	Susceptible	No zone of Inhibition	Resistant	9mm	Resistant
4	Isolate4	14mm	Resistant	17mm	Susceptible	No Zone of Inhibition	Resistant	10mm	Resistant
5	Isolate 5	12mm	Resistant	17mm	Susceptible	28mm	Susceptible	10mm	Resistant
6	Isolate 7	No zone of Inhibition	Resistant	16mm	Susceptible	27mm	Susceptible	No zone of Inhibition	Resistant
7	Isolate 8	16mm	Resistant	17mm	Susceptible	No true zone of Inhibition	Resistant	15mm	Resistant
8	Isolate 11	17mm	Resistant	27mm	Susceptible	18mm	Susceptible	16mm	Resistant
9	Isolate 14	10mm	Resistant	30mm	Susceptible	26mm	Susceptible	15mm	Resistant

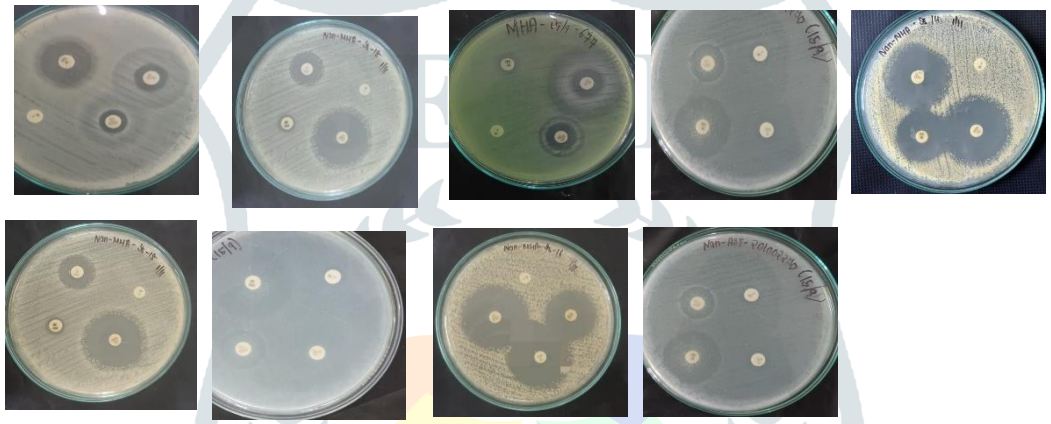


Figure 7: Antibiotic Susceptibility Test for isolates positive to biofilm formation

Table 5: Age wise and gender wise distribution of *S. Aureus* in urine samples.

S. No	Age range (in years)	Total no. of urine samples.	Inpatient from total urine samples.	Outpatient from total urine samples.	No. of urine samples positive for presence of *S. aureus*	
					Female	Male
1	Below 21	3	3	0	1	0
2	21-30	9	6	3	4	0
3	31-40	7	6	1	2	0
4	41-50	3	3	0	1	0
5	51-60	5	5	0	0	0
6	Above 60	3	3	0	0	1

Graph1: Age wise and gender wise distribution of *S. Aureus* in urine samples of different groups.

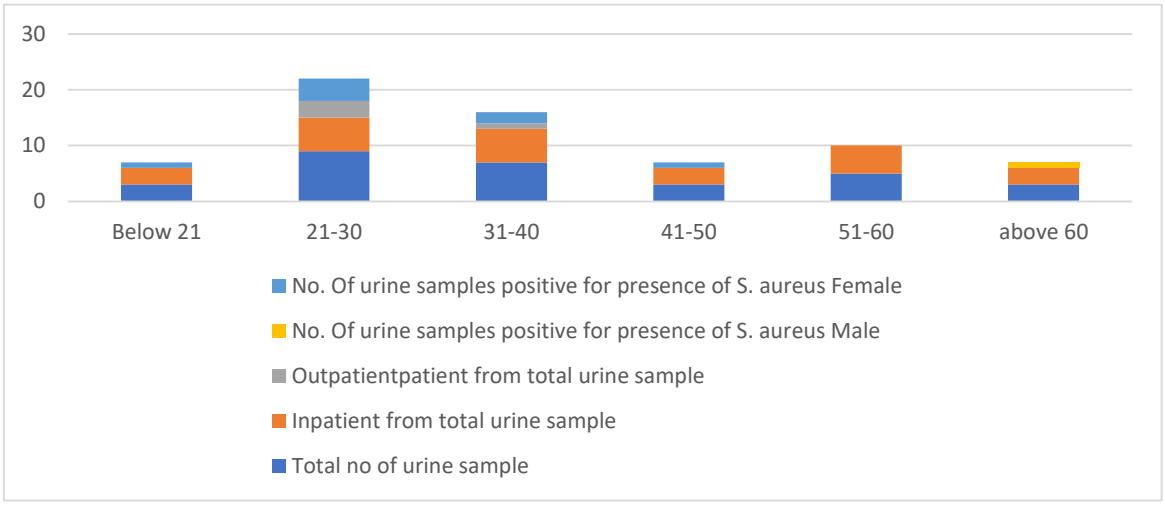
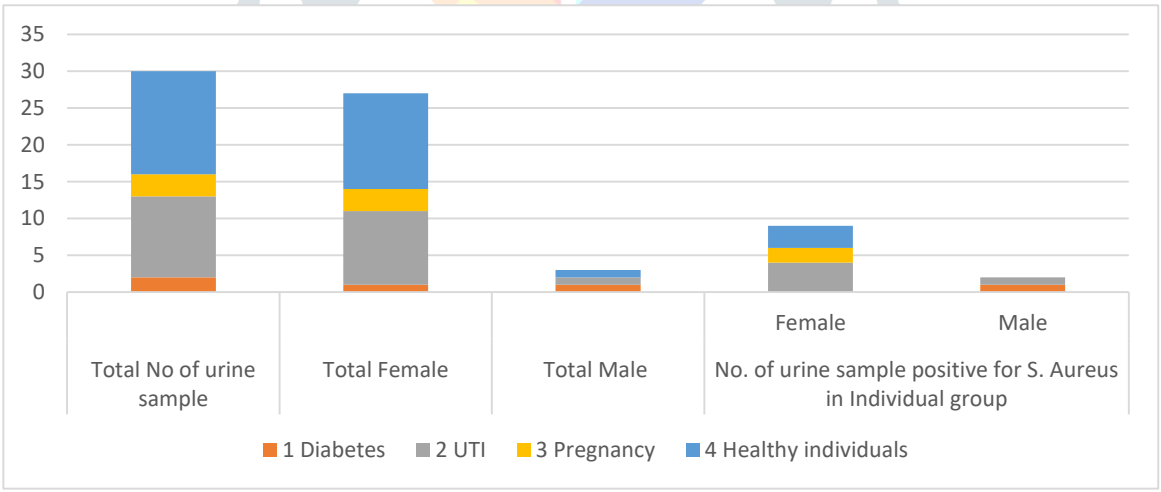


Table 6: Gender-wise distribution of *S. Aureus* in various individual groups

S. No	Individual group	Total No of urine sample	Total Female	Total Male	No. of urine sample positive for *S. Aureus* in Individual group	
					Female	Male
1.	Diabetes	2	1	1	0	0
2.	UTI	11	10	1	4	1
3.	Pregnancy	3	3	0	2	0
4.	Healthy individuals	14	13	1	3	0

Graph 2: Gender-wise distribution of *S. Aureus* in various individual groups



After 30 urine samples were cultured on Mannitol Salt Agar (MSA) media, 16 isolates with a characteristic colony morphology like that of Staphylococcus species were discovered. These isolates' colonies were convex, medium in size, yellow, and had a somewhat mucoidal look. To verify the presence of *S. aureus*, additional biochemical investigation was carried out. The total 16 isolates were subjected to morphological characterization using Gram staining, which showed that all of them had Gram-positive characteristics. These included cocci-shaped cells that were clustered together and had a colour that was violet. Under a microscope, each isolate showed unique Gram-positive characteristics. Seven out of sixteen isolates tested positive for the IMViC test, according to biochemical identification using the assay. Positive IMViC test findings showed positive citrate utilization, positive Voges-Proskauer (VP) test results, positive methyl red test results, and negative indole synthesis. The results of the sugar utilization test revealed that the bacterial samples were exposed to substrates such as glucose, sucrose, mannitol, and mannose. The purpose of this evaluation was to evaluate the isolates' ability to produce gas and acid. A favourable outcome for *S. aureus* is defined by the dye's green colour changing to yellow and little to no gas formation over time. The presence of the catalase enzyme and agglutination or coagulation are indicated by the positive catalase and coagulase tests, which offer important information about the physiological and metabolic characteristics of the *S. aureus* isolates under investigation.

The tube method was used to study *S. aureus* biofilm production, revealing that positive isolates undergo further analysis. Nine out of sixteen isolates were positive for biofilm formation. Antibiotic susceptibility testing revealed resistance against penicillin, while *S. aureus* was found to be sensitive against gentamicin. The study examined the age and gender distribution of *S. aureus* in urine samples, finding a higher prevalence among females aged 21-30, based on age and gender. The study found that *S. aureus* is more prevalent in females with urinary tract infections, based on risk factors such as diabetes and pregnancy.

IV. CONCLUSION:

This study examines *S. aureus* isolates from urine samples, revealing typical colony morphologies and positive biofilm formation, linked to antibiotic resistance. Females aged 21-30 showed strongest resistance, especially those with urinary tract infections. The study emphasizes antibiotic treatments and surveillance for infection management.

V: ACKNOWLEDGMENT

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VI. REFERENCES:

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