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"Comparative evaluation of in-vitro methods : Tissue culture plate method and tube method for the detection of biofilm formation by *Staphylococcus aureus* isolates from various clinical samples"

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ABSTRACT

Background Biofilms are defined as microbially derived sessile communities characterized by the cells to the substratum or to each other irreversibly attached. They are embedded in matrix of extracellular polymeric substances (EPS) which they have produced and exhibit an altered phenotype with respect to growth rate gene and gene transcription . Biofilm are associated with many medical conditions including indwelling medical condition devices, dental plaque, upper respiratory tract,infections,peritonitis and urogenital infection.

Objectives: Compare and evaluate in-vitro methods: tissue culture method and tube method for the detection of biofilm formation by *Staphylococcus aureus* isolates from various clinical samples.

Material and methods: The present study was carried out from January 2023 to December 2024 in the Department of Microbiology, Jawaharlal Nehru Medical College, KAHER Belagavi. A total of 145 *Staphylococcus aureus* isolates which were isolated from various clinical isolates received in the Department of Microbiology, Jawaharlal Nehru Medical College at, KAHER, received from KLE Dr. Prabhakar Kore Hospital, Belagavi.

Result: A total of 145 *Staphylococcus aureus* isolates were tested for biofilm production by Tissue culture plate (TCP) and Tube method (TM). Tissue culture plate method was considered as gold standard which showed 25(17%) of *Staphylococcus aureus* isolates to be strong biofilm producers and 53(36%) as moderate biofilm producers, and 67(46%) as none or weak biofilm producers. Tube method showed 17(12%) of *Staphylococcus aureus* isolates to be strong biofilm producers, 47(32%) as moderate biofilm producers were and 81(55%) none or weak biofilm producers.

Conclusion: This study showed Tissue culture plate method to be more reliable method to detect biofilm production from *Staphylococcus aureus* isolates .

Keywords: biofilm, *Staphylococcus aureus* isolates, Tissue culture plate method, Tube method

INTRODUCTION

A biofilm can be defined Microbiologically as, suicide community typified by cells that are attached to a substrate on interface, or each other are embedded in a matrix of extracellular polymeric substances, and exhibit an

altered phenotype with regard to the growth, gene expression and protein production (1). Biofilm thickness can range from single cell layer to substantial community encased by Viscous Polymeric Mili structural analysis have shown that in some cases unique dense pillar or mushroom shaped structures can be formed by micro colony architecture of these dense biofilms however the other structures to form depending on the environmental condition. Biofilm gains cohesion and viscoelasticity from the interaction of EPS with bacterial aggregates [2]. Bacteria can therefore adhere to both biotic and abiotic surfaces. One of the main factors contributing to chronic, persistent infection is the development of pathogenic biofilm [3]. Dr. Stanley Wall has demonstrated, using Bacillus subtilis from soil, that a protein called Deg benefits the individual microorganisms to determine whether or not to create a biofilm. Staphylococci that produce biofilm commonly colonize catheters and medical equipment and can result in illnesses linked to foreign bodies [4].

To evaluate the impact on biofilm phenotype, some researchers have supplemented brain- heart infusion (BHI) broth with sucrose or trypticase soy broth (TSB) with glucose.Nonetheless, some have provided a thorough explanation of how S. aureus biofilms rely on sodium chloride (NACL). Their quantitative interpretation and classification according to the biofilm formation criteria, however, lacked clarity and could not be repeated in all types of laboratory environment. There is an urgent need for straightforward consensuguideline in vitro biofilm synthesis. By clinical isolates of *Staphylococcus aureus* is direly needed [5].

There are several ways to find evidence of biofilm formation, these consist of Tissue culture plate (TCP), Congo red agar (CRA), Tube method (TM)[6]

Hence this study was undertaken to compare Tissue culture plate method (TCP) and Tube method (TM) in biofilm detection by S.aureus.

MATERIALS AND METHOD

Study center: Department of Microbiology, KAHER's Jawaharlal Nehru Medical College, Belagavi.

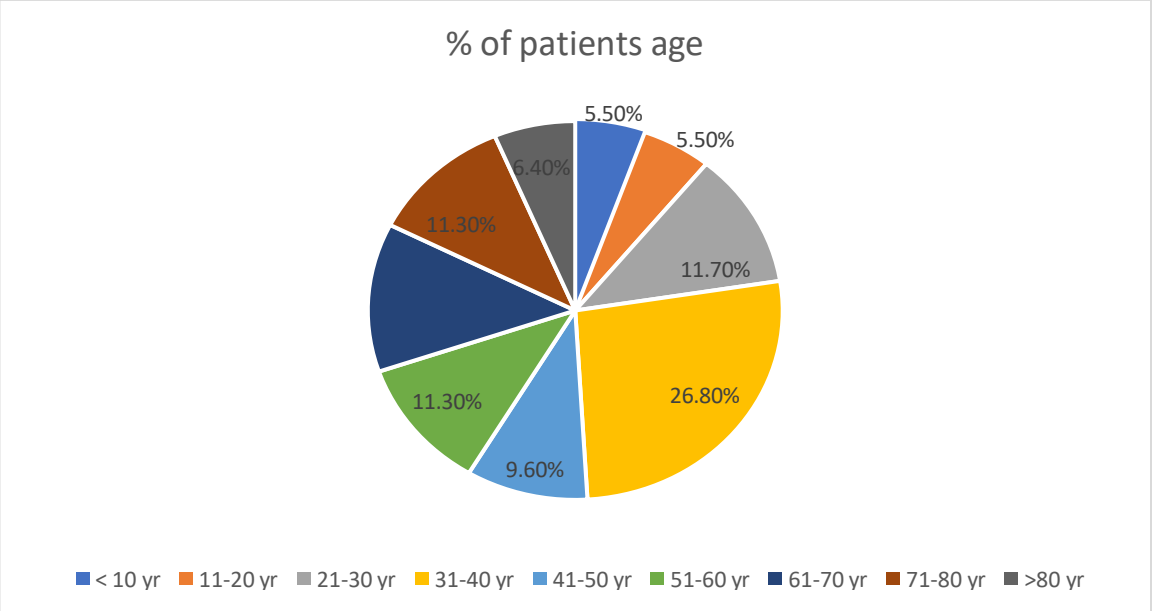
Source of Data: All isolates of *Staphylococcus aureus* from various clinical samples received at the Department of Microbiology, Jawaharlal Nehru Medical College, KAHER Belagavi from KLE, Dr Prabhakar Kore Hospital.

Sample size: 145 *Staphylococcus aureus* isolates from various samples
Study period: One year cross sectional study year (January 2023 – December 2023)
Study design: A cross-sectional study.
Inclusion criteria: : All the isolates of *Staphylococcus aureus* isolated from various clinical samples received at the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi.
Sampling procedure: Systematic sampling technique
Statistical Analysis: Prevalence was calculated and expressed in percentage
Results and Discussion: A total of 145 *Staphylococcus aureus* isolates were tested for biofilm production by Tissue culture plate (TCP) and Tube method (TM). Tissue culture plate method was considered as gold standard which showed 25(17%) of *Staphylococcus aureus* isolates to be strong biofilm producers and 53(36%) as moderate biofilm producers, and 67(46%) as none or weak biofilm producers. Tube method showed 17(12%) of *Staphylococcus aureus* isolates to be strong biofilm producers, 47(32%) as moderate biofilm producers were and 81(55%) none or weak biofilm producers.

Table No.1: Age wise distribution of patients (n= 145)

Age group	**Number of patients**	**PERCENTAGE**
<10yrs	8	5.5%
11-20 yrs	8	5.5%
21-30 yrs	17	11.7%
31-40 yrs	39	26.8%
41-50 yrs	14	9.6%
51-60 yrs	16	11.3%
61-70 yrs	18	12.4%
71-80 yrs	16	11.3%
≥80 yrs	9	6.4%
TOTAL	**145**	**100%**

Staphylococcus aureus was commonly found to be isolated from young adults ranging from age 31yrs 40yrs: 34(26.56%), followed by age group of 31yrs - 40yrs 16(13.91%). Least were isolated from =20 years & =80 years with 10.0% & 6.4% respectively.



Graph No. 1: Age wise distribution of patients in % (n=145)

Table No. 2: Gender wise distribution of isolates

	No. of patients	**Percentage**
MALE	66	41.51%
FEMALE	79	54.48%
Total	145	%

Isolation of *Staphylococcus aureus* was predominantly found to be from females (54.78%), compared to males (45.21%) with female to male ratio 1:0.8

Graph No. 2: Gender wise distribution of isolates

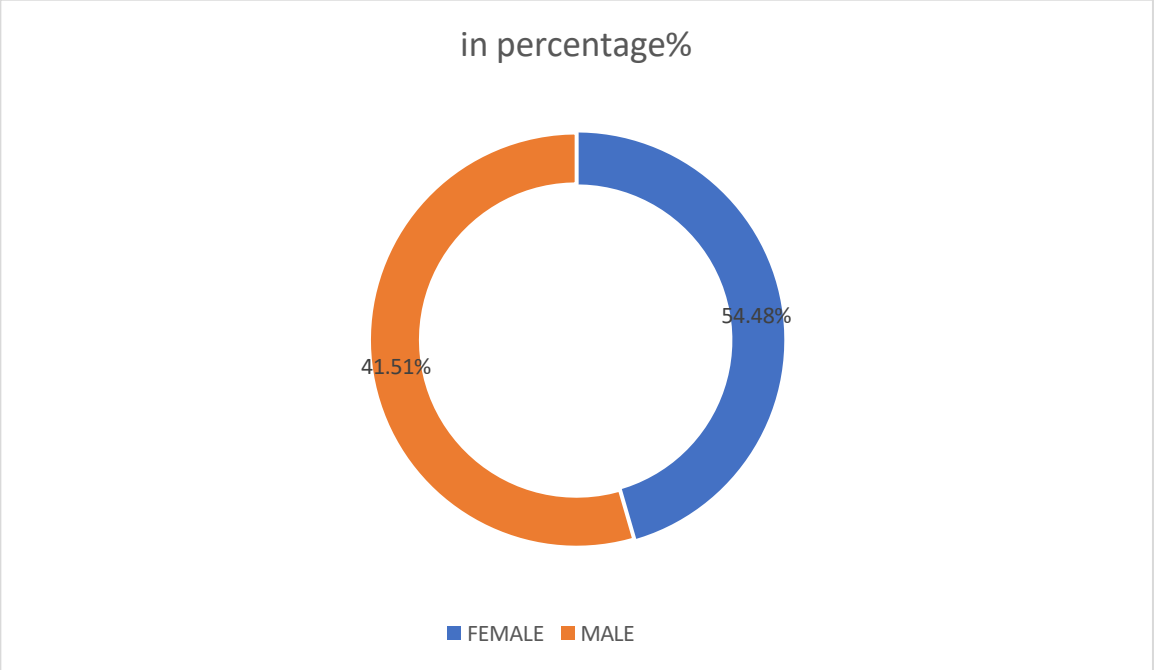
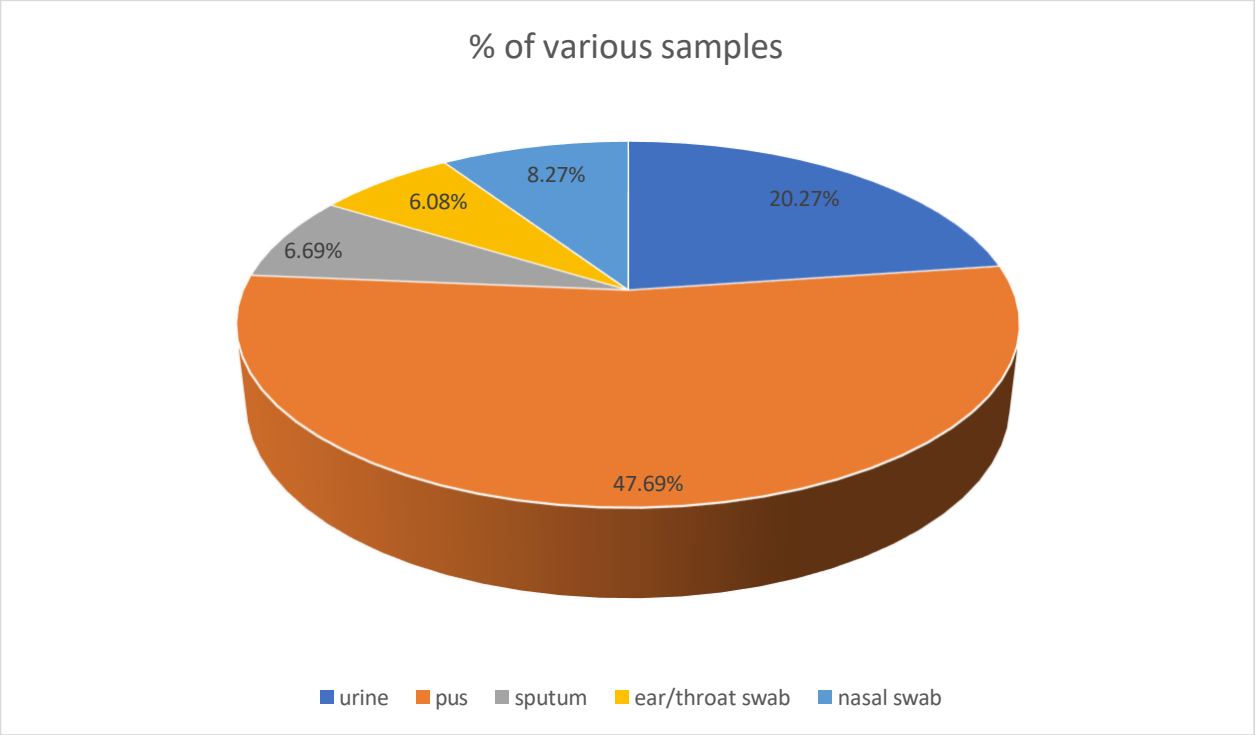


Table No.3: Distribution of *Staphylococcus aureus* in various clinical samples

Types of Samples	**Number of Samples**	**PERCENTAGE**
Urine	29	20.27.%
Pus	69	47.69%
Sputum	09	6.69%
Ear/throat swab	19	6.08%
Nasal swab	12	8.27%
Blood /others	07	4.82%
TOTAL	145	100%

Graph No.3: Distribution of *Staphylococcus aureus* in various clinical samples



Majority of then Staphylococcus *aureus* isolates in this study were from from pus sample 70(60.86%), followed by urine 20.27%.

	Biofilm formation	**Tissue culture plate**	**Tube method**
Clinical isolates N= 145	Highly adherent	25 (17%)	17(12%)
	Moderate	53(36%)	47(32%)
	Weak /non biofilm	67(46%)	81(55%)

No 4. Screening of Staphylococcus aureus isolates for biofilm formation by TCP,TM and CRA methods

The statistical value of tube method and Congo red agar method and tissue culture plate for biofilm detection(n=145)

Screenig method	Sensitivity	specificity	Positive p value	Negative p value	accuracy
TCP	97.1	97.5	98.6	95.1	97.2
TM	73.6	92.6	93.4	66.6	82.7

Biofilm production by bacteria are responsible for many recalcitrant infections and difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate. In our study, we have found that the majority of biofilm producing isolates of S.aureus were from pus sample which is similar to the study findings from the study done by Dolan et al. Tissue culture plate showed higher percentage of biofilm production in S.aureus compared to Tissue culture method , findings of which are similar to the study findings of Mathur et al and Bose et al . In contrast to this Ruzicka et al, in their study found Tube Method as a better method for for biofilm detection than tissue culture method.

CONCLUSION

Tissue Culture Method in comparison with Tube method is more sensitive and specific method in detection of biofilm production by S. aureus. And thus, incorporation of Tissue Culture Method for biofilm producing S.aureus as a routine in Culture section would help in early detection and treatment thus help in reducing mortality and morbidity and also inappropriate antibiotic usage by the clinicia

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