

# Evaluation of New Glass Slide and Petri Dish Methods of Biofilm Testing in Uropathogenic Bacteria and Their Comparison with Test-Tube Method

Biswas M<sup>1</sup>, Bhattacharyya S<sup>2\*</sup>, Banik A<sup>3</sup> and Jilani G Md<sup>4</sup>

<sup>1</sup> MSc. Biotechnology student, Swami Vivekananda Institute of Modern Science, Sonarpur, West Bengal.

<sup>2,3</sup> Associate Professor, Microbiology, AIHH&PH Kolkata.

<sup>4</sup> Professor, Department of Biotechnology, Swami Vivekananda Institute of Modern Science, Sonarpur, West Bengal.

\*Corresponding Author: Bhattacharyya S, Associate Professor, Microbiology, AIHH&PH Kolkata.

DOI: <https://doi.org/10.58624/SVOAMB.2023.04.029>

Received: May 22, 2023 Published: June 26, 2023

## Abstract

Biofilms are produced by many uropathogens and they render the drugs ineffective even if antibiotics are effective against the bug in vivo. In this study we evaluated the biofilm forming potential of the uropathogenic bacteria by the test tube method and compared 2 new methods with it, which were the glass Petri dish method and glass slide method. We found that the glass petri dish method was as good as, if not better than Test tube method to detect biofilms in uropathogenic bacteria. The slide method was neither very sensitive specific. Also, biofilms in test tube method could be observed microscopically. So these can be explored further by researchers later on.

**Keywords:** Uropathogens, biofilm, slide, petri dish, test tube.

## Introduction

A urinary tract infection (UTI) is a collective term for infections that involve any part of the urinary tract. It is one of the most common infections in local primary care. The incidence of UTIs in adult males aged under 50 years is low, with adult women being 30 times more likely than men to develop a UTI (1). UTIs are very common, mainly in women and people assigned female at birth. About 20% of people will have a UTI at some point during their lives. Men and people assigned male at birth can also get UTIs, as well as children, although they only affect 1% to 2% of children (2). Urinary tract infection (UTI) caused by uropathogens is the most common infectious disease and significantly affects all aspects of the quality of life of the patients. Although, uropathogens are increasingly becoming antibiotic-resistant, which threatens the only effective treatment option available-antibiotic, resulting in higher medical costs, prolonged hospital stays, and increased mortality (3). Midstream urine samples are collected in a wide mouth sterile container from study subjects who have not received antimicrobials within previous fifteen days. Then the bacterial uropathogens are isolated and tested for antimicrobial drug resistance pattern. Women are at greater risk of developing a UTI than are men. Women get UTIs more often because a woman's urethra (the tube from the bladder to where the urine comes out of the body) is shorter than a man. This makes it easier for bacteria to get into the bladder. If an infection is limited to the bladder, it is called cystitis, and can be painful and annoying. But serious health problems can result if a UTI spreads to the kidneys (4). A urinary tract infection is a very common type of infection in your urinary system. It can involve any part of our urinary system. Bacteria especially *E. coli* are the most common cause of UTIs. Symptoms include needing to urinate often, pain while peeing and pain in our side or lower back (5). A biofilm can consist of a single microbial species or a combination of different species of bacteria, protozoa, archaea, algae, filamentous fungi and yeast that strongly attach to each other and to biotic or abiotic surfaces (6). A Biofilm is an assemblage of the microbial cells that is irreversibly associated with a surface and usually enclosed in a matrix of polysaccharide material. Biofilm is composed primarily of microbial cells along with extracellular polymeric substance (EPS) (7).

The various isolates were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Acinetobacter baumannii* and *Enterococcus faecalis* etc. In the healthcare settings, biofilms have been shown to develop on medical device surfaces, dead tissues (e.g., sequestra of bones), and inside living tissues (e.g., lung tissue, teeth surfaces).

Biofilms growing in food processing environments may lead to spoilage of food, which in turn can cause serious public health risk to consumers and serious economic consequences (8).

The effects of biofilms are seen primarily in 4 ways by facilitating the emergence of antimicrobial drug resistance, generating chronic infections, the modulation of host immune response, and the contamination of medical devices (9).

Biofilm of bacteria can be detected by Test tube method (TM), Congo Red Agar (CRA) method, Microtiter plate (MtP) assay, plate counting of biofilm-embedded bacteria (sessile bacteria), PCR to detect the gene responsible for production of the matrix, mass spectrometry (MS), Confocal Laser Scanning Microscopy (CLSM), Scanning Electron Microscopy etc (10).

## Materials and Methods

**a. Type of study:** Laboratory based observational study.

**b. Time of study:** March 2023 to May 2023 (2 months).

**c. Place of study:** Department of Microbiology, Bidhan Nagar campus, All India Institute of Hygiene and Public Health, Kolkata.

**d. Methodology:** Midstream urine samples from the different hospitals were collected. The samples were inoculated on culture media (CLED) in the laboratory for the isolation of the microbes. Then CLED plates were incubated overnight and then colonies were identified next day by Gram stain and other standard biochemical tests (Indole production, phenotypic tests like motility on semisolid agar, Citrate utilisation, Urease positivity and reaction on TSI/Triple sugar Iron agar). Then the colonies were passed in peptone water with 1% glucose overnight in test tube and sterile borosilicate petri dish with two (2) sterilized glass slide in incubator. After over night incubation, borosilicate test tube was discharged by rinsing thrice with normal saline. Then borosilicate sterile test tube, one glass slide and borosilicate sterile petri dish was stained with alcoholic safranin for 1 minute. Another glass slide was stained with Gram stain. After staining, the borosilicate test tube was rinsed thrice with NS to discharge stain. The stained borosilicate sterile test-tube, both slide and petri dish focussed under the microscope to see the detailed structure. Then biofilm formation in bacteria pathogens were testified and compared. Drug susceptibility was done for the isolates, on Mueller Hinton agar by Kirby-Bauer's disk diffusion method.

## Results

There were thirty-three (33) isolates from parent institute.

There were five (5) isolates from private hospitals.

There were seventeen (17) isolates from government teaching hospitals.

There were thirty-seven (37) isolates from OPD.

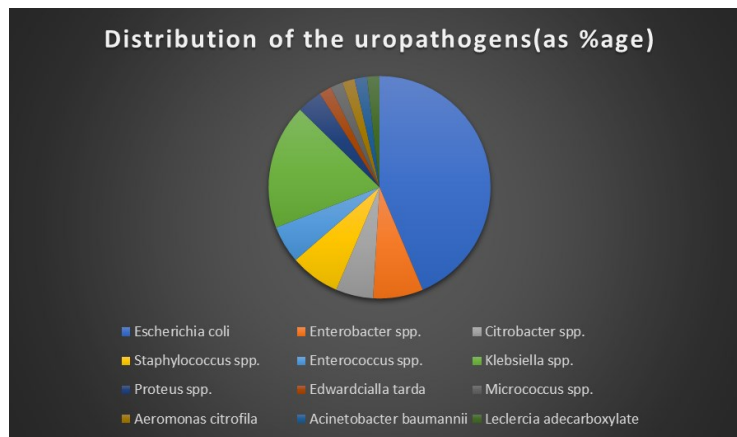
There were thirteen (13) isolates from IPD.

Six (6) isolates were such that their source (OPD/IPD) was unknown.

Total fifty-six (53) samples were collected from different places. Out of all these, common uropathogen was *Escherichia coli*. It was twenty-four (24) in number. *Klebsiella pneumoniae* was the second commonest uropathogen in it. Other uropathogens such as *Enterobacter cloacae*, *Staphylococcus aureus*, *Enterococcus durans*, *Enterococcus faecalis*, *Micrococcus* spp, *Proteus vulgaris*, *Citrobacter freundii*, *Citrobacter koseri*, *Acinetobacter baumannii*, *Aeromonas hydrophila*, *Edwardsiella tarda* and *Leclercia adecarboxylata* were also found in samples. Out of all isolates, there were nineteen (19) isolates were bacteria from different species.

**Table 1:** Commonest uropathogens in descending order.

	Name of Uropathogens	Total number
1.	<i>Escherichia coli</i>	24
2.	<i>Klebsiella pneumoniae</i>	8
3.	<i>Enterobacter cloacae</i>	3
4.	<i>Staphylococcus aureus</i>	3
5.	<i>Klebsiella oxytoca</i>	2
6.	<i>Citrobacter koseri</i>	2
7.	<i>Proteus sp.</i>	2
8.	<i>Proteus vulgaris</i>	1
9.	<i>Staphylococcus sp.</i>	1
10.	<i>Enterococcus durans</i>	1
11.	<i>Enterococcus faecalis</i>	1
12.	<i>Enterococcus spp.</i>	1
13.	<i>Micrococcus spp.</i>	1
14.	<i>Enterobacter aerogenes</i>	1
15.	<i>Citrobacter freundii</i>	1
16.	<i>Edwardsiella tarda</i>	1
17.	<i>Aeromonas hydrophila</i>	1
18.	<i>Acinetobacter baumannii</i>	1
19.	<i>Leclercia adecarboxylata</i>	1



**Fig 1:** Distribution of the uropathogens (as percentage).

**Table 2:** Percentage distribution of uropathogens.

	Distribution of the uropathogens	In percentage (%)
1.	<i>Escherichia coli</i>	42.85
2.	<i>Klebsiella spp.</i>	17.85
3.	<i>Enterobacter spp.</i>	7.14
4.	<i>Candida spp.</i>	7.14
5.	<i>Staphylococcus spp.</i>	7.14
6.	<i>Enterococcus spp.</i>	5.35
7.	<i>Citrobacter spp.</i>	5.35
8.	<i>Proteus spp.</i>	3.57
9.	<i>Edwardciella tarda</i>	1.78
10.	<i>Micrococcus spp.</i>	1.78
11.	<i>Aeromonas hydrophila</i>	1.78
12.	<i>Acinetobacter baumannii</i>	1.78
13.	<i>Leclercia adecarboxylate</i>	1.78

### In Test tube Method

Out of all isolates which were positive for biofilm in TT method, thirteen (13) were from OPD.

Out of all isolates which were positive for biofilm in TT method seven (7) were from IPD.

Out of all isolates which were positive for biofilm in TT method, four (4) were from unknown source.

Out of all isolates, there were fourteen (14) isolates from different species which were biofilm producer by Test tube method.

Out of 53 samples, 25 samples showed biofilm on Test tube method which were seen from naked eye. In these 25 positive isolates, common uropathogen was *Escherichia coli* showed most biofilm positive in test tube like total seven (7) in number. 2<sup>nd</sup> most common uropathogens were *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* showed biofilm positive in test tube. Other uropathogens were such as *Staphylococcus* sp., *Enterococcus faecalis*, *Proteus* sp., *Proteus vulgaris*, *Edwardsiella tarda*, *Acinetobacter baumannii*, *Leclercia adecarboxylata* etc. showed biofilm positive in test tube by test tube method.

### Sensitivity and Specificity

Sensitivity: Sensitivity is the ability to find out the true positive and eliminate the false negative.

Specificity: Specificity is the ability to find out the true negative and eliminate the false positive.

Sensitivity and specificity of the Glass slide method as a compared to Test tube (TT) method: There were fourteen (14) isolates were biofilm producer by TT method.

Sensitivity and specificity of the Glass slide method as a compared to TT method:

Specificity of slide method=  $TN / (TN + FP) \times 100$

$$= 12 / (12 + 14) \times 100$$

$$= 46.153\%$$

$$\approx 46\%$$

Here, TN= True negative

FP= False positive

TN=12

FP=14

Sensitivity of slide method =  $TP / (TP + FN) \times 100$

$$= 12 / (12 + 16) \times 100$$

$$= 42.857\%$$

$$\approx 43\%$$

Here, TP= True positive

FN= False negative

TP=12

FN=16

Sensitivity and specificity of the Borosilicate petri dish method as a compared to Test tube (TT) method: There were fourteen (14) isolates were biofilm producer by TT method.

Sensitivity and specificity of the Borosilicate petri dish method as a compared to TT method:

Specificity of the Borosilicate petri dish method =  $TN / (TN + FP) \times 100$

$$=3 / (3+20) \times 100$$

$$=13.04\%$$

$$\approx 13\%$$

Here, TN= True negative

FP= False positive

TN=3

FP=20

Sensitivity of the Borosilicate petri dish method =  $TP / (TP+FN) \times 100$

$$=18 / (18+3) \times 100$$

$$=85.71\%$$

$$\approx 86\%$$

Here, TP= True positive

FN= False negative

TP=18

FN=3

**Table 3:** Table showing comparison of biofilm testing two methods.

	Name	Specificity	Sensitivity
1.	Test tube method to Glass slide method	46.153%	42.857%
2.	Test tube method to Borosilicate petri dish method	13.04%	85.71%

**Matrix pattern seen in the uropathogens:**

*Glass slide method*

Rhomboidal matrix/crystals: Mostly seen in *Escherichia coli*.

Needle-shaped matrix/crystals: Mostly seen in *Escherichia coli*, and also in *Staphylococcus* spp. and *Klebsiella pneumoniae*.

*Borosilicate petri dish method*

Pentagonal matrix/crystals: Mostly seen in *Escherichia coli*.

Matrix is irregular shape: Mostly seen in *Escherichia coli*.

Needle-shaped matrix/crystals: Mostly seen in *Escherichia coli*, and also *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter cloacae* etc.

Rhomboidal- shaped/crystal: Mostly seen in *Enterobacter aerogenes*.

**Shape and clustering pattern:**

*In glass slide method*

*Escherichia coli*: As bacilli in clusters as well as irregular pattern.

*Klebsiella pneumoniae*: Mostly as GNB in clusters, and two (2) isolates appeared as coccoid form.

*Staphylococcus aureus*: Always as cocci in clusters.

*Candida* spp.: Mostly as yeasts in clusters, intertwined matrix was present.

*In borosilicate petri dish method*

*Escherichia coli*: As bacilli was shown in clusters as well as cocci-bacilli in cluster. Sometimes it also shown adherence bacilli in small cluster. It also shown cocci in chain pattern. Sometimes *E. coli* appeared as coccoid form in petri dish, which further hints at the possibility of slow metabolism in biofilm-associated bacteria.

*Proteus vulgaris*: As coccobacilli pattern was shown in it.

*Klebsiella pneumoniae*: As coccobacilli pattern as well as cocci in cluster was shown in it.

*Klebsiella oxytoca*: Bacilli in cluster was shown in it.

*Citrobacter freundii*: Adherence of bacteria in undefined pattern was shown in it.

*Staphylococcus aureus*: As cocci in irregular cluster pattern was shown in it.

## Discussion

Biofilms are formed by many medically important bacteria, especially the so-called ESKAPE pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterococcus* spp.) Biofilm formation renders uropathogens resistant to many antimicrobials, due to the release of the exopolymeric matrix formed of extracellular DNA, proteins and polysaccharides, which prevents the contact of these antimicrobials with the microbes (5). Also, these biofilms activate efflux pumps, by which the antibiotics are expelled from bacterial cells even if they succeed in reaching the bacterial cells after penetration of matrix. There are many methods for studying bacterial and fungal biofilms in vitro, like Test tube method, Microtitre plate method, Congo Red Agar method, and Confocal laser scanning microscopy. Most of these methods are most costly and very difficult to perform. Hence, cheap and easy methods are needed to study the uropathogens Hence the slide method seems to be appropriate and useful. So, by the above study we were able to detect and identify the pathogenic microbes in urine samples which form biofilm in vitro. So, we observed that the borosilicate Petri dish method is very good and sensitive and the glass slide method is very easy to perform and observe. Different adherence patterns could be observed for the bacteria in forming biofilms. More studies are needed in this important area of public health. Microbiology since this is very important and has implications for therapeutics. In one method we will be able to see the structure of the pathogenic microbes which form biofilms of the feature and characteristic of the microbes.

The Petri dish method is a new and easy method for biofilm observation. We can see structure of biofilm forming microorganisms, matrix shape and pattern. Glass slide method is also very easy and simple method of biofilm study, but may suffer from lack of specificity and too much false positivity. As far as we know, this type of study has not been carried out by any researchers before. However, only adherence pattern may not be indicative of biofilm formation over Petri dish. As far as we know these methods have not been tried earlier for testing biofilms. Specific clustering patterns of bacteria should be more important for terming as biofilm. This is a very interesting and new observation and can show a new path in Medical Microbiology and Biotechnology. More such studies are needed in this interesting area.

## Conclusion

Biofilms cause resistance to many antimicrobial agents. The after-effects of biofilm produced on indwelling medical devices are recurrent, untreatable infections and possible removal of medical device. To overcome chronic and recurrent infections, it is important to detect biofilms of microorganisms, determine antibiofilm activity of agents, and determine antibacterial activity of agents against biofilm-embedded microorganism with the appropriate methods by clinical microbiologist and biofilm researcher microbiologist. Identification of genes involved in biofilm formation and measurement of gene expression as a result of antibiofilm and antibacterial activity of agents can be advantageous in biofilm studies (14). Uropathogenic bacteria and yeasts form biofilm readily and there should be easy and simple methods to study biofilms in vitro. Here, the borosilicate petri dish method was found to be almost as good as the test tube method for biofilm study in vitro and can be explored more. It is simple and easy to do and informative. However, the slide method was not so good and non-specific. More such studies are needed in this aspect.

## Abbreviations

CLED: Cystine Lactose Electrolyte Deficient

EPS: Extracellular Polymeric Substance

IPD: Inpatient Department

NS: Normal/isotonic saline.

OIF: Oil Immersion Field

OPD: Outpatient Department

TT: Test-tube

UTI: Urinary Tract Infection

w/v: Weight/volume.

## Conflict of Interest

The authors declare no conflict of interest.

## References

1. Tan CW, Chlebicki MP. Urinary tract infections in adults. *Singapore Med J*. 2016 Sep;57(9):485-90. doi: 10.11622/smedj.2016153.
2. UTI(<https://my.clevelandclinic.org/health/diseases/9135-urinary-tract-infections>); Last accessed 19.5.23
3. Linlong Lila Yangyang, Jiali Yang, Xiang Xie, Huan Chen. The immune responses to different Uropathogens call individual interventions for bladder infection, Volume 13-2022.
4. Ahmed S S, Shariq A, Alsalloom A A, Babikir I H, Alhomoud B N. Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *Int J Health Sci (Qassim)*. 2019 Mar-Apr;13(2):48-55. PMID: 30983946; PMCID: PMC6436442
5. UTI(<https://my.clevelandclinic.org/health/diseases/9135-urinary-tract-infections>); Last accessed 19.5.23
6. Muhammad M H, Idris A L, Fan X, Guo Y, Yu Y, Jin X, Qiu J, Guan X and Huang T; Beyond Risk: Bacterial Biofilms and Their Regulating Approaches; Volume 11 - 2020 | <https://doi.org/10.3389/fmich.2020.00928>.
7. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis*. 2002 Sep;8(9):881-90. doi: 10.3201/eid0809.020063. PMID: 12194761; PMCID: PMC2732559.
8. Carrascosa C, Raheem D, Ramos F, Saraiva A, Raposo A. Microbial Biofilms in the Food Industry-A Comprehensive Review. *Int J Environ Res Public Health*. 2021 Feb 19;18(4):2014. doi: 10.3390/ijerph18042014. PMID: 33669645; PMCID: PMC7922197.
9. Vazquez J A, Manavathu E K; Medical Importance of Microbial Biofilms in the Management of Infectious Diseases; Feb26,2020.
10. Hassan A, Kaleem F, Omair M, Iqbal M, Usman J, Khalid A; "Evaluation of different detection methods of biofilm formation in the clinical isolates" doi: 05.03.2011

**Citation:** Biswas M, Bhattacharyya S, Banik, Jilani G Md. Evaluation of New Glass Slide and Petri Dish Methods of Biofilm Testing in Uropathogenic Bacteria and Their Comparison with Test-Tube Method. *SVOA Microbiology* 2023, 4:2, 35-41.

**Copyright:** © 2023 All rights reserved by Bhattacharyya S, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.