ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 16, Issue, 12, pp.30887-30891, December, 2024
DOI: https://doi.org/10.24941/ijcr.48157.12.2024

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STUDY OF BIOFILM FORMATION IN INDWELLING CATHETERS FROM PATIENTS WITH CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

[1*]Selvi, I., [2]Thirumalaikolundu Subramanian, P. and Uma. A.[3]

[1*]Assistant Professor, Department of Microbiology, SUT Academy of Medical Sciences, Vattappara, Thiruvananthapuram, Kerala; [2]Professor, Department of Medicine, Trichy SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India; [3]Professor & HOD, Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, Trichy, Tamil Nadu, India

ARTICLE INFO

Article History:
Received 14th September, 2024
Received in revised form
27th October, 2024
Accepted 20th November, 2024
Published online 26th December, 2024

Key Words:
Biofilm, Catheter Associated UTI, Indwelling Urinary Catheter, Bacteria.

**Corresponding author:*
Selvi

ABSTRACT

Background: Biofilms are communities of microorganisms that are enclosed in a matrix of exopolymers, contributing to chronic and recurring infections, particularly in indwelling medical devices such as urinary catheters, and have developed resistance to numerous antimicrobial drugs. Biofilms plays a crucial role in the pathogenesis of Catheter associated urinary tract infection (CAUTI), which in turn responsible for 40% of health care associated infections. ***Objective:*** The objective was to identify biofilm using phenotypic methods in urinary catheter isolates and compare these methods to choose a sensitive method with good reproducibility. ***Method:*** A total of 55 culture positive urinary isolates were identified by standard microbiological methods from 126 hospitalized patients catheterized for more than 48 hours and subjected to biofilm detection by phenotypic methods such as Tissue Culture Plate (TCP), Congo Red Agar (CR) and Tube Adherence (TA) method. ***Results:*** Out of 55, 15 (27.2%) showed biofilm formation by TCP method. Maximum biofilm production was seen in *Escherichia coli* (49%) followed by *Klebsiella pneumoniae* (27.2%). CR detected biofilm production in 45.4%, TA in 34.5% and TCP in 27.2% isolates. CR and TA methods were compared to TCP, as it is considered gold standard method and they both demonstrated a sensitivity of 86.6% and specificity of 65% and 72.5% respectively. ***Conclusion:*** TCP is a quantitative and reliable method for detection of biofilm with good reproducibility.Detection of biofilms can be recommended for chronic and recurrent infections before institution of empirical antibiotics especially in resource limited settings to effectively prevent health care associated infections.

Copyright©2024, Selvi 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.*

Citation: Selvi, I., Thirumalaikolundu Subramanian, P. and Uma. A. 2024. "Study of biofilm formation in indwelling catheters from patients with catheter-associated urinary tract infections*". International Journal of Current Research*, 16, (12), 30887-30891.

INTRODUCTION

Biofilms are communities of microorganisms encased in an extracellular polysaccharide matrix that promotes adhesion to surfaces. They are ubiquitous in nature can be seen in medical settings such as indwelling medical devices, dental plaque, upper respiratory tract infectionsand urogenital infectionsas well as in non-medical contexts such as industrial or potable water system piping and also frequently encountered in natural aquatic systems (Donlan, 2002). Biofilms serve as a significant virulence factor, offering a protected environment for organisms to endure and significantly alter protein metabolism and gene expression, thus leading to the development of resistance to antimicrobial therapy (Hassan *et al*, 2011 and Charankaur *et al*., 2013). Biofilm formation along catheter surface is the most important cause of bacteriuria (Nicolle, 2014).

Furthermore, prolonged catheterisation itself is a risk factor for biofilm formation in indwelling catheters, potentially resulting in complications such as pyelonephritis, renal failure, and bacteremia (Donlan 2001, and Warren 2001) and also studies says that approximately 26% of hospitalised patients with indwelling urinary catheters for 2 to 10 days develop bacteriuria (Lee *et al*, 2013 and Chenoweth *et al*, 2013). Approximately 70–80% of catheter-associated urinary tract infections (CAUTIs) are attributable to the use of an indwelling urinary catheter, rendering CAUTIs among the most common healthcare-associated infections (HAIs) (Nicolle, 2014; Jacobsen *et al*., 2008). Recent prevalence surveys reveal that 27% of patients with indwelling devices in an 1800-bed hospital in a state in India (Kumar *et al*, 2014) and 23.6% in 183 US hospitals (Magill *et al*, 2014) are the primary contributors to healthcare-associated infections (HAIs). The Centers for Disease Control and Prevention (CDC, 2013) defines a catheter-associated urinary tract infection (CAUTI) as the presence of an indwelling urinary

catheter for more than two calendar days, accompanied by significant bacteriuria of more than 10^3CFU/ml (with no more than two microorganisms), and at least one clinical sign or symptom such as fever greater than 38°C, suprapubic tenderness, or costovertebral angle tenderness. CAUTI is significantly associated with increased morbidity, mortality and health care costs (Trautner BW 2004). A diverse array of uropathogens are responsible for colonisation of catheter such as *Escherichia coli, Klebsiella spp., Proteus, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Stickler DJ 1996 and Subramanian P *et al* 2012). Early detection and management of biofilm-forming microbes can be one of the essential steps towards prevention and management of health care associated infections. Hence, it is necessary to develop an effective strategy for the care of devices and improvement of patient care. The current study aims to detect the biofilm formation in indwelling catheter among patients with catheter-associated urinary tract infections using simple and reliable phenotypic methods, such as, Tissue Culture Plate (TCP), Congo Red Agar (CR), and Tube Adherence (TA) methods.

MATERIALS AND METHODS

Place and duration of the study: The study was conducted in Department of Microbiology, SRM Medical college and Hospital, Trichy from May 2016 to Oct 2017.

Selection of isolates: In this prospective study, 126 hospitalized patients over the age of 18 years, catheterized for more than 48 hours are included. After disinfecting the port of foley's catheter with alcohol swab, 3ml of urine sample was aspirated and transported to laboratory without delay. The urine sample after noting the macroscopic appearance, subjected to wet mount and inoculated on CLED media by standard loop and incubated at 37°C for 18 hours aerobically. Urine culture was done semi-quantitively and a count of ≥ 10^3 CFU/ml was considered as significant. These isolates were identified using routine biochemical tests followed by antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per CLSI guidelines (CLSI, 2015). All culture positive urinary isolates were tested for biofilm formation by three phenotypic methods and also evaluated the reliability of these methods in order to determine most suitable screening method.

Ethical committee approval: Institutional ethical committee approval obtained. Patient's Informed consent was taken prior to collection of the data and clinical isolates. Biofilm detection was done by following methods:

Tissue culture plate (TCP) method (Nabajit Deka, 2014): Overnight culture of the isolate from nutrient agar plate is inoculated into Trypticase soy broth (TSB). The primary inoculums are then inoculated in TSB with 1% glucose prepared in 1:100 dilutions and loaded into 96 wells flat bottom microtitre plate. Plates are covered and incubated at 37ºC for 24 hours in aerobic condition, the well are then decanted and washed three times with Phosphate buffer saline (PBS). After washing, fixed with methanol for 15 minutes. Then the wells are decanted and stained with crystal violet followed by distilled water. Optical density of each well is measured at 490 nm using an automated ELISA plate.

Calculation for optical density for detection of biofilm
Cut off OD=Negative control (3 standard deviation +mean)
Positive control= Average
Strong biofilm= more than 4 cut off OD value
Moderate =2 cut off OD up to 4 cut off OD value
Weak biofilm= 0 cut off OD up to 2cut off OD value

Congo Red Agar (CR) Method (Mathur T, 2006): The medium composed of Brain heart infusion broth (37 gm/l), sucrose (5gm/l), agar number 1 (10 gm/l) and Congo red dye (0.8 gm/l). Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121°C for 15 minutes. Then it was added to autoclaved Brain heart infusion agar with sucrose at 55°C. Plates were inoculated with test organism and incubated at 37°C for 24 to 48 hours aerobically. Positive result was indicated by black colonies with a dry crystalline consistency. Weak slime producers usually remained pink while dark colonies in absence of a dry crystalline colonial morphology indicated as indeterminate result.

Tube adherence (TA) method (Mathur T, 2006): A loopful of test organism from overnight culture was inoculated into10 ml of Trypticase soy broth with 1% glucose. Broths were incubated at 37°C for 24 hours. The cultures were decanted and tubes were washed with phosphate buffer saline pH7.3.The tubes were dried and stained with 0.1% crystal violet. Excess stain was washed with deionized water. Tubes were dried in inverted position and observed for biofilm formation. Biofilm Production was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined and amount of biofilm formation was scored as 0-absent/weak, 1-moderate, 2-strong.

RESULTS

A total of 126 catheterised patients participated in the present study; 47 (37.3%) were female and 79 (62.6%) were male. The mean age for males and females was 68 years and 66 years, respectively. The predominant age group affected was 61-70 years (34.9%), followed by 71-80 years (21.4%) and 51-60 years (19%). Among 126 CAUTI isolates, 55 (43.6%) had significant bacteriuria, while growth was not observed in 71 (56.3%) isolates. Among 55 culture-positive urine isolates, the predominant organisms were Gram-negative (98.1%). The most frequently isolated organism was *Escherichia coli* (49%), followed by *Klebsiella pneumoniae* (27.2%). *Citrobacter spp.* and *Pseudomonas aeruginosa* each accounted for 7.2%, *Proteus mirabilis* for 5.4%, and *Enterobacter spp*. for 1.8%. Among Gram positive organism only one *Staphylococcus aureus* (1.8%) was isolated (Table1). In the TCP method, among the 55 isolates tested for biofilm formation, 10 (18.1%) were identified as strong biofilm producers, 5 (9.0 %) as moderate producers, and 40 (72.7%) as weak or non-biofilm producers (Figure 1(a)). The predominant species involved in biofilm production were *Escherichia coli* (29.6%), followed by *Klebsiella pneumoniae* (26.6%) and *Citrobacter spp.* (25%) (Table 2). In the Congo Red agar (CR) method, 25 (45.4%) isolates were identified as biofilm producers, while 30 (54.5%) were recognised as non-biofilm producers (Figure 2(b)). In contrast, the Tube Adherence (TA) methoddisplayed 19 (34.5%) as biofilm producers and 36 (65.4%) as non-biofilm producers (Figure 3(c)) and (Table 3 and 4). Considering TCP as gold standard, data from CR and TA methods are compared (Table5).Parameters like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)and

accuracy were calculated. True positives were biofilm producers by TCP, CR and TA method. False positive were biofilm producers by CR and TA method and not by TCP method. False negative were the isolates which were non-biofilm producers by CR and TA but were producing biofilm by TCP method. True negatives are those which were non biofilm producers by all the three methods. Comparative analytical study of CR and TA methods, with respect to TCP method was done. Both CR and TA demonstrated similar sensitivity of 86.6%. TA showed higher specificity of 72.5% as compared to 65% shown by CR. Accuracy of both CR and TA methods are 70.9% and 76.3% respectively (Table6).

Table.1 Spectrum of organisms isolated

Organism	Isolates	Percent
Escherichia coli	27	49%
Klebsiella pneumoniae	15	27.2%
Citrobacter spp.	4	7.2%
Pseudomonas aeruginosa	4	7.2%
Proteus mirabilis	3	5.4%
Enterobacter spp.	1	1.8%
Staphylococcus aureus	1	1.8%
Total	55	100%

Table 2. Organism wise distribution of biofilm production

Organism	Total isolates	Biofilm producers	Percent
Escherichia coli	27	8	29.6%
Klebsiella pneumoniae	15	4	26.6%
Citrobacter spp.	4	1	25%
Pseudomonas aeruginosa	4	0	---
Proteus mirabilis	3	0	---
Enterobacter spp.	1	1	100%
Staphylococcus aureus	1	1	100%
Total	55	15	

Table.3 Detection of biofilm production by different phenotypic methods

Method	Biofilm producers	Percent
Congo red agar	25	45.4%
Tube method	19	34.5%
Tissue culture plate method	15	27.2%

Table 4. Screening of the isolates for biofilm formation by Tissue Culture Plate, Congo Red Agar and Tube Adherence methods

No. of Isolates	Biofilm formation	TCP (n)	CR (n)	TA (n)
55	Strong	10	19	7
	Moderate	5	6	12
	Weak/none	40	30	36

Table 5. Comparison of Congo Red agar method (CR) & Tube Adherence method (TA) with Tissue Culture Plate method (TCP)

CR method	TCP method		Total
	Positive	Negative	
Positive	13	14	27
Negative	2	26	28
Total	15	40	55

TA method	TCP method		Total
	Positive	Negative	
Positive	13	11	24
Negative	2	29	31
Total	15	40	55

Table 6. Diagnostic parameters of Tube method and Congo Red Agar method for biofilm detection

Screening method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
CR	86.6%	65%	48.1%	92.8%	70.9%
TA	86.6%	72.5%	54.1%	93.5%	76.3%

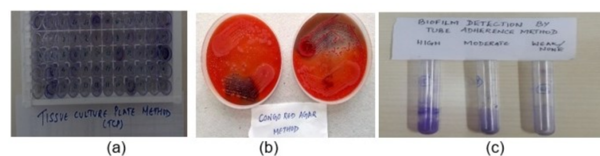

Figure 1. Screening of biofilm by TCP, CR and TA method

DISCUSSION

Catheter-associated urinary tract infection (CAUTI) is among the most prevalent healthcare-associated infections (HAIs), constituting up to 40% of all HAIs (Nicolle, 2014 and Chenoweth 2014). Organisms that produce biofilms are accountable for numerous persistent infections, particularly in patients with indwelling medical devices, and these organisms exhibit considerable resistance to various antimicrobial agents (Mulla *et al*, 2011). Furthermore, several studies (Tenke P (2004); Donalan (2002); Hassan (2011)) have demonstrated that bacteria within a biofilm can withstand antibiotic concentrations 1000 times greater than their planktonic counterparts. Biofilms may be composed of a single or multiple species, depending on the device and its duration of use in the patient. Urinary catheter biofilms may initially be composed of single species buton prolonged catheterisation can have multiple species (Stickler, 1996 and Majumder MI *et al*, 2014). In this study, there were only few samples yielding more than one type of bacteria in urine culture, since it was insignificant in number, those isolates were excluded from the study. The study demonstrated that males were primarily impacted, which is in accordance with the results of previous research (Kazi *et al*, 2015 and Kulkarni *et al*, 2014). Although females are at a greater risk for having urinary tract infections than males, additional risk factors such as advanced age, diabetes mellitus, and chronic kidney disease, which are more prevalent in males, may contribute to the increased incidence.

Escherichia coli was the most commonly isolated pathogen in both urine isolates and biofilm (49% vs 29.6%), followed by *Klebsiella pneumoniae* (27.2% vs 26.6%). In a similar study by (Majumder MI 2014), the predominant isolate and biofilm producers were *Escherichia coli* (60% vs 72%), followed by *Klebsiella spp.* (13% vs 18%). This study identified one isolate each of *Citrobacter, Enterobacter,* and *Methicillin-resistant Staphylococcus aureus* as biofilm producers, while isolates of *Proteus mirabilis* and *Pseudomonas aeruginosa* were non-biofilm producers. Diverse methodologies are available for evaluating biofilm formation. In the present study, a total of 55 clinical isolates from catheterised urine samples of patients diagnosed with CAUTI were assessed for their biofilm formation abilities using in vitro phenotypic methods: Tissue Culture Plate (TCP), Congo Red Agar (CR), and Tube Adherence (TA) methods. These methods have been chosen for their simplicity in conventional laboratory settings. The present investigation identified biofilm development in 25 (45.4%) isolates using the Congo Red agar method, in 19 (34.5%) isolates via the Tube method, and in 15 (27.2%)

isolates through the Tissue Culture Plate method among the urine isolates. This biofilm detection pattern resembles that of (Tayal *et al* (2015)), who identified biofilm formation in 40.8% of samples using the Congo Red agar method, 37.96% using the Tube method, and 27% with the Tissue Culture Plate method in uropathogens from mid-stream urine samples. The CR method indicated that 45.4% of the subjects were biofilm producers; however, various studies reported differing results, such as Nabajit Deka (2014) at 20%, while Sayal *et al.* (2014) and Mathur *et al* (2006) observed significantly lower rates of 9.17% and 6.8%, respectively. Nonetheless, our findings closely align with those of Ruzicka *et al.* (2004), which reported 43.5%. The tube adherence approach identified 34.5% of isolates as biofilm producers and demonstrated a little greater accuracy of 76.3% compared to the CR method, which had an accuracy of 70.9%. However, prior studies (Mathur T (2006), Shaymaa HM (2008), and Hassan A (2011)) indicate that TA is not a reliable general screening test for identifying biofilm-producing isolates, as distinguishing between moderate, weak, and non-biofilm producers proved challenging due to discrepancies among various observers. This study found that CR exhibited a sensitivity of 86.6% and a specificity of 65%, aligning with the findings of Fatima K *et al* (2011) (67.65% vs 89.13%) and Deotale *et al.* (2015) (80% vs 71.4%) whereas TA demonstrated a sensitivity of 86.6% and a specificity of 72.5%, similar to the findings of Mishra *et al.* (2015) (80.7% vs 66.7%) and Deotale *et al.* (2015) (70% vs 85.7%) in comparable with method. Based on the results, the TCP method is a precise and reproducible screening technique. It can also be used as a trustworthy quantitative tool to determine the formation of biofilms and has the benefit of having less subjective error because it uses an ELISA reader to read biofilm formation.

CONCLUSION

Bacteria prefer to colonise and proliferate as biofilms on surfaces, particularly on indwelling medical devices like urinary catheters, which are the most prevalent equipment utilised in hospitals. They are accountable for numerous persistent and resistant infections that might result in heightened morbidity, extended hospital stays, and higher economic burden. The Tissue Culture Plate (TCP) method is an accurate and quantitative technique for assessing biofilm formation, suitable for regular usage in microbiology laboratories, especially in chronic infections, to significantly mitigate healthcare-associated infections.

REFERENCES

Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002 Sep;8(9):881-90.

Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis. 2011 Jul-Aug;15(4):305-11.

CharanKaur D, Khare A S. Biofilm formation and antibiotic susceptibility pattern in MRSA strains in a tertiary care hospital. Indian J Basic Appl Med Res 2013;1:37-4.

Nicolle LE. Catheter associated urinary tract infections. Antimicrob Resist Infect Control. 2014 Jul 25;3:23.

Rodney M. Donlan, Biofilm Formation: A Clinically Relevant Microbiological Process, *Clinical Infectious Diseases*, Volume 33, Issue 8, 15 October 2001, Pages 1387–1392.

Warren JW. Catheter-associated urinary tract infections. Int J Antimicrob Agents. 2001 Apr;17(4):299-303.

Lee JH, Kim SW, Yoon BI, Ha US, Sohn DW, Cho YH. Factors that affect nosocomial catheter-associated urinary tract infection in intensive care units: 2-year experience at a single center. Korean J Urol. 2013 Jan;54(1):59-65.

Chenoweth C, Saint S. Preventing catheter-associated urinary tract infections in the intensive care unit. Crit Care Clin. 2013 Jan;29(1):19-32.

Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008 Jan;21(1):26-59.

Kumar A, Biswal M, Dhaliwal N, Mahesh R, Appannanavar SB, Gautam V, Ray P, Gupta AK, Taneja N. Point prevalence surveys of healthcare-associated infections and use of indwelling devices and antimicrobials over three years in a tertiary care hospital in India. J Hosp Infect. 2014 Apr;86(4):272-4.

Magill SS, Edwards JR, Bamberg W, Beldaus ZG, Dumyati G, Kainer MA, Lynfield R, Maloney M, McAllister-Hollod L, Nadle J, Ray SM, Thompson D, Wilson LE, Fridkin SK: Multistate point-prevalence survey of health care-associated infections. N Engl J Med 2014, 370:1198–1208.

Centers for Disease Control and Prevention (CDC). National Healthcare Safety Network (NHSN) Report, Data Summary for 2011, Device-Associated Module, Atlanta: CDC. Retrived from http://www. cdc. gov/nhsn/PDFs/dataStat/NHSN-Report-2011-Data-Summary. pdf website. 2013.

Trautner BW, Darouiche RO. Catheter-associated infections: pathogenesis affects prevention. Arch Intern Med. 2004 Apr 26;164(8):842-50.

Stickler, D. J. (1996). Bacterial biofilms and the encrustation of urethral catheters. *Biofouling*, *9*(4), 293–305.

Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J. 2012;5(7):344-8.

Clinical and Laboratory Standards Institute (2015) Performance Standards for Antimicrobial Susceptibility Testing; 25th Informational Supplement. CLSI Document M100-S25, Clinical and Laboratory Standards Institute, Wayne, PA.

Nabajit Deka Comparison of Tissue Culture plate method, Tube Method and Congo Red Agar Method for the detection of biofilm formation by Coagulase Negative *Staphylococcus* isolated from Non-clinical Isolates. *Int.J.Curr.Microbiol.App.Sci*(2014) 3(10) 810-815.

Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of Staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol. 2006 Jan;24(1):25-9.

Chenoweth CE, Gould CV, Saint S. Diagnosis, management, and prevention of catheter-associated urinary tract infections. Infect Dis Clin North Am. 2014 Mar;28(1):105-19.

Mulla SA and Revdiwala S 2011. Assessment of biofilm formation in device associated clinical bacterial isolates in a tertiary care hospital. Ind J Patho Microbiol., 5(3): 561-564.

Tenke P, Riedl CR, Jones GL, Williams GJ, Stickler D and Nagy E 2004. Bacterial biofilm formation on urologic

devices and heparin coating as preventive strategy. Int J Antimicrob Agents., 23(1):67-74.

Majumder MI, Ahmed T, Hossain D, Ali M, Islam B, *et al*. (2014) Bacteriology and Antibiotic Sensitivity Patterns of Urine and Biofilm in Patients with Indwelling Urinary Catheter in a Tertiary Hospital in Bangladesh. J BacteriolParasitol 5: 191.

Kazi MM, Harshe A, Sale H, Mane D, Yande M, *et al*. (2015) Catheter Associated Urinary Tract Infections (CAUTI) and Antibiotic Sensitivity Pattern from Confirmed Cases of CAUTI in a Tertiary Care Hospital: A Prospective Study. Clin Microbiol 4: 193.

Kulkarni, Dr & Talib, Shaikha & Naik, Dr & Kale, Dr. (2014). Profile of Urinary Tract Infection in Indwelling Catheterized Patients. IOSR Journal of Dental and Medical Sciences. 13. 132-138.

Tayal Ruchi, Baveja Sujata and De Anuradha. Comparison of Phenotypic Methods for the Detection of Biofilm Production in Uro-Pathogens in a Tertiary Care Hospital in India. Volume 4 Number 9 (2015) pp. 840-849.

Sayal, Pallavi & Singh, Kanwardeep& Devi, Pushpa. (2014). Detection of bacterial biofilm in patients with indwelling urinary catheters. *CIB Tech J Microbiol*.. 3. 2319-38679.

Ruzicka F *et al*. 2004. Biofilm detection and clinical significance of Staphylococcus epidermidis isolates. Folia Microbiol ; 49(5):596-600.

Shaymaa, H., Moteeb. Quantitative and qualitative assays of bacterial biofilm produced BY Pseudomonas aeruginosa AND Klebsiella spp.. Journal of university of Anbar for Pure science, (2008).;2(3).

Fatima Khan, Indu Shukla, Meher Rizvi, Tariq Mansoor and S.C. Sharma, 2011. Detection of Biofilm Formation in *Staphylococcus aureus*. Does it have a role in Treatment of MRSA Infections? *Trends in Medical Research, 6: 116-123.*

Deotale VS, Attal R, Joshi S, Bankar N. Correlation between biofilm formation and highly drug resistant uropathogens (HDRU). International Journal of Current Research and Review. 2015 Jan 15;7(2):61.

Mishra, S.K., Basukala, P., Basukala, O. *et al.* Detection of Biofilm Production and Antibiotic Resistance Pattern in Clinical Isolates from Indwelling Medical Devices. *Curr Microbiol* 70, 128–134 (2015).

****