Original Article

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Microbiology Section

Evaluation of Phenotypic Methods for Detection of Biofilm Formation in Uropathogens

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ABSTRACT

Introduction: Urinary tract infection (UTI) is one of the leading causes of morbidity encountered in clinical practice. Biofilm produced by the urinary pathogens leads to recurrent and recalcitrant UTI there by contributing to longer stay in hospital and increased cost of treatment.

Aim: The present study was conducted to evaluate Congo Red Agar method (CRA) and Tube Method (TM) in detection of biofilm formation in uropathogens with respect to Tissue Culture Plate method (TCP).

Materials and Methods: Study was conducted in the Department of Microbiology from May 2016 to May 2017. Midstream clean catch urine collected from patients with symptoms of UTI was processed by standard methods. A total of 264 randomly selected urinary isolates were subjected to biofilm detection by CRA method, tube method and TCP method. TCP was considered as gold standard. Results were expressed in terms of percentages, sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV).

Results: Biofilm was detected in 105 (39.77%) isolates by TCP method, in 124(46.97%) by CRA method and 101(38.26%) by tube method. CRA method and TM method showed a sensitivity of 80% & 63.81%, specificity of 75.47% & 78.62%, PPV of 68.29% & 66.34%, and NPV of 85.11% and 76.69% respectively.

Conclusion: Congo red agar and tube methods can be considered for detection of biofilms in resource constraint conditions.

Keywords: Congo red agar method, Tissue culture plate method, Tube method, Urinary Tract Infection

INTRODUCTION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population [1]. Biofilm producing bacteria cause recurrent and chronic UTI there by contributing to longer stay in hospital and increased cost of treatment [2, 3]. Biofilm formation is the major virulence determinant of uropathogens. Biofilms promote development of antimicrobial resistance by retarding diffusion of antimicrobials and facilitating plasmid exchange thus enabling dissemination of antimicrobial resistance [4, 5]. Detection of biofilm producer strains will guide the clinician in modifying antibiotic therapy for better clinical management [6] and also help in designing adequate control measures as the isolates are also resistant to variety of disinfectants [3]. This emphasizes the need to screen all clinical isolates for biofilm production.

Though there are many methods for detection of biofilm, there are indecisive results observed in various studies [7]. There is no standard protocol for the detection of biofilm production [8]. With this background, the present study was undertaken to evaluate the Congo Red Agar method (CRA) and Tube Method (TM) in detection of biofilm formation by uropathogens considering Tissue Culture method (TCP) as gold standard.

MATERIALS AND METHODS

The present Cross Sectional study was conducted in the Department of Microbiology, Adichunchanagiri Institute of Medical Sciences and Adichunchanagiri Hospital and Research Centre, from May 2016 to May 2017. Institutional ethical committee clearance and informed consent was obtained for the study.

Sample size calculation and sampling: Sample size was calculated using CDC-Epi Info software, version 7.2 [Population size 20000, Expected frequency 24% ,acceptable margin of error 5% and confidence level 95%] [9,10,11]. A total of 264 urinary isolates were selected for the study by random sampling.

Inclusion criteria: Male and female patients of all age groups with symptoms of UTI attending various outpatient departments and admitted in wards of hospital were included in the study.

Exclusion criteria: Repeated isolates from the same patient and patients who were on antibiotic therapy or had history of antibiotic intake within one week prior to sample collection were excluded from the study.

Midstream clean catch urine was collected and processed as per standard protocol without delay in the laboratory. Specimens were inoculated on to MacConkey agar plate and 5% sheep blood agar plate using standard loop technique. The inoculated plates were incubated aerobically at 37ºC for 24 -48 hours. Isolates were identified by standard microbiological methods [12]. Isolates with significant bacteriuria were subjected to biofilm detection by CRA method, TM and TCP method.

Tube method: A loopful of the isolate from the agar plate was inoculated into a glass tube containing 5 mLof trypticase soya broth with 1% glucose and was incubated at 37ºC for 48 hours. After decanting, each tube was stained with 0.25% Safranin. Tubes were rotated to ensure uniform distribution of stain and then each tube was decanted and drained by placing upside down. Visible film lining the inner wall and the bottom of the tube was considered as positive [13]. The absence of a film or the mere presence of a ring at the liquid air interface was interpreted as a negative result [14]. The amount of biofilm formed was scored as weak, moderate and strong [7]. Each test was interpreted by two different observers.

Congo red agar method: The urinary isolates were streaked on to the Congo red agar plate (Hi Media, Mumbai, India) and were incubated aerobically at 37ºC for 24-48 hours. The appearance of black colonies with dry crystalline consistency was taken as positive for slime production. Isolates producing very dark coloured colonies were interpreted as strong biofilm producers. Those bacteria forming black colonies were considered as moderate biofilm producers and those producing almost black colonies were noted as weak biofilm producers [15]. Isolates forming red colonies were considered as non-biofilm producers [14]. Each test was interpreted by two different observers.

Tissue culture plate assay: A suspension of the isolate equivalent to the McFarland 0.5 turbidity standard was prepared in Muller-Hinton broth (Hi Media Mumbai, India) for each strain. 100 µL from each bacterial suspension was inoculated on to 96 well tissue culture microtitre plates and was incubated at 37ºC for 24 hours. The medium was then removed and the wells were washed three times with sterile distilled water.150 µL of crystal violet was added to each well and was left for 45 minutes at room temperature. The dye was then removed and was followed by five washings with sterile distilled water. The contents were removed and the wells were washed three times with sterile distilled water. To each well, 150 µL of crystal violet was added and were kept at room temperature for 45 minutes. The dye was decanted and the wells were washed five times with sterile distilled water. Then the wells were stained with 200 µL of 95% ethanol for three minutes. From each well, 100 µL of ethanol was transferred to another microtitre plate. Shimadzu absorption Spectrophotometer was used to find out the optical density of ethanol dye suspension. Optical density was measured at 540 nm [14,16]. Optical density cut off value (ODc) was calculated by taking

Optical density cut-off value (ODc)=Mean optical density (OD) of negative control+3X standard deviation (SD) of negative control [13]. Isolates with optical density ≤ODc were considered as non-biofilm producers. Isolates with optical density ≤2 X ODc were interpreted as weak biofilm producers, isolates with optical density between 2 to 4 X ODc were taken as moderate producers of biofilm and those isolates with optical density >4 X ODc were considered as strong biofilm producers [8].

ODs of negative controls-0.014, 0.016, 0.045, 0.018, 0.009 and 0.020

Mean ODs of negative controls =0.021

1SD=0.012, 3SD=0.037

ODc = Mean + 3SD

= 0.021 + 0.037

= 0.058

ODc = 0.06

Non biofilm producers : ODs ≤ ODc --- (≤0.06)

Weak biofilm producers : ODs <2 X ODc ---- (< 0.12)

Moderate biofilm producers : ODs 2X ODc ≤4 X ODc --- (0.12 - 0.24)

Strong biofilm producers: ODs >4 X ODc ---- (>0.24)Tests were performed in triplicates. *S.epidermidis* ATCC 35984 and *S.epidermidis* ATCC12228 were used as positive and negative controls respectively [5].

STATISTICAL ANALYSIS

The data were expressed in terms of percentages and proportions. The Chi-Square test [Table/Fig-1a and 1b] was applied to compare variables. The value of P <0.05 was considered as statistically significant. The data obtained in CRA method and Tube method was compared with that of TCP method. Sensitivity, Specificity, PPV and NPV were calculated considering TCP as gold standard method [17,18].

	Outcome1 (Negative)	Outcome 2 (Positive)	Total
Group 1(TCP)	159	105	264
Group 2(TM)	163	101	264
Total	322	206	528

[Table/Fig-1a]: Chi-Square test. (p= 0.72)

	Outcome 1 (Negative)	Outcome 2 (Positive)	Total
Group 1 (TCP)	159	105	264
Group 2 (CRA)	140	124	264
Total	299	229	528

[Table/Fig-1b]: Chi-Square test. (p=0.095)

RESULTS

Out of total 264 isolates studied, *E. coli* (43.2%)was the major isolate followed by Klebsiella species (16.3%), Enterococci species(14%), methicillin resistant Coagulase negative Staphylococci(7.2%), Gram negative non-fermenting bacilli (other than *Pseudomonas aeruginosa* and Acinetobacter species)

Methods	Biofilm producers No (%)				Non biofilm producers No (%)	Total No (%)
	Strong	Moderate	Weak	Total		
Congo red agar method	40 (3.26) n=124	47 (37.90) n=124	37 (29.84) n=124	124 (46.97) n=264	140 (53.03) n=264	264 (100)
Tube method	15 (14.85) n=101	22 (21.78) n=101	64 (63.37) n=101	101(38.26) n=264	163 (61.74) n=264	264 (100)
Tissue culture plate method	58 (21.97) n=105	47 (17.80) n=105	159 (60.23) n=105	105 (39.77) n=264	159(60.23) n=264	264(100)

[Table/Fig-2]: Biofilm detection by different phenotypic methods.

(6.1%), Enterobacter species (4.2%), Citrobacter species(2.7%), *Ps.aeruginosa*(1.5%). Acinetobacter species, Coagulase negative Staphylococci and Proteus species constituted 1.1% of isolates each. Providencia species (0.8%), *S.aureus*(0.4%) and Morganella species (0.4%) were the other isolates.

Biofilm was detected in 39.77% of the isolates by TCP method, in 46.97% of isolates by CRA method and in 38.26% of isolates by Tube method. [Table/Fig-2] shows the biofilm detection by different phenotypic methods and [Table/Fig-3] shows the sensitivity, specificity, PPV and NPV of congo red agar method and tube method as compared to tissue culture plate method. The difference between the biofilm detection rates by CRA method and Tube method were statistically not significant. [Table/Fig-4] shows the bacteriological profile of isolates and biofilm formation by the isolates.

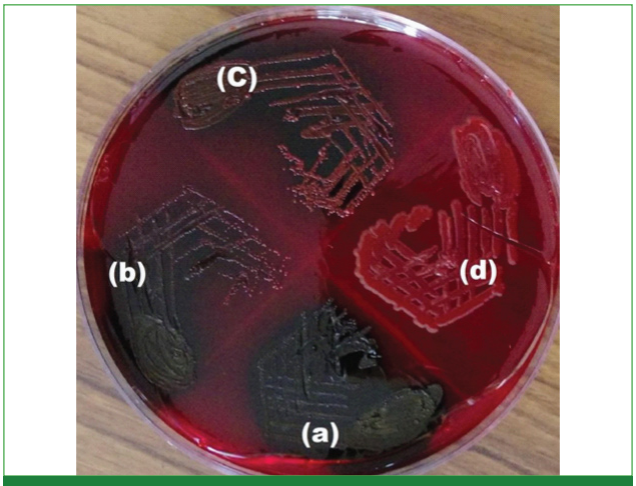
[Table/Fig- 5, 6 and 7] shows biofilm detection by Congo red agar method, Tube method and Tissue culture plate method respectively.

Biofilm detection methods	Sensitivity (%)	Specificity (%)	*PPV (%)	**NPV (%)
Congo red method	80	75.47	68.29	85.11
Tube method	63.81	78.62	66.34	76.69

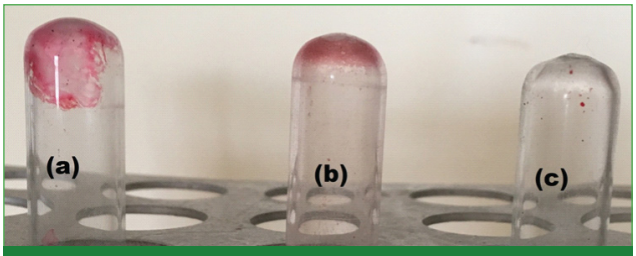
[Table/Fig-3]: Diagnostic efficiency of Congo red agar method and tube method.
Tissue culture plate method was considered as gold standard; *PPV-Positive predictive value; **NPV-Negative predictive value

Urinary isolates	Biofilm producers Number (Percentage)
E. coli	44 (42)
Klebsiella species	21 (20)
Enterococcus species	12 (11)
Gram negative nonfermenting bacilli other than Ps.aeruginosa and Acinetobacter species	10 (10)
Methicillin resistant Coagulase negative Staphylococci	6 (6)
Citrobacter species	4 (4)
Enterobacter species	3 (3)
Ps.aeruginosa	1 (1)
Acinetobacter species	1 (1)
Coagulase negative Staphylococci	1 (1)
Proteus species	1 (1)
Providencia species	1 (1)
Total	105 (100)

[Table/Fig-4]: Bacteriological profile of urinary isolates and biofilm production.



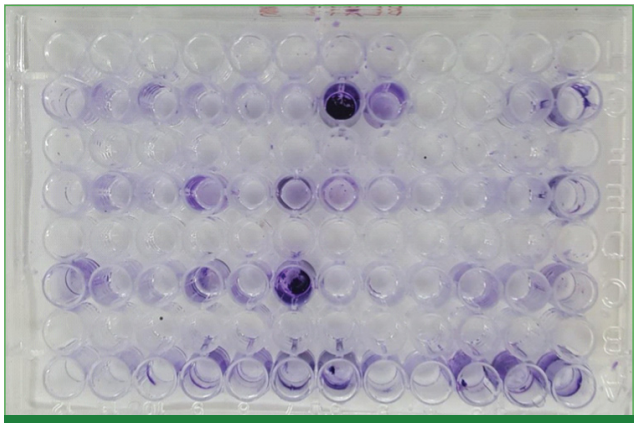
[Table/Fig-5]: Congo red agar method: (a) Strong biofilm producer; (b) Moderate biofilm producer; (c) Weak biofilm producer; (d) Non biofilm producer



[Table/Fig-6]: Tube method: (a) Strong biofilm producer; (b) Moderate biofilm Producer; (C) Non biofilm producer

DISCUSSION

UTI is considered as the most common bacterial infection worldwide causing significant morbidity and loss of work place productivity [19,20]. UTIs have become a serious health threat with 250 millions of cases reported annually with much recurrence rate and chronicity [21]. There is an increasing trend in the antimicrobial resistance among uropathogens and is attributed to formation of biofilms [11,17]. Biofilms producing pathogens play an important role in causing potentially fatal



[Table/Fig-7]: Tissue culture plate method.

and persistent infections [7]. Hence its detection should be mandatory in a laboratory set up [22]. Biofilm detection can help the clinicians to formulate prompt effective therapeutic measures thereby help in reducing the morbidity and mortality associated with biofilm producing bacterial infections [13,23,24].

In the present study, *E. coli* (43.2%) was the major isolate followed by Klebsiella species (16.3%). This is in concordance with study by Kabir et al [21]. Many authors also found *E. coli* as the predominant isolate in their studies [3-5,24-26]. *E. coli* is associated with majority of UTI because of its virulence factors like adhesins (type I, *P fimbriae*) [5]. In the present study, majority of the biofilm producing isolates were E.coli (42%) followed by Klebsiella species (20%).These findings are in agreement with other studies [3,5,21]. However, Abdagire et al [25] found higher biofilm production by *S. aureus* (60.15%) followed by *E. coli* (39.58%). There are various methods for detection of biofilm formation. Previous studies done on phenotypic methods showed variable results regarding the suitable method that can be used for screening biofilm producers in the clinical specimens. In the present study, biofilm was detected

Authors (year)	Tissue culture plate method (%)	Tube method (%)	Congo Red agar method (%)
Bose et al [23] (2009)	54.19	42.46	6.15
Saroj et al [27] (2012)	56	48	72
Munesh et al [28] (2013)	47.9	-	72.9
Nabajit et al [31] (2014)	83	57	20
Tayal et al [2] (2015)	27	37.96	40.88
Pallavi et al [26] (2017)	69.91	53.09	9.73
Samidurai et al [24] (2017)	45.71	42.86	42.86
Present study	39.77	38.26	46.97

[Table/Fig-8]: Biofilm detection by different methods observed in various studies.

Authors (years)	Tube method				Congo red agar method			
	Sensitivity (%)	Specificity (%)	PPV* (%)	NPV** (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Mathur et al [29] (2006)	73.6	92.6	93.4	66.6	6.8	90.2	66.6	25.3
Bose et al [23] (2009)	76.27	97.56	97.36	77.66	8.25	96.34	72.72	47.02
Adilson et al [32] (2010)	100	100	-	-	89	100	-	-
Hassan et al [13] (2011)	73	92.5	94	66	11	92	73	37
Tayal et al [2] (2015)	94.59	83	-	-	94.59	81	-	-
Chandana et al [11] (2015)	71.8	88.8	-	-	12.7	86.2	-	-
Pragyan et al [17] (2016)	81	95.1	93.3	85.6	16.8	93.9	67.9	57.3
Triveda et al [18] (2016)	45.9	82.14	42.31	67.65	25	46.67	9.86	29.9
Pallavi et al [26] (2017)	74.70	96.85	97.87	65.42	11.24	98.43	93.33	36.13
Ram et al [6] (2017)	97.30	100	100	95.24	-	-	-	-
Sufia et al [33] (2018)	57.89	92.73	89.19	68	28.07	92.73	80	55.43
Present study	63.81	78.62	66.34	76.69	80	75.47	68.29	85.11

[Table/Fig-9]: Statistical evaluation of Tube and Congo red agar method in various studies.
*PPV-Positive Predictive value; **NPV-Negative Predictive value

in 46.97% of the urinary isolates by CRA method followed by 39.77% using TCP method and 38.26% by Tube method. This is in concordance with study by Golia S et al., [27] where, CRA method detected more biofilms followed by TCP method and Tube method. Where as in a study by Tayal RA et al., [2], CRA method detected 40.8% of biofilms followed by Tube method (37.96%) and TCP method (27%). In the present study TCP was considered as gold standard method as in studies by others [2,13, 27-30]. The biofilm detection by Tube method and CRA method in the present study was not statistically significant as compared to TCP method (P=0.72 and P=0.095 respectively)In studies by Taj Y et al., [7], Bose S et al., [23] and Deka N et al [31], CRA detected only 3.47% , 6.15% and 20% of biofilms respectively. Solmaz et al [8] found results of CRA method similar to TCP method. [Table/Fig-8] shows the biofilm detection by different methods observed in various studies. Sensitivity and specificity of CRA method was found to be 80% and 75.47% respectively. Oliveira A et al., [32] found higher sensitivity and specificity (89% and 100% respectively and Gupta MK et al., [28] 73% sensitivity. Whereas, others have reported a lesser sensitivity of 11% [13]. The variations observed in CRA method in various studies might be due to the fact that different studies used modifications in the media with greater sugar content and few studies also had increased the duration of incubation promoting greater biofilm production by the bacteria [7,29,32]. In the present study six colour reference scale was used to interpret diverse colonies grown on CRA media. Colour scale was also adopted in other studies [32]. However few studies considered black colonies with dry crystalline consistency as biofilm producers [5,7,13]. In the present study subjective errors observed while interpreting almost black and biofilm negative isolates.

In the present study tube method detected biofilm formation in 3.26% of urinary isolates which is almost similar to TCP method (39.77%).Tube method showed a sensitivity of 63.81% and a specificity of 78.62%. Oliveira A et al., [32] found 100 % sensitivity and specificity by tube method. Higher sensitivity and specificity were also noted by Mathur T et al [29], Bose S et al., [23] and Sayal P et al [30]. Observer differences were noted while differentiating weak biofilm producers from non-biofilm producers. Mathur T et al., [29] and Tayal RA et al., [2] also reported subjective errors in tube method. [Table/Fig-9] shows the statistical evaluation of biofilm detection methods in different studies. The variations observed in various studies might be because of the differences in the sources from which the strains were isolated and differences in the methodology employed in the study. The limitations of the study being the small sample size because there were no data available from our region regarding the prevalence of biofilm among bacteria, we considered expected frequency as quoted in research article and because of time constraints we could not do the pilot study. Twelve isolates were lost during subculture. We could not compare the biofilm formation rates among different isolates as isolates number were not equal and clinical correlation of the isolates was done.

CONCLUSION

Early identification and evolving effective control strategies against potentially pathogenic biofilm forming uropathogens can be one of the essential steps towards the prevention and management of problematic UTIs. There are accurate biofilm detection methods like molecular methods. A suitable method which is cost effective easy to do and requiring less technical expertise is the need of the hour. TCP method is the most suitable specific reproducible, reliable method with the advantage of the both qualitative and quantitative analysis and with no subjective errors. Considering the ease of dong the test, rapidity and cost effectiveness, CRA method and tube method can be considered for biofilm detection in resource constraint conditions.

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REFERENCES

[1] Razak SK, Gurushanthappa V. Bacteriology of urinary tract infection and antibiotic susceptibility pattern in a tertiary care hospital in South India. Int J Med Sci Public Health 2012;1:109-12.
[2] Tayal RA, Baveja SM, De AS. Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Int J Health Allied Sci 2015;4:247-52.
[3] Mohammed EA, ElShalakany AH. Detection of biofilm formation in uropathogenic bacteria. Egyptian Journal of Medical Microbiology 2015;24 (1):49-57.
[4] Trivedi H, Sao S, Murthy R, Sagrikapradhan. Prevalence of biofilm formation in uropathogens.World Journal of Pharmaceutical Research 2017;6(6):1665-75.
[5] Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections. J Clin Diagn Res 2012;6(9):1478-82.
[6] Reddy KRM. Tube adherence test as a screening tool for detection of biofilm formation among Staphylococcus aureus. Int J Curr Microbiol Appl Sci 2017;6(8):1325-29.
[7] Taj Y, Essa F, Aziz F, Kazmi SU. Study on biofilm forming properties of clinical isolates of Staphylococcus aureus. J Infect Dev Ctries 2012;5(6):403-09.
[8] Moghadam SO, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin resistant *Staphylococcus aureus* isolated from burn patients Iran. J Infect Dev Ctries 2014;8(12):1511-17.
[9] CDC Epi Info version 7.2.Available at https://www.cdc.gov/epiinfo/pc.html. Accessed on April 2nd 2018.
[10] Khan AM. Sample size estimation. In: Gupta P, Singh N(Editors). How to write the thesis and thesis protocol-A premier for Medical, Dental and nursing courses.1st ed. New Delhi: Jaypee Brothers Medical publishers(p) Ltd;2014pp.83-92.
[11] Devaraj C, Sajjan A.Comparision of three different methods for the detection of biofilm in Gram positive cocci and Gram negative bacilli isolated from clinical specimens. J Pharm Sci & Res 2015;7(11):952-55.
[12] Collee JG, Fraser AG, Marmion BP, Simmons A. Editors. Mackie and McCartney Practical Medical Microbiology.14th ed. Edinburgh(UK):Churchill Livingstone; 1999.

[13] 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; 15(4):305-11.
[14] Murugan K, Usha M, Malathi P, Al-Sohaibani AS, Chandrasekaran M. Biofilm Forming Multi Drug Resistant Staphylococcus spp. Among Patients with Conjunctivitis. Pol J Microbiol 2010;59:233-39.
[15] Ferreira AA, Tette PAS Mendonca RCS, Soares ASC, Carvalho MM. Detection of exopolysaccharide production and biofilm related genes in Staphylococucus species isolated from poultry processing plant. Food Sci Technol (Caminas) [online]2014;34(4):710-16.
[16] Esteban J, Manso DM, Spiliopoulou I, Ampuero JC, Roblas RF, Foka A, et al. Biofilm development by clinical isolates of Staphylococcus spp. From retrieved orthopedic prostheses. ActaOrthopaedica 2010;81:674-9.
[17] Panda PS, Chaudhary U, Dube SK, Comparison of four different methods for detection of biofilm formation by uropathogens. Indian J Pathol Microbiol 2016;59(2):177-79.
[18] Triveda L, Gomathi S. Detection of biofilm formation among the clinical isolates of Enterococci: An evaluation of three different screening methods. Int J Curr Microbiol App Sci 2016;5(3):643-50.
[19] Tayal RA, Baveja SM, De AS. Comparison of phenotypic methods for the detection of biofilm production in uropathogens in a tertiary care hospital in India. Int J Curr Microbiol App Sci 2015;4(9):840-49.
[20] Zheng JX, Bai B, Lin ZW, Pu ZY, Yao WM, Chen Z et al. Characterization of biofilm formation by Enterococcus faecalis isolates derived from urinary tract infections in China. Journal Med Microbiol 2018; 67:60-67.
[21] Shahidul KM, Asma A, Farahnaaz F, Sunjukta A. Determination of antibiotic resistance pattern of biofilm producing pathogenic bacteria associated with UTI. Int J Drug Dev & Res 2013;5(4):312-19.
[22] De A, Deshpande D, Baveja SM, Taklikar S. Detection of biofilm formation in bacteria from cases of urinary tract infections, septicemia, skin and soft tissue infections and post-operative infections by Congo Red Agar method. J Acad Med Sci 2012;2:46-47.
[23] Bose S, Khodke M, Basak S, Mallick SK. Detection of biofilm producing Staphylococci: Need of the hour. J Clin Diagn Res 2009;3:1915-20.
[24] Nalayini S, Vasanthapriyan MR, Anuradha N, Moorthi DA.A study on the detection of biofilm formation by multidrug resistant uropathogenic isolates in a tertiary care hospital. J Evolution Med Dent Sci 2017;6(80):5641-46.
[25] Abdagire NV, Chincholkar VV, Kulkarni DM, Nilekar SL, Birajdar SV. Biofilm production among uropathogens and their antibiogram. Int J Pharm Bio Sci 2014;5(1):261-66.
[26] Sayal P, Sandhu R, Singh K, Devi P. Slime production among uropathogenic bacterial isolates: Evaluating different phenotypic detection methods. Annals of Pathology and Laboratory Medicine 2017;4(1):48-53.
[27] Golia S, Hittinahalli V, Karjigi SK, Reddy KM. Correlation between biofilm formation of uropathogenic Escherichia coli and its antibiotic resistance pattern. Journal of evolution of Medical and dental Sciences 2012; 1(3):166-75.
[28] Gupta MK, Gahlot R, Nigam C, Kumar V. Biofilm: Detection methods and correlation with antimicrobial resistance in Staphylococci. National Journal of Laboratory Medicine 2013;2(2):07-10.
[29] 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. Ind J Med Microbiol 2006;24(1):25-29.
[30] Sayal P, Singh K, Devi P. Detection of bacterial biofilm in patients with indwelling urinary catheters. CIB Tech Journal of Microbiology 2014;3(3):09-16.
[31] Deka N. 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):810-15.
[32] Oliveira A, Cunha MLRS. Comparison of methods for the detection of biofilm production in coagulase negative Staphylococci. BMC Research Notes 2010;3:260. Available at https://www.ncbi.nlm.nih.gov/pmc/articles/pmc 2973941/. Accessed on December 12, 2017.
[33] Siddhiqui S, Afreen U, Kotgire S. Evaluation of biofilm formation by three different methods and its antibiogram with special reference to Indwelling Medical Devices from a tertiary care hospital. Annals of Pathology and Laboratory Medicine 2018;5(2):171-76.

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