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Biofilm formation and antibiotic resistance of uropathogenic *E. coli* isolated from urinary tract of catheterized patients

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Abstract

The present study have been carried out on (100) urine samples were collected from catheterized patients from (3) hospitals at Baghdad city for a period of ten months ,all samples were directly cultured on conventional laboratory media, (83) samples were positive for bacteriological culture, Hi Crome (M1335) UTI medium have been used for differentiation between the isolated bacteria. Antibiotic sensitivity test was performed for uropathogenic *E. coli* (UPEC) by Kirby-Bauer disc diffusion method then the percentage of multi-drug resistance have been estimated. Detection of biofilm formation have been done for (UPEC) by spectrophotometric assay (TCP) ;optical density hve been calculated for the bacterial cells that can produce biofilm for each isolate. Results showed that (60) isolates were belonged to the bacterium *E. coli* (72.3%),multi-drug resistance appeared : (60) isolates were resistant for all antibiotics (100%) and associated in resistance to four antibiotics (100%), (54) for two antibiotics (90%), (51)for three antibiotics (85%), (34) for two antibiotics(56.66%), (12) for two antibiotics (20%). Highly resistance was towards cefotaxime, aztreonam, vancomycin and erythromycin. UPEC were sensitive to nitrofurantoin (100%), imepenem and amikacin (80%). Biofilm producing UPEC were (53) isolates (88.33%) distributed among weak producer(36), moderate(15) and strong(2): 67.92%, 28.30%, 3.77% respectively.

Keywords : UTIs, Catheter, UPEC, Biofilm, Multi-drug resistance.

Introduction

Biofilm is a microbiologically derived sessile community characterized by cells irreversibly attached to a substratum or to each other and embedded in a matrix of extracellular polymeric substances that they have produced (Donlan and Costerten, 2002). The formation of biofilms is mediated by mechanical, biochemical and genetic factors (Balasubramanian *et al*., 2012) .Biofilms enhance the virulence of the pathogen and have their potential role in various infections, they are currently estimated to be responsible for over 65% of nosocomial infections (NI) and 80% of all microbial infections (Romling and Balsalobre, 2012).

Urinary tract infections (UTIs) are defined as diseases which are caused by a microbial invasion of the genitourinary tract, that extends from the renal cortex of the kidney to the urethral meatus ,they represent the most commonly acquired bacterial infections and they account for an estimated 25-40% of the NI, the risk of developing urinary tract infections increases significantly with the use of indwelling devices such as catheters (Bagshaw and Laupland, 2006).

The urinary catheters are tubular latex or silicone devices which when inserted may readily acquire biofilms in the inner or outer surfaces (Niveditha *et al*., 2012). Bacterial biofilms play an important role in UTIs being responsible for persistence of infections causing relapses and acute prostatitis (Soto, 2014). The resistance of biofilm cells to antimicrobial agents is a clinically important feature, bacteria forming biofilms are difficult to eradicate due to the antimicrobial resistance phenotype that this structure confers being combined therapy recommended for the treatment of biofilm - associated infections (Hoiby *et al*., 2012). Catheter associated urinary tract infection (CAUTI) is a common health care associated infection worldwide resulting from wide spread use of urinary catheter and inappropriate antibiotics use (Majumder *et al*., 2014). The pathogenesis of CAUTI is related to the susceptibility of inert catheter material to microbial colonization and formation of pathogenic biofilm, its early detection prevents various hazards as well as economic impact (Trautner and Darouiche, 2010; Majumder *et al.,* 2014)

E. coli from the initial bacteria that cause UTIs particularly in patients with long-term indwelling catheters, uropathogenic *E. coli* (UPEC) is responsible for more than 80% of all the UTIs and it cause both symptomatic and a symptomatic bacteriuria (Niveditha *et al*., 2012).

Materials and Methods

A total of 100 urine samples have been collected from patients of all age groups and both sexes with a urinary catheters suffering from symptoms of UTIs, for ten months period from 3 hospitals at Baghdad city. Samples were collected under complete aseptic conditions with a sterile syringes then transported to a sterile urine containers.

Isolation and Identification:

1-One ml of urine have been cultured on each of nutrient, blood and MacConkey agar, incubated at 37°C/24hrs.

2- All the growing isolates were cultured on HiCrome medium, incubated at 37°C/18-24hrs, if the colonies appeared dark pink to reddish that means the isolate is *E. coli.*

3-Vitek 2 was used in confirmatory diagnosis of *E. coli* isolates.

Antimicrobial Sensitivity: Isolates were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method according to (CLSI,2011). Fourteen antibiotic discs were used in this study: piperacillin(30 µg), cefotaxime(30 µg), imipenem(10µg), gentamycin (10µg), ciprofloxacin (10 µg), nalidixic acid (30 µg), aztreonam (30µg) ,azithromycin(15 µg) ,amikacin (10µg), nitrofurantoin(30µg), ceftriaxone (30µg), amoxicillin-clavulanic acid (30µg), vancomycin(10 µg), erythromycin(15µg).

Detection of biofilm formation: All the isolates were screened for biofilm production by tissue culture plates (TCP) method according to (Stepanovic *et al*., 2007); flat-bottomed 96 well clear polystyrene ,tissue culture treated microtiter plate were inoculated with 200 µl of bacterial suspension corresponding to 0.5 McFarland ,incubated at 37°C for 24hrs, the contents of each well were removed by decantation and each well washed 3 times with 300 µl of normal saline, the remaining attached bacteria were heat fixed by exposing them to hot air at 60°C for 60sec, crystal violet stain was added to each well (15 min), excess stain wash rinsed off by decantation ,plates were washed, 95% ethanol was added to each well, after(30min), optical density (OD) of stained adherent bacterial films were measured at 630nm by using ELISA reader. Results were averaged and standard deviation was calculated, the cutoff was defined as three standard deviations above the mean ODc (Tenorio *et al*., 2003). Each isolate was classified as follows ;

weak biofilm producer OD= 2 × ODc, moderate biofilm producer 2×ODc≤ OD=4×ODc, strong biofilm producer OD ≥4 × ODc .

Statistical analysis : The results were expressed by percentages(%) as well as means and standard deviation were calculated for optical density values in biofilm production experiment.

Results and Discussion

This study have been done to isolate and detect the biofilm forming *E. coli* in patients with catheter associated UTIs. One hundred urine specimens have been collected from catheterized patients for a period of ten months from three hospitals at Baghdad city.

Eighty-three of the specimens were positive for bacteriological culture on HiCrome (M1335) UTI agar. This medium is recommended for the detection of urinary tract pathogens. Where HiCrome UTI agar has broader application as a general nutrient agar for isolation of various microorganisms, it facilities and expedites the identification of some Gr- and Gr+ bacteria on the basis of different contrasted colony colors produced by reactions of specific enzymes of certain- genus or species-with two chromogenic substrates (Parveen *et al.*, 2011; Ghanwate, 2012).

Collection of bacterial isolates from catheters have been showed multi-species catheter colonization; (60) isolates were identified as *E. coli* (pure colonies), followed by (7) isolates belonged to *Klebsiella pneumonaie*, (5) were identified as *Staphylococcus aureus*, (5) for *Proteus* sp. (2) for *Pseudomonas aeruginosa*, (4) were considered as a mixed infections ; *Enterococcus* sp + *S.aureus*, other two cultures were for *E. coli* + *S. aureus*, as shown in Table (1).

Table (1): Uropathogens Isolated from Catheterized Patients

Isolate	Number	Percentage%
E. coli	60	72
K. pneumoniae	7	8.4
S. aureus	5	6
Proteus sp.	5	6
Ps. aeruginosa	2	2.5
Enterococcus sp. +S. aureus	2	2.5
E. coli +S. aureus	2	2.5
Total	83	100

Our results were focusing on pure isolates of *E. coli* , isolates of other species have been excluded

,identification results showed that these colonies appeared as a dark pink to reddish, these results were compatible with results of(Poulomi et *al*., 2007) and (Jayshri *et al*., 2013) about colony characteristics and colors on HiCrome UTI agar, the chromogenic substrates are specifically cleaved by the enzyme β-D-galactosidase so the colonies appeared pink in color.

UTI in catheterized patients can occur in several ways; organisms can migrate into the bladder through the mucoid film that forms between the epithelial surface of the urethra and catheter as well as the contamination of the urine in the drain bag can allow organisms to access the bladder (Simon and Robertson, 2008).

UTIs is one of the most common bacterial infections in human , a patient was said to be suffering from a catheter-associated urinary tract infections(CAUTI) if he was catheterize and if he developed one or more of the following conditions, that is fever (temp≥38°C) without any other known cause, urgency or suprapubic tenderness, with the urine culture showing more than 10 colony-forming units or more/ml of urine, with not more than two types of organisms. A CAUTI was also considered when the urine showed pyuria (more than 10 leukocytes/ml of urine) (Niveditha *et al*., 2012). Trautner and Darouiche (2010) have been reported that the pathogenesis of CAUTI is related to the susceptibility of inert catheter material to microbial colonization.

Results of this study were agreed with the finding of (Salih and Al-Ani, 2013) they concluded that *E. coli* was the most common uropathogenic followed by *Klebsiella* sp., also (Niveditha *et al*., 2012) have been reported that the majority of UTIs causes were referred to UPEC (70%), this percentage was near to the isolation rate in our study (72%). *E. coli* was the most frequently isolated pathogen (60%) in the study of Majumder *et al*. (2014). As well as Hassin (1991) revealed that incidence of *E. coli* in UTIs was (74%). Momtaz *et al*., 2013 reported that *E. coli* is the most important cause of UTIs, because of different virulence factors that contributes to colonization and invasion of this bacterium, also results were compatible with the results of Svanborg and Godaly, (1997) and Hedlund *et al*. (2001) who documented that *E. coli* is responsible for more than (80%) of all the UTIs.

Balasubramanian *et al.* (2012) have been documented that seven species of microbes were isolated from the indwelling catheters, also they indicated that high colonization of *E. coli* in catheters may be attributed to encrustation and blockage that led to urine retention and caused pyelonephritis, septicemia and endotoxic shock, as well as they confirmed that the catheter has to be replaced if biofilm formation was noticed . Bacteria isolated from UTIs may originates from the skin of patients or health care workers, tap water and other sources in environment (Donlan, 2001).

E. coli is the most common causes of nosocomial infections ,and that may be common cause colonization in indwelling medical catheters ,UPEC survive in hospital environment despite unfavorable conditions such as dessication, nutrient starvation and antimicrobial treatment, it is hypothesized that their ability to persist and exhibit virulence ,is a result of their capacity to colonize catheters ,thus they are responsible for increased morbidity and mortality in UTIs patients (Donlan and Costerton, 2002; Revdiwala *et al*., 2012).

Antimicrobial sensitivity: *E. coli* isolates have been examined against 14 antibiotics as remembered above in materials and methods, it had been noticed that tested isolates were appeared multi-drug resistant to most used antibiotics, as shown in the following Table (2).

Table(2): Multi-drug resistance of UPEC isolates

Antibiotic code	No of antibiotics	No. of isolates	Percentage %
CTX,ATM,VA,E	4	60	100
CIP,CRO	2	54	90
PRL,NA,AMC	3	51	85
CN,AZM	2	34	56.66
IPM,AK	2	12	20
F	1	0	0

CTX ; Cefotaxime ATM ; Aztreonam VA ; Vancomycin E ; Erythromycin CIP ; Ciprofloxacin CRO ; Ceftriaxone PRL ; Piperacillin NA ; Nalidixic acid AMP ; Amoxicillin-clavulanic acid CN ; Gentamycin AZM ; Azithromycin IPM ; Imipenem AK ; Amikacin F ; Nitrofurantoin.

As appeared from the results that nitrofurantoin is the most effective antibiotic in UTIs specially for catheterized patients because all UPEC were sensitive to this antibiotic, so it is the successful antimicrobial agent followed by imipenem and amikacin, (80%) of the isolates were susceptible to these drugs, while all the isolates were resistant to ceftriaxone, aztreonam, vancomycin, erythromycin (100%). Abdallah *et al*. 2011 have been confirmed that imipenem and amikacin were the most effective antibiotics against gram-negative bacterial isolates that is in agreement with our results about *E. coli* sensitivity to those two drugs. Sharma *et al*. (2009) were indicated that amoxyclavulanic and nitrofurantoin were the most effective antibiotics for *E. coli* growing as biofilms but non- biofilm producer *E. coli* were sensitive to nitrofurantoin,

amoxyclavulanic and ceftizoxime.

Biofilm formation: The pathogenesis of CAUTI is related to the susceptibility of inert catheter material to microbial colonization and formation of pathogenic biofilm. Bacterial biofilms are often associated with long-term persistence of organisms in various environments and they display dramatically increased resistance to antibiotics (Anderson *et al*., 2003).

Table (3): Categorization of biofilm producing *E. coli*

Biofilm	Grade	No. isolates	Percentage(%)
producing isolates	Strong	2	3.77
	Moderate	15	28.30
	Weak	36	67.92
		Total =53	88.33
Non producers		7	11.66
		Total=60	100

Strong biofilm producer OD ≥4 × ODc . Moderate biofilm producer 2×ODc≤ OD=4×ODc . Weak biofilm producer OD= 2 × ODc

The results have shown that from(60) *E. coli* isolates for UTIs in catheterized patients; (53) isolates were biofilm producer: (36) were weakly biofilm productive isolates with a percentage of (67.92%) followed by (15) isolate were moderate in their production (28.30%), only (2) isolates were strongly formed biofilm (3.77%). Seven isolates were non biofilm producers (11.66 %). Sharma *et al*. (2009) have been documented that uropathogenic strains of *E. coli* account for (70-95%) of the UTIs, significant biofilm productive *E. coli* isolates were (67.5%) while our percentage was (88.33%) by the same method (TCP) ,also they concluded that bacteria that invade the bladder cells and grow into structural colonies known as biofilms may be responsible for many recurrent UTIs.

Study of Hassan *et al*. (2011) concluded that the (TCP) method is a more quantitative and reliable assay for the detection of biofilm forming microorganisms as compared to tube method and Congo red agar test, and it can be recommended as a general screening method for detection of biofilm producing bacteria in laboratories. The risk of developing UTIs increases significantly with the use of indwelling devices such as catheters (David and Stickler, 2008). A variety of bacterial species that colonize catheters can produce biofilms and these biofilms induce serious complications ,isolated cases of single-species biofilm were observed ,but most biofilms contained mixed bacterial communities containing up to five species causing biofilm mixed infections, prolonged UTI can facilitate the development of catheter biofilm, the risk of UTI is related to the length of time the catheter in place (Macleod and Stickler, 2007). In patients with long- term indwelling catheters, catheter must be changed at 10-12 week intervals, contaminated urine can, therefore be, flowing through individual catheters for period of 3 months, thus catheters provides attractive sites for bacterial colonization: the biofilm bacteria thrive in their matrix gel and the gentle flow of warm nutritious urine (David and Stickler, 2008). Sritharan and Sritharan (2004) were found that the majority of biofilm producing bacteria was from urine followed by urinary catheter tips (26.3%) as well as they reported significant association of biofilm producing with urinary catheters. Certain organisms of these biofilm produce urease, which hydrolyzes urea in the patients urine to ammonium hydroxide, the elevated pH results in precipitation of minerals, these minerals containing biofilms form encrustations and consecutive obstruction that may completely block the inner lumen of the catheter (Balasubramanian *et al*., 2012).

Diseases involving bacterial biofilms are generally chronic and difficult to treat because bacteria in biofilm are most resistant to antimicrobial agents, recurrent urinary tract infections caused by UPEC represent classical biofilm disease problem, such infections may be difficult to treat as they exhibit multidrug resistance (Sharma *et al*., 2009). Strategies for prevention of CAUTI are really measures to delay the onset of bacteriuria and no strategy can effectively prevent bacteriuria and CAUTI indefinitely in a person who is chronically catheterized (Trautner and Darouiche, 2010). The most effective strategy for treating these infections may be removal of the biofilm contaminated device (Donlan, 2001).

Ghanwate (2012) was demonstrated in her study about UPEC in a bacteriuria patients that percentage of isolation from urine samples was (51%), disagreed with our results (72%),(52%) were

found to be positive for biofilm formation while (88.33%) were biofilm producers in the present study that indicates biofilm formation in catheters is highly stronger than its formation in bacteriuria, this chronic nature of some UTIs is being attributed to the ability of *E. coli* to form a biofilm and this ability render it resistant to conventional antimicrobial therapy ,the ability to form a biofilm is often considered to be a virulence-associated trait. In contrast (Ferrieres *et al*., 2007) have been reported that asymptomatic bacteriuria(ABU) strains significantly were better biofilm formers than UPEC strains in human urine. Therapy against UTI should be guided by antimicrobial susceptibilities as urinary isolates are developing resistance to commonly used antibiotics (Majumder *et al*., 2014). Microbial biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy, this also helps in the spread of antibiotic resistance in nosocomial pathogens by increasing mutation rates and by the exchange of genes which are responsible for antibiotic resistance (Lynch and Robertson, 2008). Revdiwala *et al*. (2012) referred to different mechanisms of drug resistance for *E. coli* isolates included ;production of extended spectrum β lactamases (ESBL) and carbapenemases (carbapenems hydrolyzing enzymes).

Our results have been agreed with the results of Pramodhini *et al.* (2012) regarding high frequency of *E. coli* isolated from UTIs of catheterized patients, percentage was (70%) near to that of present study (72%),but the biofilm producing isolates were(63%) lower when compared to (88.33%), as well as(80%) of the biofilm producing isolates showed multidrug –resistance while in our study all the biofilm producing isolates(53/60) were multidrug-resistance (100%) that may be attributed to the numerous or high number of antibiotics we used (14) compared to five antibiotics only which they used. Biofilm producing bacteria are responsible for many recalcitrant infections and are notoriously difficult to eradicate, they exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilm, decreased growth rate and expression of resistance genes (Kim, 2001). Table (4) have been revealed positive relation between antibiotic resistance and biofilm production of UPEC isolates in the present study.

Table (4): Antibiotic resistance of biofilm producer and non-producer UPEC isolates

Antibiotic	Biofilm				Total No=60	Resistance percentage (%)
	Biofilm producing isolates(n=53)	%	Biofilm non producer(n=7)	%		
Cefotaxime	53	88.33	7	11.66	60	100
Aztreonam	53	88.33	7	11.66	60	100
Vancomycin	53	88.33	7	11.66	60	100
Erythromycin	53	88.33	7	11.66	60	100
Ciprofloxacin	51	94.44	3	5.55	54	90
Ceftriaxone	50	92.6	4	7.4	54	90
Piperacillin	46	90.2	5	9.8	51	85
Nalidixic acid	44	86.3	7	13.7	51	85
Amoxicillin-clavulanic acid	44	86.3	7	13.7	51	85
Gentamycin	33	97.05	1	2.95	34	56.66
Azithromycin	29	85.3	5	14.7	34	56.66
Imipenem	12	100	0	0	12	20
Amikacin	9	75	3	25	12	20
Nitrofurantoin	0		0	0	0	0

As shown in Table (4) that(60) isolates were resistant for all antibiotics, they were identical in their resistance to four antibiotics , it's apparent from the results the ability of these isolates to produce biofilm (88.33%) versus (11.66%) not biofilm producers, while (54) isolates were shared the resistance to two antibiotics in addition to the first previous four, (94.44, 92.6, 90.2, 86.3, 86.3, 97.05, 85.3, 100 and 75)% the resistance of UPEC biofilm producers isolates towards :(ciprofloxacin, ceftriaxone, piperacillin, nalidixic acid amoxicillin-clavulanic acid, gentamycin, azithromycin, imipenem, amikacin) respectively, whereas the resistance of non- biofilm producers were 5.55, 7.4, 9.8, 13.7, 13.7, 2.95, 14.7, 0,25 and 0.0 % respectively.

Biofilm producing isolates displayed relatively high resistance against tested antibiotics than non-producer, an elevated expression of the efflux pump and physiological heterogeneity plays an important role for the development of antibiotic resistance in biofilm bacteria by affecting the rate of growth, metabolism, interbacterial quorum signals,the accumulation of toxic products and changing in the local microenvironment (Majumder *et al*., 2014).

The study conducted by (Matija *et al*., 2008) demonstrated that(53%)strains of UPEC were biofilm producing,there was significant correlation between biofilm production and resistance to multiple antibiotics such as ampicillin, cotrimoxazole, nalidixic acid and norfloxacin as also shown by Suman *et al*. (2007), as well as resistance of biofilm forming UPEC to nalidixic acid was demonstrated by Solo *et al*. (2007). Vidal *et al.* (1998) reported mutant strains of *E. coli* which form a thick adherent biofilm visible with naked eye, this mutant acquired the ability to colonize hydrophilic (glass) and hydrophobic(polystyrene) surfaces and to form aggregation clumps, observation by electron microscopy revealed the presence at the surfaces of the mutant bacteria of fibrillar structures looking like the particular fimbriae designated curli, curli are morphological structures of major importance for inert surface colonization and biofilm formation and demonstrate that their synthesis is under the control of two component regulatory system. Study of Hancock *et al*. (2012) demonstrated that biofilm formation can be impaired by the addition of divalent metal ions, such as zinc and cobalt which inhibits iron uptake by virtue of their higher -than-iron affinity for the master controller protein of iron uptake. these results support that iron uptake is needed crucial for biofilm formation, and thereby , targeting these uptake system might be an effective way to eradicate biofilms caused by infectious strains. We concluded from present findings that UTI particularly CAUTI must be detected and treated and managed with the proper and suitable methods to lower the economic cost in hospitals.

Conclusion

Correlation was observed between biofilm production and multi-drug resistance of UPEC and further studies are needed to find correlation between biofilm producer isolates and their resistance to antibiotics. Also safe usage techniques of indwelling catheters are suggested.

References

Abdallah, N.M.A.; Elsayed, S.B.; Mostafa, M.M.Y. and El-gohary, G.M. 2011. Biofilm forming bacteria isolated from urinary tract Infection, relation to catheterization and susceptibility to antibiotics. Inter. J. Biotechnol. Molecul. Biol. Res., 2(10): 172-178.

Anderson, G.C.; Palermo J.J.; Schilling J.D.; Roth, R.; Heuser J. and Hultgren, S.J. 2003. Intracellar bacterial biofilm –like pods in urinary tract infections.Science.,301:105-107.

Bagshaw, S.M. and Laupland, K.B. 2006 . The Epidemiology of the intensive care unit acquired urinary tract infections. Curr. Opin. Infect. Dis., 19: 67-71.

Balasubramanian, A.; Chairman, K.; Ranjit, Singh, A.J.A. and Alagumathu, G. 2012. isolation and identification of microbes from biofilm of urinary catheters and antimicrobial susceptibility evaluation. Asian Pacific J. Trop. Biomed., S1780-S1783.

CLSI (Clinical and Laboratory Standards Institute). 2011. Performance Standard for Antimicrobial Susceptibility Testing; Twenty –First Informational Supplement., M100-S21.31(1)

David, J. and Stickler, M.D. 2008. Bacterial biofilms in patients with indwelling urinary catheters: catheter biofilms. Nat. Clin. Pract. Urol. CME., 12(5): 117-123

Donlan, R.M and Costerton, W. 2002 . Biofilms :Survival mechanism of clinically relevant microorganisms. Clin. Microbiol. Rev., 15(2): 167-93.

Donlan, R.M .2001 . Biofilms and device-associated infections. Emerging Infectious Diseases., 7(2): 277-281.

Ferrieres, L.; Hancock, V. and Klemm, P. 2007. Biofilm exclusion of uropathogenic bacteria by selected asymptomatic bacteriuria *Escherichia coli* strains. Microbiol., 153(6): 1711-1719.

Ghanwate, N.A. 2012. Biofilm eradication studies on

uropathogenic *E. coli* using ciprofloxacin and nitrofurantoin. Int. J. Pharm. Biomed. Res., 3(2): 127-131.

Hancock, V.; Dahl, M.; Klemm, P. 2010. A bolition of biofilm formation in urinary tract *Escherichia coli* and *Klebsiella* isolates by metal interference through competition for FUR. Appl. Environ. Microbiol.,76(12): 3836-3841.

Hassan, A .; Usman, J. ; Kaleem, F.; Omair, M.; Khalid, A. and Iqbal, M. 2011. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz. J. Infect. Dis., 15(4) Salvador July /Aug.

Hassin, S.K.R. 1991 . Studies on urinary tract infections. Bangladesh. Medical. J., 20:29-32.

Hedlund, M.;Duan, R.D.; Nilsson, A.; Svensson, M.; Karpman, D. and Svanborg, C. 2001. The fimbriae ,the transmembrane signaling ,and the cell activation. J. Infect. Dis., 183: S 47-S50.

Hoiby, N.; Bjarnsholt, T.; Girskov, M.; Molin, S. and Ciofu, O. 2012. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents., 35(4): 322-332.

Jayshri ,P.; Payal, R.D.; Sanjay, R.; Afros, B.; Bimal, C. and Parul, D.S. 2013. Utility of UTI CHROMagar media for rapid identification of uropathogens. NHL J. Med. Sci., 2(1).

Kim, L. 2001. Riddle of biofilm resistance. Antimicrob. Agents. Chemother., 45(4): 999-1007.

Lynch, A.S. and Robertsen, G.T. 2008. Bacterial and fungal biofilm infections .Annu. Rev. Med.,59:415-428.

Macleod, S.M. and Stickler, D.J. 2007. Species interactions in mixed-community crystalline biofilms on urinary catheters. J .Med. Microbiol.,56:1549-1557.

Majumder, M.I. ; Ahmed, T.; Hossain, D.; Ali, M.; Islam, B. and Chowdhury, N.H. 2014. Bacteriology and antibiotic sensitivity patterns of urine and biofilm in patients with indwelling urinary catheter in a tertiary hospitals in Bangladesh. J. Bacteriol. Parasitol., 5(3): 191-196.

Matija, R.; Manca, M.P.; Breda, Z. and Darja, Z.B. 2008. Biofilm formation in indwelling catheters. J. Med. Microbiol., 57: 1329-1334.

Momtaz, H.; Karimian, A.; Madani, M.; Safarpoor Dehkordi, F.;Ranjbar, R. *et al.,* 2013. Uropathogenic *Escherichia coli* in Iran; Sero-group distributions, virulence factors and antimicrobial resistance properties. Ann. Clin. Microbiol. Antimicrob.,12:8

Nivedatha, S.; Pramodhini, S.; Umadevi, S.; Kumar, S. and Stephen, S. 2012. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs) .J. Clin. Diagn. Res., 6(9): 1478-1482.

Parveen, R.; Saha, S.K.; Shamshuzzaaman, S.M. ; Rasid, A.L.; Chowdhury, A. and Muazzam, N. 2011. Detection of uropathogens by using chromogenic media(HiCrome UTI Agar),Cled Agar and other conventional media. Faridpur. Med. Coll. J., 6(1): 46-50.

Poulomi, N.; Thakur, A.R. and Ray, C.S. 2007. Characterization of bacterial strains isolated through microbial profiling of urine sample.Journal of Biological Sciences. ,7(1):44-51.

Pramodhini, S.; Nivedatha, S.; Umadevi, S.; Kumar, S.; Stephen, S. 2012. Antibiotic resistance pattern of biofilm –forming uropathogens isolated from catheterized patients in Pondicherry, India. Australian. Medical.J., 5(7): 344-348.

Revdiwala, S.; Rajder, B.M. and Mulla, S. 2012. Characterization of bacterial etiologic agents of biofilm formation in medical devices in critical care setup. Critical. Care Res. Practice., 6.

Romling, U. and Balsalobre, C. 2012. Biofilm infections ,their resistance to therapy and innovative treatment strategies. J. Int. Med., 272(6): 541-561.

Salih, M.T. and Al-Ani, N.F. 2013. Microbiological aspects in biofilm produced by some uropathogens isolated from patients with indwelling bladder catheters. Raf. J. Sci., 24(1): 1-16.

Sharma, M.; Aparna, S,; Yadav, S. and Chaudhary, U. 2009. Biofilm production in uropathogenic *Escherichia coli* . Indian. J. Pathol. Microbiol., 52(2): 294.

Simon A.L. and Robertson, G.T. 2008. Bacterial and fungal biofilm infections .Annual Review of Med., 59 :415-28.

Solo, S.M.; Smithson, J.A. ; Martinez, J.P. ; Horcajada, J. and Mensa, V. 2007. Resistance of biofilm UPEC for nalidixic acid. J. Urol., 177: 365-368.

Soto, S.M. 2014. Importance of biofilm in urinary tract infections : new therapeutic approaches. Adv. Biol., 13.

Sritharan, M. and Sritharan, V. 2004. Emerging problems in the Management of infectious diseases. The Biofilm Med. Microbiol.,22: 140-142.

Stepanovic, S.; Vokovic, D.; Hola, V.; Bonaventara, G.; Djukic, S.; Cirkovic, I.and Ruzicka F. 2007. Quantification of biofilm in microtiter plates overview of testing conditions and practical

recommendations for assessment of biofilm production by Staphylococci, APMIS., 115(8): 891-899.

Suman, E.; Jose, J. Varghese, S. and Kotian, M.S. 2007. Study of biofilm production in *Escherichia coli* causing urinary tract infection .Indian J. Med. Microbiol., 25:305-6.

Svanborg, C. and Godaly, G. 1997. The bacterial virulence in urinary tract infections. Infect. Dis. Clin. North Am., 11:513-29.

Tenorio, E.; Saeki, T.; Fujita, K. *et al*. 2003. Systematic characterization of *E. coli* genes/ORFs affecting biofilm formation. FEMS. Microbiology. Letters., 225(1): 107-114.

Trautner, B.W. and Darouiche, R.O. 2010 . Role of biofilm in catheter-associated urinary tract infections. Am. J. Infect. Control., Oct.25.

Vidal, O.; Longin, R.; Prigent-Combaret, C. Dorel, C. Hooreman, M.and Leieun, P. 1998. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: Involvement of a new ompR allele that increases curli expression. J. Bacteriol., 180(9): 2442-9.