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# ASSESSMENT OF BACTERIAL BIOFILM FORMATIONS IN URINARY CATHETERS; CASE CONTROL STUDY

**Zena N. Aljalelli*[1], Muataz Alani[2]**

[1]Clinical Microbiologist, Alkhansaa Teaching Hospital Directorate of Health of Ninavah.
[2]Consultant Surgeon, Alkhansaa Teaching Hospital, Hospital Directorate of Health of Ninavah.

**\*Corresponding Author: Zena N. Aljalelli**

Clinical Microbiologist, Alkhansaa Teaching Hospital Directorate of Health of Ninavah.

**ABSTRACT**

**Objectives:** To conduct bacteriological identification that causing biofilm in urinary catheters; using morphological and biochemical methods. **Materials and method:** The study was conducted during the period from Oct 2017 to July 2020. Bacteriological samples were collected from patients admitted to different Teaching Hospitals in Mosul city. Patients are those with urinary tract catheterization . A total of (106) bacteriological samples were collected. The studied subjects were (53) patients,(33 males and 20 females). The age of the patients was ranged between 1-80 years (mean46.82 ± 337). A control subjects are those who are admitted to the hospital for other causes and had short term catheterization, All samples were cultured on different media for full bacteriological investigation and estimation of total and viable counts. **Results:** In the present study a total of 131 microorganisms were isolated. Gram negative bacteria were more frequently encountered than the gram positive ones among all samples. Urinary catheters yielded 74 microorganisms while 57 bacterial isolates were obtained from urine samples. The most frequent gram negative isolate was *E.coli* for both urinary catheters and urine cultures, while *E.faecalis* and *Staph. Epidermids* were the dominant gram positive bacteria. The most effective antimicrobial agent against gram negative bacterial isolates was Imipenem, while ciprofloxacin was the top agent for gram positive isolates. The estimation of total and viable counts out of 53 tested samples 49 (92.6%) showed a total count ranging between ( 150-300 x$10^6$) bacteria, while the viable count was ranging between( 120-250 x$10^4$ ) bacteria only. The remaining 4 cases (7.4%) yielded a clear suspension and showed no growth on blood agar. **Conclusions:** The bacteria formed biofilm within the medical devices are mainly gram negative. Imipenim is the most effective agent against gram negative isolates, while Vancomycine, represented the drug of choice for gram positive isolates.

**KEYWORD:** urinary, bacteria, biofilm, catheterization.

## INTRODUCTION

In the natural environment, bacteria predominantly exist in the matrix enclosed sessile communities referred to as biofilm.[1] It is estimated that 99.9% of bacteria in nature are attached to a surface in form of a biofilm.[2] The biofilm is a structural community of bacterial cells envelope in a self-produced polymeric matrix and adherent to an inert or living surface.[3] The biofilm protects cells from deleterious conditions, as attack by the mammalian immune system.[1] Therefor the biofilms are more resistance to immune clearance mechanisms and antibiotics compared to planktonic cells.[4] Organisms within biofilm can withstand nutrient deprivation, pH changes, oxygen radicals and disinfectants better than planktonic organisms.[5]

Over the past decade bacteria in the form of biofilms have been recognized as important causes of variety of human infections.[6] Urinary tract infections are the most common infections in patients with a chronic indwelling bladder catheter, bacteruria is essentially unavoidable in this patient group.[7] The aims of the present study are to conduct bacteriological identification of bacteria causing biofilm using morphological and biochemical methods. Also to determine the antibiogram profile of isolate microorganisms.

## PATIENTS AND METHODS

The present work was carried out in the Diagnostic Bacteriology Laboratory, Department of Microbiology, Mosul College of Medicine. The clinical samples were

collected in the period from October 2017 to July 2020. A total of 131 bacteriological samples were collected from the patients admitted to different Teaching Hospitals in Mosul city including Al-Jumhori ICU and Al- Sabonjji ward, and Al- Batool Teaching Hospitals. The samples consisted of 53 urinary catheters swabs, 53 urine samples. Urinary catheter swabs and urine samples were collected from the same patient in those with urinary tract infections and on catheterization for at least 10 days to 2 weeks, The studied subjects comprised of (33) males and (20) females. The age of the patients was ranged between 1-80 years(mean 46.82 ± 337).

A control subjects comprising 33 individuals, who admitted to the hospital for any cause and had short period of 1-2 days. A midstream urine samples or urine from urinary catheters were collected in sterile, wide–mouthed, screw capped plastic container for microbiological study including culture and sensitivity test.

In the current study different culture media were used. The estimation of total count of bacteria constituted the biofilm in medical device was done. The number of the viable microorganisms in the original 1ml was counted as follows: **Number of colonies in 0.1 ml x (10) x dilution factor.** The isolates were tested for their sensitivity to selected antibacterial agents using the standard disc diffusion method. (Bauer et al, 1966). The plates were incubated at $37^0$ C for 18-24 hrs. The resultant inhibition zone diameter for each disc was measured and compared with the control measure 8. Eighteen antimicrobial discs for susceptibility were used (Table1). All discs were purchased from Oxoid(U.K.).

**Table 1: Antibacterial agents used for testing the sensitivity of the different isolates.**

| Antibiotic | Conc.(ug/disk) | Antibiotic | Conc.(ug/disk) |
|---|---|---|---|
| Ampicillin | 10ug/disc | Erythromycin | 15 ug/disc |
| Carbenicillin | 25ug/disc | Gentamicin | 10 ug/disc |
| Cefotaxim | 10ug/disc | Imipenem | 100 ug/disc |
| Cefoxitin | 30ug/disc | Piperacillin | 100 ug/disc |
| Ceftazidime | 30 ug/disc | norflaxacin | 5 ug/disc |
| Ceftriaxone | 30 ug/disc | Tetracycline | 30 ug/disc |
| Cephalothin | 30 ug/disc | Ticarcillin | 75 ug/disc |
| Chloramphenicol | 30 ug/disc | Tobramycin | 10 ug/disc |
| Ciprofloxacin | 5 ug/disc | Vancomycin | 30 ug/disc |

**Statistical analysis**
Statistical analysis was done by Microsoft office Excel 2003, Mann Whitney test and Z-test were used to evaluate the present data.

**RESULTS**
The present study enrolled 53 patients with urinary catheters tubes, of whom were 33 males and 20 were females.

The age of patients ranged from 1-80 years(mean 46.8±2.337SD). The highest frequency of the catheterization was noted among the age group (61-70) years. The studied patients were on catheterization for a period of 10-30 days.

The examined patients on urinary catheters (12f -22f and made of latex) which comprising 53 (56.4%) patients with 53 (56.4%) urine samples. The most common underlying urological conditions recorded in the studied patients were benign prostatic hypertrophy (41.5 %), urinary stones (22.6 %), burn (9.4%), tumors (7.4%) urethral obstruction (5.7%), trauma to the bladder(3.8 %) ,ureteric reimplant (3.8%) nephrectomy(1.9%) neurogenic bladder (1.9%), pelvic ureteric junction PUJ obstruction (1.9%).

A total of 131 bacterial isolates were obtained from both urinary catheters and urine samples .Urinary catheters yielded 74 (56.5%) microorganisms while 57 (43.5 %) bacterial isolates were obtained from urine samples in addition 3 candida spp. were detected. Statistically there was no significant difference in the number of bacteria isolated from urinary catheter and urine samples P value>0.05.

Gram negative bacteria were more frequently encountered (63;85.1%) than the gram positive ones (11;14.9%) in urinary catheter cultures and (49;86%) and (8;14%) in urine cultures respectively.

Among patients (53 urinary catheter swab samples), positive culture was noted in 51(96.2%) of the cases. Pure cultures were obtained in 24(45.3%) patients, while mixed culture isolates were the dominant which found in 27(50.9%) patients,. However, no growth was seen in the remaining 2(3.8%) cases. Statistically there was significant difference between mixed and pure cultures (P value <0.05).

Out of the tested 53 urine patients samples 48(90.6%)yielded positive culture isolates, of which 34(64.2%) showed pure cultures, while mixed growth were obtained in14(26.4%) samples ,and no bacterial growth was seen in the remaining 5 (9.4%)cases. Statistically there was no significant difference between mixed and pure cultures(P value >0.05).

**Table 2: The percentages of pure and mixed bacterial isolates from urinary catheter swab biofilms and urine samples.**

| Microorganisms | Urinary catheter (53) | | | | | | Urine(53) | | | | | | Total isolates | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | pure species | | Mixed species | | Total | | pure species | | Mixed species | | | |
| | NO | % | NO | % | NO | % | NO | % | NO | % | NO | % | No | % |
| **Gram positive** | | | | | | | | | | | | | | |
| *E. faecalis* | 4 | 7.5 | 1 | 4.2 | 3 | 11.1 | 3 | 5.7 | 2 | 5.9 | 1 | 7.1 | 7 | 5.3 |
| *Staph. epidermidis* | 4 | 7.5 | 1 | 4.2 | 3 | 11.1 | 3 | 5.7 | 1 | 2.9 | 2 | 14.3 | 7 | 5.3 |
| *Staph. aureus* | 3 | 5.7 | 1 | 4.2 | 2 | 7.4 | 2 | 3.8 | 1 | 2.9 | 1 | 7.1 | 5 | 3.8 |
| | 11 | 14.9 | | | | | 8 | 14 | | | | | | |
| **Gram negative** | | | | | | | | | | | | | | |
| *E.coli* | 19 | 35.8 | 11 | 45.8 | 8 | 29.6 | 15 | 28.3 | 11 | 32.4 | 4 | 28.6 | 34 | 26 |
| *Klebsiella.spp* | 14 | 26.4 | 4 | 16.7 | 10 | 37 | 9 | 17 | 5 | 14.7 | 4 | 28.6 | 23 | 17.6 |
| *Pseudomonas spp.* | 12 | 22.6 | 1 | 4.2 | 11 | 40.7 | 9 | 17 | 4 | 11.7 | 5 | 35.7 | 21 | 16 |
| *Proteus.spp* | 6 | 11.3 | 1 | 4.2 | 5 | 18.5 | 5 | 9.4 | 2 | 5.9 | 3 | 21.4 | 11 | 8.4 |
| *Enterobacter.spp* | 5 | 9.4 | 1 | 4.2 | 4 | 14.8 | 5 | 9.4 | 3 | 8.8 | 2 | 14.3 | 10 | 7.6 |
| *Citrobacter spp.* | 3 | 5.7 | 1 | 4.2 | 2 | 7.4 | 3 | 5.7 | 2 | 5.9 | 1 | 7.1 | 6 | 4.6 |
| *Acinetobacter.spp* | 2 | 3.8 | 1 | 4.2 | 1 | 3.7 | 1 | 1.9 | 1 | 2.9 | 0 | 0 | 3 | 2.3 |
| *Chryseobacterium spp.* | 2 | 3.8 | 1 | 4.2 | 1 | 3.7 | 2 | 3.8 | 2 | 5.9 | 0 | 0 | 4 | 3.1 |
| | 63 | 85.1 | | | | | 49 | 86 | | | | | | |
| **Total** | 74 | 56.5 | 24 | 18.3 | 50 | 38.2 | 57 | 43.5 | 34 | 26 | 23 | 17.6 | 131 | 100 |

Pure vs mixed cultures in urinary catheters p-value 0.000
Pure vs mixed cultures in urine p-value0.036
urinary catheters vs urine in pure cultures p-value 0.001
urinary catheters vs urine in mixed cultures p-value 0.001

The most frequent isolate was *E. coli* (35.8%) in urinary catheter swab cultures and (28.3%) in urine sample cultures, followed by *Klebsiella spp.*(26.4 %) in case of urinary catheter swab cultures and (17%) for urine cultures, while *Pseudomonas spp.* represented(22.6 %) in case of urinary catheter swab cultures and (17%) for urine cultures. Other gram negative baciteria such as *Proteus.* (11.3%) *Enterbacter spp.* (9.4%) *citrobacter spp.*(5.7 %), *chrysobacterium indologenes* (3.8%)and *acinetobacter spp.*(3.8%)were isolated from urinary catheter cultures, while in urine cultures the incidence of the above mentioned microorganisms was (9.4%), (9.4%), (5.7%), (3.8)and (1.9 %) respectively.

Regarding the gram positive bacteria, E.faecalis and Staph. epidermids were the dominant isolates in both urinary catheter and urine samples their percentage were (7.5%)for each in urinary catheters and 5.7% in urine samples. Staph. aureus ranked in the third place and constituted 5.7%in urinary catheters and 3.8% in urine specimens.)

The main bacterial species isolated in pure form was E.coli (45.8%) in urinary catheter cultures and(32.4%) in urine cultures, while the main bacteria identified in mixed growth was Pseudomonas spp. (40.7%) in urinary catheters and (35.7%)in urine cultures.

The control group represented 66 individuals who were on catheterization for a short duration ranged between(1-2) days. those on urinary catheter which comprising 33(50%) with their 33 urine samples Statistically there was significant difference between mixed and pure cultures(P value <0.05).On the other hand for urine cultures 30% represented pure infection while,(15.2%) showed mixed infections and (54.5%) yielded negative growth. Statistically there was significant difference between mixed and pure cultures(P value <0.05).

In case of urinary catheter cultures a total of 8 microorganisms were isolated from the control group of which 25% were gram positive and 75% were gram negative. The predominant isolates were E.coli and Pseudomonas spp. each represented 6.1% followed by Klebsiella (3%)spp. For urine cultures, 15 microorganisms were detected of which E.coli was the dominant (12.1%), followed by Klebsiella spp. (9.1%), Pseudomonas spp.(6.1 %), and Enterobacter spp.(3%) as shown in Table.7. For gram positive bacteria the two predominant isolates were E. faecalis and Staph. epidermids (3%) for each in case of urinary catheter cultures. In urine cultures gram positive bacteria represented 26.7% and gram negative ones were 73.3%(Table 7). The most frequent isolate was E. faecelis (9.1), followed by Staph. epidermids (3%),. A total of 10 Microorganisms were detected.

The antibiogram profile of the bacterial isolates was determined against a panel of antibacterial agents and the following results were obtained, The most effective antibiotic against gram negative bacterial isolates from urinary catheter was imipenem 69%, followed by norfloxacin 59%, ciprofloxacin 53% and gentamycin 35.8%. While for urine samples isolates the percentages were 85.1%, 76.7%,73.7%,60.1% respectively.

The least effective antibiotic in both urinary catheters and urine samples was cephalaxine (0.7%, 3.9% respectively),while chloramphenicol showed a 0.0% in both cases. In addition, the most effective antibioticc against gram positive bacteria was ciprofloxacin (63.9% )for urinary catheter isolates and 88.9% for urine sample isolates, followed by chloramphenicol 55.6%,amoxicillin 47.2% and vancomycin 44.4% for urinary catheter isolates ,while for urine sample isolates the percentages were 77.8%,50% and 50% respectively. The least effective antibiotics for urinary catheter isolates were ceftriaxion and cephalixin 8.3% for each drug ,while for urine sample isolates the least effective agent was cephalixin 11%.

## DISCUSSION
Biofilm, which cover and secures bacteria, has been demonstrated on urinary catheter. Organisms contained within the biofilm appear to be well protected from mechanical flow of urine, host defense, and even antibiotics.

In the present work, out of 53 urinary catheter samples 50 (96.2%)cases yielded positive bacterial growth which comprising wide range of microorganisms. This result was in agreement with that of another study in which incidence of positive bacterial growth was 100%.[9] The frequency of pure culture in this study was 45.3%, while mixed culture was 50.9%. Concerning the 33 control group of short duration urinary catheterization, 24.3 % yielded positive culture of which 18.2 % represented pure infection, while 6.1% showed mixed infection; this result can be explained on the basis that the longer time of catheterization the higher the diversity shown by the biofilm microbial community in other word patients with long term catheterization are commonly infected by complex communities of bacteria with a dominance of gram negative rods this was documented by other investigators.[10]

In case of urine culture, positive bacterial growth constituted 90.6% while negative culture represented 9.4%, this result was in accordance with that of other workers who reported 98% for positive culture and 1.8% for negative ones.[11]

A total of 131 bacterial isolates were obtained from both urinary catheters and urine samples. The number of microorganisms isolated from urinary catheter samples is higher than that obtained from urine samples. This result can be explained on the basis that administration of systemic antibiotics may kill planktonic bacteria within the urine and reduce the initial rate of catheter-associated bacteriuria, that is to say; it does not, however eradicate sessile biofilm bacteria. In the current study various gram negative and gram positive bacteria were detected from urinary catheter samples, gram negative bacteria constituted 85.1%. This result was in contrast to that of another study.[12] In which the incidence of gram positive bacteria was 59.5%. This variation may be due to the underlying disease that patients suffered, their socio-economic status, and optimal cultural method used .In the present work E.coli was the most common isolated microorganism(35.8%), followed by Klebsiella spp.(26.4%) and Pseudomonas spp. (22.6%). This result was in contrast to that of other study.[13] In which Pseudomonas spp. were the predominant isolates from urinary catheters, represented 35.9%, followed by E.coli (31%) and Klebsiella spp.(17.9%). Such difference may be explained on basis of variation in the sample size, resistance to anti microbial drugs and type of patients enrolled.

Concerning the gram positive bacteria E.faecalis constituted 7.5%, which was much lower than that (24.7%) reported in other study.[14] It is well known that Staph. epidermidis is frequently associated with urinary tract infection and usually shows high resistance to the treated antibiotics. However, the isolation rate of this microorganism in the current study was only 7.5% which was in contrast to that (24.3 %) obtained by other workers.[15] Moreover, Staph. aureus is usually has encountered in the urinary tract infections which was quietly clear in the present work where this microorganism constituted 5.7% of the total cases while higher incidence(10.4%) was reported by other investigators.[13] These discrepancie may be due to the variations in the endemicity of this microorganism at different geographical localities, or related to alteration in the immunity of the patients.

Regarding the gram negative bacteria isolated from urinary catheters of control group, E.coli and Pseudomonas spp. each showed incidence of 6.1%, followed by Klebsiella spp. (3%). This result confirmed that short duration of catheterization yielded a low variety and less numbers of accumulated bacteria.[11]

Almost the same bacteria encountered in urinary catheters were recovered from urine samples. The gram negative bacteria showed 86% and gram positive reported 14.9% of the total examined samples.

The most frequent isolate was E.coli which constituted 28.3%, although higher percentage (40%) was reported by another study.[16] However, another work conducted by[17] reported a low isolation rate (15%), this difference in isolation of E.coli may be attributed to the variation in the sample size, hygiene measurement followed by the patient, frequent use of antibiotics or different local resistance of microorganisms to antimicrobial agent.

Furthermore, different gram positive bacteria were isolated such as E. faecalis which represented 7.5 % out of the examined cases. This result was somewhat lower than that (11.1%) reported by16.

Among the isolated Staphylococcal species, Staph aureus constituted 3.8% of the total urine culture isolates, and this result was proportion to those(4.3%-7.4 %) reported by other researchers 15,[18] respectively. In short term catheterization common bacterial spp. such as E.coli was isolated from urine samples. Other common organisms were Pseudomonas spp, Klebsiella spp. Protus spp. Staph epidermidis and Enterococci.[19]

According to the antibiogram study of gram positive bacteria, it was evident that the most effective antibacterial agent was vancomycine, this result was in accordance with that of other study.[20] Ciprofloxacine has been found to be more effective against gram positive bacteria isolated from urinary catheters and urine samples.

In the current work the least effective antibioticsagainst bacteria isolated from urinary catheter and urine samples were cephalexine and ceftriaxone. This can be explained by the high consumption of often inappropriately prescribed antibiotics, combined with crowding, multiple pathology and frequent use of invasive device.

## CONCLUSION
The bacteria formed biofilm within the medical devices are predominantly gram negative (85.1%) and to lesser extent (14.9%) gram positive in case of urinary catheter. The percentage of microorganisms isolated from both urine and urinary catheters are the same in 65.5 % of the cases. E.coli represented the most common isolate among gram negative bacteria in urinary catheters. Negative urine culture does not necessarily exclude urinary catheter colonization Imipenim is the most effective agents against gram negative isolates, while. Vancomycine, Ciprofoxacine and Chloramphynicol represented the drug of choice for gram positive isolates.

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