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In terms of biofilm inhibitory concentration and minimal biofilm eradication concentrations, the role of microbial biofilm in upper versus lowers urinary tract infections

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ABSTRACT

This study has been undertaken for detection the most common microorganisms isolated from infected JJ stents and infected calculi in upper urinary tract and their representative urine samples from lower urinary tract and their ability to produce biofilm quantitatively. Furthermore, depending on Biofilm inhibitory concentration (BICs) and Minimal biofilm inhibitory concentration (MBECs) for determine the biofilm antibiogram for biofilm producer study isolates to the selected antimicrobial agents.One hundred and thirty specimens obtained from Sixty five patients admitted to Urology Department in Al-Ramadi Teaching Hospital during the period from April through December 2008. They include 94 specimens taken from 47indwelling double J ureteric stents in addition to renal stones and representative urine samples obtained from 18 patients with renal calculi. Quantitative biofilm formation assay and biofilm antimicrobial susceptibility test was achieved.Out of 47 upper end of JJ stent, 19 (40.4%) were positive for culture. Out of 24 yield microorganisms 19 (79.2%) were biofilm producer isolates. Out of 47 lower ends of JJ stent, 16 (34%) were positive for culture. Of these, 16 microorganisms were biofilm producer isolates. Also, 5 renal stones were positive for culture and all the yielded bacterial isolates were biofilm producers. The biofilm cells were required 50-100 times the MIC values for ciprofloxacin in both of JJ stent and infection stones while with cefotaxime, the study sessile were required 50-500 X, 50-100 X MICs valuefor JJ stents and infection stones respectively. Further, the biofilm producer isolates cells were required 10- 100 times the MIC values for amikacin in both of two specimens. The study concluded that *Klebsiella pneumonia* and *Candida albicans* were the most common microorganisms isolated from infected JJ stent while *Klebsiella pneumonia*was the predominant in struvite stone followed by *Proteus mirabilis*. No significant difference observed between upper and lower urinary tract infections regarding biofilm formation. Further, with the increase in duration of insertion, double J stent will be more predisposing to microbial colonization. Furthermore, the biofilm producer study isolates were required lower concentration of amikacin to remove bacterial biofilm from JJ stents and infection stones in comparison with ciprofloxacin and cefotaxime.

Key Words: Upper versus lower UTI, microbial biofilm, BICs, MBECs

الملخص باللغة العربية

أجريت الدراسة على 130 عينة مستحصلة من 65 مريض دخلوا إلى ردهة الجراحة البولية في مستشفى الرمادي التعليمي للفترة من نيسان إلى كانون الأول 2008حيث شملت 94 عينة اخذت من 47 قسطرة (JJ)بالإضافة حصى الكلى والادرار ل 18 مريض يحملون حصى الكلية.انجزت طرق انتاج الغشاء الحيوي الكمية واختبار الحساسية الدوائية لهذا الغشاء. من مجموع 47 قسطرة JJ في الجزء العلوي اظهر 19 (40,4%) من العينات نتائج موجبة للزرع وكانت 19 (79,2%) عزلة بكتيرية منتجة للغشاء الحيوي من مجموع 24 عزلة. اما في الجزء السفلي للقناة البولية فكانت 16 (34%) عينة موجبة للزرع من مجموع 47 قسطرة JJ واظهرت 16 كائن حي مجهري قدرته على انتاج الغشاء الحيوي. اظهرت خمسة حصيات كلى نتائج موجبة للزرع لبكتريا منتجة جميعها للغشاء الحيوي.احتاجت خلايا البايوفيلم من 50−100 ضعف التركيز المثبط الادنى من السبروفلوكساسين لتثبيط الغشاء الحيوي البكتيري في كلا من قسطرة JJ والحصيات المتجرثمة فين حين كانت التراكيز من 50−500 و 50−100ضعف التركيز المثبط الادنى كفيلة بإزالة الغشاء الحيوي من قسطرة JJ والحصيات المتجرثمة وعلى التوالي باستخدام مضاد السيفوتاكسيم بينما احتاج مضاد الاميكاسين الى تراكيز اقل لتثبيط وازالة الغشاء الحيوي البكتيري. تستنتج الدراسة بأن الكلبسيلا الرئوية والمبيضات المعزولة من قسطرة (JJ) الأكثر شيوعاً أما في الحصوات المتجرثمة فكانت الكلبسيلا الرئوية الأكثر سيادة يليها بكتريا المتقلبات. ان النتيجة الأكثر بروزاً كانت بأنه مع مرور الفترة الزمنية لإدخال قسطرة (JJ) الى الحالب فأن الأخيرة ستكون أكثر عرضة للاستيطان الميكروبي المؤدية بالنهاية للإصابات قسطرة (JJ) المتزامنة مع الغشاء الحيوي. استنتجت الدراسة في اختبار فحص حساسية المضادات الميكروبية بأن العزلات الدراسة المنتجة لهذا الغشاء احتاجت اقل التراكيز من الاميكاسين لازالت الغشاء الحيوي من قسطرة (JJ) والحصوات المتجرثمة بالمقارنة بمضادي السبروفلوكساسين والسيفوتاكسيم .

INTRODUCTION

It is well realized that a biofilm is defined as the accumulation of microorganisms and their extracellular products to form a structured community on a surface (1). It is well realized that device-related biofilm infection result from the multifaceted interaction of bacterial, device, and host factors. Of these three factors, bacterial factors are probably the most important inthe pathogenesis of device-associated infection, whereas device factors are the most amenable to modification with the objectiveof preventing infection(2). Microbial adhesion and biofilm formation on medical devices represent a common occurrence that can lead to serious illness and death (3).

The majority of JJ stents are used temporarily, particularly instone-forming patients. Sometimes, the stent may also be a permanent solution, especially in patients with malignant ureteral obstruction (4).The initial step in encrustation of any urinary drainage device appears to be bacterial colonization and the formation ofa layer of microorganisms that accumulates on the surface,along with their by-products (5).The presence of this layer, called “biofilm,” in combination with elevation of urinary pHand changes in electrolyte composition, is responsible for crystalformation and stent encrustation (6). The development of encrustationscan cause stent obstruction with impaired urine flow,which can compromise patient care and may lead to pyelonephritis, sepsis, and shock (7).

On the other hand, there are several types of renal stones based on the type of crystals of which they consist. The calcium oxalate stones, uric acid stones, struvite stones, the driving force behind struvite stones is infectionof the urine with urease-producing bacteria. The urease hydrolyses urea, resulting in ammoniacalurine, alkalinity and stone formation and people with certain

metabolic abnormalities may produce calcium phosphate stones or cystine stones (8)

Due to importance of biofilm related infections, the treatment is performed by prolonged and high dose of antimicrobial therapy as well as elimination of infected medical device which is cornerstone of successful therapy (9).This is critical if the devices are in place for long periods of time and/or are essential for the patient's lifewith the selection of antibiotic-resistant organismsbecoming inevitable. The result is increased morbidityand mortality of patients requiring the insertion or placement of an implantable medical device (10)

One practical implication of this is that traditional diagnostic techniques that detect planktonicorganisms that generate minimum inhibitory concentrations (MIC) ofantibiotics to kill or inhibit the organisms fail to providethe clinician with data on what concentration ofantibiotic will eradicate the biofilm. Thus, new biofilm antibiogram parameters like BICs and MBECs is much more valuable (11). Thus, this study has been undertaken for detection the most common microorganisms isolated from infected JJ stents in patients with ureteric obstruction and infected calculi obtained from patients with obstructive uropathy during pyelolithotomy.

Further, quantitative determination of bacterial biofilm formed in infected renal stones and double J stents. Furthermore, depending on BICs and MBECs parameters, for detection of biofilm antimicrobial susceptibility test for biofilm producer study isolates to three selected antimicrobial agents (Ciprofloxacin, Cefotaxime, and amikacin).

PATIENTS AND METHODS

Study patients and specimen collection:

This study includes one hundred and thirty (130) specimens obtained from Sixty five (65) patients admitted to Urology Department in Al-Ramadi Teaching Hospital and carried out during the period from April through December 2008. The patients were of different sex and the mean of age was 43.68 with standard deviation (18.34). Full informative history had been taken directly from the patient or his parents or relatives, and the informations were arranged in formula sheet . All studied specimens were assigned in to upper and lower urinary tract samples. They include 94 specimens taken from 47 indwelling JJ ureteric stents with their upper end representing upper urinary tract specimen and their lower end representing the lower urinary tract specimen, 18 renal stones were obtained from the renal pelvis during pyelolithotomy (open surgery for renal stone) and at the same time a catheter collected bladder urine sample was taken for each patient.

The specimens under this study were taken by an expert urologist in the theater. The JJ stents were basically used for treatment of ureteric obstruction. The cause of ureteric obstruction and duration of JJ insertion were reviewed and included in the informative formula sheet. All the JJ stent were removed using urethrocystoscopy done under general anesthesia with complete aseptic technique with both ends stored in a sterile closed capped containers containing 10 ml of sterile normal saline and send to the laboratory for further bacteriological processing. The renal stone were removed from patients with obstructive uropathy. The stones were placed in sterile closed capped containers contained sterile normal saline or urine aspirated from renal pelvis.

Processing of samples and Laboratory investigation:

Under sterile conditions catheter collected urine samples, and JJ stent were cultured immediately into the culture media. The culture media used were nutrient agar, MacConkey agar, blood agar, sabouraud dextrose agar and chocolate agar. The streaked

culture media were incubated at 37 °C for 24-48 hr.

Regarding to renal calculi, the removed stones were cultured immediately before and after crushing for the stones into the appropriate culture media mentioned previously. After crushing the stone in a sterile holder, dilution of the crushed stone were made according to criteria laid down by Baron, and associates (10). The culture was done from each dilution and the number of colonies were counted and they compared to those from original sample before dilution (10). All the study samples were bacteriologically identified and confirmed by biochemical test according to the criteria laid down by Baron, *et.al* (10)

Biofilm Study:-

Quantitative assay of biofilm formation:

Microtiter plate assay:

Adhesion and biofilm formation was determined by using a spectrophotometric method described by Mireles and associates**,** (11) as in the following: Twenty-five µl overnight bacterial growth in brain heart infusion broth added to the wells of sterile flat bottom of microtiter plates. Then, 175 µl of fresh sterile brain heart infusion broth added (medium containing 2% glucose) and incubated at 37 °C up to 24 hours or 48 hours. The planktonic cells were aspirated and the wells washed 3 times with sterile distilled water.Afterthat, the plates were inverted and allowed to dry for 30 min. at room temperature and 200 µl of (1%) crystal violet was added to each the well for30 min.The wells subsequently washed 3 times with sterile distilled water to wash off excess crystal violet. The wells were allowed to dry at room temperature for 15 minutes and the crystal violet bounded to biofilm was extracted with 200 µl of 95% ethanol .Finally, the absorbance of the extracted crystal violet was measured at (540 nm- 550nm) with automatic microplates reader.

The isolates were classified according to biofilm production depending on the criteria laid down by Christensen, *et. al*., (1985) as following: Strong producer more than 0.24; weak producer between 0.120-0.24 while non-producer less than 0.120.

Antimicrobial susceptibility for planktonic cells:

Broth macrodilution method (MIC method):

Three antimicrobial agents used for planktonic and biofilm study belonged to the following groups: Ciprofloxacin (flouroquinolones), Amikacin (aminoglycosides), and Cefotaxime (third generation Cephalosporins).Twenty eight isolates of different bacteria were included. The bacterial standardization was performed according to McFarland turbidity standard (12).

Procedure:

Antimicrobial agents stock solutions were filter sterilized and prepared at concentration (1000µg/ml). Different antibiotic concentration (0.5-32µg/ml) were prepared in 5 ml of Mueller-Hinton broth and transferred to sterile capped tubes.
At least 4-5 morphologically similar colonies were inoculated into Mueller-Hinton broth and incubated at 37°C until the viable turbidity was equal to the 0.5 McFarland, (about 108 CFU/ml). After that, the suspension was diluted 1:100 and certain volumes transferred to the tubes containing antibiotic dilutions, to reach a final cell concentration of (about 105 CFU/ml). Controls were represented by two tubes; one of them contained broth only and the other contained broth plus microorganism. Then the tubes were incubated overnight at 37°C.The result of MICs were interpretated as the lowest concentration of antimicrobial agents which inhibits visible bacterial growth after overnight incubation (13)

Biofilm antimicrobial susceptibility test:

Biofilm formation by study isolates on catheter segments:

The method used for bacterial biofilm formation on catheter segments was described by Ohgaki, (14) Ishida, *et. al*.(15) Briefly, the tested bacteria incubated in brain heart infusion broth overnight at 37 oC .Then 10 µl of overnight culture was added to 500 µl of sterile media in which catheter segments (1cm2) were inoculated, and subsequently incubated overnight at 37 ^{o}C. After that washing of the segments was achieved by sterile media (3-4) times to remove weakly attached bacteria. Then segments were resuspended with sterile media and vortexed vigorously for 2 min which was considered as controls.

Bactericidal activity of antibacterials against biofilm forming sessile cells:

To determine the bactericidal activity of selected antibiotics against the sessile cells, the catheter segments were incubated with the organism as described above, were taken out, washed gently with sterile media or saline and subsequently transferred to saline containing a given antibiotic with distinct concentrations (10X, 50X, 100X, 500X and 1000X) at which X represented the MIC of mentioned antimicrobial agents against planktonic cells which was previously detected. After that the tubes were incubated for 24 hr. at 37^{o}C (15).

Extraction and quantification of biofilm bacteria:

After exposure of tested organisms to the desired concentrations of antibiotic, they were transferred to 10 ml of fresh brain heart infusion broth and stirred vigorously with a vortex mixer for 2 min. for dispersion sessile or adherent cells. Then, the suspension was diluted and plated on nutrient agar plates for bacterial colony counting and compared with original bacterial count before exposing to antimicrobial agents (15).

Detection of biofilm inhibitory concentrations (BICs) and minimal biofilm eradication concentration (MBECs):

A- Biofilm Inhibitory Concentration (BIC):

After incubation the tubes for 24hr. at 37 ^{o}C, the biofilm inhibitory concentration was detected and defined as the lowest concentration of antimicrobial agents which inhibits bacterial biofilm growth on a surface of catheter. It was represented by the clearance of broth medium consisting (1cm) catheters and the required concentrations of antimicrobial agents (16).

B- Minimal Biofilm Eradication Concentration:

After plating the diluted suspension into agar plates and counting the number of bacterial colonies, MBEC was determined. MBEC was defined as the lowest concentration of antibiotic or biocide capable of killing biofilm producer bacteria. It was represented by disappearing of colonies of biofilm producer organisms on the culture plates (16)

Statistical analysis:

All data were analyzed using the SPSS statistical program (statistical Package for the Social Science) version 14.0. Statistical significance was taken with p value < 0.05 and 0.001 . The significant differences were detected by using either the goodness fit test within chi-square test or independent sample-test. All the study graphics (bar chart, scatter diagram or dot chart) were done by using Microsoft Excel XP (17), (18), (19)

RESULTS

Out of 47 upper end of JJ stent, 19 (40.4%) were positive for culture and 28 (59.6%) were negative. Out of 24 microorganisms 19 (79.2%) were biofilm producer isolates while 5 (20.8%) were non biofilm producers. Out of 47 lower ends of JJ stent, 16 (34%) were positive for culture and 31(66%) were negative. 16 microorganisms were biofilm producer isolates while 5 were not.

Out of 94 JJ stent samples, distributed in to 47 (50%) were from upper end of the stent and 47(50%) were from lower end of stent. Among 94 JJ stents, 35 (37.2%) were infected. With regard to the duration of JJ stents used, among 35 (37.2%) infected ones, 2 (11.1%) in the duration range from (1-30) day, 8 (30.8%) were with (31-60) day, 18 (42.9 %) were with (61-90) day and 7 (87.5%) in duration of insertion more than 90 day. Figure (1).

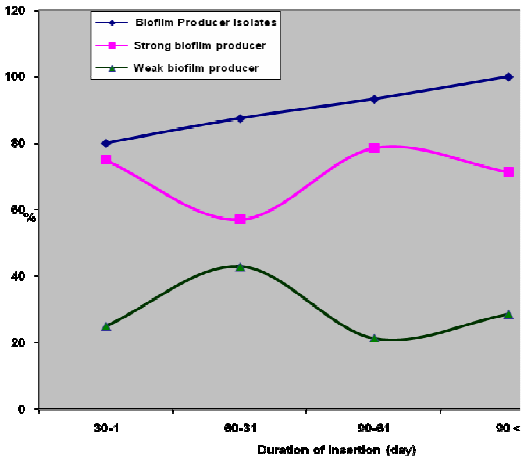

Figure (1): Biofilm production versus duration of insertion (days) for patients with infected JJ stent

Among infected JJ stent, 10 (22.2%) *Klebsiella pneumonia* and 10 (22.2%) *Candida albicans* were the most common isolated microbes from JJ stent. Out of 10 *Klebsiella pneumonia* the biofilm was produced strongly in 6 (60%) isolates, and weakly in 2 (20%), while the others were non biofilm producer isolates. Regarding to *Candida albicans*, out of 10 the biofilm was produced strongly in 6 (60%) isolates and weakly in 2 (20%) while the others were non biofilm producer isolates. Also, *Staphylococcus epidermidis* in 8 (17.9%), 5 (62.5%) isolates from upper end of stent and 3 (37.5%) isolates from lower end of stent, out of 8 *Staphylococcus epidermidis* the biofilm was produced strongly in 5 (62.5%), and weakly in 2 (25%), while the other was non biofilm producer isolates. out of 6 (13.3%) *Staphylococcus aureus* the biofilm was produced strongly in 3 (50%) and weakly in 2 (33.3%) while the others were non biofilm producer isolates. out of 6 (13.3%) *Pseudomonas aeruginosa* the biofilm was produced strongly in 2 (33.3%) and weakly in 2 (33.3%) while the others were non biofilm producer isolates. Out of 5 (11.1%) *Escherichia coli*, the biofilm was produced strongly in 2 (40%) and weakly in 1(20%) while the others were non biofilm producer isolates.

In this study, 5 renal stones were positive for culture and 13 were not and all the isolates were biofilm producers. At the same time out of 18 catheter urine sample obtained from lower urinary tract 4 (22.2%) were positive. Of these specimens, biofilm was produced in 3 (75%) isolates.

Out of 18 stones, 5 (27.8%) were infection stones while the other 13 (72.2%) were non infection stones from upper urinary tract. out of 18 urine specimens obtained from bladder with infection stones, 4 (22.2%) were infected urine with infection stones, 14 (77.8%) were non infected urine with non-infection stones and infection stones. Among infection stones *Klebsiella pneumonia* were 5 (71.4%), 3 (60%) from upper urinary tract and 2 (40%) obtained from bladder urine, followed by 4 (28.6%) *Proteus mirabilis*, were 2 (50%) from upper urinary tract and 2 (50%) obtained from bladder urine. Out of 5 Klebsiella pneumonia the biofilm was produced strongly in 3 (60%) and weakly in 1 (20%) and other non-biofilm producer, out of 4 *Proteus mirabilis* the biofilm was produced strongly in 3 (75%) and weakly in 1 (25%).

In this study, mixed microbial infection was found in ten cases in patients with obstructive uropathy due to renal stones at which the fungus, *Candida albicans*

was shared with both of*Klebsiella pneumoniae* and with *Staphylococcus epidermidis* in four cases and with *Pseudomonas aeruginosa* in two cases.

Quantitative biofilm production (Spectrophotometric assay):

In the Quantitative biofilm formation assay, a spectrophotometric method was done with the presence of glucose. In the most scientific method, spectrophotometric assay with ELISA reader our result showed that out of 20 (27.8%) isolates of *Klebsiella pneumonia*, 14 (19.4%) of *Staphylococcus aureus*, 10 (13.9%) of *Staphylococcus epidermidis*, 10 (13.9%) of *Candida albicans*, 6 (8.3%) of *Escherichia coli*, 6 (8.3%) of *Pseudomonas aeruginosa*, 5(7%) of *Proteus mirabilis* and 1 (1.4%) of *Streptococcus faecalis* biofilm phenomenon was observed in 16 (80%), 11 (78.6%), 9 (90%), 8 (80%), 3 (50%),4 (66.7%), 5 (100%) and 1 (100%) respectively.

Antimicrobial susceptibility test for planktonic cells:

Broth macrodilution technique:

In this part of study, antimicrobial susceptibility test for selected antimicrobial agents against logarithmic phase planktonic cells of study isolates obtained from patients with double J stent and infection stones was detected by detection of MICs according to the criteria laid down by National Committee for Clinical Laboratory Standard, (2001) by using an international quality isolate of *Pseudomonas aeruginosa* American Type Culture Collection (ATCC 27853) and isolate of *Staphylococcus aureus* American Type Culture Collection (ATCC 25923). Our result showed that MICs were 6.5± 7.14 , 22± 12, 6± 2.3 16± 0.0, 32± 0.0, 4± 0.0, 7.2± 6.5, 10± 4.0 and 6± 2.3 for ciprofloxacin, cefotaxime and amikacin respectively against logarithmic phase planktonic cells of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* obtained from patients with JJ stent. Furthermore, our result showed that MICs were 14.4± 3.6, 19.2± 12.1 and 8.8± 4.38 for ciprofloxacin, cefotaxime and amikacin respectively against logarithmic phase planktonic cells of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones.

Regarding biofilm antimicrobial susceptibility test, as far as ciprofloxacin is concerned, the BIC of ciprofloxacin against study isolates (A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) obtained from patients with JJ stent were 50 (50 XMIC), 800 (50 XMIC), 200 (50 XMIC), 160 (10 XMIC), 800 (50 XMIC), 80 (10 XMIC), 100 (100 XMIC), 100 (100 XMIC), 800 (50 XMIC), 400 (50 XMIC) µg/ml, respectively. Further, the MBEC of 500 (500 XMIC), 8000 (500 XMIC), 2000 (500 XMIC), 16000 (1000 XMIC), 8000 (500XMIC), 800 (100X MIC), 1000 (1000 XMIC), 1000 (1000 XMIC), 8000 (500XMIC), 4000 (500 XMIC) µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of ciprofloxacin, against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of ciprofloxacin against study isolates (A22, A24, A25, A26 and A27) were 800 (50X MIC), 1600 (100X MIC), 400 (50X MIC), 800 (50X MIC), 800 (50X MIC) µg/ml respectively. On the other hand, the MBEC of 8000 (500X MIC), 16000 (1000X MIC), 4000 (500X MIC), 8000 (500X MIC), 16000 (1000X MIC) µg/ml were enough to eradicate biofilm of above mentioned isolates respectively.

The result of third generation cephalosporins, cefotaxime against sessile cell of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* obtained from patients with JJ stents, the BIC of cefotaxime against study isolates(A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) were 800 (100X MIC), 1600 (50X MIC), 1600 (100X MIC), 3200 (100X MIC), 3200 (100X MIC), 1600 (50X MIC), 4000 (500 X MIC), 1600 (100X MIC), 400(50X MIC), 800 (100X MIC), µg/ml

respectively. On the other hand, the MBEC of 4000 (500X MIC), 8000 (500X MIC), 8000 (500X MIC), 32000 (1000X MIC), 16000 (500X MIC), 32000 (1000X MIC), 4000 (500X MIC), 16000 (1000X MIC), 8000 (1000X MIC), 4000 (500X MIC), µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of third generation cephalosporins, cefotaxime against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of cefotaxime against study isolates (A22, A24, A25, A26 and A27) were 400 (50X MIC), 3200 (100X MIC), 1600 (100X MIC), 1600 (50X MIC), 400 (50X MIC), µg/ml respectively. On the other hand, the MBEC of 8000 (1000X MIC), 32000 (1000X MIC), 8000 (500XMIC), 32000 (1000X MIC), 4000 µg/ml were enough to eradicate biofilm of above mentioned isolates respectively.

The result of amikacin against sessile cell of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* obtained from patients with JJ stents, the BIC of amikacin against study isolates (A1, A7, A10, A11,A12, A13, A14 , A15, A20 and A22) were 400 (50X MIC), 200(50X MIC), 40 (10X MIC), 400(50X MIC), 200 (50X MIC), 400 (100X MIC),400 (50X MIC), 80 (10X MIC), 400 (50X MIC), 200(50X MIC), µg/ml respectively. On the other hand, the MBEC of 4000 (500X MIC), 2000 (500X MIC), 4000 (1000X MIC), 8000 (1000X MIC), 2000 (500X MIC), 4000 (1000X MIC), 4000 (500X MIC), 8000 (1000X MIC), 4000 (500X MIC), 2000 (500X MIC), µg/ml were enough to eradicate biofilm from catheters for above mentioned isolates respectively.

The result of amikacin against sessile cell of *Proteus mirabilis* and *Klebsiella pneumonia* obtained from patients with infection stones , the BIC of amikacin against study isolates (A22, A24, A25, A26 and A27) were 400 (100X MIC), 800 (100X MIC), 800 (100X MIC), 160 (10X MIC), 80 (10X MIC), µg/ml respectively. On the other hand, the concentrations of 4000 (1000X MIC), 8000 (1000X MIC), 4000 (500X MIC), 16000 (1000X MIC), 8000 (1000X MIC), µg/ml were enough to eradicate biofilm of above mentioned isolates respectively. Table (1)

DISCUSSION

It is well realized that the prognosis of catheter-associated infection is complicated due to the occurrence of chronic or recurrent UTIs, complicated UTI, and pyelonephritis. If left untreated, these infections can lead to abscess formation, renal obstruction, and scarring and eventually will lead to bacteremia, sepsis, and, possibly, death. These infections are difficult to treat due to the presence of biofilms and crystals that protect uropathogens from proper treatment. Only after the complete eradication of biofilm and crystals in the urinary tract can a catheter-associated infection be eliminated (20).

It is well know that ureteric stent, is a thin tube inserted into the ureter to prevent or treat obstruction of the urine flow from the kidney. The presence of biofilm in particular with urease-producing bacteria, leads to the hydrolysis of urea, an elevation of urinary pH and the deposition of struvite and calcium phosphate encrustation on these stents. Clinically, encrustation and infection of indwelling stents are associated with pain, haematuria, blockage and sepsis (21). In this study, patients with obstructive uropathy who are submitted to JJ ureteric stent were one of the study groups. Among this group, a total of 94 JJ stent samples in 47 patients were studied, half of them from the upper end of urinary tract and the other half from the lower end.

Regarding to the infection associated with JJ stents and it's relation to the duration of insertion, our result revealed that out of 35 indwelling JJ stents studied, 2 were with (1-30) day duration of insertion, 8 were with (31-60) day duration of insertion , 18 were with (61-90) day duration of insertion and 7 were with > 90 day duration of insertion. In a study designed by Kliś and co-workers (22), these researchers documented that stent cultures were negative in all patients that had their stent kept for shorter than 30 days. There were 25%

positive stent cultures in case of the stents maintained for the period longer than 30 days, but not longer than 90 days, and 45% in case of catheters kept for longer than 90 days. In another study designed by Riedl and associates (23), 93 ureteral stents from 71 patients were examined. Nine patients with permanent ureteral stenting due to malignant ureteral obstruction, and 62 patients with temporary ureteral stents. Bacteruria and bacterial stent colonization were found in all patients with permanent stents. In patients with temporary stents, colonized stents were found in 69.3%.

Kumar and associates (24) recommended that early removal at 2 weeks is advisable and the short duration of stenting is effective and saves the cost of repeat hospitalization at a later date. Laube and associates[25] mentioned that the stent is a double-edged weapon and it may behave as a friend or an enemy.

Table (1): The biofilm inhibitory concentrationsand minimal biofilm eradication concentration for amikacin against biofilm producing isolates among study specimens.

Isolate no.	Colony count for control (CFU/ml)	Biofilm Inhibitory Concentration (BIC)			Minimal Biofilm Eradication Concentration (MBEC)		
		No. of folds higher than MIC	Conc. µg/ml	Colony count CFU/ml	No. of folds higher than MIC	Conc. µg/ml	Colony count CFU/ml
The result of JJ stent specimen							
***Staphylococcus epidermidis*(A1, A7, A13) &*Staphylococcus aureus*(A15)**							
A1	66 X10^5	50X	400	34 X10^2	500X	4000	7
A7	80X10^5	50X	200	57X10^2	500X	2000	2
A13	91X10^5	100X	400	33	1000X	4000	Zero
A15	46 X10^5	10X	80	39	1000X	8000	Zero
***Pseudomonas aeruginosa* (A10 ,A14) , *Escherichia coli* (A11,A12)&*Klebsiella pneumonia* (A20, A22)**							
A11	33 X10^6	50X	400	31	1000X	8000	Zero
A12	57X10^5	50X	200	43	500X	2000	Zero
A10	30X10^5	10X	40	33	1000X	4000	Zero
A14	61 X10^6	50X	400	50 X10^2	500X	4000	Zero
A20	80X10^5	50X	400	40X10^2	500X	4000	2
A22	91X10^5	50X	200	34X10^2	500X	2000	3
The result of infection stones							
pneumonia* (A24,A25,A26) *Proteus mirabilis* (A22, A27)*Klebsiella							
A22	77X10^5	100X	400	33	1000X	4000	Zero
A24	90X10^6	100X	800	40X10^2	1000X	8000	2
A25	80X10^5	100X	800	34X10^2	500X	4000	1
A26	57X10^5	10X	160	33	1000X	16000	Zero
A27	66X10^5	10X	80	43	1000X	8000	Zero

The organisms commonly contaminating these devices and developing biofilms are *Escherichia. Coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis* and other gram-negative organism. In this study, out of 94 JJ stent sample, 35 (37.2%) were infected. Among infected JJ stent, the most commonly isolated microorganisms were *Klebsiella pneumonia* and *Candida albicans* followed by *Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. In other study designed by Yeniyol and co-workers (26) these researchers revealed that out of 11 patients, bacterial colonization of JJ stents were observed. In 10 of them both stent and urinary cultures were positive, and showed identical microorganisms; 80% *Escherichia coli*, 10% *Escherichia coli* + *Candida*, 10% *Klebsiella*. Kehinde and associates, (27) showed that of 250 patients requiring JJ stent insertion was investigated microbiologically prior to stent insertion and on the day of stent removal. One hundred four stents (41.6%) were culture positive and 146 (58.4%) were culture negative. The commonest isolates were *Escherichia coli, Enterococcus* spp., *Staphylococcus* spp., *Pseudomonas*, and *Candida* spp. Also, Meir and co-workers, (28) showed that out of 82, 58 (70.7%) were culture positive and 24 (29.3%) were culture negative .The commonest isolates were *Staphylococcus* (coagulase negative and positive), *Enterococci* and *Proteus*.

With regard to other study specimen group, renal calculi, Margel and associates (29) documented that renal calculi pathogens are one of the predisposing factors for infectious events. Intra-operative stone culture may be essential in directing the antibiotic regimen postoperatively and should be routinely used. Infected stones function as a sanctuary for organisms and may attenuate the effects of antibiotics against them causing persistent infection (30). Incorporation of urea splitting bacteria within the developing struvite stones as well as calcium oxalate stones that had become secondarily infected results in a focus of infection that is resistant to conventional antimicrobial therapy and manifested clinically by repeated urinary tract infections caused by the infecting organism, therefore complete removal of all the infected stones material is considered to be essential for the eradication of persistent Bacteriuria associated with the infected renal calculi (31).

Margel and associates (29) revealed that out of 75 patients, 33 (44%) had sterile urine and stone cultures while both urine and renal stones were colonized in 23 patients (30.7%). Also, a colonized stone culture associated with a sterile urine culture was found in 19 patients.

Our study showed that out of 18 patients, 5 (27.8%) had infected stones cultures while 13 (72.2%) patients were with sterile urine and stones cultures. Among infection stones, *Klebsiella pneumonia* was the most predominant which was isolated from 5 (71.4%) cases, 3 (60%) of them from upper urinary tract and 2 (40%) obtained from bladder urine followed by *Proteus mirabilis* 4 (28.6%), half of them from upper urinary tract and 2 (50%) obtained from bladder urine. In 4 (80%) of them, the urine and stone cultures showed similar pathogens. A colonized stone culture associated with a sterile urine culture was found in 1 (20%) stone.

Studies of biofilm formationhave primarily focused on biofilms formed by a single speciesof microorganism (32). However,biofilms are thought to be composed of more than one species.Although implant-associated infections involving both bacteria and fungi are not uncommon (33), mixed species biofilms of this type have been studied relatively little. In this study, mixed microbial infection was found in ten cases in patients with obstructive uropathy at which the fungus, *Candida albicans* was shared with both *Klebsiella pneumoniae* and *Staphylococcusepidermidi*s in four cases and with *Pseudomonas aeruginosa* in two cases.

Adam and associates (34) showed that extensive interactions between *Staphylococcusepidermidi*s and *Candida*

albicans have been demonstrated in biofilms containing both organisms. In mixed fungal-bacterial biofilms, both staphylococcal strains showed extensive interactions with *C. albicans*. The researchers concluded that the extracellular polymer produced by *S. epidermidis* RP62A could inhibit fluconazole penetration in mixed fungal-bacterial biofilms. Conversely the presence of *C. albicans* in a biofilm appeared to protect the slime-negative staphylococcus against vancomycin. Overall, the findings suggest that fungal cells can modulate the action of antibiotics, and that bacteria can affect antifungal activity in mixed fungal-bacterial biofilms. *Pseudomonas aeruginosa* and *Candida albicans* are consistently identified as some of the more important agents of nosocomial infections. In light of the recent information regarding device-associated nosocomial infections, understanding the nature of *P. aeruginosa* and *C. albicans* infections is increasingly important. These two microorganisms demonstrate: (1) an ability to form biofilms on the majority of devices employed currently, (2) increased resistance/tolerance to antibiotics when associated with biofilms, (3) documented infections noted for virtually all indwelling devices, (4) opportunistic pathogenicity, and (5) persistence in the hospital environment (35).

Regarding to quantitative biofilm production, the study results showed that *Proteus mirabilis* was the most common microbial isolate produced biofilm phenomenon (crystalline biofilm)5 (100%), followed by *Staphylococcus epidermidis*, *Candida albicans*, *Klebsiella pneumonia, Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* respectively. The tissue culture plate method was found to be most sensitive, accurate and reproducible screening method for detection of biofilm formation by microorganisms and has the advantage of being a quantitative model to study the adherence of microorganisms on biomedical device (36).

With regard to antimicrobial susceptibility test for planktonic cells, numerousstudies have now demonstrated that biofilm-grown microorganismshave an inherent lack of susceptibility to antibiotics, whereasplanktonic cultures of this same organism do not(37). This resistance is lost once thebiofilm is reverted to conditions that permit planktonic growth. The innate tolerance of microbial biofilms to antibiotictherapy has led to problems in their eradication and inthe management of patients with device-related infections. This difference in antibiotic susceptibility betweenplanktonic and biofilm populations of the same organism may resultfrom differences in the diffusion of antibiotics or muchmore complex changes in the microbial physiology of the biofilm (38). The slow growth of biofilm bacteria and exopolysaccharide or glycocalyx acting as a barrier to the penetration of antibacterial agents are considered responsible for failure of antimicrobial therapy of these infections (39).

In the field of antimicrobial susceptibility test for sessile cells of study bacterial isolates obtained from JJ stentand renal stones, our result revealed that the biofilm cells were required 50-100 times the MIC values for ciprofloxacin obtained for the same isolates in logarithmic phase of planktonic cells in each of the two specimens. Regarding to cefotaxime, our result showed that the sessile cells of study bacterial isolates obtained from JJ stent were required 50-500 X MIC values for cefotaxime in comparison with those observed with other study specimen, infection stones which required 50-100 MIC to eliminate bacterial biofilm for the same antimicrobial agent with high statistical significant differences.

On the other hand, the biofilm cells wererequired 10- 100 times the MIC values for amikacin against bacterial isolates obtained from JJ stent and infection stones.The high concentrations of quinolones were used to treat the preformed biofilms because 1) these concentrations would be expected to reach the biofilms when therapeutic doses of quinolones are infused through the vascular catheters and, 2) using such high concentrations would

minimize the exposure of very large inoculum of bacteria in the biofilms to sub-inhibitory concentrations of the quinolones (40).It is well known that flouroquinolones are indeedvery effective in stopping the growth of a biofilm (41). Atthe same time, restricted diffusion can protect the biofilm froma degradable antimicrobial. Retarded diffusion will decrease theconcentration of the antibiotic entering the biofilm, helpingan enzyme like β-lactamase destroy the incoming antibiotic (42). A restricted penetrationof this small molecule coupled to its destruction by the microbialcells was apparently responsible for resistance. It can be expectedthat any mechanism of antibiotic destruction or modification (likeacetylation of aminoglycosides) will be especially effective whencoupled with a diffusion barrier of the biofilm. The MIC for biofilm cells is higher than that for planktoniccells (42).

The administration of a minimal biofilm eradicating concentration (MBECs) greater than the MIC of planktonic cells has been stressed in order to prevent bacterial biofilm cells from expressing antibiotic-resistant factors (33).

The study concluded that *Klebsiella pneumonia* and *Candida albicans*were the most common microorganisms isolated from infected JJ stent. Also, among infection stones, *Klebsiella pneumonia* was the most predominant microorganism followed by *Proteus mirabilis*. Mixed biofilm species consist largely of *Candida albicans* and other bacteria like *Klebsiella* spp., *Staphylococcus epidermidis* and *pseudomonas aeruginosa* play an important role in the formation of biofilm on JJ stent. Further, with the passage of time (duration of insertion), double J stent will be more predisposing to microbial colonization and eventually leading to biofilm associated JJ stent infection. Furthermore, In biofilm antimicrobial susceptibility test, the biofilm producer study isolates were required lower concentration of amikacin to remove bacterial biofilm from JJ stents and infection stones in comparison to ciprofloxacin and cefotaxime.

REFERENCES

1. Costerton, JW., Cheng, K J., Geesey, GG., Ladd, T I., Nickel, J C., Dasgupta, M. and Marrie, TJ.(1987). Bacterial biofilms in nature and disease. J. Ann. Rev. Microbiol.(41):435–464.

2. Darouiche, RO. (2001). Device-associated infections: a macroproblem that starts with microadherence. J. Clin. Infect. Dis. (33):1567–1572.

3. Habash, M. and Reid, G. (1999). Microbial biofilms: their development and significance for medical device-related infections. J. Clin. Pharmacol. (39): 887-898.

4. Riedl, CR., Witkowski, M., Plas, E. and Pflueger, H.(2002). Heparin coating reduces encrustation of ureteral stents: A preliminary report. Int .J Antimicrob Agents.(19):507–512.

5. Choong, S., Wood, S., Fry, C.and Whitfield, H. (2001). Catheter associated urinary tract infection and encrustation. Int. J. Antimicrob. Agents.(17):305–310.

6. Stickler, D., Ganderton, L., King J., Nettleton, J and Winters, C.(1993) *Proteus mirabilis* biofilm and the encrustation of urethral catheters. J. Urol Res(21):407–412.

7. Warren, JW (1994). Catheter associated bacteriuria in long-term care facilities.J. Infect. Control Hosp. Epidemiol. (15):557-562.

8. Griffith, DP. and Osborne, CA. (1987). Infection (ureaase) stones. J. Mineral and electrolyte metabolism. (13):278–85.

9. Fux, CA., Stoodley, P., Hall- Stoodley, L. and Costerton, JW. (2003). Bacterial biofilms: adiagnostic and therapeutic challenge. Expert. Rev. Anti-infect. ther., (1):667-683.

10. Baron, EJ., Peterson, LR. and Finegold, SM. (1994). Bailey and Scott'

s Diagnostic Microbiology. Toronto: C.V. Mosby Company . 9th edition .

11. Mireles, JR., Toguchi, A and Harshy, RM (2001). *Salmonella enterica* serovar *typhimurium* swarming mutent with altered biofilm forming abilities: Surfactin inhibits formation . J. Bacteriol. (183): 5848-5854.

12. Vandepitte, J., El-Nageh, M., Tikhomiros, E. and Stelling, JM. (1996). Guidelines for antimicrobial resistance surveillance. World Health Organization. Alexandria, Egypt.

13. Ferraro, MJ., Craig, W A., Dudly, M N., Ehopoulos, GM., Hecht, DW. (2000). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard, 5 th edition. NCCLS. (20): 1-24.

14. Ohgaki, N. (1994). Bacterial biofilm in chronic airway infection. J. Jpn. Assoc. Infect. Dis., (68): 138-151.

15. Ishida, H., Ishida, Y., Kurosaka, Y., Otani, T., Sato, K. and Kobayashi, H. (1998). In vitro and In vivo activities of levofloxacin against biofilm-producing *Pseudomonas aeruginosa*. J. Antimicrob. Agents. Chemother. (42): 1641-1645.

16. Ceri, H., Olson, ME.,Morck, DW. and Storey, DG(2006). Minimal Biofilm Eradication Concentration (MBEC) Assay: Susceptibility Testing for Biofilms. London. Taylor and francis group. 136-142

17. Glantz, Z. and Stanton, A. (2002). Primer of biostatistics: Biostatistics and clinical practice. 5th edition.229-239

18. Chap, TLE. (2003). Introductory biostatistics. John Wiley and Sons. Publication. 423-510

19. Simon, SD. (2006). Statistical evidence in medical trials. What do the data really tell us? Oxford University Press. 501-509

20. Jacobsen, SM., Stickler, DJ., Mobley, HLT. and Shirtliff, ME. (2008). Complicated Catheter-Associated Urinary Tract Infections Due to *Escherichia coli* and *Proteus mirabilis*. J. Clinical microbial reviews. (21):26-59.

21. Choong, S. and Whitfield, H. (2000). Biofilms and their role in infections in urology. J. BJU International. (86): 935-941.

22. Kliś, R., Korczak-Kozakiewicz, E. and Różański, W. (2006). Urinary tract infections and bacterial colonization of Double-J catheters. J. Urologia polska. (62): 93-513.

23. Riedl, CR., Plas, E., Hübner, WA., Zimmerl, H., Ulrich, W. and Pflüger, H. (1999) . Bacterial colonization of ureteral stents. J.Endocrinol. (36): 53-59.

24. Kumar, A., Verma, BS., Srivastava, A., Bhandari, M., Gupta, A. and Sharma, R. (2000). Evaluation of the urological complications of living related renal transplantation AT a single center during the last 10 years: Impact of the double-J stent. J. The Journal of Urology. (164): 657- 660.

25. Laube, N., Kleinen, L., Bradenahl, J. and Meissner, A. (2007). Diamond-Like Carbon Coatings on Ureteral stents—A New Strategy for Decreasing the Formation of Crystalline bacterial Biofilms? J. urology. (177): 1923-1827.

26. Yeniyol, CO., Tuna, A., Yener, H., Zeyrek, N., Tilki, A. and Coskuner, A. (2004). Bacterial colonization of double J stents and bacteriuria frequency. J. International Urology and Nephrlogy.(34): 199-202.

27. Kehinde, E O.,. Rotimi, VO., Al-Hunayan, A., Abdul-Halim, H., Boland, F.and Al-Awadi, KA. (2004). Bacteriology of urinary tract infection associated with indwelling J ureteral stents. J Endourology. (18): 891-896.

28. Meir, D. B., Golan, S., Ehrlich Y.and. Livne, P.M. (2009). Characteristics and clinical significance of bacterial colonization of ureteral double-J stents in children. J. Pediatric Urology.

29. Margel, D., Ehrlich, Y., Brown, N., Lask, D., Livne, PM. and Lifshitz, DA. (2006). Clinical implication of routine stone culture in percutaneous nephrolithotomy—a prospective study . J. Urology. (67): 26-29.

30. Schwartz, BF. and Stolle, ML. (1999). Non-surgical management of infection – related renal calculi . J. Urol. Clin. North Am. (26): 765-78.

31. Riad, EM., Roshdy, M., Ismail, M A., El-Leithy, TR., EL. Ghoubashy, S., El Ganzoury, H., El Baz, AG. and Kamel, AI.(2008). Extracorporeal Shock wave Lithotripsy (ESWL) Versus Percutaneous Nephrolithotomy (PCNL) in the Eradication of Persistent Bacteruria Associated with Infected Stones. J. Basic and Applied Sciences, (2): 672-676.

32. Kolter, R., and Greenberg, EP. (2006). The superficial life of microbes. J. Nature (441):300-302.

33. Costerton, JW., Stewart, PS. and Greenberg EP.(1999). Bacterial biofilms: a common cause of persistent infections.J. Science(284):1318-1322.

34. Adam, B., Baillie, GS. and Douglas, LJ. (2002). Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. J. Antimicrobial agents. (51) : 344–349.

35. Pierce, GE. (2005). *Pseudomonas aeruginosa*, *Candida albicans*, and device-related nosocomial infections: implications, trends, and potential approaches for control. J. Industrial Microbiology and Biotechnology. (32): 309-318.

36. Mathur, T., Singhal, S., Khan, S., Upadhyay, DJ., Fatma, T. and Rattan, A.(2006). Detection of biofilm formation among the clinical isolates of Staphylococci: An evaluation of three different screening methods. Ind. J. Med. Microbiol. (24): 25-29.

37. Larsen, T., and Fiehn, NE.(1996). Resistance of *Streptococcus sanguis* biofilms to antimicrobial agents. APMIS (104):280-284.

38. Davies, DG., Parsek, MR., Pearson, JP., Iglewski, BH., Costerton, JW. and Greenberg EP. (1998). The involvement of cell-to-cell signals in the development of bacterial biofilms. J. Science (280):295-298.

39. Huang, C., Xu, KD., McFeters, GA. and Stewart, PS.(1998). Spatial pattern of alkaline phosphatase expression within bacterial colonies and biofilms in response to phaophate starvation. J. Appl. Env. Microb.(64): 1526–1531.

40. Yassien, M. and Khardori, N.(2001). Interaction between biofilms formed by *Staphylococcus epidermidis* and quinolones. J. Diag. Microbiol. Infect. Dis. (40): 79-89.

41. Brooun, A., Liu, S. and Lewis, K. (2000). A dose-response study of antibiotic resistance in *Pseudomonas aeruginosa* biofilms. J. Antimicrob. Agents Chemother. (44):640-646.

42. Lewis, K. (2001).Riddle of Biofilm Resistance . J. Antimicrobial Agents and Chemotherapy. (45) : 999-1007.