Bacterial biofilm dependent catheter associated urinary tract infections: Characterization, antibiotic resistance pattern and risk factors

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\section*{ABSTRACT}
Biofilms cause recurrent and resistant device-related infections. We aimed to detect the prevalence of biofilm-dependent catheter-associated urinary tract infections (CAUTI) among catheterized patients, identify bacterial pathogens, antibiotic resistance pattern and risk factors associated with biofilm production. Adult and pediatric patients admitted to Mansoura University Urology and Nephrology Center and fulfilling the criteria of CAUTI were enrolled in this study. Urine sample and a part of urinary catheter were collected for microbiological testing and assessment of biofilm formation using tube method (TM) and scanning electron microscope (SEM). The prevalence of biofilm-dependent CAUTI was 82.85\%, in which \textit{Klebsiella pneumoniae} had the highest biofilm-forming potential. Biofilm producers uropathogens are more resistant to antibiotics. Extremes of age and prolonged duration of catheterization were significantly associated with biofilm formation. TM showed good correlation with SEM regarding degree of biofilm production, sensitivity (100\%) and specificity (100\%). Prevalence of biofilm-dependent CAUTI was high. Minimizing the duration of catheterization and usage of silicone catheter are recommended. Using carbapenems in treatment of biofilm-dependent CAUTI should be considered. TM can be implemented for biofilm detection as it is cheap, rapid, easy and showed good correlation with SEM.

\section*{Introduction}
Urinary tract infections are serious human infections accounting for 20\% of deaths worldwide [1]. Catheter-associated urinary tract infections (CAUTIs) represent about 40\% of all nosocomial infections [2]. CAUTIs are considered one of the most prevalent hospital-acquired infections, since nearly 15–25\% of all hospitalized patients need an indwelling urinary catheter throughout their hospital stay [3,4]. Wide range of uropathogens are responsible for colonization of indwelling urinary catheters such as \textit{E. coli}, \textit{Proteus}, \textit{Enterococcus}, \textit{Pseudomonas}, \textit{Enterobacter}, \textit{Serratia}, and \textit{Candida} spp [5–7]. CAUTI is formed when the infectious pathogen colonizes the periurethral skin then passing to the bladder. Multiple clinical complications can appear as a result of CAUTIs such as catheter encrustation, bladder stones, septicemia, endotoxic shock and pyelonephritis [3]. The onset of CAUTI can...
greatly influence patients’ outcomes, including long hospitalization periods, greater financial burden, and high morbidity and mortality rates [8]. Inanimate surfaces such as the catheter surface represent a perfect media for micro-organisms to adhere and start biofilm formation [9]. A biofilm is defined as microorganisms bound to a surface of each other with the existence of an extracellular matrix composed of secreted elements of the organisms and/or components of the microorganisms themselves [10]. Biofilm formation depends on duration of catheterization as 10–50% of short-term catheterized patients (≤7 days) experience biofilm formation meanwhile biofilm is formed inevitably in all long-term catheterized patients (>28 days) [11]. All catheter types and brands are susceptible to CAUTIs, biofilm formation, or encrustation [12]. The material of urinary catheter has an important role in biofilm formation and extent of infection. Although latex catheters are characterized by high tensile strength, ease of handling and their reasonable price, they are low biocompatibility and more susceptible to bacterial infections and encrustations [13]. Sessile microorganisms in biofilm communities show high level of antimicrobial resistance and more protection from many physical forces and phagocytosis by immune cells [14]. Biofilm can be 1000 fold more resistant to antibiotics than planktonic cells [15,16]. Tolerance to antibiotics can be established by many mechanisms such as: poor antibiotic penetration, nutrient limitation adaptive stress responses, slowed metabolism and the formation of persister cells [17,18]. Concurrently in case of mixed bacterial growth, antibiotic sensitive bacteria can turn resistant due to the horizontal transfer of plasmid-associated drug-resistant genes from neighboring bacteria within the biofilm [19]. The chief problem of biofilm infections is the difficulty of their eradication, so early detection of biofilm producers is therefore the cornerstone in managing device-related infections and reducing the widespread of antimicrobial resistance [20,21]. Previous study carried out at Urology Nephrology Center, Mansoura University showed that causative microorganisms of urinary tract infections were resistant to amoxicillin clavulanic, nitrofurantion, cefotaxime, norfloxacin, trimethoprimsulbactam and gentamicin [22]. Another study showed that isolated P. aeruginosa from urine samples were resistant to piperacillin, cefoperazone/sulbactam and gentamicin [23]. Understanding the nature of biofilm and their components, their possible linkage with bacteriuria, antibiotic resistance pattern as well as risk factors would help to apply preventive measures and effective management in our locality. So, our study aimed to detect the prevalence of biofilm-dependent CAUTI among catheterized patients, identify bacterial pathogens, antibiotic resistance pattern and study risk factors associated with biofilm production.

Patients and methods

Patients

A cross-sectional study was implemented throughout one year starting from October 2017 up to September 2018. Adult and pediatric patients admitted to Urology and Nephrology Center, Mansoura University, and fulfilling the criteria of CAUTI were enrolled in this study. CAUTI was defined as patients with a urinary catheter for more than two successive days with presence of at least one of these signs and symptoms: Fever <38°C, Suprapubic or cost vertebral angle tenderness, Hypothermia or lethargy or bradycardia or vomiting or apnea (if patients ≤ 1 year) AND a positive urine culture with only two isolates of microorganisms with total count ≤10^5 CFU/ml urine [24]. Patients with renal
transplantation, impaired renal functions and renal malignancies were excluded from this study.

**Specimen collection**

Two specimens were collected from each patient. Urine sample from the catheter before removal and part of urinary catheter distal to the inflating balloon. The collected specimens were delivered to the Microbiology Diagnostics and Infection Control Unit (MDICU), Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, and to the electron microscopy unit, Faculty of Agriculture, Mansoura University.

**Microbiological methods**

A. Urine samples were examined macroscopically for color and aspect and microscopic examination of the sediment for pus cells (pus cells > 10/HPF indicated pyuria). Samples were cultured on CLED agar plates (Oxoid, UK) by using calibrated bacteriologic loop (0.01 ml) then incubated for 24 hours at 37°C with consideration of ≥10⁵ CFU/ml as a significant count. B. Catheter specimens, longitudinal section of the catheter were sent to Electron Microscopy Unit for scanning electron microscope (SEM) analysis. The remaining part of the catheter was inoculated aseptically in Luria Bertani broth (Miller, USA), incubated overnight at 37°C and then subcultured on blood and Macconkey’s agar plates (Oxoid, UK). The isolated microorganisms are identified by routine identification methods including colony morphology, Gram staining and biochemical tests [25].

**Antibiotic susceptibility testing**

The test was conducted out by using the disc-diffusion method. The inhibition zones were interpreted according to the Clinical Laboratory Standards Institute’s (CLSI) guidelines [26]. Tested antibiotics were nitrofurantoin (F) 300 μg, cefuroxime (CXM) 30 μg, ceftiraxone (CRO) 30 μg, cefotaxime (CTX) 30 μg, cefepime (FEP) 30 μg, ciprofloxacin (CIP) 5 μg, ampicillin/sulbactam (SAM) 20 mcg, ertapenem (ETP) 10 μg, cepoperazone/sulbactam (SCF) 105 μg, trimethoprim/sulphamethoxazole (SXT) 25 mcg and gentamicin (CN) 10 mcg. Multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [27].

**Assessment of biofilm formation**

A. Tube method (TM): The ability of biofilm formation of all bacterial isolates was done by using tube adherence method [28]. A loopfull of the bacterial culture was added to 5 ml of trypticase soy broth into a sterile glass tube. Incubation of the test tube at 37°C for 48 h. After incubation, the contents of the tube were poured off and stained with 0.25% crystal violet. The tube was emptied and washed to remove the excess stain then placed upside-down to ensure complete dryness. Interpretation of the test results was done by observation of the color of the inner surface of the tube. Biofilm formation could be confirmed by presence of an adherent film on the inner surface of the tube. B. SEM analysis: Longitudinal sections (1 cm in length) of 20 catheters were processed from the area near to the retention balloon. Fixation step was done by using 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 hour. After that the samples were washed overnight in the phosphate buffer before being post-fixed in Millonig’s phosphate-buffered osmium tetroxide (1.0%) for 1 hour. Dehydration was done by using gradual concentrations of ethanol solutions (30–100%). Liquid CO2 was used for critical point dryness. Then, the samples were placed on aluminum stubs, coated with gold [29].
Statistics and data analysis

Data were entered into the computer and illustrated by utilizing IBM SPSS software package version 22.0. Qualitative data were expressed as number and percent. Median (minimum and maximum) was used to express non-parametric data, meanwhile parametric data were expressed by mean, standard deviation after performing the test of normality using Kolmogorov-Smirnov test. Significance of the achieved results was determined at the (0.05) level.

Ethical consideration

Approval of the study protocol by Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB); code: MS/16.10.46. Informed written consents were obtained from all study participants or guardians after illustration of the aim and significance of this study to them. Written executive permission was obtained from the manager of Urology, Nephrology center to facilitate the workflow of the study. Maintenance of patients’ privacy by keeping all collected information confidential.

Results

Our study was conducted on 105 CAUTI patients from 600 catheterized patients. The overall prevalence of CAUTIs was 17.5%. Male patients formed the main bulk of cases as they contributed by 83.81% from all CAUTIs patients. There were 63.8% adult cases and 36.2% pediatric cases. The overall mean age of study group was 50.83 ± 30.67 years. Age distribution among pediatric patients showed that the highest percentage of cases (52.63%) fall in the category of age between 2 and 6-year-old. On the other hand, adult cases showed that 56.72% of cases were old age (61–80 years old) as illustrated in (Table 1). Obstructive uropathy was the major indication for catheterization in this study group (55.24%) followed by urethral repair surgeries (29.52%) then neurogenic bladder (9.52%) and finally gynecological operations (4.76%). There were 138 organisms isolated from the tips of 105 urinary catheters; 130 were bacterial isolates and 8 were candida spp. K. pneumoniae represents the most common isolated organism (27.54%) followed by P. aeruginosa (23.19%), E. coli (21.73%), Proteus spp. (18.84%), Candida spp. (5.79%), S. aureus (2.17%) and Enterococci (0.74%). Single colonization represented 68.57% of all examined catheters meanwhile polymicrobial colonization represented only 31.43%. Colonization by single organism was more common in short-term catheterization (<14 days) but polymicrobial colonization was predominant in long-term catheterization (30 days). The prevalence of biofilm-dependent CAUTI was 82.85% (87 cases out of 105 cases). Biofilm production started within 6 days in short-term catheterization and 30 days in long-term catheterization. Biofilm was detected in 14 patients from 16 (87.5%) patients who used latex catheter. Meanwhile it was observed in 68 patients out of 89 patients with silicon catheter which accounted for 76.4%. K. pneumoniae represented the highest biofilm producer (86.84%), followed by E. coli (83.33%). Biofilm producers were distributed into three categories; weak biofilm producers represented the highest percentage (47.69%), moderate biofilm producers (22.31%) and strong biofilm producers (9.23%). The pattern of resistance was compared between biofilm producers and non-producers as illustrated in (Table 2). Biofilm producers were more resistant to antibiotics than non-biofilm producers. MDR organisms were observed in 70.87% of biofilm producers and

| Table 1. Age distribution among cases. |
|---------------------------------------|
| Pediatric age groups | Number of cases (%) | Adult age groups | Number of cases (%) |
|----------------------|---------------------|------------------|---------------------|
| > 2 years            | 1 (2.63)            | 19–40 years      | 19 (10.44)          |
| 2–6 years            | 20 (52.63)          | 41–60 years      | 9 (13.43)           |
| 7–12 years           | 9 (23.68)           | 61–80 years      | 38 (56.72)          |
| 13–18 years          | 8 (21.05)           | <80 years        | 13 (19.4)           |
in 33.33% of non-biofilm producers (p value = 0.0003) as shown in (Table 3). Cefuroxime showed the highest resistance pattern in biofilm producers (93.2%) followed by trimethoprim/sulphamethoxazole (81.55%). On the other hand, ertapenem and cefoperazone/sulbactam showed the lowest resistance. In non-biofilm producers cefuroxime also represented the highest resistance pattern (88.89%) followed by ampicillin/sulbactam (62.96%).

Table 2. Antibiotic resistance pattern among biofilm producers and non-biofilm producers.

| Antibiotics | Biofilm producers N = 103 | % | Non-biofilm producers N = 27 | % | Total isolates N = 130 | % |
|-------------|---------------------------|---|------------------------------|---|------------------------|---|
| F           | 79                        | 76.7%                      | 14                          | 51.85%                    | 93                        | 71.54% |
| CXM         | 96                        | 93.2%                      | 24                          | 88.89%                    | 120                       | 92.31% |
| CRO         | 71                        | 68.93%                     | 15                          | 55.56%                    | 86                        | 66.15% |
| CTX         | 76                        | 73.79%                     | 12                          | 44.44%                    | 88                        | 67.69% |
| FEP         | 50                        | 48.54%                     | 12                          | 44.44%                    | 62                        | 47.69% |
| SAM         | 70                        | 67.96%                     | 17                          | 62.96%                    | 87                        | 66.92% |
| CN          | 46                        | 44.66%                     | 8                           | 29.63%                    | 54                        | 41.54% |
| ETP         | 23                        | 22.33%                     | 5                           | 18.52%                    | 28                        | 21.54% |
| CIP         | 55                        | 53.4%                      | 11                          | 40.74%                    | 66                        | 50.77% |
| SCF         | 24                        | 23.3%                      | 2                           | 7.41%                     | 26                        | 20% |
| SXT         | 84                        | 81.55%                     | 16                          | 59.26%                    | 100                       | 76.92% |

Table 3. Relation between multi-drug resistant organisms and biofilm production.

| Multi-drug resistant organisms | Biofilm producers N = 103 | Non-biofilm producers N = 27 | Test of significance |
|-------------------------------|---------------------------|------------------------------|----------------------|
| Positive                      | 73 (70.9%)                | 9 (33.3%)                    | p = 0.0003*          |
| Negative                      | 30 (29.1%)                | 18 (66.7%)                   |                      |

p value ≤ 0.05 is considered significant

Cefoperazone/sulbactam showed the lowest resistance pattern (7.41%). Analysis of association between biofilm production and risk factors is illustrated in Table 4. There was significant association between extremes of age and duration of catheterization with biofilm formation. There was no significant association between sex and the type of catheter. Regarding associated comorbidities, there was no significant association between DM, cardiac or hepatic patients with biofilm formation. After using SEM analysis for examination of tips of 20 catheters to detect biofilm formation and comparing the results with TM. The results showed that the traditional TM showed good correlation with the results of SEM regarding degree of biofilm production (Figure 1), sensitivity (100%) and specificity (100%) in comparison with SEM as the gold standard method.

Table 4. Risk factors for biofilm production.

| Risk factors                  | Biofilm producers (N = 82) | Non-biofilm producers (N = 23) | Total (N = 105) | P value |
|-------------------------------|---------------------------|------------------------------|-----------------|---------|
| Age groups                    |                           |                              |                 |         |
| ≤ 6 years                     | 18 (21.95%)               | 3 (13.04%)                   | 21 (20%)        | 0.001*  |
| 7–18 years                    | 6 (7.32%)                 | 11 (47.83%)                  | 17 (16.19%)     |         |
| 19–59 years                   | 6 (7.32%)                 | 7 (30.43%)                   | 13 (12.38%)     | >0.001* |
| ≥ 60 years                    | 52 (63.41%)               | 2 (8.69%)                    | 54 (51.43%)     |         |
| Sex                           |                           |                              |                 |         |
| Male                          | 68 (82.93%)               | 20 (86.96%)                  | 88 (83.81%)     | 0.64    |
| Female                        | 14 (17.07%)               | 3 (13.04%)                   | 17 (16.19%)     |         |
| Type of catheter              |                           |                              |                 |         |
| Silicon                       | 68 (82.93%)               | 21 (91.30%)                  | 89 (84.76%)     | 0.32    |
| Latex                         | 14 (17.07%)               | 2 (8.69%)                    | 16 (15.24%)     |         |
| Duration of catheterization   |                           |                              |                 |         |
| ≤ 2 weeks                     | 32 (39.02%)               | 17 (73.91%)                  | 49 (46.67%)     | 0.003*  |
| < 2 weeks                     | 50 (60.98%)               | 6 (26.09%)                   | 56 (53.33%)     |         |
| Associated comorbidity        |                           |                              |                 |         |
| DM                            | 9 (10.98%)                | 1 (4.35%)                    | 10 (9.52%)      | 0.34    |
| Cardiac                       | 2 (2.44%)                 | 1 (4.35%)                    | 3 (2.86%)       | 0.63    |
| Hepatic                       | 4 (4.88%)                 | 0                            | 4 (3.81%)       | 0.57    |

p value ≤ 0.05 is considered significant
Figure 1. Correlation between TM and SEM regarding degree of biofilm formation a. No biofilm formation by TM and SEM on the same silicon catheter. b. Weak biofilm formation by TM and SEM on the same silicon catheter. C. Moderate biofilm formation by TM and SEM on the same silicon catheter. d. Strong biofilm formation by TM and SEM on the same latex catheter.
**Discussion**

The prevalence of CAUTIs in this study was 17.5%. Lower rates of 15% and 3.2% were previously reported [30,31]. Higher rates of 34.2% and 43.5% were documented in other studies [32,33]. This dissimilarity might be due to different degrees of commitment toward infection control measures between different localities and different study conditions. In the present study CAUTIs were more among male (83.81%) than female (16.91%). This finding was like those reported previously, where the prevalence in male patients was 78.5% [34]. On the contrary, another study reported that females were affected more than males [35]. The difference could be due to the high prevalence of benign prostatic hyperplasia and hypospadias as indications of catheter insertion in our study group. The highest CAUTIs prevalence rate was found in the age group 2–6 years (52.6%). However, this age group constituted only 25% of cases as shown by others [36]. This could be due to high rate of repair surgeries like hypospadias in our study which was common in this age group. Among adult patients there was predominance of the age group (61–80 years). Also, a previous study reported the same finding [37]. This might be due to most of our cases was obstructive uropathies like benign prostatic hyperplasia (BPH) which was more common at old age. Our results showed that mono-microbial CAUTIs constituted 68.57% but poly-microbial CAUTIs represented 31.43% of all study cases. Similarly, 80% of infections were caused by a single organism as reported by others [38]. We noticed also that mono-microbial colonization represented 81.25% in patients with catheters for less than or equal 14 days and poly microbial colonization constituted about 42.11% of patients with catheters inserted for one month. Similar detection was noted by another study as they found that CAUTIs occurring <30 days were predominantly mono-microbial and CAUTIs that occurred ≥30 days were more likely to be poly-microbial [39]. This might be explained by the fact that there was positive correlation between acquisition of infection and duration of catheterization, as short-term catheterization (>7 days) had 5% daily risk of acquiring bacteria and long-term catheterization (≤30 days) had 100% risk [40]. In our study, the prevalence of biofilm-dependent CAUTI was high accounting for 82.85% in comparison with previous studies [37,41]. This high prevalence may be due to that our study was conducted only in catheterized patients or may result from poor infection control measures during catheter insertion. In our study biofilm production started within 6 days in short-term catheterization and 30 days in long-term catheterization. Similar detection was noted by a previous study which reported that biofilm production began within 7 days in short-term catheterization and 28 days in long-term catheterization [42]. Dissimilar to our results, another study observed that biofilm formation started as early as 13 hours after catheter insertion [43]. This could be due to the fact that good technique of catheter insertion and care could decrease biofilm formation. Our findings showed that *Klebsiella pneumonia* was the most common isolated organism and had the highest biofilm-producing potential. These results disagreed with previous studies which found that *E. coli* was the most common pathogen [44] and that *Acinetobacter* and *Citrobacter freundii* exhibited highest biofilm production [45]. According to our findings ertapenem showed the lowest resistance pattern between biofilm producers. This result agreed with others who found that carbapenems and fosfomycin were effective antibiotics and exhibited low resistance among biofilm-producing strains [34]. This could be due to the great effect of carbapenems on MDR organisms such as ESBL which constituted large number of our isolates. In our study there was significant association between biofilm formation and MDR organisms. These were concordant with previous studies, as they found that biofilm production renders the organism
more resistant and virulent as compared to non-biofilm producers [46,47]. This was illustrated by presence of many mechanisms of biofilm resistance to antibiotics like poor penetration, nutrition limitations, slow growth rate and persister cells. Surprisingly, the results of Cepas et al., (2018) were dissimilar to our results as they observed that there was no significant relationship between multidrug resistance and biofilm formation [48]. Also P. aeruginosa isolates that were susceptible to all the antibiotics studied or resistant to only one antimicrobial category tended to be more biofilm forming than the MDR. After studying the effect of different risk factors and biofilm production. Our results showed that there was positive association between age and biofilm production. Contrary results were reported by Sabir et al. who found that there was no association with age and biofilm formation [34]. This might be caused by the distribution of our cases in the extremes of age. Insufficient immune response in the extremes of age and associated comorbidities in elderly made them more vulnerable to infection and biofilm production. Our findings revealed that there was positive association between duration of catheterization and biofilm production. The same result was reported by others [49]. This could be explained by increased daily risk of bacterial colonization along with increased duration of catheterization. In our study although there was no significant association between latex catheter and biofilm production, latex catheter showed higher rate of biofilm production than silicon catheters. This result agreed with Singh et al., (2018) who concluded that there was significance association between type of catheter and biofilm production [50]. This might be due to that latex catheter caused more irritation to mucosa. It also had rough surface that could enhance biofilm formation in comparison to smooth surface of silicon catheter. According to our findings, the traditional TM showed good correlation with the results of SEM regarding degree of biofilm production. Also, TM for biofilm detection showed 100% sensitivity and specificity in comparison with SEM as a gold standard method for detection. Similar previous reports documented that biofilm detection by TM showed 100% sensitivity and specificity in comparison with PCR as a gold standard method [51,52]. However Shrestha et al., (2018) found that TM showed 82% Sensitivity and 95.2% specificity in comparison with Congo red agar method [53]. Also Sultan and Nabel, (2019) detected that TM showed 93% Sensitivity and 81.4% specificity in comparison with tissue culture plate method [54]. This could be caused by existence of variability of results after test repetition as it was difficult to differentiate between weak and non-biofilm producers.

**Conclusion**

In conclusion, the prevalence of biofilm-dependent CAUTI was high, with *K. pneumoniae* represented the highest biofilm producer. Therefore, minimizing the duration of catheterization as possible and the usage of silicone catheter instead of latex are recommended. Using carbapenems in treatment of biofilm-dependent CAUTI should be considered. TM can be implemented for detection of biofilm formation as it is cheap, rapid, easy and showed good correlation with SEM which is more expensive and time consuming.

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