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Investigating the Impact of Catheterization on the Urinary Infection Prevalence

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Urinary tract infections are the main hospital infections, which are often caused through catheterization. Uropathogenic bacteria can be a potential cause of urinary tract infection using forming biofilms in catheters. The purpose of this research is to study the biofilm formation of *Klebsiella pneumoniae* isolated from catheter-related urinary tract infections. The research was conducted on 110 patient urine samples. Samples were divided into 2 groups with non-catheter-related and catheter-related urinary infections. Identification and isolation of *Klebsiella pneumoniae* were investigated by performing biofilm formation and biochemical tests using the method of microtiter plate. 40 and 70 samples were related to non-catheter-related and catheter-related infections, respectively. For *Klebsiella pneumoniae* isolates is related to urinary infection caused by catheterization, 13.3% formed weak biofilm, 26.7% moderate biofilm, and 60% strong biofilm. For *Klebsiella pneumoniae* isolates related to urinary tract infection without a catheter, 23.3% formed weak biofilm, 36.6% moderate biofilm, 33.3% strong biofilm, and two isolates did not form any biofilm. The utilization of catheters in hospitalized people increases the risk of infection of the urinary tract, which is related to various reasons such as the catheterization duration, the catheter type, and the form biofilms ability of uropathogenic bacteria. The biofilm formation power of bacteria during the catheter increases the infection risk and causes antibiotic resistance of isolates and lack of patients proper treatment.

Keywords: Urinary Infections, Catheterization, *Klebsiella pneumoniae*, Uropathogenic bacteria.

1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections that affects 150 million people worldwide every year. Urinary tract infection can be one of the important causes of mortality in male infants, elderly men, and women of any age (1, 2). Serious complications of urinary tract infection include frequent recurrences, kidney damage in young children, pyelonephritis with sepsis, premature birth and complications due to frequent use of antimicrobial compounds, high-level antibiotic resistance, and problems such as colitis caused by *Clostridium difficile* (3, 4).

Uncomplicated UTIs usually affect people who are healthy and have no neurological or structural disorders of the urinary tract. These infections are divided into lower infections (cystitis) and upper infections (pyelonephritis) (5). Complicated infections with agents that

compromise the urinary tract or host defenses. Urinary obstruction, urinary retention caused by neurological disease, immunosuppression, kidney failure, pregnancy, kidney transplant, and the presence of foreign bodies such as static counters, catheters, or other urinary drainage devices can be mentioned among them (6, 7).

Studies have shown that up to 40% of all hospital-acquired infections worldwide and more than 80% of hospital-acquired urinary tract infections are usually associated with catheterization, especially catheter-associated UTIs (CAUTIs) (6). The most common Gram-negative uropathogenic bacteria in CAUTI cases include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, *Citrobacter freundii*, and *Providencia* (8, 9). Although the catheter or tube is generally an important medical device, its long-term use for hospitalized patients causes the bacteria that cause hospital infections to enter the bladder and create a biofilm along the surface of the catheter (10, 11).

Uropathogenic bacteria use different mechanisms for survival in response to bladder stress, including food poverty and immune system responses. By forming a biofilm and causing morphological changes, they can persist and cause frequent infections (12). Studies have shown that the antibiotic resistance of mature bacterial biofilm is 10 to 1000 times that of planktonic bacteria, and the bacteria in biofilms can resist phagocytosis and it is very challenging to eliminate them (13). Biofilms are microbial communities of cells attached to surfaces that are self-produced and embedded in an extracellular polymeric matrix (13).

Due to the increase in the number of antibiotic resistance, severe infections, and the increasing difficulty in effective treatments, *Klebsiella pneumoniae* has attracted the attention of the world as an infectious microorganism and one of the important causes of catheter-related urinary tract infections (14). *Klebsiella pneumoniae* is the cause of several diseases, including pneumonia, bloodstream infections, meningitis, and surgical site infections, and usually this bacterium is isolated from medical devices (15), and compared to planktonic infections, the treatment of infection with the strain *Klebsiella pneumoniae* can form biofilms more difficult (16). The present study aims to investigate the ability of biofilm formation in *Klebsiella pneumoniae* isolates isolated from canter-related urinary tract infections.

2. Materials and Methods

2.1. Statistical population

This descriptive cross-sectional study was done on 110 urine samples. The studied subjects were two groups of patients. The first group of patients were admitted to surgery and intensive care units, and the second group of patients with urinary tract infections who had no history of hospitalization. Urine samples were collected through the catheter using an aseptic method or by piercing the catheter tube with a needle and syringe in patients with urinary tract infections in the urinary tract and involved in short-term catheterization (17). Counter-associated asymptomatic bacteriuria (CA-ASB) was generally diagnosed when the bacterial count was greater than 10^5 CFU/ml in a urine sample appropriately collected from an asymptomatic patient.

2.2. Isolation and identification of *Klebsiella pneumoniae*

To identify *Klebsiella pneumoniae* isolates, samples were cultured directly on McConkey agar medium. After 24 hours of the greenhouse at 37 °C, biochemical detection of bacteria was done based on common laboratory tests, such as sulfide indole motility medium, triple sugar iron agar, and methyl-red/Voges-Proskauer (MR-VP).

2.3. Investigation of biofilm formation

The biofilm formation power of *Klebsiella pneumoniae* isolated from the urine samples of two patient groups was investigated by the microtitre plate method. For this purpose, trypticase soy agar culture medium containing 2% glucose and broth trypticase medium containing 2% glucose were also prepared. Bacterial samples were cultured linearly on trypticase soy agar containing glucose 2 and kept in a greenhouse at 37 degrees Celsius for 24 hours. From the colonies grown on this culture medium, they were removed with a loop and taken again on the tryptic culture medium to the broth containing 2% glucose, so that the bacterial suspension is equivalent to half of McFarland's turbidity. 200 microliters of bacterial suspension were taken and placed in 96-well microtiter plates and kept in a greenhouse at 37 degrees Celsius for 24 hours. The colonies grown on this culture medium were removed with a loop and were taken again on the trypticase culture medium containing 2% glucose so that the bacterial suspension was equivalent to half McFarland turbidity. 200 microliters of bacterial suspension were taken and placed in 96 well microtiter plates and kept in a greenhouse at 37 degrees Celsius for 24 hours. After incubation, all the wells of the microplate were aspirated and then the wells were washed with 200 microliters of phosphate saline buffer to remove all cells that were not attached. Adhered bacteria were fixed with 200 microliters of 99% methanol for 25 minutes. Then the wells were dried in the air and stained with 200 microliters of 2% crystal violet for 20 minutes. After this time, the wells were washed again with sterile distilled water and dried in the air. 200 microliters of 33% glacial acetic acid were added to the wells and kept for 15 minutes until all the remaining dyes were dissolved in the acid. Finally, to read the OD results, an ELISA reader with a wavelength of 570 nm was used. This test was repeated three times for each bacterial sample and a well containing an empty culture medium, which was the trypticase culture medium containing 2% glucose, was taken as a negative control. To analyze and observe the ability of the strains to form biofilms, the method of determining the classification criterion of optical absorption values or ODC was used (18).

2.4. Statistical analysis

Data was collected and entered into the database using Microsoft Excel 2013. Statistical analysis was done in SPSS23. The chi-square test was utilized to compare data in terms of the difference in biofilm formation ability between two groups of bacteria isolated from patients with catheter-related and non-catheter-related urinary infections. A significance level was considered less than 0.05.

3. Results

3.1. Results of *Klebsiella pneumonia* patients and isolates

In this descriptive cross-sectional study, out of 110 referred patients, 70 patients had catheter-related urinary infections, and 40 patients had non-catheter-related urinary infections. Among the studied clinical samples, 30 isolates of *Klebsiella pneumoniae* were identified and 30

isolates of *Klebsiella pneumoniae* isolated from catheter-related patients were randomly selected to conduct the study.

Table 1. Determination of biofilm type of *Klebsiella pneumoniae* isolates based on ODc values.

Strong biofilm	$OD > 4 \times ODc$
Medium biofilm	$2 \times ODc < OD \leq 4 \times ODc$
Weak biofilm	$ODc < OD \leq 2 \times ODc$
Lacking the biofilm	$OD \leq ODc$

3.2. Results of biofilm formation of *Klebsiella pneumoniae* isolated from catheter-related urinary infection and non-catheter-related urinary infection

Examining the results of the standard deviation (SD) in the microtiter plate method (0.0172) and OD cut-off (0.1647) of *Klecilla pneumoniae* isolates isolated from catheter-related urine samples (Table 2) showed that 18 isolates (60%) were strong biofilms, 8 isolates (26.7%) formed moderate biofilm, and 4 isolates (3.13%) formed weak biofilm.

Table 2. Interpretation of biofilm formation results of *Klebsiella pneumoniae* isolates isolated from urine samples associated with the canter.

The power of biofilm formation	Results of determination of average OD values	Calculation of ODc amount
Lacking the power of biofilm formation	$OD \leq 0.1647$	$OD \leq ODc$
Weak biofilm	$0.1647 < OD \leq 0.3294$	$ODc < OD \leq 2 \times ODc$
Medium biofilm	$0.3294 < OD \leq 0.6588$	$2 \times ODc < OD \leq 4 \times ODc$
Strong biofilm	$0.6588 < OD$	$4 \times ODc < OD$

A total of 30 (100%) *Klebsiella pneumoniae* isolated from urine samples associated with the catheter could form biofilm. Also, the examination of the results of the standard deviation (SD) in the microtiter plate method (0.00381) and OD cut-off (0.0645) of *Klebsiella pneumoniae* isolates isolated from urinary samples unrelated to the catheter (Table 3) showed that 10 isolates (33.3%) formed a strong biofilm, 11 isolates (36.6%) formed an average biofilm, and 7 isolates (23.3%) formed a weak biofilm, and 2 isolates did not form any biofilm.

Table 3. Interpretation of the results of biofilm formation of *Klebsiella pneumoniae* isolates isolated from urine samples not related to the catheter.

The power of biofilm formation	Results of determination of average OD values	Calculation of ODc amount
Lacking the power of biofilm formation	$OD \leq 0.0645$	$OD \leq ODc$
Weak biofilm	$0.0645 < OD \leq 0.1291$	$ODc < OD \leq 2 \times ODc$

In total, 28 (93.3%) *Klebsiella pneumoniae* isolated from urine samples not related to the counter could form biofilm. Comparing the results of biofilm formation of *Klebsiella*

pneumoniae isolates in two groups related to the catheter and not related to the catheter showed that the power of strong biofilm formation in the isolates related to the catheter (60%) is more than that of the unrelated isolates (33.3%). However, statistical analysis did not show a significant difference between the strength of strong biofilm formation in the isolates of the two groups ($p \geq 0.05$). In addition, the isolates with weak biofilm formation power had a higher percentage in samples not related to the tube. 2 isolates not associated with the catheter could not also form biofilm.

4. Discussion

Among the most common hospital infections are urinary tract infections, pneumonia, and bacteremia. Urinary tract infection is a type of inflammatory response of the urinary tract to the invasion of pathogenic microorganisms and is caused by gram-positive and gram-negative bacteria and sometimes by fungi. Urinary tract infection is responsible for 45% of hospital infections and 80% of these infections are caused by catheterization and use of urinary counters (19). Studies have shown that urinary infection increases by 5% for every day that the tube or catheter stays in place (20). Catheters are one of the predisposing factors to urinary infections, which may lead to the spread of blood and sepsis in the patient, so the duration of hospitalization for patients increases by up to 4.5 days, and with the increase in patient mortality. It is accompanied (21). One of the effective factors in the pathogenicity of bacteria is the ability to form a biofilm, which can act as a determining factor in the strength of strains of bacteria causing urinary tract infections, such as *Klebsiella pneumoniae*, for longer survival in the urinary tract and resistance to antibiotic treatments.

In the present study, 63.3% of the referring patients had catheter-related urinary tract infections, and these patients were hospitalized in different departments of the hospital. In Maharjan et al.'s study, the prevalence of catheter-related urinary tract infection in Nepalese patients was reported as 9.61% (22), which was similar to the review study conducted by Nicolle in 15 developing countries (23).

In a study conducted by Elsous on 128 patients with urinary tract infections, he reported the prevalence of catheter-related urinary tract infections to be 3.28% (24). In comparison, the prevalence of catheter-related infection in Egypt, Britain, and Turkey was reported as 1.3%, 6.12%, and 17% respectively in 2012 (25-27). In 2013, Clancy and colleagues showed that both Gram-negative and Gram-positive bacteria could form biofilms. Gram-negative bacteria, such as Escherichia coli, Proteus mirabilis, *Cleciella pneumoniae*, and *Pseudomonas aeruginosa*, are among the most important bacteria that can form biofilms (28).

In a study conducted by Patric et al. on *Klebsiella pneumoniae* bacteria, it was shown that 54.5% of the samples could form a strong biofilm. In that study, it was shown that there is a significant relationship between bacterial adhesion to surfaces and biofilm formation (29). In a study conducted by Seifi et al. on the strength of *Klebsiella pneumoniae* strain biofilm formation, 61% of these strains isolated from urinary tract infection, 33% strong biofilm, 52% medium biofilm, 8.5% weak biofilm, and 4.6% lacked biofilm strength (30). In a study by Nirwati et al. in Indonesia, out of 148 isolates in terms of biofilm, 63.85% formed biofilm and 94.29% had weak biofilm (31).

In the present study, *Klebsiella pneumoniae* isolated from catheter-related urinary infections had stronger biofilm formation than *Klebsiella pneumoniae* isolated from non-catheter-related urinary infections. In a study conducted by Singh et al. on the urine samples of patients with catheter-related urinary tract infections, it was shown that all Gram-negative bacterial isolates,

including *Klebsiella pneumoniae* isolated from the samples, can form biofilms (32). In addition, in the study of Salah Al-Hobiashy et al., among the bacterial isolates isolated from catheter-related urinary tract infections, Escherichia coli bacteria had 60% and *Klebsiella pneumoniae* had 1.57% ability to form biofilm (33).

The use of catheters in hospitalized patients increases the risk of urinary tract infection, which depends on various reasons, including the duration of catheterization, the type of catheter, and the ability to form biofilms of bacteria associated with urinary tract infections. The power of biofilm formation by bacteria during the catheter increases the risk of infection and will lead to the emergence of antibiotic resistance of isolates and the lack of proper treatment of patients. To prevent the establishment of bacteria and their ability to stick and connect to surfaces, including the surface of hospital catheters, solutions such as investigating the mechanisms of connection and formation of bacterial biofilm, as well as preparing catheters with a special composition structure that can prevent the attachment of bacteria, can be effective.

5. Conclusions

The purpose of this research is to study the biofilm formation of *Klebsiella pneumoniae* isolated from catheter-related urinary tract infections. For *Klebsiella pneumoniae* isolates is related to urinary infection caused by catheterization, 13.3% formed weak biofilm, 26.7% moderate biofilm, and 60% strong biofilm. For *Klebsiella pneumoniae* isolates related to urinary tract infection without a catheter, 23.3% formed weak biofilm, 36.6% moderate biofilm, 33.3% strong biofilm, and two isolates did not form any biofilm. The utilization of catheters in hospitalized people increases the risk of infection of the urinary tract, which is related to various reasons such as the catheterization duration, the catheter type, and the form biofilms ability of uropathogenic bacteria. The biofilm formation power of bacteria during the catheter increases the infection risk and causes antibiotic resistance of isolates and lack of patient's proper treatment.

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