Investigation Of Biofilm Composition Of Clinically Isolated Bacteria From Patients With Urinary Tract Infection

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Abstract :
130 urine samples were taken from delegates and inpatients at Ba'aqubah Teaching Hospital and Muqdadiya General Hospital between June 2022 to October 2022 from different age groups ranging between 6-50 years, 110(84.6%) were with growth while 20 (1.54 %) were Non growth distributed between 71 (64.5%) samples for females and 39 (35.3%) samples for males, and the samples were transferred directly to the laboratory for the purpose of implanting and diagnosing samples whom had UTIs that were persistently active in both sexes and across a range of age categories. Bacterial strains that caused these pathological referrals were identified and isolated. the following distribution: The most prevalent bacteria were Staphylococcus aureus 10 (9.2%), followed by Proteus mirabilis 20 (18.2%), Pseudomonas aeruginosa 20 (18.2%), Klebsiella pneumonia 15 (13.6%), and Escherichia coli 30 (27.2%), which is thought to be the main cause of urinary tract infections.
When evaluating a group of biofilm-producing bacteria isolated from patients with urinary tract infections, only 30 specimens (30) that demonstrated multiple antibiotic resistance (MDR) were chosen. The ELISA technique revealed more accuracy when examining the composition of the biofilm, and all isolates were 30 (100%) producing the biofilm in comparison to Congo red and the method of adhesion to pipes. Regarding the pipe adhesion method and the Congo red method, 19 (36.3%) isolates of Staphylococcus aureus, Staphylococcus epidermidis, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and E.coli produced biofilms at rates of 100%, 80%, 60%, 60%, and 20%, respectively. This study sought to evaluate the potential of bacteria isolated from persons with recurrent urinary tract infections UTI and linked with kidney stones to build biofilm. It was discovered that the first approach is more accurate in indicating the degree of membrane formation.

Introduction

The presence of microorganisms in urine samples identifies urinary tract infection (UTI), one of the most prevalent and widespread bacterial infections that can affect people of all ages and genders[1]. It is the second most common health issue after respiratory illnesses. Because the majority of them have virulent agents that can penetrate the urinary system urinary tract, Enterobacteriacea, Escherichia, Klebsiell, Proteus, Enterobacter, Pseudomonas, Staphylococcus epidermidis and Staphylococcus aureus are the most frequent causes and pathogens associated with urinary tract infection [2]. Bacteria often create biofilms, which are communities of microorganisms, particularly bacteria, that maintain an organized strategy of growth and reproduction on any surface for their survival. Biofilm is a very complex microbial ecosystem. It can stick to a range of surfaces, including plastic and metal, whether they are living things or inanimate objects. Bacterial survival is promoted by growth in biofilms because, once established, biofilms are challenging to eradicate[3]. When creating antibiotic regimens for biofilms, consideration must be given to the multi-stage formation of biofilms, which has specific properties. It is influenced by a number of elements, such as the type of bacterium, the characteristics of the surface on which the microbe will be established, environmental factors, and the gene expression of genes involved in biofilm development[4]. One advantage of living in a biofilm over

bacteria that are free to float around and interact with other organisms in their environment is that the former is protected and has these abilities. As cells deep within the biofilm are protected by dense inner tissue and the outside layer of cells, resistance to chemical additives and antibiotics rises [5]. Utilizing three different techniques—the ELISA method, the Congo red method, the pipe adhesion method, and the optimal traisa—this study sought to examine the capacity of bacterial isolates obtained from diuretic samples of patients with primary tract inflammation to form biofilms as an important virulence factor.According to a report from the National Nosocomial Infections Surveillance System, 97 percent of cases of urinary tract infections were linked to urinary catheters, making them one of the most common causes of nosocomial infections (National Nosocomial Infections Surveillance System, 2002) [3]. It is well known that the presence of biofilms is one of the factors that promote infection processes, with an assumption that biofilm presence accounts for 60% of infection processes. These infections have been linked to a number of biofilm-producing agents, such as bacteria, fungus, and protozoa. [6]

2. Materials and methods

2.1. Sample collection

From the beginning of June 1/6/2022 until the end of October 1/10/2022, 130 urine samples were taken from patients with urinary tract infections UTIs of both sexes at Baqubah Teaching Hospital and Muqdadiya General Hospital. The samples were divided between 71 (64.5%) samples for females and 39 (35.3%) samples for males, with the samples being sent directly to the laboratory for testing.

2.2. Isolation and diagnosis:

The isolates were purified on the Nutrient agar medium in a planned manner and incubated all dishes at a temperature of 37 ° C for 24 hours aerobically. Tests and diagnostic tests were then conducted, including phenotypic and biochemical tests, and the isolates under study were confirmed by the Vitec device.

2.3. Detection of biofilm composition of bacterial isolates under study:

There are many methods used by clinical microbiologist [6]. To detect and measure microbial biofilms as shown in Table Tables (2-1).

Table (2-1). Methods used to detect biofilms

Method	Aim
Microtiter plate (MtP)	Quantitative Detection of Biofilms by MicroELISA
Congo red agar(CRA)	Qualitative Detection by monitoring colony discoloration
Tube method (TM)	Qualitative detection by monitoring the biofilms lining the bottom and walls of the pipe

2.3.1. Biofilm detection by microplate reader Microtiter plate (MtP)

The biofilm composition was detected using the 96-hole sterile MTP microtiter plate method as per the manufacturer's instructions Bio Tek (USA).

2.3.2. Detection of biofilms by the Congo red method (CRA)

The browning of pollination colonies on the medium of Congo red agar (CRA) is regarded as a qualitative diagnostic for the detection of microorganisms generating biofilms.By combining (0.8) g of Congo-red red pigment, (36) g of sucrose, and (37) g of the heart-brain solid mix medium (BHI), the Congo medium is created. Following pollination of the growing medium, the dishes are incubated for 24 hours at 37°C. The colonies may be identified by their shape, which shows that the colonies in the shimmering black color are producing strong biofilms while the colonies in the red hue are not. [7].

2.3.3. Detection of biofilms by pipe adhesion method(TM)

The developing bacterial colonies were transferred to glass test tubes containing a medium (TSB) Tryptic soy broth added to glucose at a concentration of 1 and incubated at a temperature of 37 ° C for 24 hours after the end of the incubation period in order to conduct this test for the qualitative detection of microorganisms that produce biofilms. The samples were placed into the tubes, which were then washed with Phosphate Buffer Saline, dried, and colored for three minutes with 1% crystalline violet dye, with the excess dye being emptied out and the tubes being rinsed with distilled water. Ion-free The tubes are then dried upside down to monitor the development of biofilms on the inner walls and pipe bottoms. [8].

3. Results and discussion

130 samples were collected from incoming and hospitalized patients, as the number of isolates that gave positive growth was 110 (84.6%), while the number of isolates that did not show bacterial growth was 20 (15.3%), and positive isolates were distributed to 71 (64.5%), a sample of females and 39 (35.3%) a sample of males as in the Table (3-3):

Table (3-1): Frequency of patients with UTI According to Age and Gender

Age	Frequency	Percent %	P-Value
≤ 29	48	% 43.6	P > 0.05.
≥ 30	62	% 56.4	
Total	110	100.0	-
Gender	Frequency	Percent %	P-Value
Female	71	%64.5	P > 0.05.
Male	39	%35.3	
Total	110	100.0	-

After isolation and diagnosis in this study, six types of bacteria were isolated, and each of the coli bacteria was diagnosed. E 30 (27.2 %) P. mirabilis 20 (18.2%), P. aeruginosa 20 (18.2%), K. penumonia 15 (13.6%) Staphylococcus epidermidis 15 (13.6%) and the least common was Staphylococcus aureus 10 (9.2%) as shown in Table (3.2)

Table (3.2): Types of bacterial isolates isolated from urinary tract infections

bacterial isolates	NO of isolates	Percentage
Escherichia coli	30	27.2 %
Proteus mirabilis	20	18.2 %
Pseudomonas aeruginosa	20	18.2%
Klebsiella. Pneumoniae	15	13.6%
Staphylococcus epidermidis	15	13.6 %
Staphylococcus aureus	10	9.2%
Total	110	100%

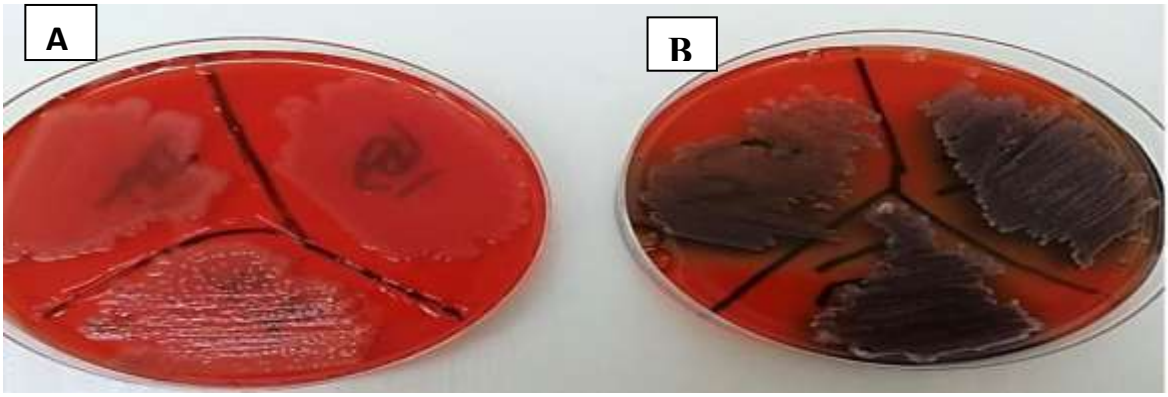
3.1. test Microtiter plate (MtP)

All of the bacterial isolates under study, including E. coli, P. mirabilis, K. pneumoniae, P. aeruginosa, S. epidermidis, and S. aureus, produced biofilms to varying degrees, including strong formation, medium composition, and weak formation, according to the study's findings after testing the isolates. As shown in Table (3.3)

3.2. Test Congo-red method (CRA)

The result of the CRA test was the appearance of colonies in black on the medium of the Congo red agar indicating the formation of the biofilm of all bacterial isolates, and it was found that one isolate belonging to the bacteria E.coli is unable to form the biofilm, so its colonies appeared in red as shown in Table (3.3).

Figure (3-1). Screening of Bacterial strains for biofilm formation on Congo red agar showing (A) negative result (B) strongly positive



3. 3. Test Tube Method (TM)

According to the study's findings, S. aureus, P. aeruginosa, E. coli, P. mirabilis, K. pneumoniae, and S. epidermidis were all susceptible to biofilm formation to varying degrees. as shown in Table (3.3). The MtP is the most efficient method for finding biofilms. As for the TM method, the degree of biofilm formation can be determined with the naked eye depending on the estimate of the thickness of the membrane formed on the inner surface of the tubes. As for the CRA method, it is a simple, easy and inexpensive examination, but it gives a result that is not sensitive to the efficiency of biofilm formation and thus only determines the ability of bacteria to produce biofilm or not. In general, all these methods can be used to investigate biofilm production qualitatively rather than quantitatively.

Table (3.3) Susceptibility of bacterial isolates isolated from urinary tract infections to biofilm production and percentages

production method								bacterial isolates
Percentage	TM	Percentage	CRA	Percentage	ELISA			
					W	I	S	
%40	2	%20	1	%100	1	1	3	E.coli
%60	3	%60	3	%100	1	1	3	P.mirabilis
%60	3	%60	3	%100	1	1	3	K.pneumoniae
%80	4	%60	3	%100	0	1	4	Ps.aeruginosa
%60	3	%80	4	%100	0	1	4	S. epidermidis
%80	4	%100	5	%100	0	0	5	S.aureus
%63.3	19	%63.3	19	%100	30			Total

Through this study, it was found that most of the patients were the main cause of their injuries is E.coli bacteria for the samples under the current study, and the study also showed that the incidence of

urinary tract infection in women is higher than men, where the percentage was 64.5% of patients are females while 35.3% are males, and this is consistent with many studies that the incidence of urinary tract infection is higher in women than men in general around the world, due to hormonal changes. which occur as a result of aging, as well as immunosuppression, as well as pregnant women[9]. These findings are consistent with those of Irbasmantini et al. (2023) [10]. It discovered that females are more susceptible than males are, and discovered that 89% of the isolates were E. coli, the primary cause of urinary tract infections, and that 95% of isolates formed biofilms[12]. Which indicated the findings of her study that Proteus and Klebsiella are the primary causes of urinary tract infections, but it is consistent with numerous studies that demonstrate that E. coli is the primary cause of it as a pathogenic pathogenic, and that E. coli UPEC (Uropathogenic) is the primary cause of urinary tract infections and is accountable for 80% of cases. Whether they are gathered or obtained from hospitals, the fact that they produce the so-called Bacteriurea and have a variety of virulence factors, such as adhesion components and poisons such (haemolysin), Because it is linked to urinary system pathogenicity, it is known as UPEC [13]. Three distinct approaches have been used to study the biomembrane that bacteria create, which is crucial to their pathogenicity[14]. According to the MtP, CRA, and TM methods, it was discovered that S. aureus and P. aeruginosa had a high ability to form biofilms, while E. coli, P. mirabilis, K. pneumoniae, and S. epidermidis had a medium ability to do so. Studies have shown that bacteria vary in their capacity to form biofilms. the type of infection, the nature of the generated organism, and the surrounding environmental factors, including temperature and Ph. For instance, bacteria isolated from individuals with prostatitis are better able to thicken and form biofilms than bacteria isolated from individuals with cystitis. [15].

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