Bacteriological Study of the Bacteria Cause Urinary Tract Infection of Patients Admitted to Cardiac Care Unite a Baqubah General Teaching Hospital

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Abstract

Background: Urinary tract infection patient Urinary Tract Infection (UTI) one of commonest types of patient admitted to CCU (cardiac care unit) of other medical wards this is occur directly from contact to infected hand or material or during catheterization.
Objective: To evaluation common bacterial cause UTI in patients admitted Cardiac Care Unite to show the antimicrobial agent and bacteria resistant and production the biofilm.
Patients and Methods: Collection of samples from urine aseptically for culture.Isolation and identification of uropathogens using biochemical tests and testing ability of these bacterial isolation for virulence production and testing the antimicrobial susceptibility test.
Results: It is A total of 135 catheter samples were collected from patient (135) catheter samples from patients in CCU at Baqubah General Teaching Hospital for the period from 1st November 2016 to 1st March 2017 frommales and female, and the samples and cultured on the medium blood agar and MacConkey agar. Then growing bacterial farms subjected to microscopic and biochemical tests for the diagnosis of bacteria. Escherichia coli with a ratio 31.8% , and (20)isolations of Proteus mirabilis with a ratio 18.2%, 16 isolates of Klebsiella pneumonia, (14) isolations of Pseudomonas aeruginosa with a ratio 12.7% .The Antimicrobial sensitivity is investigated for (9) antibiotics from different groups including; The results show a high resistance to most of the antibiotics under study, and resistance all isolates ware of Aztreonam ,Cefotaxime , Co-Trimoxazol with ratio 95% , Naldixic acid with ratio 100%, Tetracycline 75% , Gentamycin 70% and Antimicrobial sensitivity to antibiotics to Amikacin 45% followed by Ciproflaxcin 50% and tobramycin 80% . All kinds of bacteria were produced in biofilm in the ELISA method with a ratio 100% ,while Congo red with a ratio 50%.
Conclusion: It was observed that multiple antibiotic resistance was common among local isolates of the bacteria under study and Biofilms are important in protecting the bacteria inside the catheter from antibiotics.
Key words: Gram Negative bacteria, Antibiotics ,Biofilm ,Urinary Tract Infection , cardiac care unit.
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Introduction

Urinary Tract Infections are one of the most common health problems in most countries of the world, followed by Respiratory Tract Infection [1]. UTIs are all age groups of males and females. One of the main causes of infant death is what referred by [2], as well as older males are also infected because most of them are exposed to prostates infection, which causes slow discharge of the bladder from the arteries, which cause inflammation of the urinary tract [3]. Older women also suffer from urinary tract infection largely because of hormonal changes due to aging, immunosuppression [3], pregnant women, and patients with chronic diseases such as diabetics or Immunosuppressed or weakened in immunization due to HIV infection or cancer. All of the above increases the risk of urinary tract infection [3]. UTI is the most common infection of hospital Nosocomial Infections by 20% - 30% in the cardiac care unit [4]. Bacteria, the main cause of UTIs, are 95% [5] and usually bacteria that live in the Digestive Tract, as well as in the vagina or around the urethra which are at the forefront of the urinary tract. Most of these bacteria enter the urethra and are transferred to the bladder and kidneys. Clinical injury is usually caused by Gram-positive bacteria and Gram positive bacteria despite fungi, viruses and parasites [6]. It can also be caused by a common cause of urinary tract infection non-bacterial hemorrhagic infections include hemorrhagic cystitis, for example, with viruses such as adenovirus [7].Common pathogenic bacteria pathogens in the intensive care unit include Gram Negative bacteria such as Escherichia, Klebsiell, Proteus, Enterobacter, Pseudomonas, Serratiaspp and Gram positive bacteria such as Streptococci , Enterococcus sp.and Staphylococcus [8].

The main cause of Uropathogen in the world is Escherichia coli (U P E C), which is 80% -85% according to [9]. Antibiotic resistance by the bacteria that cause urologic infections has become known throughout the world, which has been increasing, especially for the widely used antibiotics. This has led to resistance of bacteria to these antibiotics by developing defensive mechanisms against them, and there is a correlation between increased resistance and antibiotic use by patients ,These bacteria may produce Virulence Factors such as biofilm ,Which is related to the presence of the catheter because it is a suitable environment for germs, which are suitable for the formation of the biofilm[10].

Patients and Methods

Use media such as MacConkey agar, Blood agar, nutrient agar, and the of the Muller-Hinton agar, types of antibiotics; Aztreonam, Cefotaxime, Gentamycin, Amikacin, Tobramycin, Naldixic acid, Ciprofloxacin and Tetracycline. Co-trimoxazole agents are used , as shown in Table 3, and the biofilm production in the environment of the Congo-Red method , the environment is prepared as it mentioned in [11].

Isolation and Diagnosis of Bacteria

It is a total of (135) collected from patients in the intensive care unit at Baqubah General

Teaching Hospital for the period from 1st November 2016 to 1st March 2017, and were directly grown on the blood agar and MacConkey for the purpose of distinguishing between positive isolates and Gram-negative isolates were purified on the center of nutritious agar in a planning manner, and then the identification, phenotypic, and biochemical tests were performed for bacterial isolates. The bacterial isolates under study were identified according[12]. The colonies were initially identified as dependent on the phenotypic traits, including the shape, color, texture, smell, and size of the colonies on the central blood agar and MacConkey centers. In addition to its ability to analyze red blood cells on the blood agar center, the bacterial isolates under study were subjected to microscopic examination by taking a small swab of the colony and transmitting it to the glass slide and dye it with a gram dye to see the type of pigment and the shape of the cells and the way they are collected [13].

Antibiotic susceptibility test

The sensitivity of isolates under study was tested for 9 antibiotic agents, they were distributed among the β-Lactam antibiotic that included Aztreonam, Cefotaxime, also from Aminoglycosides antibiotics group, Gentamycin, Amikacin, Tobramycin, Quinolone Naldixic acid, Ciprofloxacin, and other anti-tetracycline such as Co-Trimoxazol anti-folic acid was used to measure the diameter of the inhibition area for the collection of antibodies used in the study compared to the [14]. The sensitivity test was performed using the antibiotic pill method Kirby –boure test [15].

1.Pour 5 ml of nourishing broth into 2-3 colonies of 24-hour pure bacterial farms.

2.Bake well, and incubate at 37 ° C for 24 hours.

3.The growth curve was measured using the standard turbidity constant to give an approximate number of 1.5 × 108 cells / ml.

4.Transfer 0.1 mL of bacterial suspension and spread to the center of Akar Muller Hinton, leave the dish for 5 minutes at room temperature until the plant is dried.

5.Transfer antibiotic tablets to the middle surface of the plant using sterile forceps at a rate of 9 tablets per dish.

6.The dishes were incubated at 37 ° C for 24-18 hours. After measuring the diameters of the inhibition zones around each disc, the sensitive bacteria (S), R (R), or intermediate (I).

Biofilm production Test

a)Congo-red Method

A pure single colony was transferred to the nutrient agar medium to a test tube containing 5 ml of the prepared salt solution. After a good mixing with the carburetor, the mass was compared to the prepared McFarland's[0.5],the prepared Concorde fertilized and the dishes was incubated at 37 °C for 24-48 hours.The result is positive when the colonies appear black with a dry crystalline density, while the negative result remains the colonies pink [16].

b)Biofilm production by ELISA test

1.(4 - 3) colonies of bacteria at the age of 48-24 hours, and then vaccinated tubes

container (10-5) ml of heart-brain diffusion broth placed in the incubator at 37 ° C for 24 hours, hour.

2.Centrifuge the tubes to get rid of the medium and get the bacterial suspension. Wash the cells with sterile normal saline solution twice.

3.Place 180 microliters of the flat-bottomed tissue culture plate, each for two isolates and then add 20 microliters from the microbial suspension to the holes for each isolation. Three holes were left in control, with 200 microliters placed from the center of the heart-piercing broth and the non-pollinated brain.

4.Cover the dish with a clean paper and a Para Film and incubate at a temperature of 37 ° C for 24 hours.

5.The medium available in the drillings was removed by a micropipette installed at 200 microliters. The drill was then washed once using the physiological saline solution at a rate of 300 µl and using the ELISA-washer launcher.

6.Formulation of 10% Formaldehyde in 6.2.2.3 (200 µl) for each hole to fix the bacterial membrane of the bacterial isolates on the internal surfaces of the plate and leave the dish for 30 minutes at room temperature 25 ° C , and formaldehyde was removed from all the holes using a micro-pipette installed at 200 µl.

7.Turn the dish over a clean piece of gauze and set upside down to the next day at room temperature.

8.The crystal violet, recorded in 4.2.2.3, has been added at a rate of 200 µl per hole for the production of the resulting biofilm.

9.Read the optical density using the ELISA reader at a wavelength of 590 nm.

Results

The following bacteria are identified such as *E. coli, P. mirabilis, P. aeruginosa, K.penumonia*, after isolated and purified from agar samples based on microscopic characteristics of bacterial cells and morphological and plant Biochemical test including colony size, color, quenching, , biochemical test which used for the purpose of diagnosing each bacterium and conforming to the approved diagnostic systems [15] as shown in table (1) After isolation and diagnosis, four types of bacteria were isolated. As shown in Table 2, Escherichia coli 35 isolates were 31.8%, 20 isolates were Proteus mirabilis with ratio 18.2% , 16 Klebsiella pneumonia isolates with ratio 14.5%, 14 Pseudomonas aeruginosa with ratio 12.7% table (1).

Table (1): Microscopic and biochemical diagnosis of bacteria isolated from from UTIs.

Klebsiella pneumoniae	Pseudomonas aeruginosa	Proteus mirabilis	Escherichia coli	Isolates Testes
-	-	-	-	Gram stain
+	+	+	+	Catalase Test
-	+	-	-	Oxidase test
-	-	-	+	Indol production test
+	-	+	+	Methyl- red- test
+	-	-	-	Vogesproskauer- test
+	+	-/+	-	Citrate utilization- test
-	+	+	+	Motility Test
+	-	+	-	Urea hydrolysis- (urease test)

Table (2): Frequencies and percentages of bacterial types.

Bacteria	No.	%
Escherichia coli	35	31.8%
Proteus mirabilis	20	18.2%
Klebsiella. Pneumoniae	16	14.5%
Pseudomonas aeruginosa	14	12.7%
Total	85	77.27%

The results showed a clear variation in the extent of the response of isolates under study to the antibiotics used as shown in table(3).

Table (3): Antibiotics sensitivity test for bacteria isolated from urinary tract infection.

%	Total No.	P. aeruginosa		K. pneumoniae		P. mirabilis		E. coli		Antibiotics
		%	No.	%	No.	%	No.	%	No.	
100	20	100	5	100	5	100	5	100	5	Aztreonam
45	9	40	2	60	3	60	3	20	1	Amikacin
75	15	100	5	80	4	80	4	40	2	Gentamicin
95	19	100	5	100	5	100	5	80	4	Naldixic acid
100	20	100	5	100	5	100	5	100	5	Co-Trimoxazol
100	20	100	5	100	5	100	5	100	5	Cefotaxime
70	14	60	3	80	4	100	5	40	2	Tetracycline
50	10	40	2	60	3	60	3	40	2	Ciprofloxacin
80	16	80	4	60	3	100	5	80	4	Tobramycin
79.4	143	80	36	82.2	37	88.8	40	66.6	30	Total

All *E.coli* isolates are resistant to antimicrobial Co-Trimoxazol, Cefotaxime, Aztreonam, Naldixic acid, Tobramycin, Ciprofloxacin, with ratio 100% , 100% , 100% , 80% , 80% , 40 respectively . As for the investigation of the production of the biofilm as shown in table (4),. The investigation results of the biofilm

production were shown in two ways, the medium of Congo- Red (CRA) and the method of ELISA is the best methods in detecting the biological membrane was the number of positive isolates was 20 isolates with a ratio 100% and then the Congo - Red was 10 isolates 50% .

Table (4): Biofilm production by bacteria isolated from urinary tract infection.

%	Congo-Red method	%	ELISA method	Bacteria
20%	1	100%	5	E.coli
60%	3	100%	5	P.mirabilis
60%	3	100%	5	K.pneumoniae
60%	3	100%	5	Ps.aeruginosa
50%	10	100%	20	Total

Discussion

Escherichia colonies have been shown on the MacConkey Agar medium with pink color due to fermentation of lactose sugar, solid, medium-sized, convex, dry, regular and negative for oxides test, positive for catalase test, negative for urea testing, positive in test the mandolin and the red instance , negative for the Voges-Proskauer test and is unable to consume the citrate.

Proteus mirabilis bacteria is characterized by the adoption of the phenomenon of Swarming movement on the medium of blood agar as a preliminary diagnosis, as well as pale colonies on the medium of MacConkey agar because of non-fermentation of lactose sugar and consumption of peptone source of nitrogen and the production of metabolic materials increase the value of (pH), the medium, which in turn affects the Neutral red detector, makes the colonies pale colour, although this is common with other bacterial strains, but it is consistent with the phenomenon of the ripple movement [18].

As well as the smell of bacterial growth that is similar to the smell of fish on the same medium [19].Klebsiella pneumoniae bacteria was diagnosed with MacConkey agar medium in the fact that its large circular colonies, with irregular pink scales and mucus-containing capsule which were irregular, and their colonies on blood vessels were transparent and brilliant, unable to decompose blood [20].It also gave a negative result in an indole test and a positive for red Methyl test . The microscopic test showed that after a Gram stain dye was applied to a survey taken from the pure colonies on the nut medium, all the above isolates were negative to the gram chromosome as shown in table (1).

All *E.coli* isolates are resistant to antimicrobial Co-Trimoxazol, Cefotaxime, Aztreonam, Naldixic acid, Tobramycin, Ciprofloxacin, with ratio 100% , 100% , 100% , 80% , 80% , 40% respectively .

The results were consistent with [21]. The resistance of E. coli to Naldixic acid and Ciprofloxacin was 88% and 40%, respectively. , The results of the study for the antibiotic Tobramycin agree with results of [22], where the resistance ratio of E.coli of the antibiotic is 89.6%. As for the antibiotic Tetracyclline, the resistance rate was 40%. The results of the current study are consistent with the results of the study of the researcher [23] in Baghdad, where isolates were resistant to antagonism by 40% , in the anti-Gentamycin , the resistance ratio of isolates was 40% , percentage of resistance to E.coli isolates was 42% , and the Gentamycin and Amikacin were 40%. The results were close to [24]. The bacterial resistance to Amikacin 12.8% . While P.mirabilis 100% resistance against sensitivity, resistance to Co-Trimoxazol, Cefotaxime, and Naldixic Acid Tobramycin and Aztreonam and Gentamycin and Tetracyclline were all resistance by 100% as shown in table (3).

These above results were consistent with [25] which that the resistance of P. mirabilis to Cefotaxime and Tobramycin was 100% and 81% respectively. The results of Co-Trimoxazol were agreed with [26]. The resistance of the bacteria to the trimethoprim Antibiotic was 91% and it is in line with the results of the researcher [27] which the ratio of isolates to tetracycline Antibiotic was 100% . As for the antimicrobial group Amikacin , the resistance was 60% ; for Ciprofloxacin Antibiotic, the resistance was 40% ; for the sensitivity of K. pneumoniae, the resistance to the antibiotic, Cefotaxime, Aztreonam, Co-Trimoxazol, Naldixic acid 100% , Tobramycin and Tobramycin, Gentamycin,Ciproflaxcin 80% , Tetracycline and Amikacin 60% respectively. These results were agreed with [28] in her study in the city of Kirkuk on urinary tract patients where the resistance of K.pneumoniae to the anti-Co-Trimoxazol is (100%). the Tetracycline antibody was the resistance of Kl. Pneumoniae is close to that of the researcher's [29] with a resistance rate of 84% . This ratio is consistent with the findings of [30], that the rate of sensitivity of the isolates under study for Ciprofloxacin was 72.7% , while for the Amikacin Antibiotic, it was consistent with [30]. The sensitivity of isolates was in the direction of Amikacin with ratio 81.8% . For the bacteria of P.aeruginosa, the ratio of resistance to isolates for Ceftazidime, Aztreonam, Gentamicin, Co-Trimoxazol, and Naldixic acid was 100% as shown in table (3).The results agreed with [31], the resistance to Naldixic acid was 90.5% the result was agreed with [28] as the resistance of Gentamicin isolates was 100% , while the results were agreed with [32] that the isolates were resistant to antimicrobial antagonism (Co-Trimoxazol) with a ratio 100% , whereas Tobramycin and ciprofloxacin antibodies were resistant to P.aeruginosa bacteria were sensitive to 80% and 40% respectively. These results were close to [30].The antibiotics resistance

of Tobramycin was 66% and for the anti-Amikacin antibody . The isolates resistance were 34% and the tetracycline Antibiotic was the resistance percentage of P.aeruginosa bacteria is 60% . These results are in line with the results of the study conducted by the [33] in his study the a resistance ratio was 59% The resistance of certain strains and strains of bacteria, especially the intestinal strains of the Escherichia coli Proteus mirabilis, Klebsell pneumoniae Pseudomonas aeruginosa, is attributed to many antibiotics, The indiscriminate and excessive use of Antibiotics by people has an effect on their resistance to these antibiotics, as well as the possibility of some genetic mutations in sites that control the encoding of antibiotic susceptibility . the results of the present study showed that the number of E.coli isolates produced in the red membrane of the Congo- Red method was one of five isolates with a ratio 10% , while ELISA method the ratio was 100% . For P.mirabilis bacteria, the number of isolates produced by the red membrane of the Congo- Red was 3 isolates with a ratio 60% , whereas biofilm production of ELISA method in this study was 100% . Whereas P.aeruginosa bacteria, the results showed that 3 isolates with a ratio 60% and% 100% by ELISA method. The investigation results of the biofilm production were shown in two ways, the medium of Congo- Red (CRA) and the method of ELISA is the best methods in detecting the biological membrane was the number of positive isolates was 20 isolations with a ratio 100% and then the Congo - Red was 10 isolations 50% . There is a relationship between the ability of bacterial species to produce the membrane and its susceptibility to rehydration Female disease, since the bacterial species that possess biofilm resistant to many antibiotics and resistant to Opsonization and Phagocytosis as well as resistance to various environmental , in different proportions, including the production of Biofilm, which surrounds the colonies or colonies of bacterial cells to provide some kind of protection, and dose of Antibiotics and access to antibiotics bacterial cell for being a sticky substance. Conditions [34].

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