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Detection of the Capsule Production, Biofilm formation in *Escherichia coli* isolated from different sources in Kirkuk city

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ABSTRACT

This study was carried out in Kirkuk city from November 2021 to April 2022. The study included, 155 specimens of patients (50) urine specimens , (55) sputum specimens,(25) wound specimens and (25) burn specimens ,who attended Azadi Teaching Hospital in Kirkuk city. This study came to identify some virulence factors for Escherichia coli, four kinds of specimens were collected from patients, Smear samples were placed in sterile collection tubes containing transport media, and then transported to the laboratory for culture on different selective media for identification. the Bacterial isolates were diagnosed using traditional methods, the results showed that (37) bacterial isolates were biofilm-producing, by various grades, so (13) isolates formed the biofilm with a strong adherent grades,. , (18) isolates forming a biofilm with a moderately adherent, and (6) isolates forming a biofilm with a weakly adherent grades, ,as the results showed that (29) bacterial isolates had the capsule. The Antimicrobial Agents test results showed that 42 (100%) isolates were resistants to Amoxicillin, 41 (98%) isolates were resistants to Cefotaxime, 35 (83%) isolates were resistants to Cefexime, and 34 (81%) were resistants to the Cefepime,33 (78%) isolates were resistants to the Ceftriaxone,, (27) (64%) isolates were resistants to the Gentamicin, and 26 (62%) isolates were resistants to the Doxycycline (24) (57%), isolate was resistants' to the Amikacin, And 10 (24%) isolates were resistants to Imipenem ,and 16 (38%) isolates were resistants to Levofloxacin.

Introduction

Escherichia coli is G- microorganisms, facultative anaerobe, pole-forming bacterium of the genus Escherichia. It is a big and diverse group of microorganisms that are typically found in the lower digestive tracts of warm- blooded organisms(1). More of the members of this group are commensals. Other pathogenic strains are classified into two groups according to the place of infection(2). Escherichia coli, Which infects the digestive system and causes diarrhea, is designated as intestinal pathogenic Escherichia coli. (IPEC), those that cause disease at a place outside the intestine (UT, bloodstream, abdomen, joints, meninges, skin and soft tissues) are known as extra-intestinal pathogenic E. coli or (EXPEC)(3).

(UTI) is one of the common bacterial infections in humans. Although a number of bacteria can cause UTIs, greatest cases are caused by infection with (UPEC). The infection on the lower urinary tract is called cystitis, however if the infection is on the upper urinary tract, it is known as pyelonephritis(4).

This microbe's pathogenicity is due to the possession of various virulence elements ,among these elements are the possession of iron chelates, siderophores, CNF, and its possession of surface structures such as flagella, capsule, and lipopolysaccharides that confer antigenic characteristics on bacteria by creating(H) antigen and (O)antigen and (K)antigen and also possess Cilia that aid them attach themselves to the host's tissues, giving them biofilm-forming ability .

Materials and methods:

(155) samples, were obtained from patients suffering from several infection states, which included wound infections, burns, UTI, and sputum specimen. These specimens were cultured, Then the dishes were incubated at a temperature of 37°C for1days.

The phenotypic appearances of the bacterial isolates were studied after culturing the bacterial isolates. involved the appearance, range, consistency, color, edges, and elevations of the isolated bacterial colonies (6). Swabs were prepared as of the bacterial isolates that were grown on Mac-Conkey agar medium at an age of 18-24 hours. They were stained with a gram stain, then, examined under a light microscope to see the shape, arrangement and colors of the cells according to their interaction with the gram stain (7).

Detection of biofilm formation: Microtiter plate method

The researcher's method was adopted (8), which includes the following:

1- Activation of the bacterial isolates under study on blood agar medium

2- Young colonies of bacterial isolates were inoculated in BHI broth medium with 1% glucose and, incubated 37°C for1days.

3- 180 µl of fresh BHI broth medium was taken and 20 µl of bacterial growth was added at the age of 24 hours and compared with 0.5 McFarland standard turbidity.

4- 200 microliters of the bacterial suspension were transferred to a microtiter plate using negative control pits containing BHI medium alone, and incubated at 37°C for 24 hours.

5- The contents of the plate were removed and rinsed 3 times by a phosphate buffer solution to remove non-adherent cells, then air dried.

6- Then added 150 µl of methanol alcohol for twenty minutes.

7- Empty the contents of the pits and place them upside down to air dry for 30 minutes.

8- The pits were dyed by adding Crystal Violate dye at a concentration of 0.1% for 15 minutes.

9- The dishes were washed with distilled water to remove excess stains, and then dried.

10- 150 µl of (33%) acetic acid was added in each hole for (30) minutes with no mixing, then the absorbance was determined on the wavelength of 570 nm using a spectrophotometer for the ELISA Microplate reader.

Bacterial isolates were divided according to the optical density (OD) into productive (strong, moderate, weak) or non-productive for the biofilm.

OD Value	Biofilm formation
O.D570<=0.5	Non adherent
O.D570(1-2)	Moderate
O.D570 >2	Strong

A biofilm, is a bacterial population surrounded through a layer of extracellular polymeric surrounding substance which permits them to adhere to diverse surfaces (9). Current research s have displayed biofilm forming in Escherichia coli is facilitated by the appearance of curli, and cellulose ,and that it aids UPEC persist for extended periods in the urethra through cover whole communities of the bacteria with a hydrophobic, extracellular matrix (10). Biofilm production has an result on both the effectiveness of ant -microbials and the stimulating of host immune systems, which promotes to the presence of UPEC in the UT and the major dangerous signs of UTI, and antibiotic resistance (11).

Detection of Capsule Production (Wet Mount Indian Ink Staining).

According to what was mentioned in reference (12),.

It is a technique of using wet amount by placing (1)drop from Indian ink on surface of a clean slide with the loop to carriage a part of culture (grown on brain heart infusion broth). The part of culture was mixed with Indian ink on slide and enclosed by cover slip between two filter papers (to prevent collection of colony). The slide was inspected below the light microscope. The production of a pure halo un-stained with Indian ink around bacteria revealed the presence of capsule.

Results and discussion:

Biofilm formation

The ability of E. coli bacteria to produce a biofilm was detected by the (96)-hole microtiter plate method, and the results showed that a high percentage of E. coli isolates were biofilm producers, so 37 isolates had the capability to yield biofilm in diverse grades. Compared to the negative control, (13) isolates had the ability to produce strong adherent biofilms, (18) bacterial isolates had the ability to produce moderately adherent biofilms, and (6) isolates had the ability to produce weakly adherent biofilms as shown. in Table1.

Table 1: Biofilm formation of E. coli isolated from clinical samples

Unproductive		**Weak**		**Middle**		**Strong**		**source**	
%	**Number**	**%**	**Number**	**%**	**Number**	**%**	**number**		
15	3	20	4	35	7	30	6	20	Urine
0	**0**	20	1	40	2	40	2	5	sputum
10	1	10	1	50	5	30	3	10	Wounds
14.28	1	0	0	57.14	4	28.57	2	7	Burns
11.90	5	14.28	6	42.85	18	30.95	13	42	Total
Ns Chi-Square = 5.067 P-Value = 0.167		*** Chi-Square = 8.000 P-Value = 0.046**		**ns Chi-Square = 3.852 P-Value = 0.278**		**ns Chi-Square = 4.410 P-Value = 0.220**			
Ns Chi-Square = 3.424 P-Value = 0.416									

Statistical results in Table (1) showed that there was no important variance in biofilm production of E. coli bacteria in isolated samples at a probability level P-value (0.416). In this study, the degree of

biofilm production of E. coli isolates was classified according to the ability to Biofilm production is defined as strong, medium, and weak biofilm producer, respectively

Capsule production test for E. coli

The capability of bacteria Escherichia coli , to produce the capsule was detected by Indian ink and examined under a light microscope using an oil lens, where it was found that a lit area around the bacterial cells is an indication of the presence of the capsule. (80%) isolated from the urine, while (70%) appeared isolated from wound samples, while non-capsule-producing bacterial isolates were distributed (60%) in sputum (30%) in wounds (43%) in burns and (20%)) in urine as shown in Table (2).

Table (2): Capsular production of E. coli isolated from clinical samples.

Non-capsule produced		capsule produced		%	Total	Samples
%	NO	%	NO			
20	4	80	16	47.7	20	**Urine**
60	3	40	2	11.9	5	**sputum**
30	3	70	7	23.8	10	**Wounds**
43	3	57	4	16.6	7	**Burns**
31	13	69	29	100	42	**Total**
Ns **Chi-Square = 0.308 P-Value = 0.959**		****** **Chi-Square = 21.103 P-Value = 0.0007**				

The statistical results in Table (2) revealed that there was a important variance in the production of capsules by bacteria E. coli.. at the.P-value (0.0007) and showed no significant difference in the field that did not produce capsules of E. coli in the isolated samples at the level of probability.P-value (0.959)

Antibiotic sensitivity test of Escherichia coli

A sensitivity test for antibiotics was carried out using, Kirby-Baure method, by selecting (42) bacterial isolates, consisting of (20) isolates from urine,(5) isolates from sputum,(10) isolates from wounds and(7) isolates from burns for each source of infection, and by using antibiotic tablets Where (10) antibiotics were used on Agar Mueller-Hinton medium, a suspension was prepared from the pure isolates by taking (3-5) of the young colony into tubes containing Brain Heart infusion Broth, then the tubes were incubated At a temperature of (37 °C) for a period of (16- 18) hours, after that, the growth of the bacterial isolates in the tubes was compared with a tube containing MacFarland's solution, which equals 108 x 1.5 cells / ml, and then 0.1 ml was withdrawn from each bacterial suspension from the bacterial isolates, and then it was spread by a sterile Swab on the medium of M-H agar, then the dishes were went to dry for 15 minutes, and then the tablets were spread using antiseptic forceps on the surface of the agar medium ,of (6-5) tablets in one dish, and the dishes were incubated at a temperature of 37 °C and for 24 hours after that, the diameter of the tumescence area was measured A test in each tablet, and finally the measurement results were compared with what was stated in the World Health Organization measurement tables (13). The

results showed a clear variation in the resistance of the bacterial isolates, where it was 100% resistant to Amoxicillin, 98% to Cefotaxime, 83% to Cefexime, 81% to Cefepine, 78% to Ceftriaxone, 64% to Gentamicin,. resistant to Doxycycline 62%, and for Amikacin by 57% and less for each of Imipenem and Levofloxacin (38%, 42%.) .

The relationship among virulence element and antibiotic- resistance has advanced over a long period. This strong relationship depends on the microbial types, specific mechanism of resistance and virulence element, the biological role and environmental condition and immune system of the host. This is in count to the age and host susceptibility to infections (14).

The possible description to high level of resistance to this antibiotics may be as a result of it being the most commonly accessible antibiotic used as a routine treatment for infections and people readily acquiring it across the counter for self-medication in last years. This could be a likeness of use and misuse of these antibiotics in the society. This discovery is a result of the fact that outside the hospital environment the general population has easy access to various antibiotics from any pharmacy without prescription from a doctor; and availability of prescription drugs by way of medicines and can be simply bought from any pharmaceutical store. Also, careless medical regulations clue to a proliferation of counter free medicines that are available for the treatment and controlling of predominant diseases (15).

Table3: Antibiotic susceptibility of *E. coli* isolates from clinical samples

Resistance					
Total 42	**Burn 7**	**Wound 10**	**Sputum 5**	**Urine 20**	**Antibiotic**
(%57) 24	(%43) 3	(% 90) 9	(%100) 5	(%35) 7	Amikacin
(% 24)10	(%29) 2	(%40) 4	(% 80) 4	-	Imipenem
(%64)27	(%100) 7	(% 80) 8	(%100)5	(%35) 7	Gentamicin
(%62)26	(%100)7	(%70)7	(%100)5	(%35) 7	Doxycycline
(%100) 42	(%100) 7	%100) 10	(%100)5	%100) 20	Amoxicillin
(%38)16	(%75)4	(% 30) 3	(%100)5	(%20)4	Levofloxacin
(%98)41	(%100)7	(% 9 90)	(%100)5	(%100)20	Cefotaxime
(%78)33	(%100)7	(% 80) 8	(%80) 4	(%70)14	Ceftriaxone
(%83)35	(%100)7	(% 80) 8	(%100)5	(%75)15	Cefexime
(%81)34	(%100)7	(% 80) 8	(%80)4	(%75)15	Cefepine

Conclusions:

The study showed that(37) of Escherichia coli isolate have ability to biofilm production and(29) of Escherichia coli isolates have capability to form capsules.all isolates of Escherichia coli had the highest resistance to the antibiotic, Amoxicillin by (100%) and the least resistance to the Imipenem(24%) **,** this study recommends conducting several studies to discover the reasons for the increase in the resistance of *E. coli* to the antibiotic through detection of antibiotic resistance gene.

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