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Antibiotic resistance patterns and adhesion ability of uropathogenic *Escherichia coli* in children

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Abstract: This study aimed to isolation and characterization of *E.coli* from Children urine , the phenotypic detection of biofilm formation , bacteria ability to adhesion, and the detection of *fim*H gene using PCR-based molecular diagnostic methods. In order to isolate *E.coli*, 250 urine samples were collected from children in camp saad of displaced / Diyala.Fifteen isolates(30%) of *Escherichia coli* were recovered according to the bactereiological , biochemical tests , used Api20E system and VITEK 2 system for confirmation. The study found that bacterial growth in females higher than in males and the incidence of infection in children under the age of 3 years is more common than in older age groups. The sensitivity test was performed for Ten antibiotics (Ampicillin, Piperacillin/tazobactam, Cefoxitin, Cefixime, Ceftazidime, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Trimethoprim /sulfamethoxacole) Where the percentage of resistance were (93.3% , 20.0% , 26.7% , 80.0% , 80.0% , 0.0% , 0.0% , 46.7% , 20.0% , 73.3%) respectively.Minimal inhibitory concentrations (MIC) were determined for Ceftazidime,Piperacillin /tazobactam, Gentamicin and Trimethoprim/ sulfamethoxacole. The MIC values were (4-32), (4-128), (<16), (20-320)µg / mL.Some of the virulence factors were detected, that included detection of biofilm formation and isolates ability to adhesion, all isolates showed ability to produce a biofilm and adhesion .Gene *fim*H was detected by Polymerase Chain Reaction(PCR), the percentage of isolates containing the gene was 100%.

Keywords: *Escherichia coli*, virulence factors, *fim*H.

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Introduction:

Urinary tract infection is one of the most common diseases in children. It is one of the second most common problems after respiratory diseases in community- acquired infections. While urinary tract infection is considered as first among hospital-acquired diseases (Nosocomial infection) (1). Therefore, studies have increased on the factors causing these infections, especially bacteria (2). And that all diseases resulting from the settlement of microbiology in the kidney and bladder and penetration of the tissues of the urinary tract known as Urinary Tract Infections (3).The predominant organism that cause UTI is *Escherichia coli* which responsible for 60 %–90 % of these infections (4).They usually named as a uropathogenic *E. coli* (UPEC)and have many virulence factors, including adhesion, hemolysin, capsule, and Siderphores, which enable them to invade the urinary tract and in most cases, pus cells are associated with urinary tract infections. The adherence of uropathogenic*E. coli* to urothelial surface (uroepithelial cells), is a critical first step in the pathogenesis of UTI, and it is controlled by three elements: *E. coli* adhesions, host receptors, and host defense mechanisms (5).

Fimbriae type 1 is a filamentous structure covering the surface of bacterial cells. These cilia are encoded by a gene called *fim H* and the *fim H* coordinates the glycoprotein adhesion on host cell surfaces. This factor is (90%) Of pathogenic and nonpathogenic bacterial cells (6) Fimbriae type 1 plays an important role in the pathogenesis and contributes to inflammation of the intestine, although the vast majority of pathogenic and pathogenic bacteria produce this type of fimbriae (7). Fimbriae type 1 receptors are found on the surface of red blood cells in many species (8). They are composed of a fibrous structure called pilin (1-2 microns) (9). The fimbriae type 1 is encoded in *E. coli* by a *fim* gene that encodes the capillaries and auxiliary secondary protein units and genes on the bacterial chromosome. Because of the excessive use of antibiotics ,randomization and cutting of treatment after the signs of improvement on the patient emerged, the treatment of the disease become very difficult. Therefore, the appropriate antibiotic should be tested. Treatment is not random. It is based on conducting the drug sensitivity tests on the isolated germ to determine the appropriate antibiotic to eliminate them (10).

Polymerase Chain Reaction (PCR) technique is characterized by the high speed and privacy of the *E.coli* strain in clinical specimens. In order to increase the possibility of integrated and accurate diagnosis at lower cost and time, The most widely used in all parts of the world and has relied on many studies (11). The aims of this study were isolation and characterization of *E.coli* from Children urine , the phenotypic detection of biofilm formation , bacteria ability to adhesion, and the detection of *fim*H gene using PCR-based molecular diagnostic methods .

Materials and Methods:

Collection of specimens:

Atotal of (250) urine samples were collected from children with urinary tract infections in the camp saad of displaced / Diyala during the period 20-9-2016 to 10-10-2016.

Culture characteristics:

Samples were cultured immediately onto MacConkey agar and blood agar plates and then incubated at 37°C for 24 hours. For the identification of *E.coli*, the colonial appearance on simple solid media was studied, after 24 hours incubation at 37°C.The isolates were examined for their shape, size, colour, pigments, and haemolytic activity. Then transferred and streaked on MacConky agar for detecting the ability of each isolate to ferment lactose. and isolates were identified depending on their biochemical tests and the identification was confirmed by Api 20 and Vitek 2 system . The bacterial isolates were stored for long period in a medium containing 15% glycerol at low temperature.

Diagnosis and Antimicrobial susceptibility examination and MIC by VITEK 2Compact:

In clinical microbiology Vitek2 used as an auto analyzer system for the identification (ID) and antibiotic susceptibility testing (AST) of the bacteria in clinical samples(12).

However, the samples were achieved according to manufacture instructions as following:

- Use fresh 18 hour culture of organism.
- Subculture freeze dried isolates twice before setting up any Vitek 2 cards.
- Inoculate organism into 3ml Vitek Saline tube, vortex and invert the tube 2 to 3 times to uniformly suspend inoculum.
- Using the Densichek, insert and turn the tube one full rotation.
- Cards must be filled within 15 minutes of inoculum preparation.
- Card types include Identification and Antimicrobial Susceptibility Testing (AST).

1- Adherence test:

Uroepithelial cells were obtained from the healthy people urine , The urine samples were centrifuged at 2500 rpm for 10 minutes. Supernatant was discarded, the pellet was washed with normal saline and centrifuged at 2500 for 10 minutes. The process repeated three times, For adherence assay, 0.5 ml of bacterial suspension was mixed with 0.5 ml of epithelial cells suspension and the mixture was incubated at 37°C for 60 minutes. Immediately after incubation, the suspension was washed four times by centrifugation at 2000 rpm for 10 minutes, A portion of the final cell suspension was spread onto glass slide, air dried, and then fixed on the glass slide by flaming. The suspension was stained with gram stain, washed with tap water, and air dried. The adhered bacteria were examined with oil-immersion light microscopy(13).

2- Biofilm formation:

Inoculation 1 ml of Trypton soya broth with particular isolates and incubated for 24-48hrs at 37 ºC ,Those tubes were removed carefully andSafranin stain (1%) were added to each tube for 15 minutes the tubes were rinsed and let to dry at room temperature (20-25)ºC The result was read by naked eye through the formation of biofilm as a layer at the internal wall of tubes in comparison with the negative control (tube contains Trypton soya broth medium only), thickness and color of layer consider a parameter of bacterial ability for biofilm formation(14).

Extraction of total DNA and PCR amplification:

Total DNA of bacterial isolates was extracted by using Promega Genomic DNA Purification Kit. From the total DNA, the *FimH* gene was amplified using the specific primer F (5'-ATG AAA CGA GTT ATT ACC CT- 3) and R (5'-TTA TTG ATA AAC AAA AGT CAC G-3) (15) PCR reaction was carried out in a 20µl reaction containing 2.5µl of Green Master Mix (Promega, USA)2 µl of 10pmol/ µl of each primer, 4 µl of DNA template and the volume was completed to 20 µl using nuclease-free water.Thermo cycling conditions were as follows: initial denaturation at 95 °C for 5min, followed by 30 cycles of denaturation at 95 °C for 30 sec; annealing at 59 °C for45sec; extension at 72 °C for 45 sec, and a final extension cycle at 72 °C for 5 min, then the program was held at 4°C. PCR products were resolved on 1% agarose gel Gel was visualized by UV transilluminator and the image was captured by digital camera (Canon, USA).

Results and discussion:

From a total of 250 clinical specimens, 40 specimenes which

yielded positive growth ,15 isolates were identified as uropathogenic *E.coli* (30%) depending on their biochemical tests and according to AP20E system and Vitek 2 system . All isolates had ability to ferment Lactose and form large Pink colonies, smooth further identification some of the biochemical tests was performed on isolates that gave positive results for catalase test , Indol test and Methyl red but ,the isolates gave negative result for all of oxidase test, Gelatin laquification test, Urase test Voges-Proskauer test and Citrate Utilization Test. shown that in table(1) and figure (1).

Table (1): Biochemical tests for the diagnosis of *E. coli*

***E.coli* Iso.**	**Biochemical tests**								
	Gelatinase	**Motility**	**Urase**	**Citrate**	**VP**	**MR**	**Indol**	**Catalase**	**Oxidase**
	-	+	-	-	-	+	+	+	-

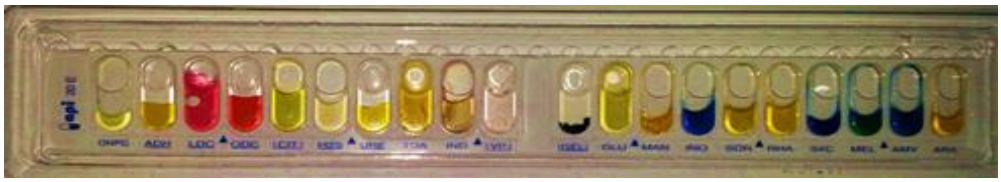


Figure (1): Biochemical testing of *E. coli* by the Api20E system

Table (2): The isolation ratio of isolated *E.coli* depending on sex

Genus	**Bacterial isolate**	**Percentage of *E. coli* (%)**
Male	7	40%
Female	8	60%
Total	15	100%

Table (2) showed that prevalence in females is higher than in males and this is Agreed with the findings of Ahmed(1). The study showed that the percentage of *E.coli* infection in children under the age of 3years is more common than in the older age groups as table (3).

Table (3): Percentage of urinary tract infection by age group of children in the camp

Age groups	**Number of samples tested (%)**	**Number of positive samples (%)**	**E.coli ratio(%)**
1-3	(40) %16	(%32.5) 13	(%20) 3
4-6	(%25.6) 64	(%17.5) 7	(%33.3) 5
7-9	(%26.4) 66	(%27.5) 11	(%20) 3
10-12	(%32) 80	(%22.5) 9	(%26.6) 4
Total	(%100) 250	(%100) 40	(%100)15

This is due to several reasons, including the incomplete development of the child's immune system ,weak body structure , As well as the incorrect methods of cleaning the anal area, starting with cleaning from the anal region towards the genital opening, which helps the transmission of bacteria to the urethra , these results are similar to Cavagnaro (16).

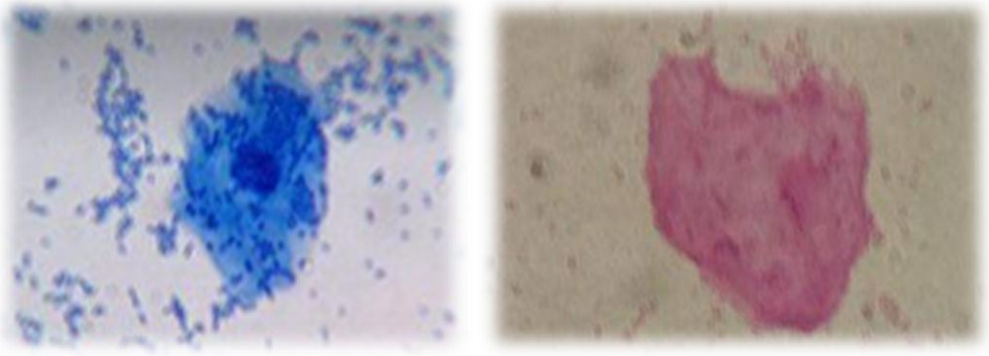
Antibiotics susceptibility Test and minimal inhibitory concentration:

All isolates under study were tested for ten antibiotics by Vitek2 Compact,

mostly of commonly used in the country for the treatment of different infections, the proportion of resistance to antibiotics Ampicillin, Piperacillin/ tazobactam, Cefoxi-tin, Cefixime, Ceftazidime, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Trim-ethoprim/ sulfamethoxacole (93.3%, 20.0%, 26.7%, 80.0%, 80.0%, 0.0%, 46.7%, 20.0%, 73.3%) respectively. The results of the present study are agreed with the findings of Al-Moussawi (17) and Al-Hammadani (18), They indicated that the resistance of *E. coli* to penicillins was 95% and 86.4%, respectively, and was not agreed with Ahmed (19), where the resistance to this antibiotic was 100%.The results of the imipenem antibiotic test (IPM) do not show any isolates resistance to this antibiotic . The results of the present study was agreed with the findings of Atar (20) in Turkey, where the resistance ratio isolates 0%, and was consistent with Al-Attar (21) in Baghdad. The resistance of bacteria to Imipenem was 0% and did not agree with Oliveira (22). The resistance to bacterial isolates of Imipenem was 52% ,with regard to Amikacin's resistance ,the results of the current study were agreed with Yaseen (23) where the resistance ratio of Amikacin was 12.8%, and did not agree with Ahmed (1) where 81.9% resistance to antimicrobial. For gentamycin, the results of the present study were agreed with those obtained by Al-Juboury (24). The percentage of *E.coli* resistant to this antibiotic was 45% and did not agree with Jaloob (25). Their isolates were 55.6% resistant. The results of Khosrow (26). which indicated that the resistance of bacteria to gentamycin was 56%, due to different systems used in the treatment and use of antibiotics. For Ciprofloxacin, Our study did not agree with Rumana (27) in Bangladesh, where their isolates were 50% resistant to antibiotic. For Trimethoprim/ sulfamethoxac-ole, the results of the current study were agreed with the findings of Al-Attar (21) in Baghdad, with a resistance ratio of 70.4%. The results of the present study did not agree with the findings of Yaseen (23) in Kirkuk, where the ratio of resistance to Trimethoprim was 44.8%.All isolates of *E.coli* showed multiple resistance to selected antibiotics in the study, multiple resistance for more than one antibiotic is one of the most medical problems because it is difficult to choose the appropriate treatment for the patient. One of the main reasons for the emergence of multiple resistance is the indiscriminate use of antibiotics without relying on the sensitivity test, which increases the chances of bacterial adaptability and resistance to antibiotics used in treatment(28). Al-Sayigh (29) states that it is very common to isolate multiple resistance strains, particularly β- lactam antibiotics in the Mazel (30). Gram-negative bacteria are resistant because their production of beta-lactam enzymes(31). The cause of the resistance may be due to a mutation in the ribosomal proteins encoded genes and thus lead to a change in the protein receptor structure on the ribosome (32). This has been identifiedMinimal inhibitory concentration (MIC) for Ceftazidime, Piperacillin /tazobactam, Gentamicin and Trimethoprim/ sulfamethoxacole The MIC to bacterial isolates under study ranged from values (4-32), (4-128),(<16),(20-320)µg / mL. Results of the current study were approaching to the results of the researcher Al-Tememy (33) for a number of antibiotics and are not close to the results of the researcher Al-Autbi(14).

Detection ability of bacteria to adhere on epithelial cells:

The results showed that all isolates 15 (100%) had the adhesion ability to epithelial cells as showed in figure (2). The adhesion ability of *E. coli* is due to many factors that help it to adhere to it, P-fimbriae and Type 1-Fimbriae, On the other hand, epithelial cells also have many nuts that help them to catch bacteria and collect bacterial cells around them (1). The effect of bacterial adhesion on host cells is an important virulence factor and represents the first and fundamental step in the process of colonization (1). In another study conducted by Al-marjani (34) on *E. coli* and isolates of children with diarrhea in a number of hospitals in Baghdad showed the possession of all isolates belonging to these bacteria adhere to epithelial cells, ie (100%) .

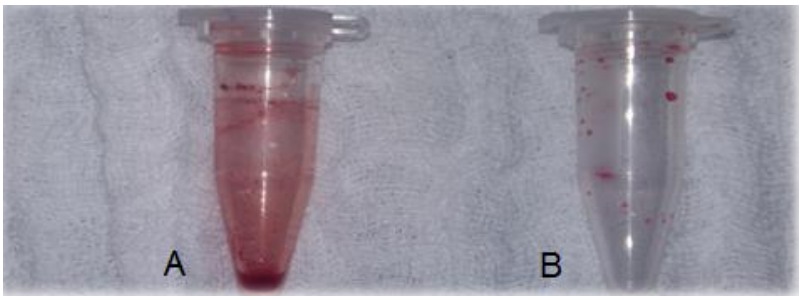


(A) **(B)**

Figure (2): The adhesion of bacterial cells to human epithelial cells.
(A) infected epithelial cell . (B) Normal epithelial cells.

Biofilm formation:

The tube method used for the detection of the ability of *E.coli* on the formation of biofilm as figure (3). Showed *E.coli* isolated from people with urinary tract infection ability to form biofilm (100%), (35) found that 90.62% of bacterial isolates isolated from UTIs were able to form the biofilm.



Figure(3): The of bacteria to the formation of biofilm
A: Positive result B: Negative result

The adhesion of bacteria to the surfaces of epithelial cells is the first step towards the formation of the biofilm, the biofilm produced by the accumulation of bacteria that is covered with a polysaccharide layer and helps

the bacteria to the adhesion (36). Table(4) shows the susceptibility of bacteria to Biofilm formation and Intensity of composition .

Table (4) The intensity of Biofilm formation in *E.coli* isolates

Biofilm formation	+++	++	+
Iso. No	6	6	3

Detection of *fim*H gene in *E. coli* isolates using PCR technique:

The results obtained in this study showed that all isolates of *E. coli* gave a positive result for *fim* (H) with 100%. as figure (4). The results obtained in this study were identical with the results of Abed (6) and Ahmed (1) who found that *fim* (H) was 90% and 100% respectively of all isolated samples from UTIs. While Abass (37) found that 71% of isolates only had the encoded gene of *Fim* (H). Ananias (15) note that 95% of*E.coli* isolates contain *fim* (H). Mihaylova (38) confirmed that 100% of the isolates contained the target *fim*(H) gene. *fim* H is one of the factors responsible for the ability of bacteria for adherence to the surfaces of epithelial cells and mucous membranes of the host cells and the necessary steps and basis in bacterial colonization. The capillaries are associated with the special receptors found on the surfaces of the epithelial cells of the urinary tract because they are a specific factor and risk of association with epithelial cells Which facilitates the presence of *E. coli* in non-intestinal tissues (1).

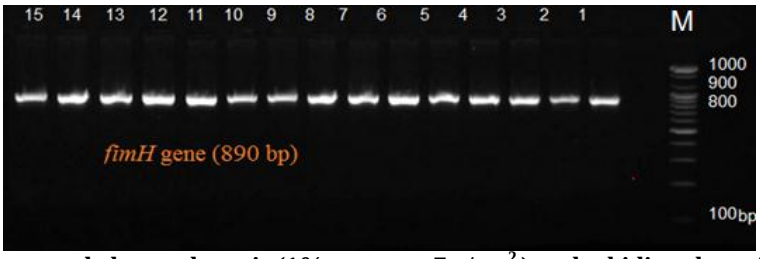


Figure (4): Agaros gel electrophoresis (1% agarose, 7 v/cm^2) and ethidium bromide staining to detect *fim*H Lane M, molecular size DNA ladder (100 bp DNA Ladder).

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