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Molecular Detection of Adhesion Gene in Uropathogenic *Escherichia coli* Isolated from Children in Iraqi Patients.

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Abstract

A total of (93) samples of urine was collected from patients ranging in age from one to ten years, including both genders, from Central Pediatric Teaching Hospital in Baghdad during the period from September 2023 and March 2024. The isolates were identified by morphological and biochemical characteristics as well as the API 20E system after the urine samples were cultured. The results showed that 59 of 93 isolates (63%) were *E. coli.* This study demonstrated that 19 samples (76%) of the *E. coli* isolates were from females and only 6 samples (24%) were from males. A selection of ten *E. coli* isolates was chosen according to their highest adherence to uroepithelial cells. The isolates' capacity to adhere to epithelial cells taken from female urine samples was examined. All of the isolates were discovered to be able to adhere to epithelial cells with a mean of (20.00±0.82, 19.00±0.82, 17.25±0.89, 17 ± 0.699025, 16.00±0.74, 14.00±0.82, 14.5± 0.67082, 13.5± 0.513701, 13.5± 0.612372, 13.00±0.70) adherent bacteria, isolates, on the other hand, had the highest capacity. Antibiotic sensitivity tests were performed on the isolates, and the results showed that all of the isolates were highly sensitive to Ciproflaxian (100%), ampicillin (90%), and gentamicin (70%), and however, higher resistance was found to Trimethoprim/Sulphamethoxazole (100%), naldixic acid (80%), Ceftriaxone (80%), and cephalexin (80%). Extraction of the genomic DNA of ten *E. coli* was carried out for genetic investigation of selected isolates. This was done by isolating them using conventional PCR and amplifying the fimH gene, which is attributed to fimbria adhesion to uroepithelial cell.

1. Introduction:

Urinary tract infections are a common and widespread disease worldwide, posing a global threat due to their frequent diagnosis among millions of individuals per year. Half of all women in the globe will get a urinary tract infection at some point throughout their lives [1]. The distribution of Uropathogenic *E. coli* strains' virulence factor-encoding genes will make it possible to identify them based on how these genes contribute to the development of the disease [2]. *E. coli* is the bacteria responsible for urinary tract infections. It enters the urinary system and travels to the bladder, causing cystitis. The spread of bacteria in the kidneys and ureters can be caused by uncontrolled conditions, resulting in acute pyelonephritis, which is a secondary infection. The infection has the potential to cause kidney damage, renal failure, and even death [3]. *E. coli* adherence to the uroepithelium may protect them from urine lavage, enabling them to reproduce and infiltrate renal tissue [4]. Type 1 pili play a significant role in the development of a UTI. The adhesion protein FimH, which is found at the distal end of the pilus, binds to glycoprotein receptors that contain trimannose and monomannose. The following cell types have type 1 fimbriae receptors: erythrocytes, intestinal cells, vaginal cells, buccal epithelium, and uroepithelial cells [5] The adhesion of (UPEC) strains to uroepithelial cells can be facilitated by the presence of pili or fimbriae, as demonstrated [6]. The aim of this study was to identify the fim H

gene, which is attributed to fimbria adhesion to uroepithelial cell.

2. Methodology:

2.1 Bacterial Isolates:

Ninety-three urine samples were collected from children with urinary tract infections attending to Central Pediatric Teaching Hospital in Baghdad between September 2023 and March 2024; their age ranged from one to 10 years. The sample were cultured on both MacConkey and Eosin methylene blue agar and incubated for 24 hours at 37^{o}C. The samples then examined microscopically using Gram stain, biochemical assays, and the API 20 system.

2.2 Adhesion Test:

The bacteria were cultured using a viable cell culture technique until the cell count was approximately 1x108 per milliliter after the cells were centrifuged and the pellet was twice cleaned with PBS [7]. The urine of healthy females was centrifuged at 1000 rpm for five minutes in order to remove uroepithelial cells.The cells were then washed three times in PBS before being resuspended in PBS [7]. The bacterial suspension and epithelial cell suspension were incubated in a shaker-incubator. Unattached bacteria were removed by centrifugation three times in PBS. The pellet was resuspended in PBS before being placed under a microscope with glass slides and allowed to air dry. Methanol:acetic acid (3:1) was used for adhering the glass slide and then coated in crystal violate. The number of bacteria adhering was counted with a compound light microscope.

2.3 Antibiotic Susceptibility Test:

The antibiotic susceptibility of *E. Coli* isolates to ampicillin, ceftriaxone, cephalexin, Ciprofloxacin, gentamycin, naldixic acid, and trimethoprim/sulfamethoxazole was tested using the disc diffusion method.

2.4 Molecular Study of DNA:

The DNA was extracted from ten isolates *E. coli* isolate using the Presto Mini Genomic DNA Kit (Geneaid, Thailand).

2.5 Amplification of FimH Gene by PCR Technique:

the FimH gene was amplified by using the specific primer as illustrated in Table 1.

The amplification conditions of the FimH gene are listed in Table 1.

2.6 Statistical Analysis

The experiment's mean standard deviation is presented in relation to the adhesion of epithelial cells and *E. coli*

Table 1. Thermal cycle conditions of the FimH gene.

Steps time	Temperature and cycle	
Initial denaturation	95^o 4min	1
Denaturation Annealing Extension	95^{o}C 30 sec 56^o 45 sec 72^{o}C 1 min	30
Final extension	72 ^{o}C 5min	1

Table 2. Primers of FimH gene used in this study [8].

Gene	Primer sequence (5'- 3')	Product size(bp)
fimH gene	F: GAGAAGAGGTTTGATTTAACTTATTG R: AGAGCCGCTGTAGAACTGAGG	504 bp

3. Result and Discussions:

3.1 Identification and Isolation of *Escherichia coli*:

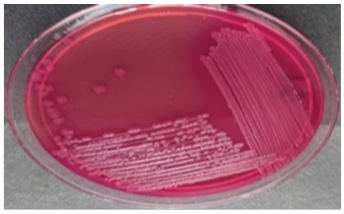
Ninety-three urine samples were examined microscopically using Gram stain, biochemical assays, and the API 20 system. The *E. coli* isolates were detected in 59 (63%) urine samples Figure 1. Following this, the samples were examined microscopically using Gram stain, biochemical assays, and the API 20 system these results agree with [9]. Only 59 isolates (63%) contained *E. coli* morphological and biochemical traits, this result agreed with [10] who found that the frequency of positive results reached 60%. Out of a total of 25 *E. coli* isolates, females had 19 (76%) and males had 6 (24%). This result is agreed with a study reported by, [11] which found that 77% of *E. coli* isolates came from females and 23% from males.

3.2 Detection of Adhesion in *E. coli:*

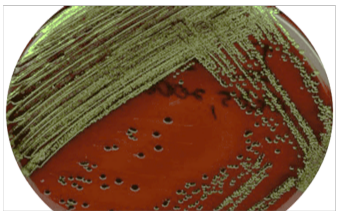
The ability of *E. coli:* to attach to epithelial cells isolated from urine was examined using the frequency of bacteria

Table 3. Reagents were added to the PCR process.

Components	volume
genomic DNA 250 ng	2
Forward primer 10 pmol/ μ	2
Reverse primer 10 pmol/ μ	2
Go Taq green Master mix (1X)	12.5
Deionized Distile Water	6.5
Final volume	25



A-



B-

Figure 1. A: Colonies of lactose fermenting *E. coli* were cultured on MacConkey agar for 24 hours at 37^{o}C. B: *E. coli* colonies on Eosin Methylene Blue Agar.

Table 4. The biochemical tests for diagnosis of *E. coli.*

Test	Result
Oxidase	+
Indole	-
Methyl red	+
Voges-proskaure	-
Citrate utilization	+
TSI test	A/ K- -
Urease test	-

distribution on epithelial cells and the mean number of bacteria adhering to twenty epithelial cells Figure 2. Bacterial cells adhere to one another and can be easily observed under a compound light microscope. The Table 5 indicates that there were varying degrees of adhesion between the isolates and female epithelial cells. The average number of adherent bacteria per epithelial cell ranged from 10.25±0.33 to 20.00±0.82 bacteria. The Table 5 indicates that there were varying degrees of adhesion between the isolates and female epithelial cells. The average number of adherent bacteria per epithelial cell ranged from 10.25±0.33 to 20.00±0.82 bacteria. It was found that isolates with high adhesive ability have mean (20.00±0.82, 19.00±0.82, 17.25±0.89, 17 ± 0.699025, 16.00±0.74, 14.00±0.82, 14.5± 0.67082, 13.5± 0.612372, 13.5± 0.513701, 13.00±0.70) bacteria/epithelial cell, so although isolate 24 has the lowest adhesive capability with mean (10.25± 0.33541) bacteria/epithelial. According

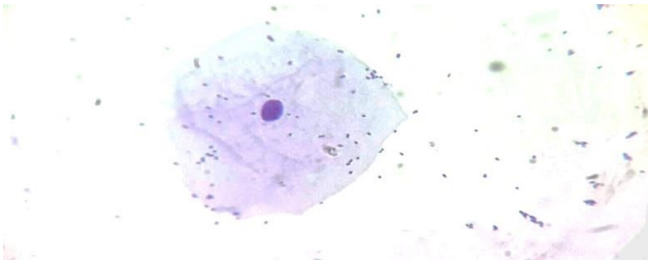


Figure 2. *E. coli* adhering to uroepithelium cell

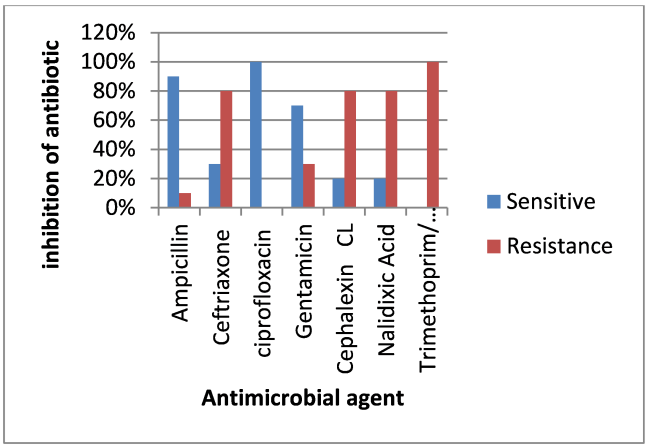


Figure 3. Antibiotic sensitivity test of *E. coli*.

to [12] the average *E. coli* adhesion on uroepithelial cells is 40 bacteria/uroepithelial cell.

3.3 Susceptibility Test for Antibiotics:

In this study, antibiotic disc diffusion was used to test ten isolates against ten antimicrobial discs according to [13] guidelines. Ciprofloxacin (100%), ampicillin (90%), and gentamycin were the most effective medications against *E. coli* (70%)as shown in Figure 3. There were differences in antimicrobial resistance. The majority of the isolates were resistant to trimethoprim/sulfamethoxazole (100%), naldixic acid (80%), Ceftriaxone (80%), and cephlexin (80%).

The resistance percentage of *E. coli* isolates to Trimethoprim/Sulfamethoxazole varied and could reach 90%, as suggested by [14] which is consistent with the present findings. Comparing the results shown in Figure 3 with the results from other studies revealed this.resistance to naldixic acid was found among the isolates (80%). These results agree with [15] who found that *E. coli* resistance reached 89%.The results of [16] for ceftriaxone and cephalexin show resistance percentages of 85% and 80%, respectively, which is in agreement with [17] for *E. coli* resistance, which was shown to reach 81%. The results as showed in Figure 3 found the number and percent of sensitive isolates of bacteria against types

Table 5. Average *E. coli.* adhesion to uroepithelialcell

The number of isolates	The ratio of *E. coli.* that adheres to epithelial cells				
	0	*1-5	6-20	>20	The average number of adhering *E. coli.* cells ± standard deviation
1	1	3	3	6	10 ± 0.49516
2	0	1	6	6	11 ± 0.76899
3	0	1	3	3	6 ± 0.49099
4	0	2	2	6	8.5 ± 0.689202
5	2	2	4	6	10.5 ± 0.443203
6	0	4	4	8	13 ± 0.707107
7	1	2	2	6	8.75 ± 0.578988
8	2	4	0	6	8.5 ± 0.645497
9	0	1	4	6	9.5 ± 0.719059
10	0	2	4	8	12 ± 0.790569
11	0	4	8	8	16 ± 0.74162
12	0	3	5	5	10.25 ± 0.33541
13	0	6	8	8	17 ± 0.699025
14	2	4	4	8	13.5± 0.513701
15	0	2	6	6	11.5± 0.694365
16	1	3	3	10	14 ± 0.829156
17	3	8	4	4	11.75± 0.440544
18	1	6	6	10	11.5 ±0.665533
19	0	8	6	6	14.5± 0.67082
20	2	2	5	12	17.25± 0.891427
21	0	5	10	10	20 ± 0.829156
22	2	2	8	6	13.5± 0.612372
23	17	8	4	127	19.25 ±0.829156
24	1	8	4	4	11.25 ±0.6033
25	0	4	4	8	13 ±0.70

of antibiotics were higher at ciprofloxacin (100%), ampicillin (90%) and gentamycin)70%). The number and percent of sensitive bacteria isolates against different antibiotics were found to be greater at ciprofloxacin (100%), ampicillin (90%), and gentamycin (70%), High sensitive to Ciprofloxacin was found among the isolates (100%) these result agree with [18] who found that the E.coli isolates were vary in their sensitive to Ciprofloxacin which could reach to 94.6%. When compared these results Figure 3 with the results for other studies, found the current results were agreed with [19] who could reach to sensitive to ampicillin reach at 90%.Results [20] for who found generally susceptible to gentamycin 89%.

3.4 Detection of *fimH* gene by PCR Technique:

Amplification of the *fimH* gene was done using specific PCR primers. The results shown in Figure 4 indicate that the gene was successfully amplified for all isolates, as evidenced by the presence of a band that has a molecular size of 508 bp. Regarding *fimH* gene these results are in agreement with a previous study by [21] who isolated *E. coli* from UTI samples and obtain the same molecular size band for *fimH* gene in their isolates. Our findings indicated a higher frequency of *fimH*, which may have referred to an essential function for the virulence genes in *E. coli* that cause urinary tract infections these result agree with [22].

4. Conclusions:

The bacteria *E. coli.* is the cause of urinary tract infections in children, particularly Iraqi children.The antibiotics that have the most effectiveness for uropothermal infections in Iraqi children are ciprofloxacin (100%), ampicillin (90%),

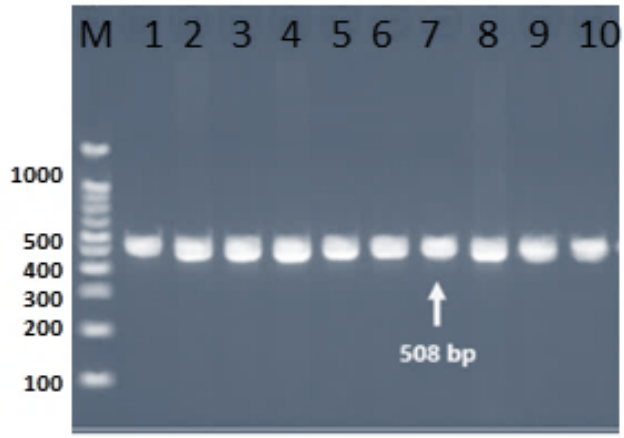


Figure 4. Amplification of *fimH* gene amplified using PCR test, amplified product (508 bps) of isolates uropathogenic *Escherichia coli.* The end product was electrophoresis on 1% agarose for 60 minutes at 110 volts

and gentamicin (70%) *E. coli* isolates showed a high rate of resistance to different antibiotics trimethoprim/ sulfamethoxazole (100%), naldixic acid (80%), Ceftriaxone (80%), and cephlexin (80%) were the antibiotics that were most resistant, and the most effective was 100% trimethoprim/ sulfamethoxazole. amplification of adhesion gene (*Fim H* gene) significantly affects the attachment of epithelial cells.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

References

[1] M. Demirci, Ö. Ünlü, and A İ. Tosun. Detection of o25b-st131 clone, CTX-M-1 and CTX-M-15 genes via real-time PCR in *Escherichia coli* strains in patients with utis obtained from a university hospital in Istanbul. *Journal of Infection and Public Health*, 12(5): 640–644, 2019, doi:10.1016/j.jiph.2019;02.017.

[2] J. Brons, S. Vink, M. de Vos, S. Reuter, Dobrindt, and J. van Elsas. Fast identification of escherichia coli in urinary tract infections using a virulence gene based PCR approach in a novel thermal cycler. *Journal of Microbiological Methods*, 169: 105799, 2020, doi:10.3168/jds.2024-24331.

[3] S. Whelan, B. Lucey, and K. Finn. Uropathogenic *Escherichia coli* (*UPEC*)-associated urinary tract infections: the molecular basis for challenges to effective treatment. *Microorganisms*, 11(9), 2023, doi:10.3390/microorganisms11092169.

[4] E. Santo, C. Macedoa, and J. M. Marin. Virulence factors of uropathogenic escherichia coli from a university hospital in ribeirão preto, são paulo, brazil. *Revista do Instituto de Medicina Tropical de São Paulo*, 48: 185–188, 2016, doi:10.1590/s0036-46652006000400002.

[5] M. S. Erjavec and D. Žgur Bertok. Extended characterization of human uropathogenic *Escherichia coli* isolates from Slovenia. *Clinical Management of Complicated Urinary Tract Infection*, 35, 2011, doi:10.18683/germs.2022.1324.

[6] A. Lundgren, van. Oostrum, P. Iturri, M. Malkoch, J. Toca-Herrara, and E. Reimhult. Adhesion of *E. coli* bacteria is force-modulated due to fimbriae-mediated surface repulsion and multivalent binding irrespective of surface specificity. *bioRxiv*, 5, 2024, doi:10.1101/2024.05.23.595589.

[7] M. Saleh Al-Dulaimi and H. Rasheed Al-Taai. Detection OF some virulence factor and antibiotics susceptibility of proteus mirabilis isolated from different clinical sources in Baquba. *Biochemical Cellular Archives*, 20(1): 1–9, 2020, doi:10.35124/bca.2020.20.1.803.

[8] H. R. Jalali, A. Pourbakhsh, F. Fallah, and G.Eslami. Genotyping of virulence f actors of uropathogenic *Escherichia coli* by PCR. *Novelty in Biomedicine*, 3(4): 177–181, 2015, doi:10.22037/nbm.v3i4.8036.

[9] H. E. Hassan, H. N Altayb, M. M El Hassan, and M. A Elmekki. Genotypic detection of the virulence factors of uropathogenic *Escherichia coli* isolated from diarrheic and urinary tract infected patients in Khartoum state, Sudan. *African Journal of Microbiology Research*, 12(9): 230–236, 2018, doi:10.5897/AJMR2017.8771.

[10] B. Dormanesh, F. Dehkordi, S. Hosseini, H. Momtaz, R. Mirnejad, M. Hoseini, and E Darian. Virulence factors and o-serogroups profiles of uropathogenic *Escherichia coli* isolated from iranian pediatric patients. *The Iranian Red Crescent Medical Journal*, 16(2), 2014, doi:10.5812/ircmj.14627.

[11] H. Staji, M. Rassouli, and S. Jourablou. Comparative virulotyping and phylogenomics of *Escherichia*

coli isolates from urine samples of men and women suffering urinary tract infections. *Iranian Journal of Basic Medical Sciences*, 22(2): 211, 2019, doi:10.22038/ijbms.2018.28360.6880.

[12] J. Danalakshmi and P. Gopinath. Detection of fimh gene for fimbrial mediated adhesion antigen in clinical isolates of *Escherichia coli*. *Research Journal of Pharmacy and Technology*, 9(9): 1427–1429, 2016, doi:10.5958/0974-360X.2016.00275.4.

[13] R. Humphries, A. Bobenchik, J. Hindler, and A. Schuetz. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing. *Journal of Clinical Microbiology*, 59(12): e0021321, 2021, doi:10.1128/JCM.00213-21.

[14] R. F. Polse, S. Y. Yousif, and M. S. Assafi. Prevalence and antimicrobial susceptibility patterns of uropathogenic *E. coli* among people in Zakho, Iraq. *International Journal of Research in Medical Sciences*, 4(4): 1219–1223, 2016, doi:10.18203/2320-6012.ijrms20160813.

[15] M. Aghazadeh, S. Sari, M. Nahaie, S. ashem, and S. Mehric. Prevalence and antibiotic susceptibility pattern of e. coli isolated from urinary tract infection in patients with renal failure disease and renal transplant recipients. *The Tropical Journal of Pharmaceutical Research*, 14(4): 649–653, 2015, doi:10.4314/tjpr.v14i4.13.

[16] Z. Abdul-hussein and A. Inssaf R. Aheema. Molecular diagnosis of diarrheagenic *E. coli* infections among the pediatric patients in wasit province, Iraq. *Journal of Pure and Applied Microbiology*, 12(4): 2229–2240, 2018, doi:doi.org/10.22207/JPAM.12.4.62.

[17] Z. Iqbal, M. Mumtaz, and A. Malik. Extensive drug-resistance in strains of *Escherichia coli* and klebsiella pneumoniae isolated from paediatric urinary tract infections. *Journal of Taibah University Medical Sciences*, 16(4): 565–574, 2021, doi:10.1016/j.jtumed.2021.03.004.

[18] F. Adzitey, S. Nafisah, and A Haruna. Antibiotic susceptibility of escherichia coli isolated from some drinking water sources in tamale metropolis of Ghana. *Faculty of agriculture, food and consumer sciences*, 8: 15, 2015, doi:10.3923/crb.2015.34.40.

[19] M. Kibret and B. Abera. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. *African Health Sciences*, 11: 40–45, 2011, doi:10.4314/ahs.v11i3.70069.

[20] E. J. Sarba, K. A. Kelbesa, M. D. Bayu, E. Z. Gebremedhin, B. M. Borena, and A. Teshale. Identification and antimicrobial susceptibility profile of *Escherichia coli* isolated from backyard chicken in and around ambo, Central Ethiopia. *BMC Veterinary Research*, 15: 1–8, 2019, doi:10.1186/s12917-019-1830-z.

[21] A. Aljanaby and Q. Alfaham M. H. Phenotypic and molecular characterization of some virulence factors in multidrug resistance *Escherichia coli* isolated from different clinical infections in Iraq. *American Journal of Biochemistry and Molecular Biology*, 7(2): 65–78, 2017, doi:10.3923/ajbmb.2017.65.78.

[22] K. Yun, H. Kim, H. Park, and K. Kim. Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. *Journal of Microbiology, Immunology and Infection*, 47(6): 455–461, 2014, doi:10.1016/j.jmii.2013.07.010.

الكشف الجزيئي عن جين الالتصاق لبكتريا *E.coli* المعزولة من الاطفال العراقيين

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الخلاصة

اوضحت النتائج 59 عزلة من بين 93 عينة (63%) كانت لبكتيريا الإشريكية القولونية. حيث اشارت النتائج أن 19 عزلة (76%) من عزلات الإشريكية القولونية كانت من الإناث و 6 عزلة فقط (24%) من الذكور. تم اختيار عشر عزلات من بكتيريا الاشريكية القولونية التي تظهر لأعلى درجة التصاقها بالخلايا الظهارية البولية تم فحص قدرة العزلات على الالتصاق بالخلايا الظهارية المعزولة من ادرار الاناث. وقد وجد أن جميع العزلات قادرة على الالتصاق بالخلايا الطلائية تبين ان العزلات تمتلك اعلى قدرة مع متوسط عدد البكتريا الملتصقة (20.00±0.82 ، 19.00±0.82 ، 7.25±0.8917±0.699025 ، 16.00±0.74 ، 14.00±0.82 ، 14.5±0.67082 ، 13.5±0.51) 3701 ، 13.5±0.612372 ، 13.00±0.70) مقارنة بالاخر.حيث تم استخدام اختبار الحساسية للمضادات الحيوية للكشف عن حساسية البكتريا ، حيث أظهرت النتائج أن جميع العزلات كانت للسيبروفلاكسيان (100%)، والأمبيسيلين (90%)، والجنتاميسين (70%)، في حين اضهرت مستوى عالي من المقاومة تجاة للتريميثوبريم. / سلفاميثوكسازول (100%)، حمض النالديكسيك (80%)، سيفترياكسون (80%)، سيفليكسين (80%). تضمنت الدراسة الجزيئية استخلاص الدنا النووي من 10 عزلات من بكتيريا الإشريكية القولونية حيث تم التحري عن الجين له القدرة على التصاق بخلايا الطلائية باستخدام جهاز تفاعل البوليمرايز المتسلسل حيث ان تضخيم جين الالتصاق يؤثر على الالتصاق بالخلايا الطلائية.

الكلمات الدالة: الاشريكية القولونية المرضية البولية، تفاعل البلمرة المتسلسل، جين الالتصاق، التهاب المسالك البولية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.