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# Bacteriological and Molecular Study of *Escherichia coli* Isolated from Urinary Catheters in Baquba Teaching Hospital

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## Abstract

One Hundred fifty-two sample of catheters and urinary tracts from patients in the Urology Unit at Baquba Teaching Hospital for the period from 15/ 7/ 2018 to 30/ 9/ 2018 for different age groups ranging from 1-80 years for both sexes, were collected. Fifteen isolates from *Escherichia coli* were subjected to microscopic and biochemical tests and the diagnosis was confirmed by using the Vitek 2 compact system the results of the detection of some virulence factors of the selected *E. coli*. The percentage of the ability of the bacteria to produce hemolycin enzyme was 53.33%, The ability of the isolates to form the biofilm was detected by Congo Red method and it was 40%. Resistance of the selected isolates to 7 antibiotics which belong to the Beta lactam group including Cefoxitin, Amoxicillin, Ephalexin, Ceftazidiume, Augmentin, Cefotaxime and Impinem. The ratios of antibiotic resistance were 86.7%, 73.3%, 60%, 73.3%, 40%, 40% and 66.7%, respectively.

The poly chain reaction (PCR) was conducted for the detection of DNA of (8) *E.coli* isolates that showed resistance to the beta lactam antibiotics using primers of the genes *bla SHV, bla*

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*CTX-M* and *bla TEM*. The results showed that all isolates were 100% found carriers of these genes.

**Keywords:** Catheter, *Escherichia coli*, Vitek 2 compact, *bla* $_{SHV}$, *bla* $_{CTX-M}$.

# دراسة بكتريولوجية وجزيئية لبكتريا الاشريشيا القولونية المعزولة من انابيب القسطرة البولية في مستشفى بعقوبة التعليمي

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## الخلاصة

جمعت 152 عينة الادرار من قسطرة المسالك البولية Catheters من المرضى الراقدين في وحدة الجراحة البولية في مستشفى بعقوبة التعليمي للفترة من 15 – 7 – 2018 ولغاية 30 – 9 – 2018 لفئات عمرية مختلفة تراوحت بين 1-80 سنة لكلا الجنسين، تم الحصول على 15 عزلة من بكتريا *Escherichia coli* التي اخضعت إلى الفحوصات المجهرية والكيميوحيوية و تم تأكيد التشخيص باستخدام نظام Vitek 2 compact system الذي اعطى نتائج تأكيدية لتشخيص عزلات بكتريا *E.coli*. أوضحت نتائج الكشف عن بعض عوامل الضراوة للعزلات *E.coli* قيد الدراسة. ان قابلية البكتريا على إنتاج انزيم الهيمولايسين بنسبة بلغت 53.33%، و تم الكشف عن قابلية العزلات على تكوين الغشاء الحيوي بطريقة احمر الكونغو وكانت بنسبة 40%. اختبرت مقاومة العزلات قيد الدراسة لـ 7 مضادات من مجموعة البيتالاكتام التي شملت Cefoxitin و Amoxicillin و Ceftazidiume و Cephalexin و Augmentin وCefotaxime و Impinem، وكانت نسبة مقاومة المضادات (86.7% و73.3% و 60% و73.3% و40% و40% و 66.7%) على التوالي.

تم اجراء التفاعل التضاعفي لسلسلة الدنا PCR لعزلات *E.coli* المقاومة لمضادات البيتالاكتام والبالغ عددها 8 عزلات باستخدام بادئ متخصص للجين *bla* $_{SHV}$ وللجين *bla* $_{CTX-M}$ وللجين *bla* $_{TEM}$ اذ أظهرت النتائج ان جميع العزلات حاملة لهذه الجينات بنسبة 100%.

**الكلمات المفتاحية:** القسطرة، *Escherichia coli*، Vitek 2 compact، *bla* $_{SHV}$, *bla* $_{CTX-M}$ .

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## Introduction

Urinary tract infection (UTI) is a health problem in many countries of the world and it is the second to respiratory tract infections [1]. The Urinary Tract Infection infects all age groups and both sexes. It also infects older males because of their exposure to prostates infection, which affect the discharge of the bladder from the urine, which causes urinary tract infection, and is significantly infected by female adults. Age is also a urinary tract infection due to which hormonal changes occur as a result of age and immunosuppression [2].

They also lack the advantage of releasing bacterial inhibitors bacteriostatic from the prostate gland [3]. Nosocomial Infections, and Hospital infections are the most common cause of urinary tract infection. In Intensive Care Units (ICUs), they range from 20 to 30%. The percentage of bacteria being the main cause of urinary tract infection is 95% [4]. The pathogens of bacterial UTI are common pathogens in hospital ICUs from negative bacteria of Gram Negative such as *Escherichia coli, Klebsiella, Proteus, Pseudomonas* and the positive bacteria of the pigment Gram Positive such as *Streptococcus, Enterococcus sp.* and *Staphylococcus* [5].

*E.coli* bacteria are more prevalent among pathogens. Many resistance genes are generated for their pathogenic strains by transferring horizontal genes to mobile genetic elements such as integrons, which possess resistant genes. Mobile genetic components such as plasmids that have an entrant that can contain re-installation genes recombination at specific locations and capable of capturing and mobilizing and (Gene cassettes) [6].

The resistance of the bacteria causing the UTI of the Antibiotics, especially for commonly used anti-retroviral antibiotics, is a close correlation between the use of these antibiotics by the patients, the increase in their resistance rates. Multidrug Resistance (MDR) is a major problem around the world. It is covered by resistance genes located on integrons [7].

## Materials and Methods

### Collection of samples

One Hundred fifty-two samples of urine and urinary tract catheters were collected from patients attending the Urology Unit at Baaquba Teaching Hospital for the period 15 July 2018 till the end of October 2018. Information about the patients was recorded including age, date of sampling, and housing. The samples were cultured on the media of blood agar and Maconky agar, the dishes were incubated at 37º C for 24 hours, after that diagnostic tests were performed using the phenotypic and biochemical methods.

### Biochemical Tests

A number of biochemical tests were carried out for the diagnosis, growing bacteria on the nutrient agar after 24 hours, which is the time required for their growth. These included the Indol test, Methyl red test, Voges - Proskaur test and Citrate Utilization test [8]. The diagnostic test was confirmed using the Vitek 2 Compact system.

### Detection of virulence factors

Hemolycin Production test the propagation route was used in drilling method well diffusion as described previously [3] to detect hemolycin productivity in the bacteria under study.

Biofilm formation test was by Congo-red method: a pure single colony was transported on the nutrient agar to test tube container with 5 ml of the Physiological Saline Solution and culture in the Congo red agar and Incubated dishes at a temperature of 37° for 24 - 48 hours. The result is positive when the colonies appear black with a dry crystalline density, but the result is negative when the pink colonies remained [3 - 9].

### Antibiotic suscepitibility Test

The sensitivity test using the Kirby power method to use 7 types of [2], and has adopted the standard specifications used previously [10]to compare the diameters of the inhibiting areas around each disk.

### Detection of genes bla *SHV* and bla *TEM* and bla *CTX-M*

### Extraction of Genomic DNA

DNA was extracted from bacterial cells in the liquid medium (nutrient broth) and using the extraction kit processed by the company (Geneaid on the instructions of its manufacturer).

### The principles used in the interaction of the primers use in PCR

The prefixes of some anti-bla *SHV*, bla *TEM* and bla *CTX-M* resistant genes were used in these *E.coli* bacteria and they were confirmed through the use of the Ncbi-genbank site, which was processed by Bioneer company in Korea as a freeze-dried product (lyophilized). [11]

**Table 1:** Sequences prefixes for genes *bla SHV bla TEM* and *bla CTX-M* to bacteria *E.coli*

| Primer Name | The initiator | The sequence | Temperature | Size (bp) |
|---|---|---|---|---|
| *bla SHV* | F | 5' CGC CGG GTT ATT CTT ATT TGT CGC '3 | 68 M | 1016 Base Pair |
| | R | 5' T CT TTC CGA TGC CGC CGC CAG TCA '3 | | |
| *bla TEM* | F | 5' CTT CCT GTT TTT GCT CAC CCA '3 | 58 M | 717 Base Pair |
| | R | 5' T AC GAT ACG GGA GGG CTT AC '3 | | |
| *bla CTX-M* | F | 5' TTT GCG ATG TGC AGT ACC AGT AA '3 | 51 M | 514 Base Pair |
| | R | 5' CGA TAT CGT TGG TGG TGC CAT A '3 | | |

### Agarose Gel Electrophoresis

The evacuation process was conducted to investigate bacterial DNA after extraction or to detect PCR reaction product, since I left samples on gel agaros 1% concentration and 70 Volt voltage for 90 minutes, followed by a screening process of jelly altsabigh alathidiom bromide and dye under UV and Volumetric directory (100 bp) [12], after the expiration of the gels was

deportations raise your electric relay plate, so check the gel with UV exposure that you put on the ultraviolet (UV gel documentation) and photo imaging device jellies [9].

## Results and discussion

### Investigation of some important virulence factors of E.coli

### Haemolysin Production

All bacterial isolates were subjected to a test screening for Hemolysin enzyme. Purified colonies growing on blood agar center isolates showed hemolysin enzyme-producing regions of glycolysis transparent about developing colonies which showed eight isolates of *E.coli* and 53.3% of hemolysin production which matches the results of other studies [13, 14].

### Biofilm formation

Biofilm formation was investigated using Congo Red Agar (CRA) and dynamic Biofilm detection. The results of the current study showed that the ratio of production of biofilm was 40%. This result is not consistent with that of Al-Muaimi [15] which showed that the number of *E.coli* producing biological membrane with Congo red method was 23 (92%) isolating and disagreed with the findings of Abuuzaimovic et al. [16] as the ratio of production of Biofilm was 78%.

### Bacterial sensitivity test of antibiotics

The sensitivity of the isolates under study was tested for 7 antibiotics distributed among the antibiotics which included Cefoxitin, amoxicillin, Cephalexin, Ceftazidiume, Augmentin, Cefotaxime and Impinem. *E.coli* cefoxitin resistance was 86.7%, which differed from the results of [17] where the ratio of insulation of resistance amounted to 26.7%. The anti-amoxicillin resistance ratio was 73.3% and this result is fairly consistent with study of Al-Khalidi [14] which was 100% counter. Resistance to anti-cephalexin was 60%. These results differed from those of Abuuzaimovic et al. [16] where the rate of resistance of bacteria was

8.33%. The anti-ceftazidiume ratio was 73.3%. This result was consistent with the [15], where the ratio of anti-resistance was 76%. The Augmentin resistance rate was 40% and this result is fairly consistent with the study in [18], which was a 20% anti-retroviral ratio. The ratio of bacteria resistance to cefotaxime was 40% and this result is close to the results of [13], as the resistance ratio was 26.7% and the ratio of bacteria resistance to impinem was 66.7%. This result differed from that of [9].

**Gene detection using PCR technology**

The PCR was conducted on the DNA of 8 E.coli isolates resistant to the beta lactam antibiotics using specific starter targeting the qualitative sequences of gene bla SHV, bla CTX-M and bla TEM for the diagnosis the resistant genes of resistance to the beta lactam antibiotic group, as Specialized prefixes used in [20] by adopting the programmer of interaction in the study, and circumstances by the researcher for the purpose of diagnosing the genes.

**Detection of gene bla TEM**

The results showed that there were 8 isolates of *E.coli* and a ratio of 100% Picture (1) was contained in the gene bla TEM depending on the emergence of a 717-pair-base pack. These results were with the findings of [15], which indicated that *E.coli* isolates contained genes bla TEM (100%) (87.5 % respectively, but did not agree with the findings of [11] and study [21] which indicated that *E.coli* isolates contained bla TEM genes by (6.8 %) (48.7%) Respectively. The difference of results between the current study and previous studies are due to the difference in the number of isolates and the different principles used as well as the location and nature of the different insulation.

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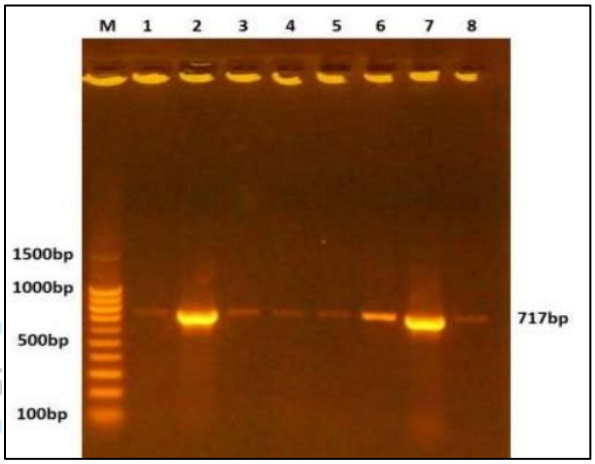
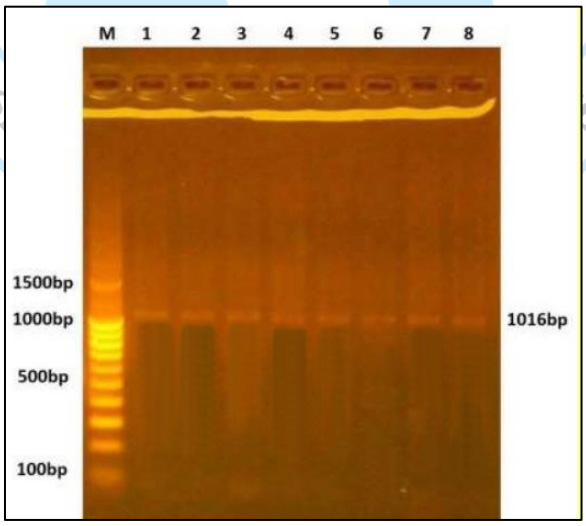



**Picture 1:** Presence of *bla* $_{TEM}$ gene species were fractionated on 1% agarose gel electrophoresis stained with *Eth.Br*. Lane1:100bp DNA marker

## Detection of gene bla SHV

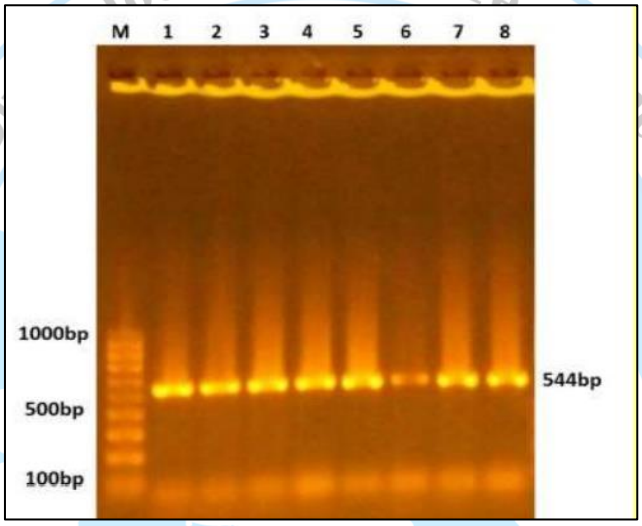
The percentage of gene bla SHV appearance was (100%) Picture (2) in (8) isolates under study. This result is different from the study in [15] and the study [11], which indicates the absence of this gene in *E.coli.* In [21] the ratio of this gene was (5.1%).




**Picture 2:** Presence of *bla* $_{SHV}$ gene species were fractionated on 1% agarose gel electrophoresis stained with *Eth.Br*. Lane1:100bp DNA marker.

**Detection of gene *bla*$_{CTX-M}$**

The appearance of gene ***bla***$_{CTX-M}$ was also (100%) Picture (3) in the isolates under study. This result is consistent with that of [20] and [22] which indicated the presence of this gene by (100%) and (88.1%) respectively, while it is not compatible with the study [21] which indicates that the ratio of gene ***bla***$_{CTX-M}$ in *E.coli* was (7.6%).




**Picture 3:** Presence of *bla*$_{CTX-M}$ gene species were fractionated on 1% agarose gel electrophoresis stained with *Eth.Br*. Lane1:100bp DNA marker.

## Conclusions

1. *Escherichia coli* bacteria are the most common bacteria in patients with urinary catheter.
2. The majority of the isolates under study possess a number of virulence factors, such as the biofilm and the hemolycin.
3. Resistance to most of the antibiotics and multiple resistance to antibiotics, was observed to be common among the selected isolates.
4. *E.coli* isolates carry genes responsible for the resistance to the antibiotics that belong to the beta lactam, such as gene *bla*$_{SHV}$, gene *bla* $_{CTX-M}$ and gene *bla* $_{TEM}$, used for detection by a specialized primer targeting the specific sequence for the purpose of diagnosing resistance genes.

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