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Unveiling the High Prevalence of Antibiotic Resistance and Quorum Sensing Genes in Uropathogenic *Escherichia coli*

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Abstract

Escherichia coli is considered one of the uropathogenic bacteria with different infection symptoms representing mild illness to acute sepsis. This study aims to detect E. *coli* in patients with urinary infection and investigate quorum sensing genes (*lux S* and *motA*) in multi-drug resistant isolates of *E. coli*. 200 urine samples were collected from patients with urinary tract infections from several hospitals in Baghdad .The antibiotics sensitivity test showed high resistance of isolates for Ampicillin (100%), Cefazolin (97%), Trimethoprim/ Sulfamethoxazole (83%), Ceftriaxone (77%), Ceftazidime and Ciprofloxacin (70% each of them), and moderate resistance of isolates for Levofloxacin (50%), Gentamicin (47%), Cefepime (40%), while low resistance Piperacillin/ Tazobactam (33%), Cefoxitin (30%), Nitrofurantoin (17%), Imipenem (10%), Ertapenem and Amikacin (7% each of them), and Tigecycline (3%). The results showed an increase percentage of infection in females was 30% in the ages 30-44 years, whereas in ages 15-29 and more than 45 years was 17%. There was a high percentage (57.11%) of resistant isolates in females which are ages 30-44 years. While the ages more than 45 years were 66.4% and ages 15-29 were 34%. While, in males, the percentage was high in ages more than 45 years (35.25%) followed by age groups 30-44 years (31.5%) and 15-29 years (31%). The prevalence percentage for *luxS* and *motA* genes in *E. coli* was 100%. In conclusion, *E. coli* isolates were multi-drug resistant due to all isolates had quorum sensing genes. Moreover, uropathogenic of *E. coli* in females was more frequent than in males due to the resistance of bacteria to antibiotics.

Keywords: *E. coli*, Quorum Sensing, *luxS*, *motA*.

الخلاصة

تعتبر الإشريكية القولونية من البكتيريا المسببة للأمراض البولية مع أعراض عدوى مختلفة تمثل مرض خفيف إلى تعفن الدم الحاد ، وتهدف هذه الدراسة إلى الكشف عن الإشريكية القولونية في المرضى الذين يعانون من عدوى المسالك البولية وفحص جينات استشعار النصاب (*lux S* و *mot A*) في العزلات متعددة المقاومة من الإشريكية القولونية. جمعت ٢٠٠ عينة بول من مرضى التهابات المسالك البولية في عدة مستشفيات في بغداد. أظهرت نتائج حساسية المضادات الحيوية مقاومة عالية للإشريكية القولونية للأمبيسيلين (١٠٠٪)، سيفازولين (97٪)، تريميثوبريم/سلفاميثوكسازول (٨٣٪) ، سيفترياكسون(٧٧٪)، سيفتازيديم وسيبروفلوكساسين (٧٠٪). ومقاومة معتدلة للإشريكية القولونية تجاه لليفوفلوكساسين (٥٠٪) ، الجنتاميسين (٤٧٪) سيفيبيم (٤٠٪)، بينما مقاومة منخفضة للإشريكية القولونية ، بيبيراسيلين / تازوباكتام (٣٣٪) ، سيفوكسيتين (٣٠٪)، نتروفورانتوين (١٧٪)، والاميبينيم(١٠٪)، والاميكاسين والارتابنيم (٧٪)، تيكيسايكلين (٣٪)، وأظهرت النتائج ارتفاع نسبة الإصابة لدى الإناث بنسبة ٣٠٪ في الأعمار ٣٠-٤٤ سنة، بينما في الأعمار ١٥-٢٩ وأكثر من ٤٥ سنة بلغت ١٧٪. وكانت هناك نسبة عالية (٥٧,١١٪) من العزلات المقاومة في الإناث اللاتي تتراوح أعمارهن بين ٣٠-٤٤ سنة. بينما كانت الأعمار أكثر من ٤٥ سنة ٦٦,٤٪ والأعمار ١٥- ٢٩ سنة (٣٤٪) بينما في الذكور، كانت النسبة مرتفعة في الأعمار التي تزيد عن ٤٥ عامًا (35.25%)، تليها الأعمار ٣٠-٤٤ عامًا (٣١,٥٪) و١٥-٢٩ عامًا (٣١٪). كانت نسبة انتشار جينات *luxS* و *motA* في الإشريكية القولونية ١٠٠٪ . كانت عزلات الإشريكية القولونية مقاومة للعديد من الأدوية لأن جميع العزلات تمتلك جينات استشعار النصاب. فضلا عن ذلك ، كانت الإشريكية القولونية المسبة لالتهاب المجاري البولية في الإناث أكثر شيوعًا من الذكور بسبب مقاومة البكتيريا للمضادات الحيوية.

INTRODUCTION

The infection in the urinary tract is occasioned by bacteria in any area of the urinary tract. Also, urinary infections can be caused by fungi and seldom by viruses [1]. *Escherichia coli* considers one of the uropathogenic bacteria with different infection symptoms representing mild illness to acute sepsis, and a death rate (20%- 40%). Sepsis occurrence and its related death increase with age disproportionately [2].

Urinary infection considers a common infection in humans. The urine tract includes the kidney, ureter, bladder, and urethra. Urinary infection includes the bladder and urethra in the lower urinary tract. Moreover, infection also comprises the ureter and kidney, and upper urinary tract. Besides, the upper urinary tract infection is more acute infected than the lower urinary tract [1]. Urinary infection is common such as cystitis. Also, there is burning of urine, and urination frequency represent common urinary infection symptom [3].

Quorum sensing of bacteria produces and liberates molecules of chemical signalling known as "autoinducers", its external concentration raises as an indicator of the rising density of cell population. When the bacteria reveal the autoinducers amount to a minimum of the threshold level for the stimulated concentration, bacteria respond by changing gene expression. Autoinducers represent the signals by which quorum sensing of bacteria connect and coincide with behaviour on a wide scale of population, so gain the capability to work as multi-cellular organisms [4].

E. coli is Gram-negative bacteria that connect with another cell by cues to stimulate the expression of particular genes for different phenotypes like the formation of biofilm, antibiotic resistance, and motility. *E. coli* signals molecules have different actions through cell-to-cell connections where it was observed to raise antibiotic resistance modulation and the inhibition formation of biofilm, and motility [5]. Biofilm plays a role in drug-resistance, and pathogenesis for several chronic diseases and does not respond to antibiotic treatment [6]. Some studies point out that in several species of bacteria, stimulation of quorum sense occurs in the biofilm activating and leading to the maturity and dismantling of biofilm. Thus, the first adhesion stage appears unsuitable for accumulating signal molecules, then after in the second step, the linked bacteria divide and generate microcolonies, increasing population intensity, so signal molecules reach suitable levels to stimulate the maturity and dismantling of biofilm in a coordinated method . When the nutrients become little and accumulate waste, the dispersion of biofilm is essential to bacteria escaping and colonizing new regions [7].

LuxS gene contributes to the quorum sensing autoinducer 2 synthesis, where autoinducer 2 in *E. coli* induces the formation of biofilm and influences the architecture of biofilm by stimulating the motility quorum-sensing regulator gene (*mqsR*) that regulates positively the expression of *motA* gene [8]. The *motA* gene is the membrane protein which has 4 membrane-crossing areas that are a formation of the proton channel [9].

This study aims to detect *E. coli* in patients with urinary infection and investigation of quorum sensing genes (*lux S* and *mot A*) in multi-drug resistant isolates.

MATERIALS AND METHODS

Sample collection

200 urine samples were collected from patients suffering urinary infection symptoms in Medical City / Baghdad during the period (2/2/2022 - 15/4/2022) performed general urine examination.

Bacterial isolates

The samples were cultivated on MacConkey Agar and incubated at 37°C for 24h. Then bacteria were diagnosed by Gram stain to distinguish between positive and negative gram stain by microscope examination. The biochemical tests (catalase, indole, methyl red, and lactose fermentation) were performed and the API 20 System. Moreover, *E. coli* was diagnosed by Vitek 2 compact system to confirm of final diagnosis.

Antibiotic sensitivity test

The antibiotic sensitivity test was performed by Vitek 2 compact system.

Molecular detection of luxS and motA genes

It was chosen 30 isolates that were more resistant to antibiotics for their DNA extraction. ABIOpure Total DNA kit /USA was used for DNA extraction. PCR was used to detect luxS gene(315bp) and motA gene (430bp) as shown in Table (1). These primers for *luxS* and *motA* genes were designed by the current study. PCR condition for these genes' initial denaturation at 95 ºC/5min 1 cycle; 35 cycles for denaturation 95 ºC/30 sec, annealing 58 ºC/30 sec, and extension 72 ºC /30sec; 1 cycle for final extension 72 ºC/7 min. The volume of

mixture reaction was 12µl of master mix (Bioneer company /Korea) ,1.5 µl for each primer, 2 µl DNA sample, and a complete 25 µl final volume by nuclease-free water. The PCR product was electrophoresed in 1.5% agarose gel at 7 V/cm for 90 minutes to detect PCR product for each gene by comparing with DNA ladder (100bp) from Bioneer company /Korea and it was visualized using Ethidium bromide stain by UV.

Table 1. Primers sequence of *luxS* and *motA* genes which were designed by this study.

Genes	Sequence primer	Size bp
luxS gene	F:5-CATACCCTGGAG CACCTG TT-3	315
	R:5TTCTTCGTTGCTGTTGATGC-3	
motA gene	F:5GGTACAGTTTTCGGCGGTTA-3	430
	R:5CGTGCGTCTCAATCTCTTCA-3	

RESULTS AND DISCUSSION

Isolation of E. coli isolates

One hundred twenty-five samples appeared positive for bacterial growth as Gram-negative bacteria. 49 isolates of Gram-negative bacteria were *E. coli* as shown on MacConkey agar and it appeared in pink color colonies and rod shape after staining with Gram stain in microscopic examination. *E. coli* showed positive results for catalase, indole, methyl red, and lactose fermentation. Also, diagnosis of *E. coli* was finally confirmed by API 20 System and Vitek 2 compact system. *Escherichia coli* is a main bacteria spp. that plays a role in occurring of urinary infections [10]. *E. coli* has a vast range of virulence genes that can increase the pathogenicity of *E. coli* and drug resistance [11].

Antibiotic sensitivity of E. coli

The results of antibiotics sensitivity showed a high resistance of *E.coli* for Ampicillin (100%), Cefazolin (٩٧%),Trimethoprim/Sulfamethoxazole (83%), Ceftriaxone (77%), Ceftazidime and Ciprofloxacin (70% each of them), and moderate resistance of E.coli for Levofloxacin (50%), Gentamicin (47%), while low resistance of *E.coli* for Cefepime(40%), Piperacillin/ Tazobactam (33%), Cefoxitin (30%), Nitrofurantoin (17%), Imipenem (10%), Ertapenem and Amikacin (7% each of them), Tigecycline (3%) as shown in Figure 1.

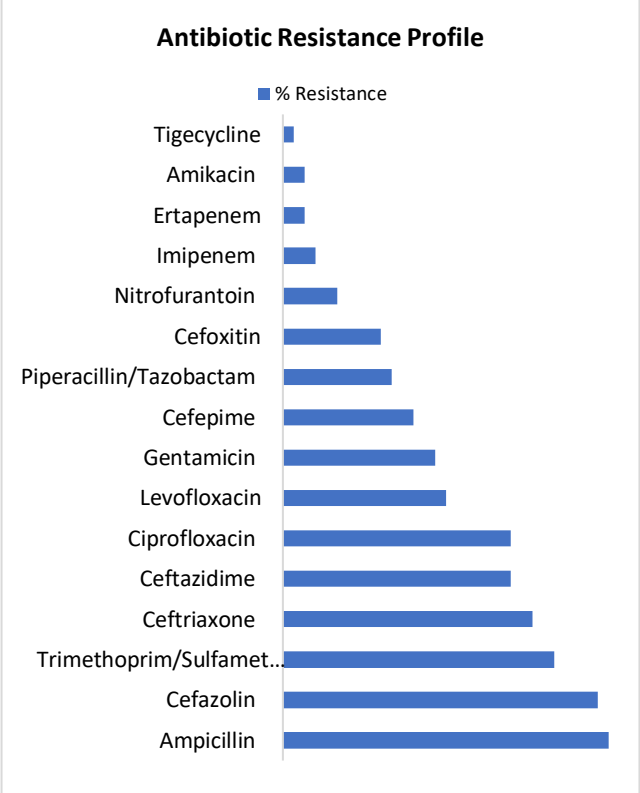

Figure 1. Resistance percentage to antibiotics in *E. coli* isolates.

In previous years, it appeared the rising rate of drug impedance and multi-resistant phenotypes observed in *E. coli* turned into a major issue around the globe [12]. The present study showed that urinary infection spread via *E. coli* in the people, it coincides with other Iraqi surveys studied. One study showed that the prevalence of *E. coli* in patients with urinary illness in hospitals of Baghdad more than other species of bacteria [13].

Distribution of urinary infection according to age and gender

The outcomes showed an increase percentage of infection in females was 30% in the ages 30-44 years, while in ages 15-29 and more than 45 years was 17% as shown in Figure 2. There was a high percentage (57.11%) of resistant isolates in females which are ages 30-44 years. While the ages more than 45 years were 66.4% and ages 15-29 were 34%. Whereas, the percentage in males was (35.25%) in ages more than 45 years, followed by the ages 30-44 years (31.5%) and 15-29 years (31%) as shown in Table 2.

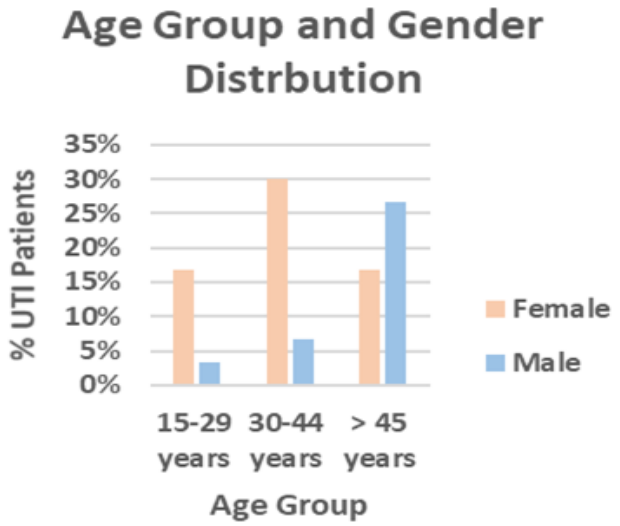

Figure 2. Distribution of urinary tract infection (UTI) according to age and gender.

Table 2. Distribution of percentage resistance isolates according to gender and age.

Age group	Gender	Resistant isolates (%)
15-29	Female	34%
	Male	31%
30-44	Female	57.11%
	Male	31.5%
>45	Female	66.4%
	Male	35.25%

Also, it observed that patients with urinary infections have of *E. coli* more than *Klebsiella, Staphylococcus aureus, Proteus, Acinetobacter, Pseudomonas, Enterobacter, Enterococcus* and *Citrobacter* [14].

This study observed a high infection in females with urinary illness than in males, this agreed with one study that observed the prevalence ratio of urinary illness in women was 73.50%, while in men was 26.50%. This is due to the different dissection of the urinary system in males and females and the nature of the physiological composition; where the length urethra is short when compared with males. Moreover, hormonal variation can stimulate infections. Also, males are extra protection because of the anatomical components of the urine system [15].

Another research demonstrated that urinary illnesses were more spread in older patients due to their immunological condition and ages. Where the urinary illness was 50-60% in patients at 60 years, while in patients at 40- 59 years was 38.55%. This high-rate prevalence can be due to several factors including old age in the excretion of the urine system and lower contraction of the muscle in the bladder which has a role in bacteria development via the lower secretion of the urine system [16].

The outcomes of this study in *E.coli* resistance toward antibiotics were approximate with one study that observed the percentage impedance of E.coli against Cefixime at 63.3%, Ceftriaxone at 61.3% and Ciprofloxacin 46.1% [17]. Ghaima et al. [14] showed that patients with urinary infection by *E. coli* was resistant to Amikacin51.2%, Ceftriaxone 42.7%, Ceftazidime 52.2%, Cefepime 33%, Ciprofloxacin 49.4%and Imipenem 4.1%. Females have a shorter urethra than males due to physiological and anatomical characteristics that increase the hazards of bacterial rise from the anus into the bladder therefore it was observed that resistance to antibiotics for isolates in females more than in males [18].

Molecular detection of luxS and motA genes

The results of quorum sensing genes (*luxS* and *motA*) showed the prevalence percentage for these genes in *E. coli* was 100% (30/30) as shown in Figures 3 and 4.

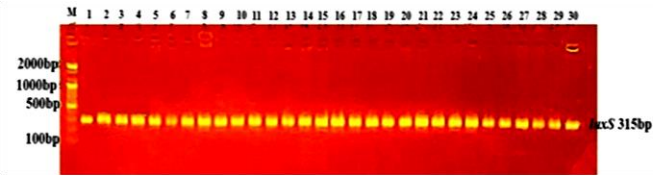

Figure 3. Electrophoresis of PCR product for *luxS* gene (315bp) in *E. coli* Lane M: DNA ladder marker (100bp).

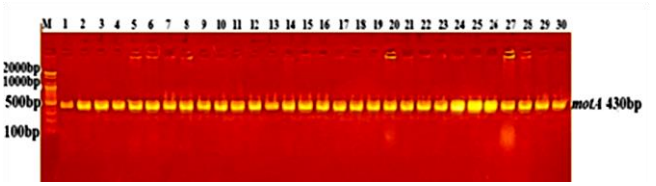

Figure 4. Electrophoresis of PCR product for *motA* gene (430bp) in *E. coli*. Lane M: DNA ladder marker (100bp).

The outcomes in this study harmonized with another study Luna-Pineda *et al.* [19] which showed in their study the isolates of *E. coli* in patients with urinary infections were multi-resistance antibiotics and also these isolates had *motA* gene. Moreover, quorum sensing is utilized to regulate the expression of genes, and many processes that contribute to virulence, like motility, and formation of biofilm, are vital for bacteria to form the phenotype of biofilm. Autoinducers are found in Gram-negative and Gram-positive bacteria. *E. coli* has Autoinducer-2, that produce via *LuxS* enzyme associated with the formation of biofilm [20]. One of studies showed *luxS*, and *motA* expression have correlated with multi-antibiotic resistance in various groups. Where the biofilm

formation has a role in the formation of multi-antibiotic resistance in *E. coli* [21].

CONCLUSIONS

E. coli isolates were multi-drug resistant because all isolates had the quorum sensing genes. Besides, uropathogenic *E. coli* in females was more frequent than in males. In addition, the infection was more frequent in ages more than 45 years.

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Ethical approval

The consent was obtained by the ethics committee of Medical educational laboratories/ Medical city/ Iraqi Ministry of Health (Ref:5214 in 1-2-2022).

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

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