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Prevalence of Ciprofloxacin resistant E. coli in urinary tract infections

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Abstract: *The resistance of uropathogenic Escherichia colito ciprofloxacin has been increased in the last decade. Our study investigated the prevalence of Ciprofloxacin resistant E. coli among Iraqi patients. The obtained bacterial isolates were tested for Ciprofloxacin resistance using disk diffusion method and the sequences of gyrA and parC of Ciprofloxacin resistant isolateswere obtained.The results revealed that about 44% of the isolated E. coliwere Ciprofloxacin resistant and the resistance was due to mutation in gyrArather than parC.*

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I. Introduction

Escherichia coli is a common inhabitant of the gastrointestinal tract of humans and animals. Most *E. coli*strains are harmless commensals of the intestinal tract. Nonetheless, others are major pathogens of humans and animals. The pathogenic *E. coli*is divided into those strains causing disease inside the intestinal tract and others capable of infection at extraintestinal sites[1].

*Escherichia coli*extraintestinal infections (viz., urinary tract infections and bloodstream infections) represent a significant public health burden worldwide[2,3]. Since the 2000s, antimicrobial resistance among *E. coli* isolates has increased dramatically contributing to the complexity in management of such infections[4].

Escherichia coli is considered as the normal bowel flora of different species of mammals and birds but some strains of *E. coli* possess pathogenic character due to the acquisition of virulent factors. Microbial characteristics associated with virulent *E. coli* include production of enterotoxin, verotoxin, colicins and siderophores, type-1 pili and motility, resistance to the lytic action of the host complement and antibiotics[5,6].

Infections due to pathogenic *E. coli*may be limited to colonization of a mucosalsurface or can disseminate throughout the bodyand have been implicated in urinary tractinfection, sepsis/meningitis and gastrointestinalinfections[7].

The present work aimed to investigate the distribution of Ciprofloxacin resistant *E. coli* amongst Iraqi patients as well as which gene is most responsible.

II. Materials and methods

Specimen collection:

Ninety-five mid-stream urine specimens were collected over a period of a five months between August and December 2016 from inpatients and outpatients at Al- Yarmouk teaching Hospital, Baghdad's medical city (Madinet Al-Teb), and Al-Kadhimiyah teaching hospital in Baghdad, Iraq. All specimens were directly streaked on the surface MacConkey agar plate, incubated overnight at 37°C.The identification of suspected colonies was assessed as described by Flournoy *etal*.[8]

Ciprofloxacin susceptibility test

Twenty-fourhours old colonies grown on nutrient agar medium were transferred into brain heart infusion broth and then incubated at 37ºC for 4 hours for obtaining turbidity standard equal to the turbidity of MacFarland tube 0.5 which is used as turbidity standard. By a sterile cotton swab a portion of bacterial culture was transferred and carefully and evenly spread on Mueller-Hinton agar medium and left for 10 min. Subsequently, Ciprofloxacin (5 µg) antimicrobial disks were placed on the agar medium with a sterile forceps and pressed firmly to ensure contact with the agar. After that, the plates were inverted and incubated at 4ºC for 18 hours.Inhibition zones developed around the disks were measured by millimeter (mm) using a metric ruler (CLSI, 2016). The isolate was interpreted as susceptible, intermediate or resistant to the antibiotic in accordance to CLSI (2016) ≥ 21, 16-20 and <16 respectively.

Polymerase chain reaction (PCR)

Bacterial Genomic DNA of all *E. coli* bacterial isolates (n=25) was extracted by Presto™ Mini gDNA Bacteria Kit (Geneaid) and AccuPower® PCR PreMix was utilized for all of the amplification reactions that was carried out using Gradient master cycler (Eppendorf, Germany).

Escherichia coli-suspected isolates were screened for the presence of *uspA* gene using specific primers uspA-F (CCGATACGCTGCCAATCAGT) and uspA-R (ACGCAGACCGTAGGCCAGAT), the reaction settings were: Initial denaturation at 95°C for 5 min followed by 30 cycle of 94°C 30 sec, 56°C 30 sec and 72°C 30 sec; following that 5 min at 72°C for final extension[9].

Ciprofloxacin resistant *E. coli* isolates were selected for the detection of potential mutations in both *gyrA* and *parC*that codefor DNA gyrase subunit A and DNAtopoisomerase IV,respectively; two sets of primers were used for this purpose:GyrA F: AAATCTGCCCGTGTCGTTGGT and GyrA R GCCATACCTACGGCGATACC for *gyrA;* ParC F: GTATGCGATGTCTGAACT and ParC: R TTCGGTGTAACGCATTGC for *parC.* The method of amplifications includedinitial denaturation at 95°C for 2 min followed by 35 cycle of 95°C 30 sec, 55.4°C 60 sec and 72°C 60 sec (Gomig et al., 2015).

The PCR products were sequenced using Sanger method and afterthat were aligned with gene sequences from National Center for Biotechnological information (NCBI) (https://www.ncbi.nlm.nih.gov/) in order to inspect for mutations.

III. Results and discussion

Bacterial isolation and identification:

All 95 urine specimens that were collected and promptly inoculated onto McConkey agar, after overnight incubation, developed bright pink or red colonies were suggested to be *E. coli*. The presumptive colony on McConkey agar for each specimen was subcultured successively onto EMB agar for presumptive identification of *E. coli*. The greenish-black colonies with metallic sheen on EMB agar (25 isolates) were presumptively selected as *E. coli.*

All isolates were stained by Gram stain in order to detect their response to Gram stain, shapes and their arrangements. All isolates negatively reacted with the stain and rod shape appearance was noticed (Table 1).

Apparently, all isolates were positive for catalase, methyl-red, and indole. Nevertheless, they gave negative results for VP and citrate.

Moreover, 52% of isolates developed clear zone on blood agar plates (β hemolysis). The invasive *E. coli*strains frequently produce virulence factors such as the hemolysins. The frequency by which hemolytic *E. coli*strains can be isolated from patient samples increases with the severity of disease[11]. In a local study done by Al-Dahmoshi*et al*. they found that 41.7% of *E. coli*produced hemolysis[12].

Detection of *uspA* gene in *E. coli* isolates

Monoplex PCR technique was carried out to detect *uspA* gene in 25 bacterial isolates thatwere identified as *E. coli*based by conventional biochemical techniques. In this assay, specific primers were used. The results showed a single band of PCR product with 884bp that represent *uspA* gene in only 24 isolates (Figure 1).

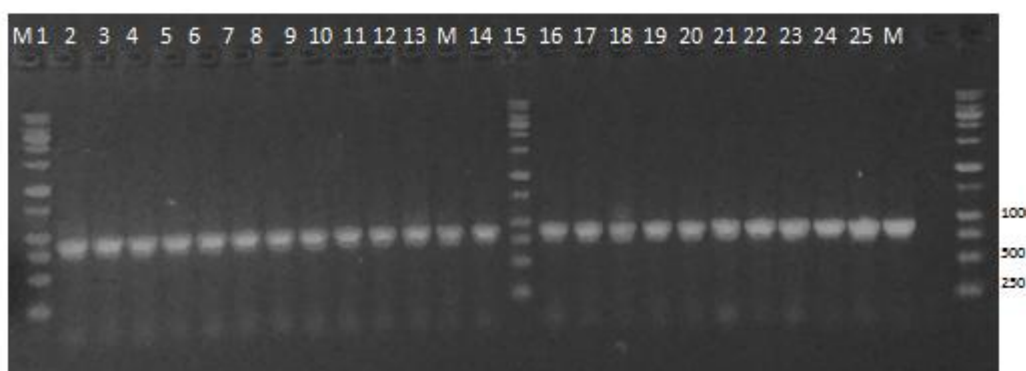

Figure no. 1: Visualization of *uspA*gene (884bp) by 2.5% agarose gel analysis. The shown bands are representative of PCR products amplified from *E. coli*isolates (lanes 1 - 25), lane M represents 250 bp DNA ladder.

The molecular identification is focused on the detection and subsequent sequencing of a specific bacterial DNA and is used broadly to identify and taxonomically classify numerous groups of microorganisms, including bacteria by the amplification of precise target region by PCR. Another approach is via the amplification of specific genes which belong to certain species; basing on certain specific features like virulence

factors or antibiotics resistance genes[13].The PCR technique is relatively considered to be fast, sensitive, and less time-consuming compared to the conventional bacteriological identification means and is broadly used to identify bacteria isolated from diverse kind of samples, including soil, food and infected human tissue[14,15].
The detection of *uspA* gene is considered to be a highly sensitive and specific method to differentiate *E. coli* from other bacteria as no amplification was observed from any of the non-*E. coli* isolates[16,17].

Ciprofloxacin susceptibility test

The Ciprofloxacin susceptibility of all *E. coli* bacterial isolates was tested using disk diffusion method. From the findings of the present study, different levels of susceptibilities to Ciprofloxacin among isolates were recorded.

About 44% of *E. coli* isolates were resistant to Ciprofloxacin while no intermediate resistance was observed among the tested isolates; moreover, 56% were sensitive.

The PCR product sequences were aligned and subsequently compared to sequences of *gyrA* and *parC*genes from NCBI, about 36 and 48 mutations in the forward and reverse strands, respectively, were identified in *gyrA* of the tested isolates; ever since *gyrA* encode for DNA gyrase, these mutationswill lead to amino acid substitutions that would alter the fluoroquinolone target protein structure and consequently the binding affinity of the enzyme by fluoroquinolone, leading to antibiotic resistance[18]. On the other hand; no mutations were recorded when comparing the sequence of *parC*from the tested isolates with sequences from NCBI and complete similarity were recorded. From the obtained results it is possible to say that Ciprofloxacin resistance in the tested isolate was due to mutation in gyrase rather than topoisomerase IV.

The findings of this study agree greatly with abdulkhaleq*et al.*[19]who found that about 40.7%. of locally isolated *E. coli* strains from Iraqi patients were resistant to Ciprofloxacin While it disagrees partially with a later study carried out by Al-Jebouri and Mdish[20]who stated that the resistance percentage of *E. coli* to Ciprofloxacin were not more than 25%.

The result disagrees with a local study by Abdul- Sahib[21]who stated that over 70% of locally isolated *E. coli*were resistant to Ciprofloxacin while no more than 70% were sensitive. Furthermore, the current finding is also incompatible with the results of Al-Fayyadh*etal.*[22]who declared that about 98.11% of *E. coli* isolates were resistant to Ciprofloxacin while 1.89% showed intermediate resistance.

Gupta *etal.*[23]stated that resistance of *E. coli* to ciprofloxacin remained low throughout 5 years evaluation study, while Schaeffer[24] stated that potential disadvantages of fluoroquinolones include cost and risk of development of resistance. Goettsch*etal.*[25]suggested that resistance to fluoroquinolones maybe expected to increase in the future.

The cause of the contradictory data considering evaluation for increasing *E. coli* resistance to ciprofloxacin may be related to the types of patients involved in each study such as geographical distribution, severity of UTI, age and prior antibiotic use[21].

IV. Conclusion

About 44% of *E. coli* isolated from urinary tract infections are resistant to Ciprofloxacin and this resistance is due mutant *gyrA* rather than *parC.*

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