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Prevalence of *chuA* gene virulence factor in *Escherichia Coli* isolated from clinical samples in AL-Diwaniyah province

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***Abstract*---**The current study aimed to investigate multidrug-resistant *E.Coli*, and (*chuA*) gene in *Es coli* which have virulence factors by PCR. Out of 150 urine and stool samples, 122 gram-negative bacteria (60 isolates belong to *E.Coli*) and 28 gram-positive bacteria were diagnosed by cultural and biochemical characteristics and API 20E System. The susceptibility test is carrying out for sixteen antibiotics drugs by diffusion method, the results showed that higher resistance to aztreonam was (81.8%), ticarcilin/clavulanic acid was (89.09%), carbencillin was (83.6%), piperacillin was (70.9%), nitrofurantoin was (67.26%). Ceftrixon was (61.8%), the most isolates demonstrated low resistance against levofloxacin (21.8%) and amikacin (43.6%). As it turned out from the study, 40 isolates of Escherichia Coli have multiple resistance to antibiotics (MDR).

Keywords*---E. coli*, *chuA* gene, multidrug resistance.

Introduction

E. coli is a typical inhabitant of the gastrointestinal tract. Some strains cause illness in the intestine and outside of the intestine, such as septicemia, diarrhea, and UTI infection [1]. *E. coli* can colonize several tissues and organs because it has many genetic materials such as virulence factors or resistance genes [2]. There are many virulence factors produced by the genes present in *Escherichia coli*, which are responsible for the possibility of these bacteria causing diseases and their resistance to the most commonly used antibiotics; in particular, the gene (chuA) can encode a heme-binding protein, is essential for the expression of outer membrane proteins implicated in the usage of heme and its protein. Another significant gene is the gene (chuB), which produces a heme-binding protein. When look at E. coli, notice that the gene chuA can encode 69-kDa protein responsible for heme absorption. According to preliminary findings, the

gene is a component of a broader locus, which seems to be extensively distributed among dangerous E. coli strains. In addition to carrying many additional virulence factors, E. coli isolates that express this gene have been shown to colonize the urinary tract and the gastrointestinal tract more significantly than bacteria missing these genes [3, 4].

Pathogenic bacteria may find it particularly beneficial to utilize heme and hemoglobin in their metabolism. These infections often release cytotoxins, which allow them to obtain access to the internal heme reserve in addition to invading the host's tissues. Cytotoxin generation and the capacity to use heme and/or hemoglobin may be effective iron acquisition throughout infection [4].

Bacteria have several mechanisms of antibiotic resistance, including The natural Resistance responsible for blocking the action of antibiotics Through the failure or inability of the opponent to reach his target because of Structural and anatomical characteristics of an organism that prevent The interaction of the antibody with the center of its biological effect may be resistance is caused by chromosomal or plasmid mutations Which carries the trait of resistance through transposon genes and is Pieces of DNA genes have the ability to move from one site to another [5]. The resistance may be due to a change in the composition of certain enzymes or their loss of function, leading to negative changes. At the site on which the antibody is working, resistance may be caused by constructing enzymes that detoxify antibiotics biology. The resistance of bacteria to β-lactam comes by its forming of β-lactam enzymes that analyze the beta-lactam ring [6]. Study aim is investigate E. Coli ability against antibiotics and the detection of *chuA* gene in the MDR isolates.

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Material and Methods

(150) urine and stool samples from patients were collected from different age groups (children, young people, and adults), including males and females. Patients range in age from 1 to 60 years. Samples were obtained from patients who consulted at AL- Diwaniyah Teaching Hospital from September 2021 to March 2022. Each patient had a case sheet containing the patient name, patient sex, patient age, and appearance of the clinical signs arranged in the questionnaire in an index. All stool and urine samples were taken from patients with UTIs and diarrhea using transport medium swabs labeled with the patient's details and transferred to the laboratory as soon as possible. Occasionally, samples are kept in transit media according to the Time of collection and then sent to the laboratory for analysis. In order to determine the presence of E.Coli, all specimens were cultured based on conventional procedures on differentiation and enrichment medium (Blood, EMB, and MacConkey agar) and afterward incubated at aerobic capacity at 37 degrees Celsius for 24 hours. According to Bergy's manual for determinative bacteriology [7], Gram-negative bacteria with purple color identification was carried out using biochemical methods (oxidase, catalase, indol, Vogues–Proskauer citrate, TSI, methyl red, urease, motility, etc.).

Antibiotics resistance assay

With the use of the disc diffusion method on Muller Hinton medium, it was possible to determine whether or not isolates were responsive to antibiotics. A panel of antibiotics was used to evaluate the antibiotic susceptibility of each isolate, with the following antibiotics included in the panel: Antibiotics such as the ones listed below are used: Amikacin AK [30g], Carbenciline PY [30g], Meropeneam MEM [10g], Ceftrixon CRO [30g], Aztreonam ATM [30g], Ciprofloxacin CIP [5g], Levofloxacine LEV [5g], Netlimicine NET [10g], Ticarciline/clavulanicacid [30g].

Small piece of bacterial suspension (made by inoculating five isolated bacteria grown on BHI media to tryptic soy broth (5) ml during 120 minutes) was added tryptic soy broth (5) ml then cultured for 120 minutes for forming the suspension with moderate turbidity (compared with McFaerland solution), bacteria were cultured on Mueller-Hinton media with discs with sufficient spacing between them to prevent overlapping of inhibition zones. Using the criteria stated by[8, after incubating the inoculation of the plates for 20 hours at 37 °C, the sensitivity and the isolates resistance against the antibiotics were examined.

Extraction of DNA

DNA was extracted from the *E. coli* isolates using the extraction kit from Bioneer company of Korean origin isAccupower® PCR-Pre Mix-Kit. The concentration and purity of extracted DNA were measured by nanodrop, then detected by gel electrophoresis.

Detection of *chuA* gene in *E. coli*

In this study, Conventional PCR was used to detect three virulence factors of *E.coli* isolates from patients with urinary tract and diarrhea infections in AL-Diwaniyah - Iraq. Table (1) shows the primers used to detect *E.Coli* virulence gene, and Table (2) shows the PCR program. PCR was done as Table (3).

Table (1): used sequences of the Primer

Gene	The Sequences	size	source
ChuA	F- GACGAACCAACGGTCAGGAT R- TGCCGCCAGTACCAAAGACA	297bp	Clermont *et al.*,[9]

Table (2): Program of PCR for gene (*ChuA*)

Primer Name	Temperature (c°)/Time				
	Initial denaturation /Time	Cycling conditions			Final Extension /Time
		Denaturation	Annealing	Extension	
ChuA	95°C\ 5min	94°C\ 1.5 min	63°C\ 1.5 min	72°C\1.5min	72°C\10 min

Statistical Analysis

Statistical analysis was carry out by Chi-square and LSD for the determination of the relationship between the variables at ($P \leq 0.05$) [10].

Results and Discussion

Identification of Sample

Depending on the gram stain, morphological features on culture media (Blood agar, MacConkey agar, Eosine methylene blue agar (EMB), and biochemical tests, Out of 150 urine and stool samples, Preliminary results showed there were 122 specimens (81.33%) were identify as gram-negative bacteria, (28) samples (18.66%) were diagnosed as gram positive bacteria as shown in figure(1). Sixty isolates of *E.Coli* bacteria from a total of 150 samples (122 gram-negative isolates and 28 isolates of gram-positive bacteria) were isolated from urine 38 (63.3%) and stool samples 22 (36.6%) as shown in table (3).

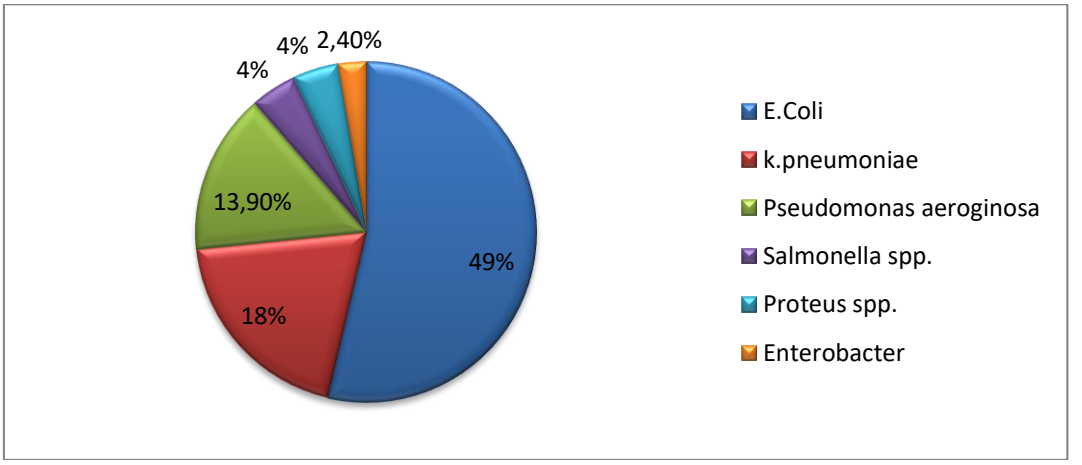

Figure (1):-percentage distribution of gram-negative bacteria isolated from urine and stool samples

It was observed through the study found that *E. coli* was the most common cause of UTIs, which constituted 49% of the total. The isolates are able to cause high infection may be due to their natural presence in the human digestive tract and from it moves to the urinary canal of the infected patient [11].

Table (3): Numbers and percentages of isolated *E.Coli* from stool and urine samples

Source	NO of samples	*E.Coli* isolates		Other bacterial isolates		X^2
		NO	%	NO	%	138*
Urine	95	38	63.3	57	63.3	
Stool	55	22	36.6	33	36.6	251*
Total	150	60	100(40%)	90	100(60%)	

*presence of significant difference at probability level 0.05

Antimicrobial susceptibility testing

The Table (4) was demonstrated that the antibiotic sensitivity results for *E. Coli* reveals higher resistance to the most multidrug resistance. The isolates resisted aztreonam ticarcilin/clavulanic acid and carbencillin, were (81.8%), (83.6%), and (89.09%) respectively. The other antibiotics, such as piperacillin, nitrofurantoin, and ceftrixon were (70.9%), (67.26%), and (61.8%) respectively. Most isolates demonstrated low resistance to the amikacin, the levofloxacin, wherever, showed (43.6%), and (21.8%) respectively. As it turned out from the study, 40 isolates of Escherichia Coli have multiple resistances to antibiotics (MDR).

Table (4): Antimicrobial susceptibility patterns of *E.Coli* (N = 60)

Antibiotic	Symbol	Resistant (%)isolates	Moderate sensitive (%) isolates	(%) Sensitive isolates
Amoxicalve	AMC	40(72.73)	12(14.55)	8(12.73)
Amikacin	AK	28(43.6)	9(12.7)	27(43.6)
Cefriaxon	CRO	34(61.8)	10(9.0)	16(29.0)
Ciprofloxacin	CIP	42(76.3)	-	18(23.64)
Levofloxacin	LEV	17(21.8)	3(5.4)	40(72.7)
Aztreonam	ATM	45(81.8)	5(3.6)	10(14.5)
Meropenem	MEM	39(70.9)	9(14.5)	12(16.55)
Tobromycine	TOB	30(54.5)	10(9.0)	20(36.3)
Netilmicine	NET	2(3.63)	12(12.7)	46(83.6)
Carbencilline	PY	46(83.6)	14(16.3)	-
Ticarcillin & Clavulanic acid	TCC	49(89.09)	6(7.4)	5(5.46)
Norfloxacin	NOR	33(60)	13(18.1)	14(21.8)
Doxycycline	DO	45(81.8)	-	15(18.1)
Pipracillin	PRL	39(70.9)	13(17.55)	8(14.55)
Nitrofurantoin	NIT	37(67.26)	11(10.91)	12(21.82)
Trimethroprime/sulfamethraxazole	SXT	40(72.37)	5(9.09)	15(18.19)
Sensitive=S 4.2 =LSD	Intermediate LSD=3.5		Resistance=R LSD=2.4	

There is a significant difference at the level of probability P< 0.05

The percentage in this study does not differ much from its counterparts, as it showed an increase in the rates of resistance to some beta-lactams. Many studies showed the presence (90%) of E. coli bacteria resistant to beta-lactams as a result of the secretion of beta-lactamases [12].

Many studies discovered that gram-negative isolates are resistant to piperacillin in 37.5% and are highly susceptible to Levofloxacin. At the same time, Mohammed [13] found that gram-negative isolates showed resistance toward levofloxacine antibiotic, which was recorded 85%. That difference of opinion with Ansari et al.,[14] found that this antibiotic was sensitive to certain bacteria. The majority of isolates showed sensitivity to Mropenem (70.9 percent). These findings are roughly in accord with Sabir [15], who discovered that E. Coli was sensitive to carbapenems in (67.5%) of their isolates. The results of the present investigation show that *E. Coli*. Isolates are resistant to Aminoglycosides, which is roughly following the findings of [16], the resistance are occur due to modifying enzymes of the aminoglycoside [17, 18]. (AMEs). [17] They isolated species from a variety of clinical sources, including fistulas from hemodialysis patients and catheters from urinary tract infections. E. coli is resisted many antimicrobials, the most Resistance to Amoxicillin (90%), Gentamicin (87.5 %), Tobramycin (40 %), Ciprofloxacin (52.5 %), and Amikacin (25 %). The results demonstrated the high resistance are associated with the resistance reaching CIP 76.3 percent, Ak (43.6 %), and Tobramycine (54,5%) [18].

The results used (cephalosporines, monobactam, and flouroquinolones) for making sensitivity test, wherever was observed in *E. Coli* has multiple resistance to antibiotics because it can form Betalactamases enzymes and reflux pump [19].

PCR technique for detection of virulence gene (*chuA*)

Molecular detection of virulence factor (*chuA*) gene was performed for the presence of a gene by polymerase chain reaction on 40 isolates of bacteria, which had the character of multiple Resistance (MDR) to antibiotics. The result revealed the presence of this gene in all isolates at a percentage (100%) in the testes isolates, as shown in figures (3, 4).

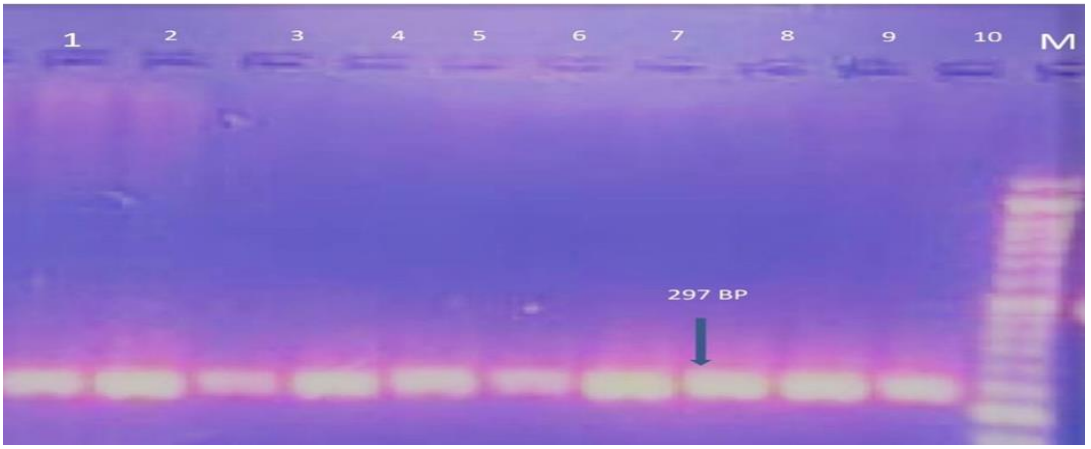

Figure (3): Detection of *chuA* gene by PCR. Lanes 1-10 are positive isolates (297 bp). The marker (100-2000 bp). (1% Agarose gel, 75 volts to 1hrours)

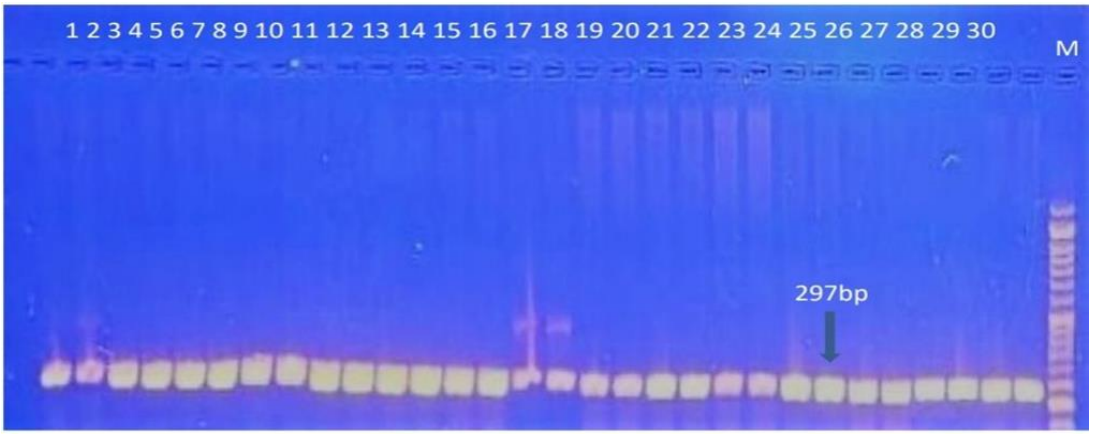

Figure (4): Detection of *chuA* gene by PCR. Lanes 1-30 are positive isolates (297 bp). The marker (100-2000 bp) (1% Agarose gel, 75 volts to 1hrours)

By comparing the findings with those of other studies, it was shown that chuA iron gene was substantially related to resistance to antibiotics such as SXT [20]. It has been hypothesized that *E. coli* could inhabit the intestine without clinical signs, but it act as etiological agents of the infections outside of the intestine, without a clear distinction between types of E. coli [21]. The presence of the gene chuA (heme receptor) supports this hypothesis.

Conclusions

E. coli isolated from fecal and urine, and it cause extraintestinal infections. These strains have virulence genes, which cause health problems. The current study demonstrated the percentage of sensitivity of the isolated samples against the antibiotics.

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