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Molecular Study of Some Virulence Genes of *Escherichia coli* Isolated from Women with Urinary Tract Infection in AL-Najaf City

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Abstract: The current study aimed to investigate the presence of (*shiA*, *sisA* and *sisB*) genes in *Escherichia coli* bacteria which responsible virulence factors by using molecular technique (PCR). Out of 500 urine samples, preliminary results showed there was a bacterial growth in 327(65.4%) specimens. It was found that 232 specimens (46.4%) were diagnosed as gram negative bacteria, 91(27.82%) of it identified as *E.coli* by cultural, biochemical characteristics, API 20E System and Vitek-2 system. while there were 95 specimens (19%) were diagnosed as gram positive bacteria and 173 specimens (34.6%) with no any growth. The susceptibility test for 13 types of antibiotics drugs were tested by using disk diffusion method, the results demonstrated that isolates of *E.coli* showed resistance to, Amoxicillin+Clavulanic acid (92.3%) Trimethoprim–sulfamethoxazol and Cefotaxime (90.1%), Aztreonam (85.71%), Ceftazidime (76.92%), Ciprofloxacin (52.74%), Amikacin (50.54%), Ceftriaxone (48.35%), Gentamicin (42.85%), levofloxcin (26.37%), Nitrofurantoin (17.58%), Imipenem(7.69%), and Meropenem (4.39%). Showing clinical and laboratory signs of urinary tract infection (UTI).

Keywords: *E.coli*, Virulence gene, UTI.

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Introduction

Urinary tract infections (UTIs) frequently occur in humans. The uropathogenic *Escherichia coli* (UPEC) is significantly associated with etiologies of UTIs, including pyelonephritis and cystitis(1). UPEC has many virulence factors that assist its colonization, invasion, and survival within the host urinary system(2, 3, 4). Interestingly, *sisA* and *sisB* homologs of *shiA* of Shigella flexneri, and play a similar role in suppressing the host defense system during UTIs (5, 6). Therefore, *sisA* and *sisB* which are located in pathogenicity islands, can be considered UPEC virulence factor genes. They are found in some but not all UPEC strains, which may explain the reported variability of inflammation among patients of UTIs(6).

Materials and Methods

Samples Collection

Five hundred midstream urine samples (MSU) were collected from women suffering from (UTI), aged between 10 to 60 years from different hospital, in AL-Najaf city (AL- Zahraa Educational Hospital for Childbirth and Children and Al Hakim General Hospital) during the period from 1st December 2018 to end April 2019.

identified as *E.coli* by cultural, biochemical characteristics, API 20E System and Vitek-2 system. The isolates were screened by (PCR) for detection of *shiA,sisA* and *sisB* genes.

Antibiotic Susceptibility testing

The disk diffusion method was used to determine antibiotic susceptibility of the isolates on Muller Hinton agar. Each isolate was tested for antibiotic susceptibility using a panel of the following antibiotics:Amoxicillin-Clavulanic acid, Trimethoprim - sulfamethoxazol, Cefotaxime, Aztreonam, Ceftazidime, Ciprofloxacin, Amikacin, Ceftriaxone, Gentamicin, levofloxacin, Nitrofurantoin, Imipenem, Meropenem. The plates were incubated at 37°C for 24 hours, and inhibitory zone diameters were measured. Interpretation of results followed criteria recommended by Clinical Laboratory Standard Institute(7).

Molecular Method

Extraction of DNA

DNA was extracted from the *E. coli* isolates using the extraction kit processed by Intron / Korea. The concentration and purity of extracted DNA was measured by nanodrope, then detected by gel electrophoresis.

Detection of urovirulence genes in *E. coli*

In this study, Conventional PCR were used for detection of three virulence factors of *E.coli* isolates from patients with urinary tract infection in AL- Najaf - Iraq. Table (1) showed the primers used for detection of UPEC virulence genes and Table (2) showed the PCR program. PCR was performed in total volume of 25µl and components are shown in Table (3).

Table (1): Primers sequences used in this study.

Virulence gene	Oligo Sequence (3′→5′) (5′→3′)	Product Size(bp)	References
shiA	TCACCTTACTGGTATGAACTC TCCAGGGCCAGACATATTCA	451	Shakhatreh, *et al.*, (1)
sisA	TTGCCCGACAGGAGAATGAC GCAGTATATGGCGTGCCTGT	360	
sisB	GAACGATAGATTATGCTTTG TCAGTACACTGAAGGCTCG	518	

Table (2): Program condition of uniplex PCR amplification for each gene in this study.

Gene	Step	No. of cycle	Temperature	Time (M:S)
shiA	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	30 cycle	94 ºC	00:45
	annealing		59ºC	00:45
	extension		72 ºC	00:50
	Final extension	1 cycle	72 ºC	10:00
sisA	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	35 cycle	94 ºC	00:45
	annealing		59ºC	00:45
	extension		72 ºC	00:50
	Final extension	1 cycle	72 ºC	10:00
sisB	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	35 cycle	94 ºC	00:45
	annealing		59ºC	00:45
	extension		72 ºC	00:50
	Final extension	1 cycle	72 ºC	10:00

Table (3): The components of the reaction mixture of uniplex PCR, final volume is 25µl.

Components	Volume
Go Taq®Green Master mix (2X)	12.5 µl
Forward primer	1.5 µl
Reverse primer	1.5 µl
DNA template	2 µl
Nuclease-free water	7.5 µl
Final volume	25 µl

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 500 urine samples, Preliminary results showed there is a growth in 327(65.4%) specimens. There were 232 specimens (46.4%) were diagnosed as gram negative bacteria, while there were 95 specimens (19%) were diagnosed as gram positive bacteria and 173 specimens (34.6%) with no any growth. Table (4).

Table (4): Prevalence of bacterial uroeathogenes isolated from women with UTI.

Bacterial growth	No. specimens	Percentage %
Gram negative bacterial	232	46.4%
Gram positive bacterial	95	19%
Negative growth	173	34.6%
Total	500	100%

Numbers and percentage of total specimens (N=500).

Antimicrobial susceptibility testing

Ninety one isolates of *E.coli* were tested for their susceptibility against 13 type of antibiotics: Amoxicillin-Clavulanic acid, Trimethoprim–sulfamethoxazol, Cefotaxime, Aztreonam, Ceftazidime, Ciprofloxacin, Amikacin, Ceftriaxone, Gentamicin, levofloxacin, Nitrofurantoin , Imipenem, Meropenem .Plates of Muller-Hinton agar were used to find the sensitivity pattern and incubated at 37°C for 24 hours. The zone of the inhibition of the bacterial growth was measured after incubation and compared with the clinical and laboratory standards institute(7).

The antibiogram for studied isolates revealed different level resistance of clinical isolates to most of antibiotice under test. It was found that 92.3% were resistance to Amoxicillin - Clavulanic acid. The present study showed a highest resistance to Trimethoprim – sulfamethoxazol and Cefotaxime 82(90.1%), also mot resistance was recorded for the antibiotice Aztreonam 77(85.71%),Ceftazidime 70(76.92%), Ciprofloxacin 48 (52.74%), Amikacin 46(50.54%), Ceftriaxone 44 (48.35%), Gentamicin 39(42.85%),

The current study demonstratated that *E.coli* possessed a low - level resistance against levofloxcin 24(26.37%), Nitrofurantoin 16(17.58%), Imipenem 7(7.69%) and Meropenem 4(4.39%) as shown in Table (5).

Table (5): percentages of antimicrobial susceptibility among 91 *E.coli* isolates.

Antibiotics	Concentration	Sensitive	Intermediate	Resistant	Percentage of resistance
Amoxicillin + Clavulanic acid	20/10 µg	7	0	84	92.3%
Trimethoprim – sulfamethoxazol	1.25/23.75 µg	9	0	82	90.1%
Cefotaxime	30 µg	9	3	82	90.1%
Aztreonam	30 µg	14	0	77	85.71%
Ceftazidime	30 µg	15	6	70	76.92%
Ciprofloxacin	5 µg	40	3	48	52.74%
Amikacin	30 µg	41	4	46	50.54%
Ceftriaxone	30 µg	42	5	44	48.35%
Gentamicin	10 µg	44	8	39	42.85%
levofloxcin	5 µg	57	10	24	26.37%
Nitrofurantoin	300 µg	71	4	16	17.58%
Imipenem	10 µg	82	2	7	7.69%
Meropenem	10 µg	87	0	4	4.39%
Chi-Square (χ^2)	---	---	---	---	9.833 **

The increasing of *E.coli* resistance to Amoxicillin-Clavulanic acid and Cefotaxime were concoradant with the results of (8) who reffered that resistance of *E.coli* to this antibiotice reached to 92%, 90.1% respectively. Trimethoprim–sulfamethoxazol show resistance,this was nearly agreed with (9) who ported a resistance percentage of (86.9%). high resistance also obseved aganist Aztreonam 77(85.71%) and Ceftazidime 70(76.92%) this results was matched with results of Zykov *et al.* (10) and Sasirekha *et al.* (11) which they found that (87%)and (75%) of *E.coli* were resistance to both respectively.

Also in the current study more than half of *E.coli* isolates were resistance to important anibiotics such as Ciprofloxacin 48 (52.74%), Amikacin 46(50.54%), Ceftriaxone 44(48.35%), Gentamicin 39(42.85%). Close to this result was reported in study carried out in Iraq by (8) Out of 50 isolation of *E. coli* showed were resistant at different rates, Ciprofloxacin and Gentamicine (46%) for both of them. Amikacin (44%).but it differs with this study of for the antibiotic Ceftriaxone (90%). The result was matched with study by Zykov *et al.* (10) and Anago *et al.* (9) who reported that many isolate of *E. coli* showed resistant to Gentamicine (42%) ,(45.2%) Respectively .Other studies have also reported similer with such as Sabir *et al.* (12). Ceftriaxone (43.3%), Ciprofloxacin (54.2%), Amikacin (12.7%) and Gentamicine (59.8%)(13) reported in his study that the percentage of *E. coli* resistance to Ceftriaxone (41%), Ciprofloxacin (77%), Amikacin (10%), Gentamicin (20%).

The isolates under study showed low resistance to some *E. coli* isolates for the antibiotic levofloxacin (26%), Other studies have also reported similar finding(14) refered that (25.8%) of *E. coli* bacteria were resistant to this antibiotic .

On the other hand the results didn't agreed with the study of Sohail *et al.* (15) which reported that resistance to this antibiotic (82%). Some isolates of *E. coli* for the samples under study were resistant to nitrofurantoin (18%). The results of the current investigation

agreed with Titilawo *et al.* (16) and Mamuye (17) who found a few isolates of *E. coli* resistant to Nitrofurantoin (19%) (20.8%) respectively. while hand the result didn't agreed with the study by Aal-Aaboda and Al-Notazy (18) who indicated that (79%) of *E. coli* isolates were resist to that antibiotics.

The current study showed low resistance to antibiotics Imipenem and Meropnem where very few isolates of *E. coli* have resistance to this antibiotic (8%) , (4%) respectively. By comparing the results with other researchers, it can be said that there is similarity with the result of Sohail *et al.* (15) which provided stating that resistance ratio of *E. coli* to Imipenem and Meropnem antibiotics is (3%).

PCR techniqne for detection of virulence genes

The results showed prevalence of virulence genes 52(58.24%)for *shiA* 44(48.35%) for *sisA* and 27(29.67%) for *sisB* (Table 6).

Table (6): Distribution of virulence genes of UPEC strains isolated from woman with UTIs in AL-Najaf-Iraq.

No. *E. coli* isolates	Virulence genes (%)		
	ShiA	***sisA***	***sisB***
91	52 (58.24%)	44 (48.35%)	27 (29.67%)

The results showed that *E.coli* strain positve to *shiA*,*sisA* and *sisB*. the UPEC virulence genes prevalence rates were 52(58.24%) ,44(48.35%) and 27(29.67%) respectively. as shown in figures (1),(2),(3).

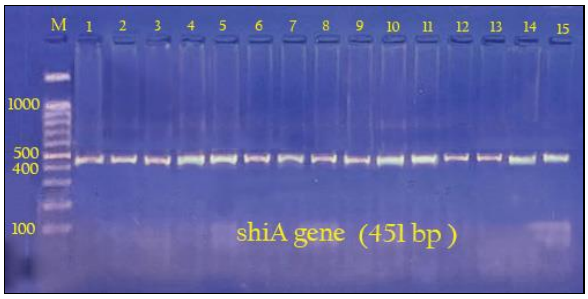

Figure (1): Gel electrophoresis of amplified *shiA* gene (451bp) from *E.coli* using conventional PCR. Agarose 2 %, 70 V/cm for 1 hrs. and 20 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15).

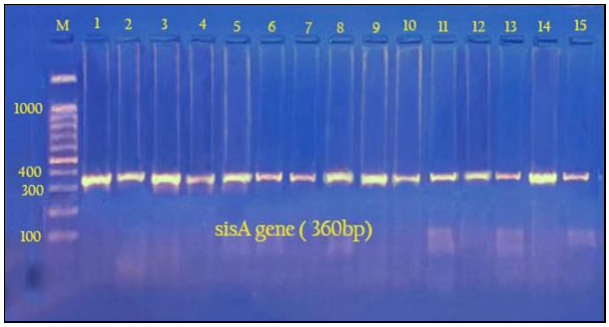

Figure (2): Gel electrophoresis of amplified *sisA* gene (360 bp) from *E.coli* using conventional PCR. Agarose 2%, 70 V/cm for 80 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15).

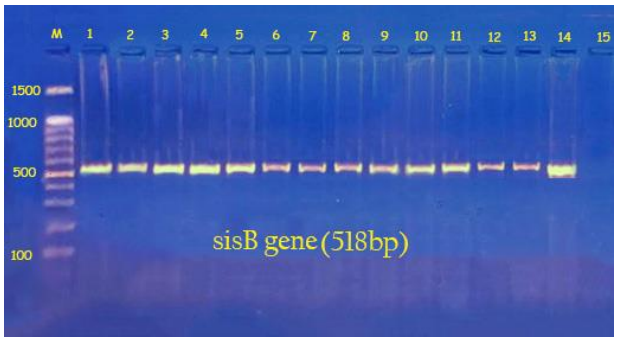

Figure (3): Gel electrophoresis of amplified *sisB* gene (518 bp) from *E.coli* using conventional PCR. Agarose 2%, 70 V/cm for 80 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-14).

By comparing the results with other researchers, the results showed Shakhatreh *et al.* (1) from Jordan ,who study the prevalence of urovirulence genes in (227) (UPEC) isolates obtained from Jordanian patients, rates were *shiA* (92%), *sisA* (72%) and *sisB* (25%). In other study by Mao *et al.* (3) who study 342 *E. coli*,to identify genes associated with UTIs in patients. The results showed *shiA* (61.11%), *sisA* (57.6%) and *sisB* (19.88%).

Interestingly, *sisA* and *sisB* homologs of *shiA* of *Shigella flexneri*, and play a similar role in suppressing the host defense system during UTIs(5, 6).

Therefore, *sisA* and *sisB* which are located in pathogenicity islands, can be considered UPEC virulence factor genes. They are found in some but not all UPEC strains, which may explain the reported variability of inflammation among patients of UTIs(6).

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