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COMPARISON OF VIRULENCE FACTORS PROFILE AND ANTIBIOTIC SENSITIVITY FOR *ESCHERICHIA COLI* BACTERIA ISOLATED FROM IRAQI WOMEN WITH URINARY TRACT INFECTIONS

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Abstract

The current study aimed to investigate the presence of certain genes in *Escherichia coli* bacteria that encoding some of virulence factors by using phenotypic and genotypic techniques by polymerase chain reaction and susceptibility test for 13 types of antibacterial drugs. The results showed that 327 specimens (65.4%) were diagnosed as bacterial growth, among 500 randomly samples, 91 isolates (27.82%) were *E. coli*. The susceptibility test for 13 types of antibacterial 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). Primers to amplify the genes encoding the virulence factors of uropathogenic *E. coli, fimH, hlyA* and *papc*, identification of uropathogenic *E. coli*. Among the isolates studied. The results demonstrated that 79 (86.81%), 62 (68.13%) and 58(63.73%) were positive respectively

Introduction

Escherichia coli is a Gram-negative, rod-shaped, flagellated and facultative anaerobic bacterium of the family Enterobacteriaceae responsible for 70% of urinary tract infections (UTIs) in human. Beside that it is responsible for many other serious infection including diarrheal infections, intra-abdominal and soft tissue infections, meningitis, pneumonia and rarely endocarditis (Cheesbrough, 2012). UTIs are mostly second common bacterial infections after respiratory tract infections, seen in primary care. (Zare *et al.,* 2018). Urinary tract infections (UTIs) is a broad term that describes microbial colonization of the urine (usually sterile) and in most cases the infection might extended to involve urethra, bladder, ureters, renal pelvis, kidney, as well as adjacent structures such as the fascia, prostate, and epididymis (Hickling *et al.,*2015).

Materials and Methods

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 polymerase chain reaction (PCR) for detection of virulence 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

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Standard Institute, CLSI (2019).

Molecular Method

Extraction of DNA

Extraction, and measured the Concentration and Purity of DNA

DNA was extracted from the *E. coli* isolates that diagnosed by vitek-2 system Using the extraction kit processed by the company Intron / Korea the results of the extraction were go without problems the concentration and purity of and with high concentration and purity extracted DNA was measured by nanodrop, than 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 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 25ìl and components are shown in Table 3.

Results and Discussion

Identification of Sample

Depending on the gram stain, morphological features on culture media (Blood base agar, MacConkey agar, Eosine methylene blue agar (EMB), and biochemical tests, Out of 500 of urine samples, Preliminary results showed there is a growth in 327(65.4%) samples.

It has been found that the most common bacteria isolates were *E.coli* 91(27.82 %), 52(15.9%) *Klebsiella spp*, 41(12.53%) *Staphylococcus spp*, 32(9.78%) *Proteus spp*, 27(8.25%) *Streptococcus spp*, 22 (6.72%) *Pseudomonas spp*, 20 (6.11%) *Enterobacter spp*, 15 (4.58%) *Acinetobacter spp*, 7 (2.14%) *Enterococcus spp*. As appeared 20 (6.11%) isolations from *Candida albicans* Fig. (1). While not growth did appear in 173 samples.

PCR technique for detection of virulence genes

The results shown prevalence of virulence genes 79 (86.81%) for *fimH* 62(68.13%) for *hlyA* and 52 (63.73%) for *papc* (Table 4). Of the *fimH* coding genes, was the most prevalently detected (79 strains), followed by *hlyA* (62 strains) and *papc* (52 strains) respectively.

The product of PCR detected by using electrophoresis.Approximately 79 (86.81%) of *E.coli* isolates gave positive results for *fimH* gene. as shown in Fig. (2)

These nearly result come together with study by Momtaz *et al.,* (2013) who found that *E.coli* have (86.17%), who study total of 123 *E. coli* strains isolated from patients with symptomatic UTIs. The patients were hospitalized or visited the emergence room at Baqiyatallah Hospital in Tehran, Iran. Also the results nearly approved with the study of Lopez-Banda *et al.,* (2014) who study the precedence of *fimH* gene among Mexican women and found that 84% of *E.coli* isolates harboring this gene.

The prevalence of *fimH* gene in *E.coli* isolate was disagreement with study of Asadi *et al.,* (2014) who study, frequency of *fimH* gene among UTI patients and found that anly 60 (51.7%) express the *fimH* gene. It also didn't agree with (Mihaylova *et al.,* 2012) Which his results showed that all *E.coli* isolates were contain *fimH* (100%). this difference may be due to the different in type and number of samples in addition to the community under is essential gene study.

fimH gene has role with in the pathogenesis of *E.coli* isolates in urinary system, this results came in line with what he mentioned by many researcher, who confirmed importance of the first type of fimbriae and its role in the induction of infection and promote the ferocity of *E. coli* bacteria in urinary tract. Such conclusion was supported by the work (Al-Khafaji 2013) in Iraq which showed the importance of the (type I fimbriae) and his relationship as a ferocity factor can participate in virulence of *E. coli* bacteria.

Furthermore the investigation showed the presence of *hlyA* gene in 62(68.13%) isolates with molecular siz (666bp). as shown in Fig. (3)

The result presented here go along nicely with the results recorded by Lee *et al.,* (2016) who found that *hlyA* gene presence in 36 (62%) in Of the 58 *E. coli* isolates, from children with UTIs. in South Korea.

In other studies reported the presence of *hlyA* gene in different proportions the result didn't agreed with this study of these researchers: Tabasi *et al.,* (2016) who found that *E.coli* have 48 (30.8%), who study conducted

Table 1: Primers sequences used in this study.

Virulence gene	Oligo Sequence (3' →5') (5'→3')	Product Size(bp)	References
fimH	F:TGCAGAACGGATAAGCCGTG R:GCAGTCACCTGCCCTCCGGTA	508	Munkhdelger *et al.*,(2017)
papC	GTGGCAGTATGAGTAATGACCGTTAATATCCTTTCTGCAGGGATGCAATA	205	(Liu *et al.,* 2015)
hlyA	GCTGCAAATAAATTGCACTCAGCCCTGCACCGATATTATCAAG	666	(Burgos,and Beutin,2010)

Table 2: 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

Table 3: Program condition of uniplex PCR amplification for each gene in this study.

Gene	Step	No. of cycle	Temperature	Time (M:S)
fimH	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	30 cycle	94 ºC	00:30
	annealing		58 ºC	00:30
	extension		72 ºC	01:00
	Final extension	1 cycle	72 ºC	05:00
hlyA	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	32 cycle	94 ºC	00:50
	annealing		55 ºC	00:50
	extension		72 ºC	00:50
	Final extension	1 cycle	72 ºC	7:00
papc	Initial denaturation	1 cycle	94 ºC	05:00
	denaturation	35 cycle	94 ºC	01:00
	annealing		55 ºC	01:00
	extension		72 ºC	01:00
	Final extension	1 cycle	72 ºC	10:00

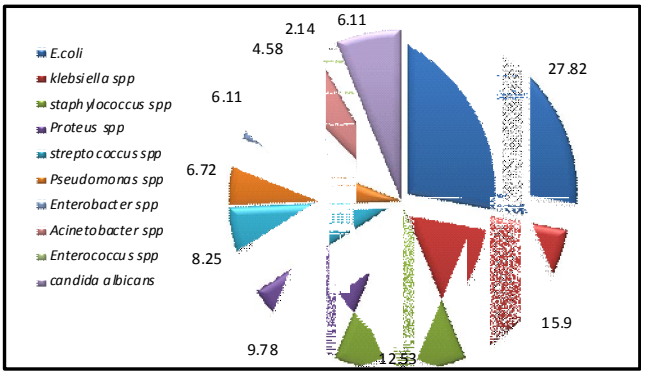

Fig. 1: Percentage of bacterial isolates of samples.

Table 4: Distribution of virulence genes of UPEC strains isolated from woman patients with UTIs in najaf-Iraq.

No. *E. coli* isolates	Virulence genes (%)		
	fimH	*hlyA*	*Papc*
91	79(86.81%)	62(68.13%)	52(63.73%)

at Pasteur Institute of Iran, a total of 156 UPEC isolated from outpatients and inpatients (symptomatic and asymptomatic UTI patients) visiting general and private hospitals in Tehran, Iran. and, Asadi *et al.* (2014) who study in Tehran, Iran, Among 60 UPEC isolates, The frequency of virulence factor was 14 (23.3%) *hlyA* gene isolates, 47 isolates were from females and 13 were from males.

Production of toxin such as hemolysin causes tissue damage facilitating bacterial dissemination, releasing of host nutrients, and may also modulate host signaling pathways affecting several processes, including inflammatory responses, host cell survival, and cytoskeletal dynamics (Wiles *et al.,* 2008).

Recent data also revealed that are most bacterial isolated are positive for *papc* gene 52 (63.73%), as shown in Fig. (4).

Stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15): Amplicons of *papC* gene.

Similar result are arrived by (Ananias and Yano.,2008),which show that (65%) of *E.coli* isolates gave positive results. It also agree with (Lopez-Banda *et al.,* 2014) who study, *E coli* isolates (108) from Mexican women, clinically diagnosed with urinary tract infection, were screened to identify virulence genes *papc* (62%).

On the other hand the Results didn't agreed with the study by Tiba *et al.,* (2013) who study, 162 Uropathogenic *Escherichia coli* (UPEC) strains from patients with cystitis were genotypically characterized the expression of *papc* gene in (32.7%) of isolates. also, a study by Zhao *et al.,* (2015) demonstrate that the prevalence *papc* gene in *E.coli* was 6.7%, which disagree with this study.

Papc gene recognized as a key determinant in promoting the virulence of *E. coli* in urinary ract infection, Lane *et al.,* (2007). The genes encoding the P pilus type were termed the *pap* genes or pyelonephritis-associated pili genes since these were typical of strains isolated from human urinary tract infections (Wright *et al.,* 2006).

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

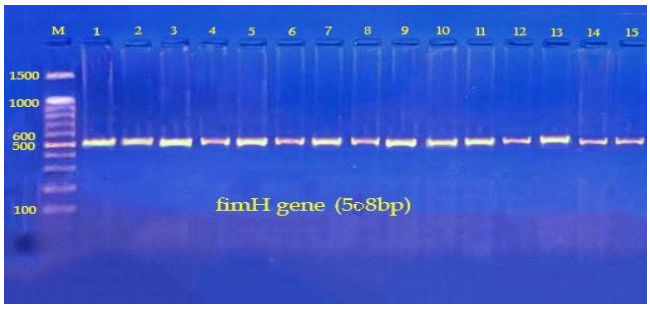

Fig. 2: Gel electrophoresis of amplified *fimH* gene (508 bp) from *E.coli* using conventional PCR. Agarose 1.5%, 70 V/cm for 1 hrs. and 20 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15): Amplicons of *fimH* gene.

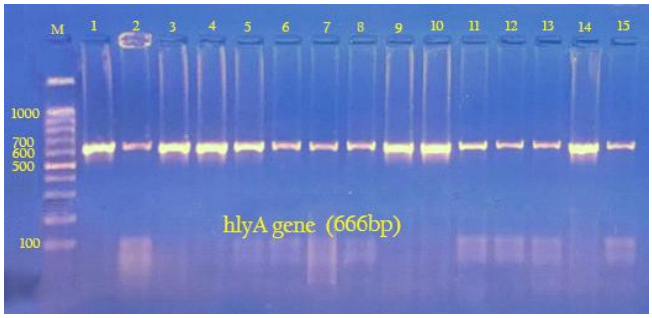

Fig. 3: Gel electrophoresis of amplified *hlyA* gene (666 bp) from *E.coli* using conventional PCR. Agarose 1.5%, 70 V/cm for 1 hrs. and 20 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15): Amplicons of *hlyA* gene.

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

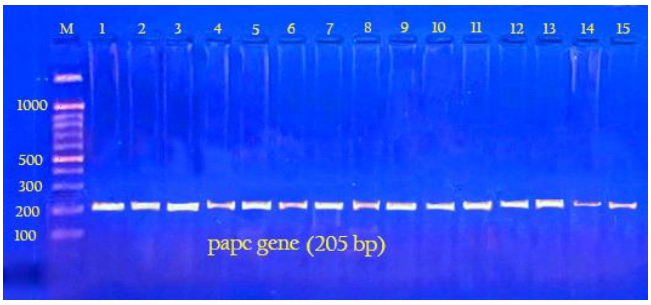

Fig. 4: Gel electrophoresis of amplified *papc* gene (205 bp) from *E.coli* using conventional PCR. Agarose 1.5%, 70 V/cm for 1 hrs. and 20 min, stained with ethidium bromide dye and visualized on a UV transilluminator. Lane (M): 100 bp DNA ladder. Lane (1-15): Amplicons of *papC* gene.

institute (CLSI.2019). The antibiogram for studied isolates revealed different level resistance of clinical isolates to most of antibiotic 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 high 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 demonstrated 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.

The increasing of *E.coli* resistance to to Amoxicillin - Clavulanic acid and Cefotaxime were concoradant with the results of (AL-faham. 2016) who referred that

Table 4-4: Percentages of antimicrobial susceptibility rate of 91 E.coli isolated.

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 (γ^2)	.....	.....	.....	.....	9.833 **

** (P<0.01).

resistance of *E.coli* to this antibiotic reached to 92%, 90.1% respectively. Suggesting that these antibiotic may not be against organisms with this particular combination. Trimethoprim –sulfamethoxazol show resistance, this was nearly agreed (Anago *et al.,* 2015) who ported a resistance percentage of (86.9%). high resistance also observed against Aztreonam 77(85.71%) and Ceftazidime 70(76.92%) there results was matched with results of (Zykov *et al.,* 2016) and (Sasirekha *et al.,* 2010) 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 antibiotics 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 AL-faham, (2016) 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 antibiotic Ceftriaxone (90%). The result was matched with study by (Zykov *et al.,* 2016) and (Anago *et al.,* 2015) reported that many isolate of *E. coli* showed resistant to Gentamicine (42%), (45.2%) Respectively.Other studies have also reported similar find with such as al (Sabir *et al.,* 2014) Ceftriaxone (43.3%), Ciprofloxacin (54.2%), Amikacin (12.7%) and Gentamicine (59.8%), (Ansari *et al.,* 2015) reported in his study that the ratio of *E. coli* resistance to Ceftriaxone (41%), Ciprofloxacin (77%), Amikacin (10%), Gentamicin (20%). In a study conducted in Zakho (Polse *et al.,* 2016) reported that resistance to Ceftriaxone (52%).

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