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Molecular analysis of virulence genes of UTI causing bacteria among pregnant women in Baghdad city, Iraq

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Abstract

UTIs consider the second common widely spread complications in pregnancy period, which if not treated it may pose a risk to the health of the pregnant woman and the fetus. Therefore, study was performed on 90 *E. coli* isolates obtained from pregnant women having acute cystitis and pyelonephritis admitted to hospitals and private clinics. They were identified using Vitek 2 automated system. Antibiotic sensitivity test were done using 19 antibiotic agents. Four virulent genes related to *E. coli* were screened. Antimicrobial sensitivity results showed that ampicillin, doxycycline, cefotaxime, cephalexin, ceftriaxone, and ceftazidime were non effective against 31% of *E. coli* isolates, while 36% of isolates could resist piperacillin and trimethoprim/ sluphomethoxazole. Only 21%, 10% and 5% of isolates could resist cefepime, tetracycline and imipenem, respectively. High percentages of isolates could resist different antibiotics such as 73% were resistant to amikacin and gentamicin, 68% were resistant to tobramycin, 89% were resistant to ticarcillin/ clavulanic acid and nitrofurantoin, and finally 94% were gatifloxacin, levofloxacin, and sulfisoxazole resistant. Genotyping results showed 90% of isolates had *fimH* gene at 508bp, while 70% of them had *kpsMTII* gene at 272bp. Also, *iroN* gene (665bp) was found among 65%, and *hly* gene (1177bp) was found among 50% of isolates. To conclude, it was obvious that *E. coli* become more virulent and resistant to antibiotics, leading to major concern on the health of pregnant women and fetus. Avoiding infections and applying different treatment regime should be taken into account.

Key words: Pregnant women, acute cystitis, pyelonephritis, *E. coli*, Virulence

1. Introduction

One of the most common and costly medical complications in pregnancy period is urinary tract infection, infect nearly 20% of all pregnant women (1, 2, 3). UTIs consider the second common widely spread complications in pregnancy period, which if not treated it may pose a risk to the health of the pregnant woman and the fetus (4). Urinary tract infections are defined as microbial colonization or inflammation of the urethra, bladder, or renal pelvis and kidneys. Women with a UTIs history having more risk of UTIs during pregnancy period due to lack of prenatal care, increased parity or age, abnormalities of urinary tract, lack of adequate fluid intake, sickle cell trait, anaemia, and diabetes mellitus (5). *E. coli* is the causative agent in about 45-85% of UTIs cases, followed *by Klebsiella spp., Proteus* spp*, Pseudomonas* spp. and *Enterobacter* spp.(6). While 15% of gram positive bacteria caused UTIs include *Staphylococci* spp., *Streptococci* spp., and *Enterococci* spp. (7). The potency of uropathogenic *E. coli* strains is presence according to the virulence factors (VFs) are detected, which are located either on chromosome or on plasmid of bacteria (8). It has been studied that weak immune system of host and having virulence genes such as *irp2, iroN*, *iuc, fim, sfa, iha, afaI, tsh, papC,* and *papG, kpsMT, iss, ompT cnf1, astA, hlyA, usp, vat, set,* and *cva/cvi* by uropathogenic bacteria like *E. coli* would lead to high intensity of UTI (9, 10). *E .coli* could acquire new virulence factors via transformation leading to be more virulent (11). Many studies have been done on uropathogenic E .coli using tools of molecular technology (12). The main purpose of this study was the detection of antibiotic resistance and virulence factors of uropathogenic *E.coli* which infect a wide range of pregnant women.

2. Materials and Methods

2.1 Bacterial isolates collection: 90 isolates of each *E. coli* isolated from pregnant women having acute cystitis and pyelonephritis admitted to hospitals and private clinics. Isolates were 99.9% *E. coli* when testing by Vitek 2 automated system.

2.2 Antimicrobial sensitivity test: *E. coli* (HB101) was used as negative control and disk diffusion method was applied according to (13) towards ampicillin (10 mcg), ticarcillin/clavulanic acid (75 mcg /10 mcg), piperacillin (100 mcg), doxycycline (30 mcg), tetracycline (30 mcg), amikacin (10mcg), cefotaxime (30 mcg), cephalexin (30 mcg), ceftriaxone (30 mcg), ceftazidime (30mcg), cefepime (30mcg), imipenem (10mcg), gentamicin (10mcg), nitrofurantoin (5mcg), sulfisoxazole (5mcg), tobramycin (10mcg), trimethoprim/ sluphomethoxazole (1.25/23.75 mcg), gatifloxacin (5 mcg), levofloxacin (5 mcg).

2.3 Genotyping assay

2.3.1 DNA extraction: Wizards kit was used to extract bacterial DNA.

2.3.2 Polymerase chain reaction process: different virulent genes were used in this study. The size and annealing time of them were mentioned in table 1. Gel electrophoresis was done using 1% of agarose at 70cm/V for 90min.

Table (1): *E. coli*'s virulent genes

Primer	Sequences	Size Product	TM	Source
fim H	**F-** TGCAGAACGGATAAGCCGTGG	508bp	65	14
	R- GCAGTCACCTGCCCTCCGGTA			
KpsMTII	**F-** GCGCATTTGCTGATACTGTTG	272bp	65	14
	R- CATCCAGACGATAAGCATGAGCA			
iroN	**F-** AAGTCAAAGCAGGGGTTGCCCG	665bp	58	15
	R- GACGCCGACATTAAGACGCAG			
hlyA	**F-** AACAAGGATAAGCACTGTTCTGGCT	1177bp	65	14
	R- ACCATATAAGCGGTCATTCCCGTCA			

3. Result

3.1 Antimicrobial sensitivity test: Results showed resistance pattern among the 90 *E. coli* isolates. Ampicillin, ticarcillin/clavulanic acid, piperacillin, doxycycline, and tetracycline were non effective against 31%, 89%, 36%, 31%, and 10%, respectively. It was also found that 73% of isolates were resistant to amikacin, 31% were resistant to cefotaxime, cephalexin, ceftriaxone, and ceftazidime while 21% were cefepime resistant. There was only 5% resistant to imipenem. Gentamicin and tobramycin were not effective against 73% and 68% of *E. coli* isolates,

respectively. There was 94% of isolates could resist gatifloxacin, levofloxacin, and sulfisoxazole, and also 89 % were resistant to nitrofurantoin. Trimethoprim/ sluphomethoxazole was not effective against 36% (figure 1).

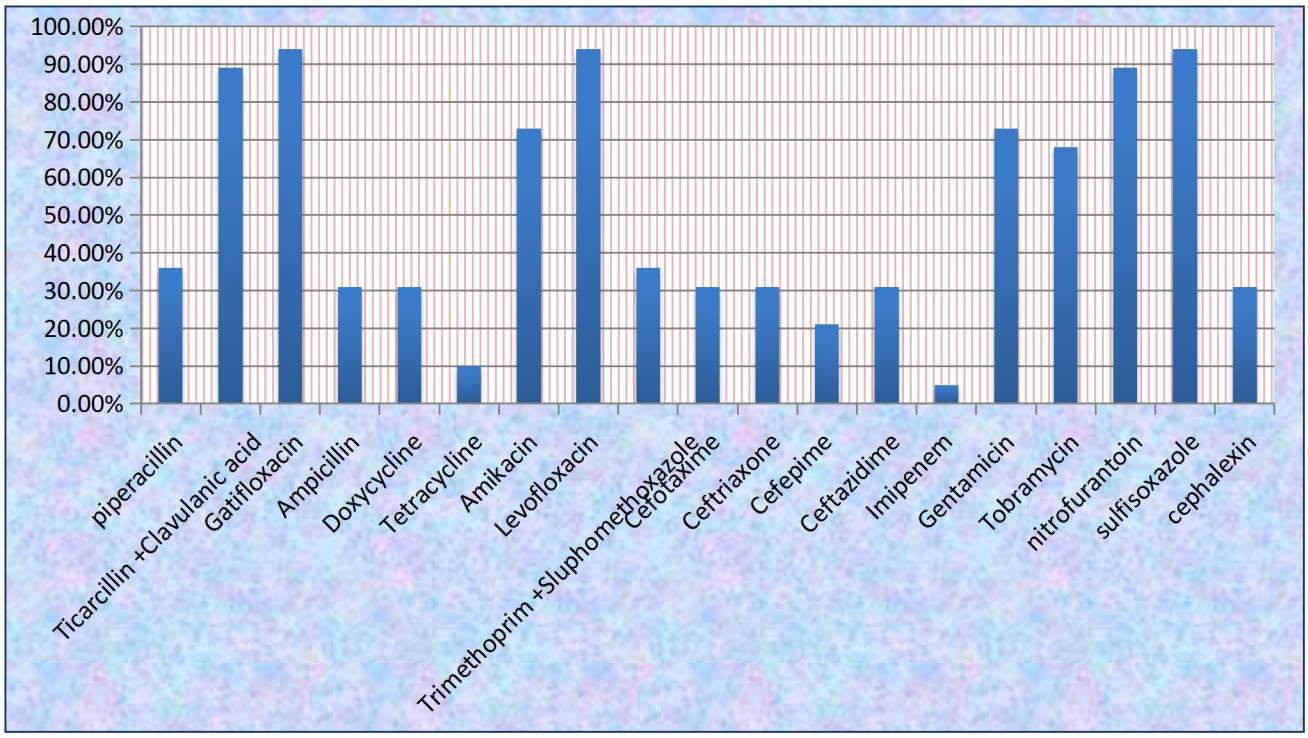

Figure 1: Resistance of 90 *E. coli* isolates towards antibiotics related to different antimicrobial classes.

3.2 Polymerase chain reaction assay: there was 90% of *E. coli* isolates had *fimH* gene at 508bp (figure 2), while 70% of them had *kpsMTII* gene at 272bp (figure 3). Also, *iroN* gene (665bp) was found among 65% (figure 4). The *hly* gene was found among 50% of isolates (figure 5).

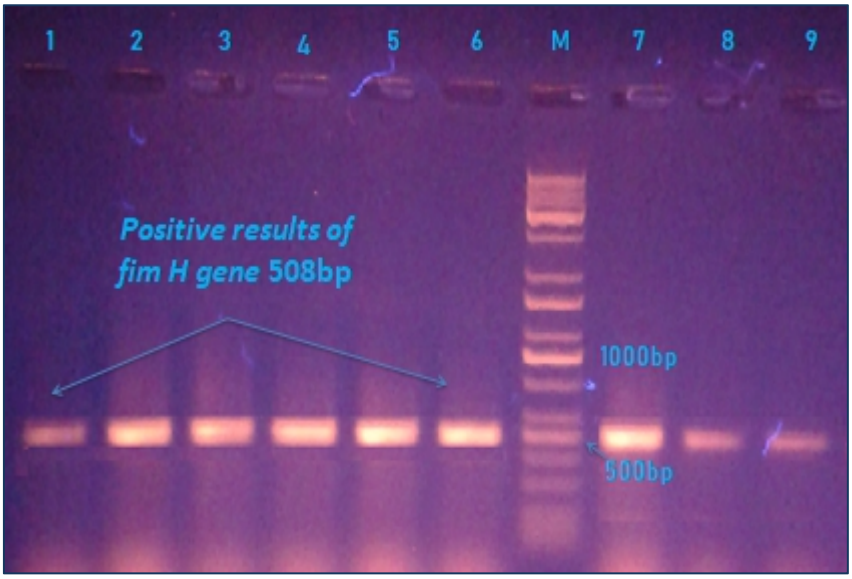

Figure 2: *E. coli* isolates had *fimH* gene at 508bp. DNA ladder was presented as M. Gel electrophoresis was done using 1% of agarose at 70cm/V for 90min.

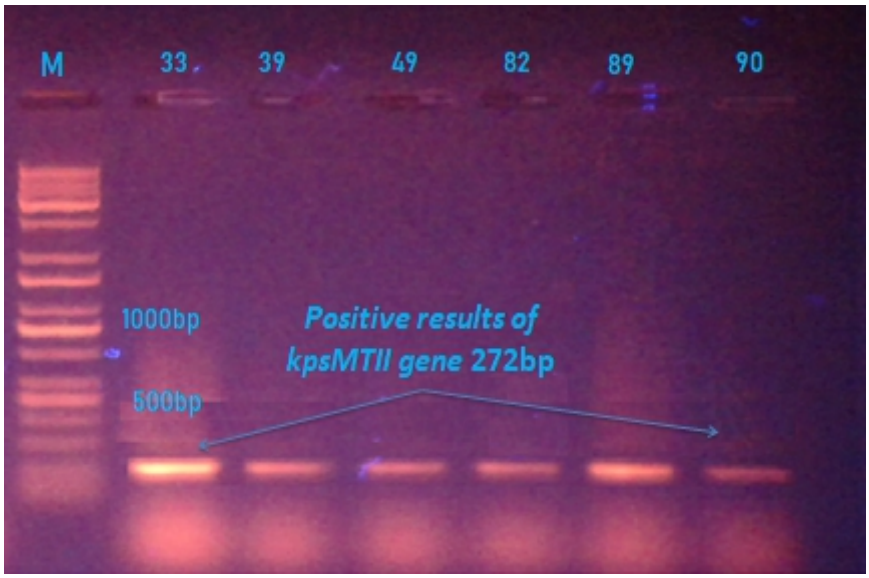

Figure 3: *E. coli* isolates had *KpsMTII* gene at 272bp. DNA ladder was presented as M. Gel electrophoresis was done using 1% of agarose at 70cm/V for 90min.

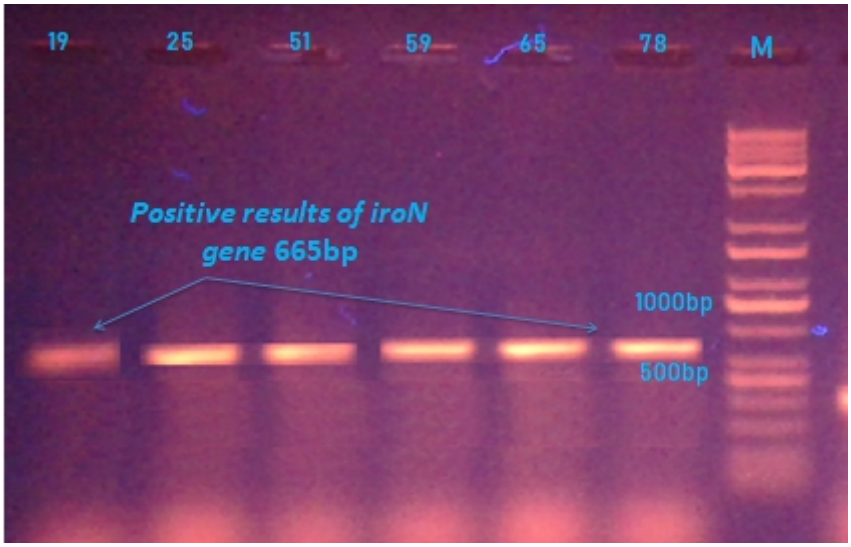

Figure 4: *E. coli* isolates had *iroN* gene at 665bp. DNA ladder was presented as M. Gel electrophoresis was done using 1% of agarose at 70cm/V for 90min.

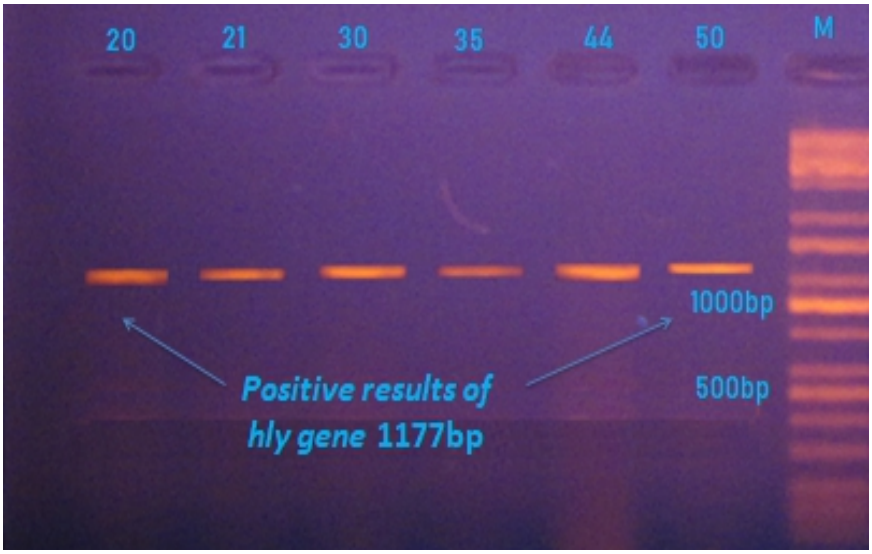

Figure 5: *E. coli* isolates had *hly* gene at 1177bp. DNA ladder was presented as M. Gel electrophoresis was done using 1% of agarose at 70cm/V for 90min.

4. Discussion

It has been stated that UTI is very common during pregnancy leading to serious health issue in both mother and fetus, especially across the developing countries like Ghana, Nigeria, Saudi Arabia, Egypt, and Iraq (16). The 90 local *E. coli* isolates obtained in this study from pregnant women sever from acute cystitis and pyelonephritis showed different antibiotic resistant levels. For example, there was a high resistance level to aminoglycoside group, beta lactamase inhibitors, quinolons, sulfisoxazole, and nitrofurantoin, which is approved by FDA to treat UTI in pregnant women (17). Also, our *E. coli* isolates resisted antibiotics related to cephalosporin class but mostly sensitive to tetracycline and imipenem. Similar results were showed by Ramos *et al.* (18), Forson *et al.* (19), Habibi *et al.* (20), Al-Mayahie *et al.* (21), Demilie *et al.* (22), AL-Zubaidi (23), Mohebi *et al.* (24), and Garofalo *et al.* (25). Twenty one of *E. coli* obtained from pregnant women with UTI were highly resistant to trimethoprim-sulfamethoxazole (80%), nalidixic acid (76%), norfloxacin (66%), ciprofloxacin (52 %), and cefalothin (85%), and they resisted gentamicin and amikacin (28%) and (23%), respectively. However, they were mostly sensitive to nitrofurantoin (14%) (26). Yeva *et al.* (27) also showed that *E. coli* isolates were extended-spectrum beta-lactamase producers and could resist many antibiotics related to beta lactams; however, all isolates were sensitive to fosfomycin. Therefore, researchers could conclude that only one dose of fosfomycin-trometamol would be useful for pregnant women. It was also shown in literature that uropathogens like *E. coli* was highly resistant towards ampicillin, co-trimoxazole, tetracycline, ceftazidime, ciprofloxacin, amoxicillin-clavulanic acid and ceftriaxone, but none of isolates could resist imipenem (28). In addition, antibiotic resistant levels like MDR, XDR, and PDR acronyms were revealed in high rate among uropathogenic *E.coli* isolated from pregnant women. Besides, Ballesteros-Monrreal *et al.* (29) reported that their isolates were resistant to various antibiotic classes so classified into 92% MDR, 7% XDR, and 1% PDR , leading to major concern of pregnant women in Mexico.

Results of polymerase chain reaction assay showed that local 90 uropathogenic *E. coli* isolates were very virulent by having different virulence genes like *fimH*, *kpsMTII, iroN,* and *hly* genes. Similar results were presented by AL-Zubaidi (23), Mohebi *et al.* (24), AL-Oqaili (26), Contreras *et al.* (30), Watt *et al.* (31), and Aljebory and Mohammad (32). It was found that 93% of uropathogenic *E. coli* isolated from hospitalized patients and private clinics had *fimH* gene, which has been considered as diagnostic marker for E. coli isolated from urinary tract infection (33). Abed *et al.* (34) revealed that 90% and 27% of *E. coli* isolates had *fim H* and *kpsMTII* genes, respectively, and they conclude that each isolate could have one or more virulent genes. It was also showed that 98% and 8% of *E. coli* isolates obtained from pregnant women had *fim H* and *hlyF* gene, respectively. Other virulent genes such as *fimA*, *sfa/foc*, *iutA*, *ibeA*, and *neuC* were found among 70% of isolates in the same study, indicating that *E. coli* become more virulent (35). It was reported that 87% of *E. coli* with K antigen isolated from pregnant women and neonates harbored *fimH* gene while only 29% of isolates had *hly* gene in addition to other virulence genes such as *sfa/foc* (37%), *ibeA* (36%), and *iutA* (82%) when tested by multiplex PCR (36). It was also found by Kaczmarek *et al.* () that 97, 83, 33, 24, 22, 12, and 7% of uropathogenic *E. coli* isolates harbored *fimH, fimA, iutA, sfa/foc, neuC, ibeA*, and *hlyF* genes (37). In addition, it was administrated that *E. coli* isolated from chronic vaginal infections had different virulence genes like *irp2, ompT, kpsMTII, cnf1, fimH, papC,* and *iroN* with high gene expression rate phenotypically (38). Many studies showed that uropathogenic *E. coli* could be more virulent if they have many virulence genes in a single isolate, for example study done by Millan *et al.* (39) reported that such isolates had *fimH, fyuA, kpsMTII, usp, PAI and papAH* in high frequency, and all 28 isolates were genotypically related in phylogenetic tree. They then concluded that these genes would enable bacteria to recurrent UTI by uptake of iron, adhere, and resist immune system of host. Furthermore, it was studied that *hly, cnf, pap* and *iroN* virulent genes were detected in high rates among *E. coli* isolates obtained from vaginal swab of pregnant women more than those were non-pregnant, presenting the threat that could be concerned when having such infection which would result in neonatal sepsis (40). It was known that the occurrence of UTI caused by *E. coli* among pregnant women would lead to premature preterm rupture of membranes, resulting in abortion. In the same study, they found that 31% and 52% of isolates had *hly* and *iroN* virulence genes located on Pathogenicity Island (41). Ballesteros-Monrreal *et al.* (29) also found that 100% of *E.coli* isolated from complicated UTI had *iroN* gene and 42% of

isolates were hemolysis producers. Researchers showed that those two genes were found on Pathogenicity Island.

Conclusion

UTIs consider has been widely spread among females especially, pregnant women, leading to health issue concern. *E. coli* as uropathogen has become more virulent by having many virulent genes besides highly resistant level towards various antibiotics classes. Therefore, it should be taken into account that pregnant women must take the appropriate antibiotics treatment to eradicate such pathogen that may lead to bad consequences during pregnancy period.

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