Prevalence of virulence factors genes of Escherichia coli isolated from pregnant and non-pregnant women with urinary tract infection in Diyala Iraq

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ABSTRACT
Uropathogenic Escherichia coli (UPEC) is an essential cause factor of complicated and uncomplicated urinary tract infections. It is in charge of approximately 80% of community gained and 50% of nosocomial infection. The current study included collection of one hundred and eighty samples among different cases (pregnant and non-pregnant women) by urinary tract infection in Al – Batoul Teaching Hospital in Baquba, during the period from November 2017 to February 2018. All isolates were identification as E. coli by using Gram – staining. EMB agar and conformation in VITEK2 compact system. It was found 96 E. coli isolates; it was carried out to 38 E. coli isolates. 18 isolates from pregnant and 20 isolates from non – pregnant women. In this study, E.coli isolates were resistance against different type antibiotics, it was pipracillin 84.38% ; tetracycline 70.83% ; Rifampicin 63.54% ; Levofloxacine 59.37% ; ciprofloxacin 54.17% ; tobramycin 47.9% ; Augmentin 39.58% ; gentamicin 35.49% ; chloramphenicol 30.21% ; Imipenem 26.04%. The molecular detection of virulence factors genes shows the prevalence of genes fimH, papC, hlyA, sfaS, iutA, and ibeA were 100%, 94.44%, 55.5%, 72.2%, 66.66%, and 22.22% respectively in pregnant case according to the mentioned tract infection. The prevalence of gene fimH, papC, hlyA, sfaS, iutA, and ibeA were 85%, 60%, 25%, 45%, 45%, and 5% respectively in non-pregnant women with urinary tract infection. All the studied strains showed 16 of virulence gene patterns, among isolates from pregnant 12 patterns, on the other hand the isolates from non-pregnant showed diversity of genes patterns.

Keywords: Escherichia coli, urinary tract infection, virulence factors genes.

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INTRODUCTION
Urinary tract infection (UTIS) is an inflammatory disturbance which occurred due to abnormal proliferation of pathogens in the urinary system, while causing changes in the normal functioning of the Kidneys and urinary tract and it's acts as one of the extreme popular diseases faced in medical practice lately (Tambekar et al., 2006). UPEC is an essential case factor of complicated and uncomplicated types of UTIS. It responsible for an approximately (80%) of community acquired and (50%) of nosocomial acquired UTIS, it is isolated from the urine of about 30% of patients experiencing urinary tract infection (Macleod and sticker, 2007). Several hours after birth, UPEC settles on the human intestine which consider as one reason of normal microbeita. However, it can cause various diseases such as diarrheal, UTI, and meningitis (Kaper et al., 2004). Antibiotic resistance of pathogens in the management of complicated and uncomplicated community acquired UTIS is a serious medical problem. However widespread use of antibiotics utilization has drove to the emergence of resistant bacteria (lartigue et al., 2007). Thus, Uropathogenic strains are confirmed to show a virulence variance especially for E. coli determinants have been related to the development of UTI. Among these factors: siderophores, toxins, capsules, fimbriae and other have been described (Ruiz et al., 2002). E. coli settlement during various anatomical sites is partially due to genome plasticity and remodelling by genetic material earning or losing process according to gained resistance or virulence factors. Thus, adaption and evolution have a horizontal transfer which consider as an essential element of E. coli to various niches (Mellata et al., 2010). Bacteria and epithelial cells is a multifactorial and complicated phenomenon which enfold several adhesins production due to the infection level, in the other hand, adherence to epithelial cells is considered to be prim reason for colonization and establishment succession; gene can be performed by encoding each of toxins siderophores, LPS, and invasions specifies the severe level of disease as well as the strains virulence (Hilbert et al., 2008). Iron is essential nutrient for the majority of bacterial species E. coli uses iron for oxygen transport and storage, DNA synthesis, electron transport and metabolism of peroxides, it has also been associated with biofilm formation due to the need of the bacteria to capture iron for growth (Reisner et al., 2006). Acute infections and recurrent infection can be
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cased by UPEC strains which are not react o popular antimicrobial treatment. UTI treatment generally includes β-lactam antibiotics, quinolones, trimethoprim/ sulfamethoxazole (Molina et al, 2011). Bacterial genome changing due to mutation or even acquisition led to resistance increment (Moura et al, 2009; Hong et al, 2009). Lately studies which attempt to characterize E.coli profile from pregnant and non-pregnant women, with described and applied tract infection by virulence gene (finH, papC, hlyA, sfaS, iutA, iheA) and their resistance to antibiotics to treatment of UTI.

MATERIAL AND METHOD
Collection of samples
During the period from November 2017 to February 2018, a total of (180) clinical urine specimens were collected for suffering patients from urinary tract infection with (15-45) year and both pregnant and non-pregnant women admitted to Al-Batoul Teaching Hospital in Baquba; 97 samples from pregnant women and 83 samples from non-pregnant women.

Identification of E.coli isolates
The specimens received were inoculated on MacConkey, Eosin Methigene Blue and blood agar plates. Then all plates were incubated a 37°C for 24 hrs. The isolates were show characteristic growth colour and identified as E.coli by using Gram- staining, conformation in VITEK2 compact system (Bio merieux / USA).

Table 1: sequence of primers of virulence genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers sequence 5 → 3</th>
<th>Ann. Temp.</th>
<th>Product Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>finH</td>
<td>F-TGC AGGA CGG ATA AGCCG TGG GGA</td>
<td>508</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-GAGTCACCCTGCGCTCC GGTATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>papC</td>
<td>F-GTGGCAGTAGAGTAGA CCGTTAGA</td>
<td>200</td>
<td>63°C</td>
<td>Abdul-Ghaffar, and Abu-Rish, 2017</td>
</tr>
<tr>
<td></td>
<td>R-ATATGCTTGTGACG CGGA TCGAATA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlyA</td>
<td>F-AACAAGGATAAGC ACTGT TCTGGCT</td>
<td>1177</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-ACCATAAACGCG TCATTCCCGTCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sfaS</td>
<td>F-GTGGATGAGCAGATTACGTG GGA</td>
<td>646</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R- CCG CCA GATTCACCCTGATTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iutA</td>
<td>F-GGCTGGACAT CATGGGAACCTGG</td>
<td>300</td>
<td></td>
<td>Lopez Banda et al, 2014</td>
</tr>
<tr>
<td></td>
<td>R-CGTCGCCGGAACG GTGA AATCG</td>
<td></td>
<td>55°C</td>
<td></td>
</tr>
<tr>
<td>iheA</td>
<td>F- AGCCAGGTTGCTGC CGCGCTGAC</td>
<td>900</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-TGGCATACCAACC AATCGGAG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR amplification
PCR assay was performed in a monoplex. It were carried out 38 E.coli isolates, 18 isolates were from pregnant and 20 isolates were from non-pregnant women amplify different virulence factors genes. finH, papC, hlyA, sfaS, iutA, iheA. PCR mixture was set up in total volume of 25 µl included Antimicrobial susceptibility Test
Ninety six of E.coli isolates and according to its resistance were tested against the following (10) antibiotic, Augmentin piperacillin, Imipenem, Tobramycin, Gentamicin, Levoflocacin, ciprofloxacin, Tetracyline, Rifampicin, chloramphenicol using VITEK2 compact system (Vitek2 Kit sensitive , Biomerieux / USA).

DNA Extraction
The DNA of thirty eight E. coli isolates that high resistant was extracted according to the instruction of the promega kit Nanodrop spectrophotometer was used for measured the DNA concentration and purity. The extracted DNA was electrophoresed by gel electrophoresis system.

Preparation the primers
Primers were prepared according to the instruction of manufactured company. The primers selected in this study shown in Table 1.
125μl of PCR green master mix , 1 μl of each primer , and 2 μl of template DNA have been used , the rest volume was completed to 25 μl with sterile nuclease-free water . The PCR thermo cycler program is described in Table 2.

Table 2: PCR thermo cycler program for DNA amplification of E.coli genes.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95°C</td>
<td>5min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>30s</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>X°C</td>
<td>30s</td>
<td>30</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>7min</td>
<td>1</td>
</tr>
</tbody>
</table>

X: annealing temperature for each primer of virulence gene as follow : fimH , papC , hlyA , sfaS , 63°C , while intA , ibeA were 55°C respectively .

RESULTS AND DISCUSSION

Isolation and identification of E.coli isolates

After performance the identification tests , it was found that in total of 96 E.coli isolates , 54 isolates from pregnant and 42 isolates from non-pregnant women , 38 (39.58%) E.coli isolates , 18 (33.33%) isolates were from pregnant patients and 20 (47.6%) isolates were from non-pregnant patients women of urine specimens in Figure 1 .

Figure 1: percentage of pregnant and non-pregnant associated E.coli.

Antibiotic susceptibility tests

From the results of the present study , various levels susceptibilities of different antibiotics among (96) isolates were observed , Table 2 . These results in recent study showed that E. coli isolates were resistant to pipracillin 84.38% , tetracycline 70.83% , Rifampicin 63.54% , Levofloxacin 59.37% , Ciprofloxacin 54.17% , Tobramycin 47.9% , Augmentin 39.58% , Gentamicin 35.42% , Chloramphenicol 30.21% , Imipenem 26.04% .

Table 2: Resistant of E. coli isolates to different antibiotic

<table>
<thead>
<tr>
<th>Antibiotic name</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin</td>
<td>84.38%</td>
<td>3.12%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>70.83%</td>
<td>3.13%</td>
<td>26.04%</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>63.54%</td>
<td>0%</td>
<td>36.46%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>59.37%</td>
<td>0%</td>
<td>40.63%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>54.17%</td>
<td>0%</td>
<td>45.83%</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>47.9%</td>
<td>0%</td>
<td>52.1%</td>
</tr>
<tr>
<td>Augmentin</td>
<td>39.58%</td>
<td>0%</td>
<td>60.42%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>35.42%</td>
<td>2.08%</td>
<td>62.5%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30.21%</td>
<td>0%</td>
<td>69.79%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>26.04%</td>
<td>2.08%</td>
<td>71.88%</td>
</tr>
</tbody>
</table>

These results were closely to the results of (Al – Ezee etal , 2019) , they found the percentage of E. coli isolates resistance to Augmentin 40% tetracycline 73.3% ; Rifampicin 66.7% , Chloramphenicol 33.3% imipenem 66.7% , gentamicin 40% and ciprofloxacin 60% . However, the study results are agreed with agreed with the results of (Lopez – Banda etal, 2014) who found the percentage of E.coli resistance of Augmentin 31.3% , Imipenem 1.9% , Pipracillin 51.6% , Gentamicin 27.8% , Tabromycin 43.5% , Levofloxacin 60% , and Ciprofloxacin 62.5% . Study results showed resistance that might has a relation to bacteria genemoe changes due to mutation or even acquisition (Moura etal , 2009 ; Hong etal , 2009).

Genetic study of E. coli isolates

DNA extraction

The DNA was extracted from E.coli isolates it were carried to 38 E.coli isolates , 18 isolates from pregnant and 20 isolates from non-pregnant women , E.coli DNA was with good quantitative and qualitative states that showed one band of DNA when analysis by the electrophoresis methods.

Detection of virulence factors genes

The infection caused by virulence factors of microorganism provides a clinician permit to anticipate the evolution of host infection. All of the genes were detected in different percentages; Results are presented in Figure 2.

Figure 2: prevalence of virulence factors genes in E. coli isolates

Detection of fimH gene in E. coli isolates
From thirty eight E. coli isolates; the monoplex PCR assay for fimH gene revealed high presence of fimH gene 35 (92.11%) in a total, 18 (100%) in pregnant patients women and 17 (85%) in non-pregnant patients women., Figure 3.

This results are agreed with the results of Al – Mayahie , 2013 who found 100% of fimH virulence gene was recorded in pregnant and non-pregnant women. Wang etal , 2002 , studied the pathogenic role of host and E. coli virulence factors in the development of E. coli bacteremia in patients with upper urinary tract infection . There was a high prevalence (92%) of the genetic determinant of fimA gene . A similar results 86.1% of fimH gene was recorded by (Lopez – Banda etal , 2014), 162 uropathogenic E.coli (UPEC) strains from patients with cystitis were genotypically characterized by PCR assay results identified 158 fimH (97.5%) (Tiba etal, 2008).

**Figure 3**: Electrophoresis of amplified PCR product for the detection of fimH gene (508bp) lanes 1-18 represent pregnant E. coli isolates and Lanes 19-38 represent non pregnant E. coli isolates respectively. Run on 1% agarose (90 min at 100 volt) stained with ethidium bromide. M: marker DNA ladder (100bp)

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

The adhesin papC fimbriae gene were showed a percentage 29 (76.32%) in a total; 17 (94.44%) in pregnant patients women and 12 (60%) in non-pregnant patients women, Figure 4.

This results closely with (Lopez – Banda etal, 2014) that found the prevalence of papC gene in his study was 62% in E. coli isolates from Mexican women. Al-Mayahie, 2013, who found 100% of papC virulence gene was recorded in pregnant and non-pregnant women. Johnson etal ,2000 , studied bacterial adhesins in patients with E.coli urosepsis and papC adhesin was (82%). In a study done by Birosova etal, 2014 , in total 201 E. coli isolates from various clinical materials (urine, vaginal and rectal swabs) were examined by PCR for the presence of pap gene was 89%.

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

The PCR assay results identified haemolysin producing gene hlyA, this study showed 15 (39.47%) in a total, 10 (55.5%) in pregnant patients women and 5 (25%) in non-pregnant patients women, Figure 5.

**Figure 4**: Electrophoresis of amplified PCR product for the detection of papC gene (200 bp). Lanes 1 – 18 represent pregnant E. coli isolates and 19-38 represent non pregnant E. coli isolates respectively. Run on 1% agarose (90min at 100 volt) stained with ethidium bromide. M: marker DNA ladder (100bp)
In study done by Karimian et al., 2012 in Iran was found in a total of 123 strains of E. coli isolated from patients with urinary tract infection were tested in PCR for detection of E. coli virulence factors; hlyA gene presence was 50.4% in isolates, while similarly isolates recovered from 75 patients, with E. coli bacteraemia caused upper UTI was 45% (wang et al., 2002). In contrast a high haemolysin percentage was recorded (25%) UPEC from patients (Tiba et al., 2008) Escherichia coli haemolysin (hlyA) is define as a pore- forming exotoxin which might participate to bacterial virulence during bloodstream infection and sepsis (Koga et al., 2014).

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

The frequency of sfaS gene in E. coli isolates, it was 22 (57.89%) in a total, 13 (72.2%) in pregnant patients women and 9 (45%) in non-pregnant women, Figure 6.

This results a greedment with (Al-Mayahic, 2013) who found the prevalence of sfaS gene was 60% in pregnant women's isolates and 50% in non-pregnant women's isolates. In study done by Lopez – Banda et al. (2014) in Mexico women clinically diagnosed with urinary tract infection.

**Detection of iutA in E. coli isolates**

In this study the detection of iutA gene coding for aerobatic siderophores revealed 21 (55.26%) in a total 12 (66.66%) in pregnant patients women and 9 (45%) in non-pregnant patients women, Figure 7.

Advan et al., 2015, confirms the prevalence 74% of iutA gene in fifty clinical E. coli isolates were previously recorded from patients suffered from urinary tract infection in Tulkarm – Palestine in contrast the study In total, 201 E. coli isolates from various clinical materials were examined by PCR for the presence of virulence factors; The aerobatic in genes were found less frequently (59%) in a total and 53% of urine samples. (Firoozah et al., 2014), and previous study showed that E. coli strains isolated from children with UTI 39.75% was positive for presence of iutA (Karimian et al. 2012).
Prevalence of virulence factors genes of Escherichia coli isolated from pregnant and non-pregnant women with urinary tract infection in Diwala Iraq

Figure 6 : Electrophoresis of amplified PCR product for the detection of iutA gene (300bp) Lanes 1-18 represent pregnant E. coli isolates and 19 – 38 represent non pregnant E. coli isolates respectively , run on 1% agarose ( 90 min at 100 volt ) stained with ethidium bromide . M : marker DNA allder (100bp).

Detection of ibeA gene in E. coli isolates

In this study the detection of ibeA gene in E. coli isolates 5 (13.16%) in a total, 4 (22.22%) in pregnant patients women and 1 (5%) in non-pregnant patients women , Figure8.

This results agreed with (Lopez – Banda etal , 2014) , who found 2.8% of ibeA related to studied Mexican women cases and are clinically diagnosed according to urinary tract infection .

Virulence gene patterns

Based on the distribution of the various targeted sequence all the studied strains exhibited 16 virulence gene patterns and showed in Table 4.

Three of virulence gene patterns specified as P2, P3, and P10 were recognized by five different gene presence (5 strains). The patterns which included strains presenting three virulence genes (P7, P9, P11, P12, and P15) were the best represented (14 strains). The association of four genes was recognized in (P4 , P5 , P6 , P8 ) patterns (10 strains) , the P13 , P14 patterns were represented by strains possessing a two genes association (4 strains) , followed by the P1 patterns , which encompassed the six genes positive strains (2 strains) and the P16 patterns , which encompassed the six genes negative strains (3 strains), the association of presents of the virulence factor patterns in relationship with the different source of the isolates , the strains isolates from pregnant patients women exhibited 12 patterns , P4 , P6 patterns were the most prevalence with 3 isolates for both patterns , followed by P2 , P5 , patterns (2 isolates ) for both patterns . Among the strains isolated from non-pregnant patients women show diversity of genes patterns. In comparison to this study , (Usein etal , 2001 ) recorded in a total of 78 E. coli strains isolated from different types of urinary tract infections all the studied strains exhibited 21 virulence gene patterns . Similar results were documented by Firoozeh etal, 2014, nineteen different virulence patterns were found among the UPEC strains regarding the frequency of virulence determinants.

![Figure 6](image)
![Figure 8](image)

Table 4: Virulence pattern identified a mong E. coli isolates

<table>
<thead>
<tr>
<th>patterns</th>
<th>Virulence genes</th>
<th>No. isolates</th>
</tr>
</thead>
</table>

Figure 8: Electrophoresis of amplified PCR produced for the detection ibeA gene (900 bp) lanes 1-18 represent pregnant E. coli isolates and 19-38 represent non pregnant E. coli isolates respectively , run on 1% agarose (90min at 100 volt) stained with ethidium bromide . M: marker DNA ladder (100bp).
REFERENCE


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