Pathogenic features of urinary *Escherichia coli* strains causing Asymptomatic Bacteriuria during Pregnancy

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Abstract

Background Asymptomatic bacteriuria is one of the common problems in pregnancy. Pyelonephritis, preterm labor and low birth weight infants have been associated with bacterial infection. Urinary tract infection (UTI) during pregnancy is frequently associated with complications. An observational cross-sectional study including investigated the prevalence of virulence genes, antimicrobial resistance, and its relationship with phylogenetic groups among *E. coli* strains isolated from pregnant women with asymptomatic bacteriuria who referred to Hafez hospital, Shiraz, Iran.

Material and Methods A total of 300 urine samples were screened for *Escherichia coli* strains. Susceptibility testing was determined by the disk-diffusion method. The phylogenetic groups and 13 virulence genes were identified by PCR. ESBL and AmpC producing isolates were detected using phenotypic methods. PCR was used to identify the *bla* TEM , *bla* SHV and *bla* CTXM genes in ESBL and AmpC-positive isolates.

Results Our results revealed that among 300 urine samples, 105 (35%) were positive for *E. coli* . The data showed that the highest and the lowest resistance rates were observed against nalidixic acid (82.1%), and imipenem (2.8%), respectively. The prevalence of ESBLs and AmpC- β -lactamase, in the *E. coli* isolates was 41% and 9.5% respectively. *bla* CTXM was the commonest genotype (93%). Phylogenetic group distribution was as follow: B1 2.8%, A 14.2%, B2 61.9%, and D 4.6%. Our result showed that most of the virulence genes belonged to group B2 and also several virulence genes such as *hlyA* , *cnf-1* , and *papGII* genes were positively associated with group B2.

Conclusion Among *E. coli* strains isolated from patients with UTIs, different features phylogroups, with special virulence factors, could cause severe infection. Awareness about the Virulence patterns distribution among Phylogenetic groups of UPEC could greatly aid in confine and prevent the development of lethal infection caused by these strains.

Background

Pregnant women are typically screened for urinary tract infections (UTIs) in early pregnancy and those with bacteriuria are treated with antibiotics[1]. Its accurate and prompt diagnosis plays an important role in reducing the course of the disease by preventing renal failure following the ascent of the infection in the upper urinary tract[2]. During pregnancy, UTI might be present as asymptomatic bacteriuria or as symptomatic infection[3]. Most infections are caused by Enterobacterales and the most common causative pathogen is *E. coli*. Uropathogenic *Escherichia coli* (UPEC) strains are responsible for 80–90% of all cases[4]. *E. coli* ability to colonize various sites is due in part to genome specific characteristics by acquisition or loss of genes encoding virulence factors and antibiotic resistance genes[5]. The interaction between UPEC and epithelial cells is effected by several factors and complex phenomenon, involving various adhesins produced according to the stage of infection while its adherence to epithelial cells plays a critical role for a successful colonization and establishment. The severity of the disease is dependent

on the expression of other genes encoding virulence factors[6]. Furthermore, an increasing trend in the spectrum and frequency of antimicrobial-resistant UTIs was observed in the past few years[7]. Not only *E. coli* resistance to various groups of antibiotics such as β lactams, aminoglycosides and fluoroquinolones can be attributed to some genes, but it also can be intrinsically resistant to the mentioned above antibiotics[8]. Phylogenetic analyses have shown that *E. coli* strains falls into four main pylogenetic groups (A, B1, B2, and D), each of which has a unique panel of genes that characterize its own evolutionary pattern. Various studies have exhibited that groups B2 and D are proportionately higher in pathogenic samples and usually more virulent, whereas groups A and B1 tend to be found at higher rates in commensal samples[9, 10]. Unlike the general population, screening for asymptomatic bacteriuria with urine culture for pregnant women should be conducted, and asymptomatic bacteriuria must be diagnosed and treated in every case. UTIs in pregnant women continue to pose a clinical problem and a great challenge for physicians. However, there are few studies on virulence factor genes (VFGs), antimicrobial resistance and pattern of phylogenetic groups amongst the *E. coli* isolated from asymptomatic pregnant women in Iran. The objective of our study was to investigate the prevalence of virulence genes, antimicrobial resistance, and its relationship with phylogenetic groups among *E. coli* strains isolated from asymptomatic pregnant women who referred to Hafez hospital, Shiraz, Iran.

Methods

Setting

Urine samples were collected from June to September 2018 from 300 pregnant women (with recurrent UTIs before pregnancy) suspected of having UTI, who had not received antimicrobials within the past two months. All of the pregnant women were in the age range 18–35 years and had referred to Hafez hospital for regular perinatal care. The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (EC IR.SUMS.REC. 96.16589).

Sample collection and identification

A midstream clean-catch urine sample were obtained from each participant. The urine samples were cultured on MacConkey agar (MA) plates, and plates were incubated in an aerobic atmosphere at 37°C for 18 h. [11]. Bacterial isolates (300) were obtained from pregnant women diagnosed and confirmed by the clinical laboratory. UTI was defined by the presence of bacteria in the urine culture ($\geq 10^5$ colony-forming units [CFU]/mL) and pyuria ($\geq 10^4$ leukocyte/mL of urine).

Confirmed *E. coli* isolates were kept frozen in tryptic soy broth (Merck , Germany) containing 20% glycerol (Merck , Germany) at - 70 °C until further experiments.

Antibiotic susceptibility testing (AST)

AST was carried out using the Kirby-Bauer disk diffusion technique as described previously, using single antibiotic disks consisting of sulphamethoxazole-trimethoprim (SXT, 25 μ g), gentamicin (GEN, 10 μ g),

cefazidime (CAZ, 30 µg), nalixidic acid (NA, 3 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 30 µg), piperacillin (PIP, 100 µg), piperacillin-tazobactam (PTZ, 100/10 µg), ampicillin (AM, 10 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), imipenem (IPM, 10 µg), aztreonam (AZT, 30 µg), ceftriaxone (CRO, 30 µg) and nitrofurantoin (NI, 300 µg) (Mast, UK). *E. coli* ATCC 25922 was used as the quality control strain for antibacterial susceptibility testing[12].

Detection of ESBLs and AmpC-Positive Isolates

ESBLs producing isolates were detected by combined disk method with clavulanic acid according to CLSI guideline[12]. The following antibiotics (Mast, UK) were used for detecting ESBLs: CAZ (30 µg), CTX (30 µg) alone as well as with 10 µg clavulanic acid. *Klebsiella pneumonia* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively. Furthermore, AmpC phenotype was specified by means of compound disk using ceftiofur (FOX), (Mast, UK) with and without boronic acid (Sigma-Aldrich Chemie, Germany) were used to detect AmpC phenotypes[13]. According to CLSI criteria, all isolates classified as resistant to ceftiofur were suspected to be AmpC producers. An increase in the zone diameter of ≥ 5 mm in the presence of boronic acid is taken to be a phenotypic confirmation of AmpC production.

Detection of virulence factors and beta-lactamase genes

DNA was extracted from one single colony of each isolate by incubation in a final volume of 100 µL of distilled water at 95 °C for 10 min followed by centrifugation. PCR was used to detect thirteen genes encoding virulence determinants that are usually associated with the *E. coli* strains responsible for extraintestinal infections: including (*fliC*, *fliD*, *fliE*, *fliF*, *fliG*, *fliH*, *fliI*, *fliJ*, *fliK*, *fliL*, *fliM*, *fliN*, *fliP*), genes[5-14]. Also PCR screening was used for the presence of different beta-lactamase genes (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTXM})(15). Each VF and β -lactamase gene was amplified with the primers described in Table 1.

Determination of *Escherichia coli* phylogenetic groups

Isolates were assigned to one of the four main phylogenetic groups of *E. coli* (A, B1, B2, and D), using the triplex PCR as described by Clermont et al. [10]. The genes *chuA*, *yjaA* and *TSPE4.C2* were amplified by PCR using the primers listed in Table 1. The *E. coli* strain ECOR62 and RS218 (B1 and B2 groups respectively) were used as controls[16].

Statistical analysis

The data was analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). The Chi-square test or the Fisher exact test was used to compare categorical variables. A P-value less than 0.05 was considered to be statistically significant.

Results

A total of 300 urine samples were screened and confirmed 105 (35%) as uropathogenic *E. coli* (CFU $\geq 10^5$ /mL).

Phylogenetic analysis

The prevalence of ABU was found to be 35%, and of the 105 *E. coli* isolates, 65 (61.9%) belonged to group B2, 22 (20.9%) belonged to group D, 15 (14.2%) belonged to group A and 3 (2.8%) belonged to B1.

Antibiotic Resistance

Over 50% antibiotic resistance was observed for Nalidixic acid (82.1%), trimethoprim/sulfamethoxazole (76.1%), ampicillin (75.9%), piperacillin (61.1%), cefotaxime (60.2%), and ciprofloxacin (51.3%) and nitrofurantoin (11.8%), gentamicin (14.2%) prevalence lower than 15%. Sensitivity values above 50% were found in imipenem (97.2%), aztreonam (88.4%), piperacillin/tazobactam (76.5%), ceftriaxone (55.3%), cefazidime (57.1%) and amoxicillin/ clavulanic acid (54.8%). Isolates that exhibited resistance to more than ≥ 3 chemotherapeutic groups were considered multi drug resistant isolates, representing 58% of the isolates. However, no statistical significant was observed among multi drug resistance and phylogenetic groups (Table2).

Prevalence of virulence and resistance genes among the isolates

Higher prevalence (above 70%) was observed for the *kpsMTII*, *fimH*, *papC*, *iutA*, *fyuA*, *traT* and *sfa/focDE* genes (100%, 90.4%, 88.5%, 83.8%, 76.1%, 72.3% and 70.4%, respectively). For *ecp* gene prevalence was close to 60% (58.1%) while the *papGII*, *hlyA*, *cnf-1* and *sfaS* genes registered prevalence lower than 15%. (14.2%, 12.3%, 8.5% and 2.8%, respectively). Table 3 shows the distribution of virulence genes regarding the phylogenetic group. Most of the virulence factors associated with the phylogenetic group B2 were identified. Among the phylogenetic groups, most of the virulence genes were found to be significantly high in groups B2 and D compared to other groups. The *kpsMTII* and *fimH* genes were widely distributed among all groups (B2 100%/100%, D 100%/81.8%, B1 100%/100%, and A 100%/86.6%, resp.). The *papC*, *iutA*, *traT*, *kpsMTII*, *fimH* and *fyuA* genes were found in isolates in all groups. The *hlyA*, *cnf-1*, and *papGII* genes were positively associated with group B2, whereas *sfaS* gene was found only in D group.

Out of the 105 *E. coli* isolates examined for β -lactamases, 41% (43) were found to be ESBL producers and 9.5% (10) were AmpC β -lactamase producers. All AmpC producers were also found to be ESBLs positive. Among the ESBL and AmpC-producing isolates, the multiplex PCR assay results indicated that 40(93%) *bla*_{CTX-M} and 34(79%) *bla*_{TEM} genes were detected in the *E. coli* isolates and *bla*_{SHV} was not found in any of the isolates.

Discussion

UTIs, are common, and women can often experience them during pregnancy. Women who've had UTIs before are more prone to get them during pregnancy. Not all UTIs cause symptoms, but in pregnancy even those without symptoms need to be treated to prevent problems later in pregnancy. Asymptomatic UTI can lead to serious problems. In this population, recurrence and treatment failure due to antibiotic resistance are major concerns. Since, these patients have had recurrent infections during their lifetimes, they could be considered as one of the sources of the community-acquired infections and also they having community acquired infection. For this reason, similarity and shared characteristics in phylogenetic groups, virulence factors and antimicrobial resistance in *E. coli* strains isolated from patients with UTI could be increased in community-acquired infections. In this study, the prevalence of asymptomatic bacteriuria in pregnant women (with recurrent UTIs before pregnancy) was 35%, which was similar to what was observed by a study in Nigeria(47.5%)[17] and a study in Ghana (42.8%)[18]. In contrast, some other studies found lower prevalence than ours, a study in Chitwan, Nepal, conducted by Neupane et al. (26%)[19] a study in Cameroon conducted by Mokube M.N. et al. (23.5%)[20] , and a study in India, conducted by Sujatha R et al. (7.3%)[21]. These difference might be due to factors including the geographical areas being investigated, the social habits of the communities, the socio-economic statuses, and standards of personal hygiene[11]. Quinolones, trimethoprim-sulfamethoxazole, and β -lactam antibiotics include penicillins and cephalosporins are the most common antibacterial drugs in UTIs' treatment[22]. Ampicillin and amoxicillin/ clavulanic acid are two mostly prescribed oral antimicrobial agents for UTI in pregnant women. Our culture results yielded 24.1% and 45.2% sensitivities to these agents, respectively. Ciprofloxacin is another drug commonly prescribed for treating ASB, and sensitivity to this drug was 48.7%, which is comparable to that of cefotaxime (40%) and ceftriaxone (55.3%). Sensitivities to imipenem and nitrofurantoin were 97.2% and 88.2%, respectively (Table4). Findings are in line with the earlier studies in Iran[6-23]. In this study, the upsurge in antibiotic resistance patterns could have been attributed by antibiotic abuse and self-medication. Also, low costs and easily accessibility of drugs could be other factors contributing to antibiotic resistance.

The findings of previous studies as well our current findings indicate that ESBL and AmpC- β -lactamase-producing isolates are typically resistant to others antibiotics such as trimethoprim/sulfamethoxazole and fluoroquinolones[13]. ESBL was detected in 43/105 (41%) of the isolates recovered from patients, and *bla*_{CTXM} was the commonest genotype (93%). Also, all of the AmpC-producing (9.5%) isolates were ESBLpositive. ESBL-producing *E. coli* showed the greatest resistance to ampicillin, amoxicillin-clavulanic acid, cefotaxime and trimethoprim-sulphamethoxazole (Table 4). This finding is in agreement with previous studies[24]. This study showed a considerable number of bacteria to harbor the *kpsMTII*, *fimH*, *papC*, *iutA*, *fyuA*, *traT* and *sfa/focDE*, respectively, which was in contrast to Sáez-López et al., but in line with Forson et al. study on pregnant women in Barcelona[23] and Ghana[18]. In our study, *kpsMTII* was the only gene found in all 105(100%) isolates. Also, 95 out of 105(90.4%) isolates harbored, *fimH* genes. *papC*, *papGII* and *ecp* (A and RB) _ the other genes related to the ability to colonize the urinary tract epithelium were detected in 88.5% , 14.2% and 58.1% of the isolates, respectively. Our results are in agreement with those found by other studies [17, 18- 23]. Also, among the tested VFs in our study, there was only *sfaS* gene at the lowest rate (2.8%). *iutA* and *fyuA* play an important role in iron acquisition

systems by up-taking the hydroxamate siderophore aerobactin[25]. The prevalence of the *iutA* (83.8%) and *fyuA* (76.1%) genes found in our *E. coli* isolates correlates with the results published by Forson et al.[18]. Significant associations were observed between SXT resistance and the presence of the siderophores *fyuA* and *iutA*. At least one of the tested siderophores was present in 98 (93.3 %) of the tested isolates. Meanwhile, of the toxin-encoding genes, *hlyA* was present in 12.3% of the isolates, while *cnf1* was detected in 8.5% of the studied strains. Positive isolates of *hlyA*, and *cnf-1* genes were susceptible to ciprofloxacin, which was in line with that of Piatti et al[26]. The distribution of the 105 *E. coli* isolates in relation to virulence genes in pregnant women revealed 76.1% (80 isolates) *E. coli* contained two or more virulence genes. The distribution of virulence genes and the phylogenetic classification are different among countries. For example, in Russia[27], UTI isolates belonged more often to phylogenetic group A. In Spain and the United States, lower percentages were recorded for the phylogenetic group D [5]; however, in the present study, the most prevalent phylogenetic group was B2 (61.9%). Our result showed that most of the virulence genes belonged to group B2 and also several virulence genes such as *hlyA*, *cnf-1*, and *papGII* genes were positively associated with group B2. All of the 65 isolates in group B2 were positive for *kpsMT KII* and *fimH* genes. Strains belonging to group B2 carry more virulence-factor genes compared to strains from other phylogenetic groups, suggesting a relationship between virulence factors and pathogenic potential. Group D contained the second highest number of *E. coli* strains. Extraintestinal pathogenic isolates from this group typically have somewhat fewer virulence factor genes and a different mix of *VFGs* than do group B2 isolates. In our study the lowest prevalence belonged to *sfaS* (2.8%) found in all 3 isolates of group D. This was in agreement with the report of López-Banda et al.[10]. Group A contains fewer virulence factors genes than group D. *E. coli* strains belong to this group that expresses *kpsMTII*, *papC*, *fimH* and *iutA* genes in high percentages. *E. coli* strains belonging to groups A and B1 do not frequently cause extraintestinal infection. These strains which are not highly virulent, generally cause disease only in hosts that are immunocompromised, and could be pathogenic in healthy hosts only if they were to acquire sufficient extraintestinal factors[28]. All 3 isolated in group B1 were *kpsMTII*, *fimH*, *papC* and *tratT* positive. No association was found between phylogroup B1 and *VFGs*.

Meanwhile, antibiotic resistance is associated with the isolates harboring certain urovirulence genes, such as *fimH* or the presence of, *kpsMTII*, *iutA* and *papC* marker in ESBL-producers.

Conclusions

Our results showed that antibiotic resistant *E. coli* strains associated with Virulence properties of asymptomatic bacteriuria in pregnant women, which enables them to attach, invade and utilize the iron acquisition systems. Taken together, our findings support the importance of some urovirulence genes (e.g., *kpsMT KII*, *fimH*, *papC*, *iutA* and *fyuA*) as a marker for developing of UTIs. These virulence genes encoding components of adhesins and iron acquisition systems were highly prevalent among UPEC isolates. To our knowledge, the present work is the first study in southwest of Iran that describe the different combinations of virulence genes in pregnant women and warrants more intensive research.

Abbreviations

ESBL: extended-spectrum β -lactamase, AST: Antibiotic susceptibility testing, UTIs: urinary tract infections, UPEC: Uropathogenic *Escherichia coli*

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

Study concept and design: SM, MM; acquisition of data and sampling: SKH; KJ analysis and interpretation of data: SM, Z.H, MH; drafting of the manuscript: SM; critical revision of the manuscript for important intellectual content:MM.; study supervision: MM.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Tables

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