JOURNAL OF MICROBIOLOGY, EPIDEMIOLOGY AND IMMUNOBIOLOGY. 2024; 101(4)
DOI: https://doi.org/10.36233/0372-9311-518
ORIGINAL RESEARCHES

Original Study Article
https://doi.org/10.36233/0372-9311-518

The molecular-genetic characteristics of uropathogenic *Escherichia coli* isolated from pregnant women with asymptomatic bacteriuria

Tatiana A. Khusnutdinova✉, Olga V. Budilovskaya, Anna A. Krysanova, Kira V. Shalepo, Anna A. Sinyakova, Alevtina M. Savicheva, Igor Yu. Kogan

D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia

Abstract

Introduction. Uropathogenic *Escherichia coli* (UPEC) are the dominant bacterial pathogens of urinary tract infections (UTIs). UPEC belong to different phylogenetic groups and have many virulence factors, the study of which, in conjuction with the assessment of their relationship with clinical forms of UTI, is necessary for a better understanding of the pathogenesis of UTI and the development of new diagnostic algorithms.

Aim: determination of the molecular genetic characteristics of uropathogenic *Escherichia coli* isolated from pregnant women with asymptomatic bacteriuria.

Materials and methods. Clinical isolates of uropathogenic *E. coli* (n = 70) from pregnant women with asymptomatic bacteriuria were included in the study. The PCR method was used to determine the belonging to phylogenetic groups and detect 15 virulence markers — genes associated with adhesion (*fimH, papC, sfa, afa, focG*); toxin synthesis (*cnf 1, hlyA, sat, vat, usp*); siderophores (*fyuA, iroN, iuc*); capsular antigen (*kpsMII*). To assess the statistical significance of differences, Fisher's exact test was used. Differences were considered statistically significant at a confidence interval of 95% ($p < 0.05$).

Results. Most of the UPEC isolates belonged to phylogroup B2 (51,4%) and were characterized by the detection of all UPEC-associated virulence factors included in this study; genes associated with adhesion (*sfa, focG*), invasins (*ibeA*), synthesis of toxins (*hlyA, cnf1, vat, usp*) and capsule (*kpsMII*), siderophores (*fyuA, iroN, hlyA*) were detected significantly more frequently ($p < 0.05$). Two or more virulence determinants were detected in 93% of isolates.

Conclusion. The identification of key determinants of virulence and/or a combination of virulence genes can be a prognostic marker for predicting the course of UTI, especially in pregnant women, and will expand diagnostic capabilities taking into account the virulent properties of the uropathogen.

Keywords: *urinary tract infections, asymptomatic bacteriuria, uropathogenic Escherichia coli, phylogenetic characteristics, virulence factors*

Ethics approval. The study was conducted with the informed consent of the patients. The study was approved by the Ethical Committee at the D.O. Ott Research Institute of Obstetrics, Gynaecology and Reproductology (protocol No. 114, December 14, 2021)

Funding source. The work was carried out as part of the fundamental scientific research FGWN-2022-0004 “Optimization of methods for prediction, prevention and treatment of “major obstetric syndromes, as well as delivery strategies in high-risk pregnant women, in order to improve obstetric and perinatal outcomes”.

Conflict of interest. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

For citation: Khusnutdinova T.A., Budilovskaya O.V., Krysanova A.A., Shalepo K.V., Sinyakova A., Savicheva A.M., Kogan I.Yu. The molecular-genetic characteristics of uropathogenic *Escherichia coli* isolated from pregnant women with asymptomatic bacteriuria. *Journal of microbiology, epidemiology and immunobiology.* 2024;101(4):462–469.
DOI: https://doi.org/10.36233/0372-9311-518
EDN: https://www.elibrary.ru/hzatlt

Оригинальное исследование
https://doi.org/10.36233/0372-9311-518

Молекулярно-генетическая характеристика уропатогенных *Escherichia coli*, выделенных при бессимптомной бактериурии у беременных

Хуснутдинова Т.А.✉, Будиловская О.В., Крысанова А.А., Шалепо К.В., Синякова А.А., Савичева А.М., Коган И.Ю.

Научно-исследовательский институт акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия

Аннотация

Введение. Уропатогенные *Escherichia coli* (UPEC) являются доминирующими бактериальными патогенами при инфекциях мочевыводящих путей (ИМП). UPEC относятся к разным филогенетическим группам и обладают множеством факторов вирулентности, изучение которых в совокупности с оценкой их связи с клиническими формами ИМП необходимо для лучшего понимания патогенеза и разработки новых диагностических алгоритмов.

Цель исследования — молекулярно-генетическая характеристика UPEC, выделенных при бессимптомной бактериурии у беременных.

Материалы и методы. В исследование включены клинические изоляты *E. coli* (n = 70), выделенные у беременных с бессимптомной бактериурией (ББУ). Методом полимеразной цепной реакции определяли принадлежность к филогенетическим группам и 15 маркеров вирулентности — гены, ассоциированные с адгезией (*fimH*, *papC*, *sfa, afa*, *focG*), инвазией (ibeA), синтезом токсинов (*cnf1*, *hlyA*, *sat*, *vat*, *usp*), сидерофоров (*fyuA*, *iroN*, *iuc*), капсульного антигена (*kpsMII*). Для оценки статистической значимости различий средних величин применяли точный критерий Фишера. Статистически значимыми считали различия при 95% доверительном интервале (p < 0,05).

Результаты. Большинство изолятов UPEC, выделенных при ББУ, принадлежали к филогруппе B2 (51,4%) и характеризовались детекцией всех ассоциированных с UPEC факторов вирулентности, включённых в настоящее исследование; достоверно чаще были обнаружены гены, ассоциированные с адгезией (*sfa, focG*), синтезом токсинов (*hlyA, cnf1, vat, usp*) и капсул (*kps*), сидерофоры (*fyuA, iroN, hlyA*). Две и более детерминанты вирулентности выявлены у 93% изолятов.

Заключение. Определение ключевых детерминант вирулентности и/или комбинации генов вирулентности может быть прогностическим маркером для прогнозирования течения ИМП, особенно у беременных, и позволит расширить возможности диагностики с учётом вирулентных свойств уропатогена.

Ключевые слова: *инфекция мочевыводящих путей, бессимптомная бактериурия, уропатогенные Escherichia coli, филогенетическая характеристика, факторы вирулентности*

Этическое утверждение. Исследование проводилось при добровольном информированном согласии пациенток, протокол исследования одобрен локальным этическим комитетом НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта (протокол № 114 от 14.12.2021).

Источник финансирования. Работа выполнена в рамках фундаментального научного исследования FGWN-2022-0004 «Оптимизация методов предикции, профилактики и лечения «больших акушерских синдромов, а также стратегии родоразрешения у беременных из групп высокого риска, с целью улучшения акушерских и перинатальных исходов».

Конфликт интересов. Спонсор не играл никакой роли в разработке исследования, сборе и анализе данных, принятии решения о публикации или подготовке рукописи.

Для цитирования: Хуснутдинова Т.А., Будиловская О.В., Крысанова А.А., Шалепо К.В., Синякова А.А., Савичева А.М., Коган И.Ю. Молекулярно-генетическая характеристика уропатогенных *Escherichia coli,* выделенных при бессимптомной бактериурии у беременных. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2024;101(4):462–469.
DOI: https://doi.org/10.36233/0372-9311-518
EDN: https://www.elibrary.ru/hzatlt

DOI: https://doi.org/10.36233/0372-9311-518

Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases. They account for up to 150 million cases worldwide each year. According to various authors, up to 50-60% of women experience an episode of UTI at least once in their lifetime [1, 2]. Clinical symptoms associated with UTIs may vary in severity depending on both the virulent properties of the pathogen and the susceptibility of the organism to infection: from asymptomatic course (asymptomatic bacteriuria) to clinically significant cystitis, pyelonephritis, up to severe urosepsis [3]. Asymptomatic bacteriuria represents bacterial colonization of the urinary tract in the absence of clinical manifestations of the disease. During pregnancy, asymptomatic bacteriuria has been considered for many years as a risk factor for pyelonephritis and adverse pregnancy outcomes (preterm labor, low birth weight, etc.) [4, 5]. Administration of antibacterial drugs for the treatment of asymptomatic bacteriuria during pregnancy may be accompanied by undesirable effects: changes in the composition of the intestinal microbiome of the pregnant woman, which subsequently determines the composition of the microbiota of the newborn; impaired development of the child's immune system [6, 7]. Furthermore, antibiotic therapy can lead to the elimination of potentially protective strains of microorganisms that prevent colonization by virulent uropathogens, thus indirectly contributing to the development of symptomatic UTIs.

The dominant bacterial pathogen among UTIs is *Escherichia coli*, a Gram-negative, motile, facultatively anaerobic bacillus belonging to the order Enterobacterales. *E. coli* is part of the commensal microbial population of the human intestine and maintains stability and homeostasis of the lumenal intestinal microbiota through symbiotic interaction with the human body. *E. coli* strains possessing certain virulence factors are able to adapt to new niches and cause a wide range of diseases of intestinal and extraintestinal localization.

E. coli associated with UTIs are known as uropathogenic *E. coli* (UPEC) [8]. UPEC possess a variety of both structural and secreted virulence factors necessary to realize their pathogenic potential in UTIs. The expression of adhesive organelles such as type 1 pili, P- and S-fimbriae allows UPEC to bind to receptors on the surface of epithelial cells in the urinary tract, colonize the uroepithelium and penetrate cells and tissues, and activate the innate immune response. In addition, S-fimbrial adhesins can be expressed by sepsis- and meningitis-associated *E. coli* (neonatal meningitis-associated *E. coli* (NMEC)). Invasins also play a significant role in the pathogenesis of neonatal meningitis, which are found predominantly in NMEC strains. An important pathogenic factor is toxins (hemolysin, cytotoxic necrotizing factor, vacuolizing vehicle toxin, secreted vehicle toxin) that damage cells and disrupt their metabolism. Production of siderophores (iron-transporting proteins) determines the ability of E. coli to capture iron, which increases viability in the urinary tract. The severity of symptomatic manifestations of UTIs is related to the acquisition and expression of virulence genes. In asymptomatic colonization of UTIs, UPEC are unable to express key virulence factors, which is probably a mechanism of adaptation to prolonged bladder persistence [9].

Based on molecular analysis, E. coli strains are divided into phylogenetic groups: A, B1, B2, C, D, E, F and G [10]. UPECs most often belong to phylogroups B2, D and to a lesser extent to groups E and F, whereas commensal strains, considered less virulent, belong predominantly to phylogroups A or B1 [11].

Numerous studies have shown an association between the presence of virulence genes and UPEC phylogroups [12–14]. However, the number of studies aimed at studying the molecular characterization and assessing the genotypic diversity of UPEC strains isolated in different manifestations of UTIs (especially in asymptomatic bacteriuria) is limited. Genetic determinants of virulence as a criterion for assessing and predicting the course of the infectious process are not currently used. Thus, an urgent direction of molecular genetic research is to study the pathogenic potential of UPEC isolated from pregnant women with asymptomatic bacteriuria to determine their clinical significance, as well as to determine the molecular basis of pathogenesis, develop new diagnostic algorithms and effective treatment methods.

The aim of the study was the molecular and genetic characterization of UPEC isolated from pregnant women with asymptomatic bacteriuria.

Materials and methods

Clinical isolates of *E. coli* (n = 70) were isolated from the urine of pregnant women with IBU who were observed by an obstetrician-gynecologist at the D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology in the years 2018–2023.

The study was conducted with voluntary informed consent of the patients, the study protocol was approved by the local ethical committee of the D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology (protocol No. 114 of 14.12.2021).

The diagnosis of asymptomatic bacteriuria was established when the same microorganism was isolated in the amount of $\geq 10^5$ CFU/mL in 2 consecutive urine samples taken at least 24 h apart, in the absence of clinical manifestations of UTI. If more than 1 microorganism was isolated, the sample was excluded from the study. Bacteriologic examination of clinical material was performed using chromogenic nutrient medium for isolation of UTI pathogens (Brilliance UTI Clarity Agar, Oxoid). The identification results were confirmed by mass spectrometry (MALDI-TOF MS, Bruker Dal-

tonics). The cultures were stored in trypticase-soy broth with addition of 30% glycerol at –70ºC.

DNA extraction was performed using the DNA-sorb AM reagent kit (Central Research Institute of Epidemiology).

The affiliation of *E. coli* strains to phylogenetic groups was determined by quadriplex-polymerase chain reaction (PCR) according to O. Clermont et al. [10].

All isolates were tested for 15 virulence markers: genes associated with adhesion (*fimH*, *papC*, *sfa*, *afa*, *focG*); invasion (*ibeA*), toxin synthesis (*cnf1*, *hlyA*, *sat*, *vat*, *usp*), siderophores (*fyuA*, *iroN*, *iuc*), and capsular antigen (*kpsMII*). Previously studied primers were used; PCR primers were synthesized by Syntol LLC [15-21]. For PCR amplification we used the Tersus plus PCR reagent kit (Eurogen) and Tertsik thermocycler (DNA-Technology). The amplicons were separated in 2% agarose gel. Data were visualized and documented using the Infinity gel documentation system (Vilber Lourmat).

Fisher's exact test was used to assess the statistical significance of differences in mean values. Differences with a confidence interval (CI) of 95% ($p < 0.05$) were considered statistically significant.

Results

Phylogenetic analysis of *E. coli* sequences isolated in pregnant women with asymptomatic bacteriuria showed that clinical isolates belonging to phylogroup B2 (51.4%) were significantly more frequent ($p < 0.05$); the remaining isolates belonged to phylogroups D, A, B1, and F (**Table 1**).

Analysis of virulence factors associated with adhesion showed that the *fimH* gene was detected in 97.1% of the strains studied; *rarC*, in 34.3%; *sfa*, in 27.1%; *focG*, in 11.4%; and *afa*, in 2.9%. The *ibeA* gene responsible for endothelial cell invasion was not detected among UPEC isolated in pregnant women with asymptomatic bacteriuria. The most common gene encoding toxin synthesis was *vat* (42.9%); *hlyA*, *cnf1*, and sat genes were detected in 21.4, 22.9, and 32.9% of isolates, respectively. The uropathogen-specific *usp* protein was detected in 57.1% of isolates. Among the genes associated with siderophore production, the *fyuA* gene was detected in 78.6% of the isolates, *iroN* in 48.6%, and *iuc* in 37.1%. The gene encoding capsule antigen synthesis (*kpsMII*) was detected in 65.7% of the studied *E. coli* strains.

Between 1 and 12 virulence genes were present in UPEC clinical isolates. None of the 70 *E. coli* strains contained all 15 virulence markers included in the study. Phylogenetic group A was significantly more frequently represented by isolates with 1 virulence gene (75%); the remaining strains were characterized by a combination of 2 (12.5%) and 5 (12.5%) genes without statistically significant differences. Isolates belonging to phylogenetic group B1 had a combination of 2 (33.3%) and 4 (50%) virulence markers in their genome without statistically significant differences; 1 (16.7%) strain had 1 virulence gene in its genome. Phylogenetic group B2 was characterized by the highest number of genes in various combinations (from 5 to 12); isolates containing combinations of 7 (11.1%), 8 (25%), 9 (13.9%), 10 (25%) and 12 (2.8%) genes in their genome were significantly more frequent. Phylogenetic groups D and F were represented by isolates in which genes encoding virulence factors were present without significant differences in combinations of 2 to 8 and 3 to 8, respectively.

Depending on the types of UPEC pathogenicity factors present, all isolates were divided into 6 clusters (**Table 2**).

Table 1. Belonging to phylogenetic groups of *E. coli* isolated from pregnant women with asymptomatic bacteriuria

Indicator	Phylogroup				
	A	B1	B2	D	F
Number of isolates	8	6	36	14	6
%	11,4	8,6	51,4	20,0	8,6
95% CI	5.1–21.3	3.2–17.7	39.2–63.6	11.4–31.3	3.2–17.7

Table 2. Frequency of detection of pathogenicity factors in uropathogenic *E. coli* isolated from pregnant women with asymptomatic bacteriuria

Pathogenicity factor	*n*	%	95% CI
Adhesion	7	10	4.1–19.5
Adhesion + siderophores	11	15,7	8.1–26.4
Adhesion + toxins	1	1.4	0.04–7.7
Siderophores + capsules	1	1.4	0.04–7.7
Adhesion + siderophores + capsules	2	2.9	0.03–12.6
Adhesion + siderophores + toxins	4	5.7	1.8–14.2
Adhesion + siderophores + toxins + capsules	44	62.9	50.5–74.1

DOI: https://doi.org/10.36233/0372-9311-518

Genetic determinants encoding 1 pathogenicity factor were identified in the genomes of 7 isolates (7%, 95% CI 4.1–19.5). The frequency of isolates containing combinations of 4 factors (62.9%, 95% CI 50.0–74.1) was statistically significantly ($p < 0.0001$) different from isolates characterized by the presence of combinations of genes encoding 2 and 3 pathogenicity factors.

The highest number of virulence genes was detected in strains belonging to phylogroup B2; *focG*, *afa*, *cnf1*, and *ibeA* genes were undetectable in phylogroup D; 8 virulence genes were detected in phylogroup F, except for *afa*, *sfa*, *focG*, *cnf1*, *hlyA*, *vat*, and *ibeA* (**Table 3**). The lowest gene diversity was detected in phylogroups A and B1. Virulence genes associated with adhesion (*sfa*, *focG*), encoding toxin synthesis (*hlyA*, *cnf1*, *vat*, *usp*), siderophores (*fyuA*, *iroN*, hlyA) and capsules (*kps*) were statistically significantly more frequent ($p < 0.05$) in isolates of phylogenetic group B2 compared to isolates of other phylogenetic groups. Statistically significant differences were found in the frequency of occurrence of the *kps* gene ($p < 0.01$) in UPEC isolates belonging to phylogenetic group A; *vat* ($p < 0.05$) and *usp* ($p < 0.001$) genes belonging to phylogenetic group D.

Discussion

Studies on the molecular and genetic characterization of uropathogenic *E. coli*, the results of which are currently available for analysis, were based on the assessment of the pathogenic potential of strains isolated in UTIs. Given the significant impact of asymptomatic bacteriuria on the development of pregnancy complications, we conducted a study aimed at investigating *E. coli* strains isolated from patients with this pathology.

Clinical isolates of *E. coli* isolated from pregnant women with asymptomatic bacteriuria belonged to 5 phylogenetic groups (A, B1, B2, D and F). It was shown that the dominant phylogroups were B2 and D (to a lesser extent) with 51.4 and 20%, respectively, which is consistent with the results of other studies [11]. According to the extended classification by O. Clermont (2013), phylogroup F is a subgroup of phylogroup B2, and *E. coli* belonging to this group are also considered uropathogenic. In our study, 8.6% of the isolates in asymptomatic bacteriuria were attributed to this phylogroup. *E. coli* belonging to phylogenetic groups A (11.4%) and B1 (8.6%) were also found to be associated with commensal isolates; this suggests that the main reservoir of *E. coli* that are able to colonize the urinary tract is the intestine.

Adhesion factors play a key role in the pathogenesis of UTIs by facilitating the attachment of *E. coli* to the uroepithelium. Overall, adhesion factors in our study were detected in isolation or in various combinations in 69 (98.6%) isolates, confirming the role of adhesins as a major urovirulence factor. According to numerous studies, the *fimH* gene is the most common adhesion gene encoding type 1 fimbriae, which was confirmed

Table 3. Occurrence of virulence genes in *E. coli* of various phylogenetic groups isolated from pregnant women with asymptomatic bacteriuria

Virulence factors	Gen	Frequency of gene occurrence, *n* (%)				
		A (*n* = 8)	B1 (*n* = 6)	B2 (*n* = 36)	D (*n* = 14)	F (*n* = 6)
Adhesins	*papC*	0	0	15 (41.7%)	5 (35.7%)	4 (66.7%)
	afa	0	0	2 (5.6%)	0	0
	fimH	8 (100.0%)	6 (10.0%)	36 (100.0%)	13 (92.8%)	6 (100.0%)
	sfa	0	0	18^{+***} (50.0%)	1 (7.1%)	0
	focG	0	0	8^{+**} (22.2%)	0	0
Invasins	*ibeA*	0	0	0	0	0
Siderophore	*fyuA*	2 (25.0%)	4 (66,7%)	33^{+**} (91.7%)	12 (85.7%)	4 (66.7%)
	iroN	0	4 (66,7%)	25^{+***} (69.4%)	3 (21.4%)	2 (33.3%)
	iuc	1 (12.5%)	3 (50,0%)	14 (38.9%)	4 (28.5%)	4 (66.7%)
Toxins	*hlyA*	0	0	14^{+***} (38.9%)	1 (7.1%)	0
	cnf1	0	0	16^{+***} (44.4%)	0	0
	sat	1 (12.5%)	0	14 (38.9%)	7 (50.0%)	2 (33.3%)
	vat	0	0	30^{+***} (83.3%)	2^{-*} (14.3%)	0
	usp	0	0	36^{+****} (100.0%)	1^{-***} (7.1%)	3 (50.0%)
Capsules	*kpsMII*	1^{-**} (12.5%)	0	32^{+****} (88.9%)	9 (64.3%)	4 (66.7%)

Note. $^{-}$ — the frequency of gene occurrence in the group is lower than the frequency of occurrence of the same gene in the overall sample; $^{+}$ — the frequency of gene occurrence in the group is higher than the frequency of occurrence of the same gene in the overall sample.
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

by the results of our study: the *fimH* gene was detected in the majority of isolates (97.1%). Virulence factors associated with the development of ascending infection (pyelonephritis) play an important role in the pathogenesis of UTIs, especially in pregnant women. The *rarC* gene (pyelonephritis-associated pili) and *afa*-adhesins (associated with the development of gestational pyelonephritis) were found in 34.3 and 2.9% of isolates in our study, respectively. The prevalence of genes encoding fimbrial adhesins *sfa* and *focG*, which are expressed by strains causing meningitis, sepsis and pyelonephritis, was 34.7 and 12.9%, respectively. The results of similar studies on the genetic determinants of virulence of UPEC isolated in asymptomatic bacteriuria show a lower prevalence of *rarC* (12.9% to 20.6%), *sfa* (8.1% to 16.9%), but a higher frequency of *afa* (34.9%) and *focG* (35.1%) genes detected [20, 22, 23].

The urinary tract can be a source of NMEC, which is one of the most common infections with high morbidity and mortality in the neonatal period [24]. The *ibeA* gene, which is one of the important virulence factors of NMEC and is responsible for endothelial cell invasion, was not detected among *E. coli* isolated from pregnant women with asymptomatic bacteriuria.

UPEC is characterized by the presence of a uropathogen-specific protein, bacteriocin-like toxin, associated with the development of pyelonephritis and bacteremia. The appearance of this virulence marker is often associated with an increase in the virulence of the strain and its survival in the urinary tract. The results of our study showed a higher detection rate of the *usp* gene (57.1%) compared to the results of similar studies, where the detection rate of the *usp* gene in UPEC isolated in asymptomatic bacteriuria was 22.6–34.4% [20, 22].

Toxin formation is characteristic of *E. coli* strains responsible for more severe forms of disease (pyelonephritis, urosepsis). The *hlyA* and *cnf1* genes associated with toxin synthesis were found in 68.2 and 63.6% of isolates from pyelonephritis cases; in 19.4 and 25.8% of those isolated in asymptomatic bacteriuria [22]. The results of our study showed a similar pattern — *hlyA* and *cnf1* genes were detected in 21.4 and 22.9% of isolates from pregnant women with asymptomatic bacteriuria. Secreted autotransport toxin (*sat*) is also a virulence factor characteristic of UPEC isolated in pyelonephritis. In our study, the detection rate of *sat* gene was 32.9%, which is significantly higher than that of L. Maniam et al. (7.5%) [20]. The *vat* gene is found in more than half of *E. coli* isolates from patients cystitis and pyelonephritis [25]. In our study, the *vat* gene was detected in 42.9% of *E. coli* isolates isolated in asymptomatic bacteriuria.

The production of siderophores, which play an important role in iron capture, increases the viability of microorganisms within the urethral tract. The presence of these virulence factors in UPEC appears to compensate for the absence of other virulence genes related to adhesion and toxin formation, and thus promotes prolonged colonization in the urinary tract without inducing an inflammatory response in the host. In the study performed, genes responsible for the synthesis of yersinebactin (*fyuA*), salmochelin (*iroN*) and aerobactin (*iuc*) were identified in 78.6%, 48.6% and 37.1% of isolates, respectively.

The capsule has a protective role against the host immune system, which promotes long-term persistence in the urinary tract. We detected the *kpsMII* gene in 65.7% of the isolates from patients with asymptomatic bacteriuria. Similar studies have shown that the frequency of this gene ranged from 38 to 73% [20, 23].

The presence of individual virulence genes in the genome is not sufficient for the realization of uropathogenic potential. Many studies suggest that several virulence factors are present in bacteria at once [22, 26]. Our data also indicate the genetic diversity of UPEC isolated in asymptomatic bacteriuria. The results of quantitative distribution of virulence factors in phylogenetic groups showed that 90% of *E. coli* isolates had two or more virulence markers. *E. coli* belonging to phylogroups B2, D, E, and F associated with UPEC contained more virulence genes than phylogroups A and B1 associated with commensal strains. Furthermore, *E. coli* belonging to phylogenetic group B2 possessed the highest number of genes in various combinations (5 to 12); genes associated with adhesion (*sfa*, *focG*), synthesis of siderophores (*fyuA*, *iroN*, *iuc*), toxins (*hlyA*, *cnf1*, *vat*, *usp*) and capsules (*kpsMII*) were found significantly more frequently ($p < 0.05$).

Numerous factors (immune response, ability to form biofilms) may influence the expression of virulence factors and the ability of UPEC isolates to adapt to persistence in UTIs. When comparing the virulence properties of UPEC isolates in asymptomatic bacteriuria and symptomatic UTIs (cystitis, pyelonephritis) in pregnant women, it was shown that E. coli isolates in asymptomatic bacteriuria and cystitis showed comparable virulence rates [23]. The determination of a large number of virulence genes in the isolate, according to the researchers, may indicate the uropathogenic potential of the pathogen, and the ability to manifest depends on the expression of this gene or a set of genes.

Conclusion

The molecular characterization and genotypic diversity of *E. coli* strains are essential for better understanding of their role in the pathogenesis of UTIs. Identification of key virulence determinants and/or virulence gene combinations may be a marker for predicting the course of UTIs, especially in pregnant women, and will enhance the diagnostic capabilities based on the virulence properties of the uropathogen

СПИСОК ИСТОЧНИКОВ | REFERENCES

1. Medina M., Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. *Ther. Adv. Urol*. 2019;11:1756287219832172. DOI: https://doi.org/10.1177/1756287219832172
2. Kot B. Antibiotic resistance among uropathogenic *Escherichia coli. Pol. J. Microbiol.* 2019;68(4):403–15. DOI: https://doi:10.33073/pjm-2019-048
3. Никифоровский Н.К., Степанькова Е.А., Сухорукова А.О. Инфекции мочевыводящих путей у беременных (обзор). *Сибирский научный медицинский журнал*. 2020;40(5):18–23. Nikiforovsky N.K., Stepankova E.A., Suhorukova A.O. Urinary tract infections in pregnancy (review). *Siberian Scientific Medical Journal*. 2020;40(5):18–23. DOI: https://doi.org/10.15372/SSMJ20200502 EDN: https://elibrary.ru/oomnqq
4. Smaill F.M., Vazquez J.C. Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochrane. Database Syst. Rev*. 2019;2019(11):CD000490. DOI: https://doi.org/10.1002/14651858.CD000490.pub4
5. Storme O., Tirán Saucedo J., Garcia-Mora A., et al. Risk factors and predisposing conditions for urinary tract infection. *Ther. Adv. Urol.* 2019;11:1756287218814382. DOI: https://doi.org/10.1177/1756287218814382
6. Заячникова Т.Е., Селезнева Н.С. Отдаленные последствия применения антибиотиков в перинатальном периоде. *Лекарственный вестник*. 2021;15(3):56–63. Zayachnikova T.E., Selezneva N.S. Long-term consequences of the use of antibiotics in the perinatal period. *Medicinal Bulletin.* 2021;15(3):56–63. EDN: https://elibrary.ru/myypir
7. Sora V.M., Meroni G., Martino P.A., at. al. Extraintestinal pathogenic *Escherichia coli:* virulence factors and antibiotic resistance. *Pathogens.* 2021;10(11):1355. DOI: https://doi.org/10.3390/pathogens10111355
8. Whelan S., Lucey B., Finn K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: the molecular basis for challenges to effective treatment. Microorganisms. 2023;11(9):2169. DOI: https://doi.org/10.3390/microorganisms11092169
9. Clermont O., Christenson J.K., Denamur E., et al. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylogroups. *Environ. Microbiol. Rep.* 2013;5(1):58–65. DOI: https://doi.org/10.1111/1758-2229.12019
10. Halaji M., Fayyazi A., Rajabnia M., et al. Phylogenetic group distribution of uropathogenic *Escherichia coli* and related antimicrobial resistance pattern: a meta-analysis and systematic review. *Front. Cell. Infect. Microbiol*. 2022;12:790184. DOI: https://doi.org/10.3389/fcimb.2022.790184
11. Rezatofighi S.E., Mirzarazi M., Salehi M. Virulence genes and phylogenetic groups of uropathogenic *Escherichia coli* isolates from patients with urinary tract infection and uninfected control subjects: a case-control study. *BMC Infect. Dis*. 2021;21(1):361. DOI: https://doi.org/10.1186/s12879-021-06036-4
12. Макарова М.А., Матвеева З.Н., Кафтырева Л.А. Интегративный подход к оценке патогенного потенциала штаммов *Escherichia coli*, выделенных из мочи. *Журнал микробиологии, эпидемиологии и иммунобиологии*. 2024;101(1):72–9. Makarova M.A., Matveeva Z.N., Kaftyreva L.A. An integrative approach to assessing the pathogenic potential of *Escherichia coli* strains isolated from urine. *Journal of Microbiology, Epidemiology and Immunobiology*. 2024;101(1):72–9. DOI: https://doi.org/10.36233/0372-9311-493
13. Казанцев А.В., Осина Н.А., Глинская Т.О. и др. Факторы вирулентности и филогенетическая характеристика уропатогенных штаммов *Escherichia coli*, выделенных на территории г. Саратова. *Проблемы особо опасных инфекций*. 2019;(4):56–60. Kazantsev A.V., Osina N.A., Glinskaya T.O., et al. Virulence factors and phylogenetic characteristics of uropathogenic *Escherichia coli* strains isolated in Saratov. *Problems of Particularly Dangerous Infections*. 2019;(4):56–60. DOI: https://doi.org/10.21055/0370-1069-2019-4-56-60 EDN: https://elibrary.ru/gplihe
14. Кузнецова М.В., Гизатуллина Ю.С. Генетические профили адгезии и адгезивная вариабельность уропатогенных штаммов Escherichia coli. *Инфекция и иммунитет*. 2021;11(3):481–90. Kuznetsova M.V., Gizatullina J.S. Genetic adhesion profiles and adhesive variability of uropathogenic Escherichia coli strains. *Russian Journal of Infection and Immunity*. 2021;11(3):481–90. DOI: https://doi.org/10.15789/2220-7619-GAP-1413 EDN: https://elibrary.ru/edkmlc
15. Basu S., Mukherjee S.K., Hazra A., et al. Molecular characterization of uropathogenic *Escherichia coli:* nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background. *J. Clin. Diagn. Res.* 2013;7(12):2727–31. DOI: https://doi.org/10.7860/JCDR/2013/6613.3744
16. Yun K.W., Kim H.Y., Park H.K., et al. Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. *J. Microbiol. Immunol. Infect.* 2014;47(6):455–61. DOI: https://doi.org/10.1016/j.jmii.2013.07.010
17. Farajzadah Sheikh A., Goodarzi H., Yadyad M.J., et al. Virulence-associated genes and drug susceptibility patterns of uropathogenic *Escherichia coli* isolated from patients with urinary tract infection. *Infect. Drug Resist*. 2019;12:2039–47. DOI: https://doi.org/10.2147/IDR.S199764
18. Momtaz H., Karimian A., Madani M., et al. Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. *Ann. Clin. Microbiol. Antimicrob*. 2013;12:8. DOI: https://doi.org/10.1186/1476-0711-12-8
19. Maniam L., Vellasamy K.M., Jindal H.M., et al. Demonstrating the utility of *Escherichia coli* asymptomatic bacteriuria isolates' virulence profile towards diagnosis and management — a preliminary analysis. *PLoS One*. 2022;17(5):e0267296. DOI: https://doi.org/10.1371/journal.pone.0267296
20. Spurbeck R.R., Dinh P.C. Jr., Walk S.T., et al. Escherichia coli isolates that carry *vat*, *fyuA*, *chuA*, and *yfcV* efficiently colonize the urinary tract. *Infect. Immun.* 2012;80(12):4115–22. DOI: https://doi.org/10.1128/IAI.00752-12
21. Tabasi M., Karam M.R., Habibi M., et al. Genotypic characterization of virulence factors in *Escherichia coli* isolated from patients with acute cystitis, pyelonephritis and asymptomatic bacteriuria. *J. Clin. Diagn. Res.* 2016;10(12):DC01–DC07. DOI: https://doi.org/10.7860/JCDR/2016/21379.9009
22. Lavigne J.P., Boutet-Dubois A., Laouini D., et al. Virulence potential of Escherichia coli strains causing asymptomatic bacteriuria during pregnancy. *J. Clin. Microbiol.* 2011;49(11):3950–3. DOI: https://doi.org/10.1128/JCM.00892-11
23. Zainel A., Mitchell H., Sadarangani M. Bacterial meningitis in children: neurological complications, associated risk factors, and prevention. *Microorganisms*. 2021;9(3):535. DOI: https://doi.org/10.3390/microorganisms9030535
24. Parham N.J., Pollard S.J., Desvaux M., et al. Distribution of the serine protease autotransporters of the *Enterobacteriaceae* among extraintestinal clinical isolates of *Escherichia coli. J. Clin. Microbiol.* 2005;43(8):4076–82. DOI: https://doi.org/10.1128/JCM.43.8.4076-4082.2005
25. Oliveira F.A., Paludo K.S., Arend L.N., et al. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. *Genet. Mol. Res.* 2011;10(4):4114–25. DOI: https://doi.org/10.4238/2011.October.31.5

Information about the authors

Tatiana A. Khusnutdinova✉ — Cand. Sci. (Med.), senior researcher, Department of medical microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, husnutdinovat@yandex.ru, https://orcid.org/0000-0002-2742-2655

Olga V. Budilovskaya — Cand. Sci. (Med.), senior researcher, Department of medical microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, https://orcid.org/0000-0001-7673-6274

Anna A. Krysanova — Cand. Sci. (Med.), senior researcher, Department of medical microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, https://orcid.org/0000-0003-4798-1881

Kira V. Shalepo — Cand. Sci. (Med.), senior researcher, Department of medical microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, 2474151@mail.ru, https://orcid.org/0000-0002-3002-3874

Anna A. Sinyakova — Cand. Sci. (Med.), senior researcher, Department of obstetrics and perinatology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, https://orcid.org/0000-0003-3094-665X

Alevtina M. Savicheva — D. Sci. (Med.), Professor, Head, Department of medical microbiology, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, https://orcid.org/0000-0003-3870-5930

Igor Yu. Kogan — D. Sci. (Med.), Professor, RAS Corresponding Member, Director, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, Russia, https://orcid.org/0000-0002-7351-6900

Author contribution: *Khusnutdinova T.A.* — research methodology and design, conducting the study; analysis and interpretation of the results, writing the manuscript; *Budilovskaya O.V., Krysanova A.A., Shalepo K.V.* — analysis and interpretation of the results, participation in the preparation of the manuscript; *Sinyakova A.A.* — data collection, participation in the preparation of the manuscript, *Savicheva A.M.* — research methodology and design, conceptualization and coordination of the study, editing the manuscript; *Kogan I.Yu.* — conceptualization of the study, editing the manuscript. All authors confirm that they meet the International Committee of Medical Journal Editors criteria for authorship, made a final approval of the version to be published.

The article was submitted 25.04.2024;
accepted for publication 17.06.2024;
published 29.08.2024

Информация об авторах

Хуснутдинова Татьяна Алексеевна✉ — к.м.н., с.н.с. отдела медицинской микробиологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, husnutdinovat@yandex.ru, https://orcid.org/0000-0002-2742-2655

Будиловская Ольга Викторовна — к.м.н., с.н.с. отдела медицинской микробиологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, https://orcid.org/0000-0001-7673-6274

Крысанова Анна Александровна — к.м.н., с.н.с. отдела медицинской микробиологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, https://orcid.org/0000-0003-4798-1881

Шалепо Кира Валентиновна — к.б.н., с.н.с. отдела медицинской микробиологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Санкт-Петербург, Россия, https://orcid.org/0000-0002-3002-3874

Синякова Анна Александровна — к.м.н., н.с. отдела акушерства и перинатологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, https://orcid.org/0000-0003-3094-665X

Савичева Алевтина Михайловна — д.м.н., профессор, зав. отделом медицинской микробиологии НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, https://orcid.org/0000-0003-3870-5930

Коган Игорь Юрьевич — д.м.н., профессор, чл.-корр. РАН, директор НИИ акушерства, гинекологии и репродуктологии им. Д.О. Отта, Санкт-Петербург, Россия, https://orcid.org/0000-0002-7351-6900

Участие авторов: *Хуснутдинова Т.А.* — методология и дизайн исследования, проведение исследования, анализ и интерпретация результатов, написание рукописи; *Будиловская О.В., Крысанова А.А., Шалепо К.В.* — анализ и интерпретация результатов исследования, участие в подготовке рукописи; *Синякова А.А.* — сбор данных, участие в подготовке рукописи; *Савичева А.М.* — методология и дизайн исследования, концептуализация и координация исследования, редактирование рукописи; *Коган И.Ю.* — концептуализация исследования, редактирование рукописи. Все авторы подтверждают соответствие своего авторства критериям ICMJE, внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 25.04.2024;
принята к публикации 17.06.2024;
опубликована 29.08.2024