We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 6

Phenotypic and Genetic Diversity of Uropathogenic *Enterococcus faecalis* Strains Isolated in the Primorsky Region of Russia

Zaitseva Elena Aleksandrovna,
Komenkova Tatiana Sergeevna,
Melnikova Elena Aleksandrovna,
Shadrin Andrey Mikhailovich and
Luchaninova Valentina Nikolaevna

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80485

Abstract

Urinary tract infection (UTI) is a topical problem of the contemporary pediatrics, pediatric nephrology, and urology. *E. faecalis* isolated from urine of children was the most important factor in development of UTIs at departments for newborns in Primorsky region. The variability of biochemical and fermentation activities of pathogenicity factors and resistance to antibiotics suggested a phenotypic heterogeneity of *E. faecalis*. It was found that uropathogenic enterococci characterized with proteolytic activity are resistant to antibacterial agents with different action mechanisms. Clinical isolates of *E. faecalis* contained two and more studied pathogenicity genes. Eleven variants of genes combinations, which code pathogenicity factors of *E. faecalis,* were identified. Uropathogenic *E. faecalis* strains attributed to ST6, ST40, ST179, ST774, and ST116 are resistant to four and more groups of antimicrobial agents. Our research confirmed high virulence properties of *E. faecalis* isolated from urine of patients with and their manifestations depending on the patient's age. Clinically significant *E. faecalis* strains have a complex of virulent properties, which allow the bacteria to materialize their pathogenic potential on all stages of the inflammation process in urinary system.

Keywords: urinary tract infections, *Enterococcus faecalis*, virulent properties, phenotypes, genotypes, newborns

1. Introduction

Urinary tract infection (UTI) is a topical problem of the contemporary pediatrics, pediatric nephrology, and urology, which takes the second or third place in the structure of children's morbidity [1, 6, 9, 12]. Risk factors of UTI in children include neonatal period, in particular premature birth, family medical history, abnormalities of urinary tract development, disruptions of urodynamics, including vesicoureteric reflux, obstructive uropathy, neurogenic bladder malfunction, urolithiasis, constipation, fecal and perianal colonization, immunocompromising conditions, including AIDS, immunosuppression therapy, frequent bladder catheterization, diabetes, sexual activity in teenagers [1, 14, 15, 18]. Newborns and infants are under high risk of UTI development, among other factors, since their immune system is not yet sufficiently developed [7]. Among the many factors, which affect development and forecast of UTI, biological properties of microorganisms, which inhabit kidney tissue, are of no small importance [10, 22, 35, 43].

Etiological structure of UTI in children has changed recently. Many studies show the growing etiological significance of *Enterococcus faecalis* [1, 6, 8, 20, 37]. Clinical significance of enterococci, which were earlier considered in different saprophytes, is being reassessed currently [4, 10].

Increase in etiological significance of enterococci is caused by many reasons, including development of antibiotic resistance, in particular, resistance to cephalosporin, widely used for treating UTIs in children [8]. Enterococci can initiate the infectious process due to their genes coding many pathogenicity factors involved in adhesion and invasion processes, development of biofilms, and hystodamaging effect (Esp, Asa1, EfaA, CylA, CylM, GelE, FsrB, etc.) [6, 13, 16, 30, 35, 42]. It is known that virulence of microorganisms depends upon the quantity of these genes [17]. Scientists from Europe, England, America, India, and Japan are doing research in this area [21, 33, 38, 39, 41]. No research related to characteristics of intraspecific genetic variability, including pathogenicity factors, in uropathogenic *E. faecalis* isolates from children having UTI, has been done in Russia until now. All the above has been determined in the topic of the research, which was undertaken in the present paper.

The aim of this research was to identify genetic variability and phenotypic features of biologic properties in uropathogenic *E. faecalis* isolates from children having urinary tract infections to assess its virulent potential and clinical significance.

2. Materials and methods

At the first stage, bacterial tests of urine samples (n = 6438) isolated from patients in cases of UTI at the multispecialty regional children's hospital from 2008 to 2016 were analyzed. Patients were aged from 3 days to 17 years.

At the second stage, biological properties and genetic variability of enterococci were studied. *E. faecalis* (n = 71) were isolated from urine of children with urinary tract infections and age from 3 days to 17 years in the diagnostic titer from 10^4 CFU/ml and higher during from 2013 to 2016. *E. faecalis* NCTC 12697 was used as the typical culture. All strains of uropathogenic

http://dx.doi.org/10.5772/intechopen.80485

Gene	Primer sequence	Product size (bp)	Reference
cylA	TGGATGATAGTGATAGGAAGT TCTACAGTAAATCTTTCGTCA	517	[11]
aggA	AAGAAAAAGTAGACCAAC AACGGCAAGACAAGTAAATA	1553	[11]
efaA	GACAGACCCTCACGAATA AGTTCATCATGCTGCTGTAGTA	705	[11]
eep	GAGCGGGTATTTTAGTTCGT TACTCCAGCATTGGATGCT	937	[5]
esp	TTGCTAATGCTAGTCCACGACC GCGTCAACACTTGCATTGCCGAA	933	[32]
gelE	ACCCCGTATCATTGGTTT ACGCATTGCTTTTCCATC	419	[34]

Table 1. Oligonucleotide primers used in the study.

E. faecalis were previously investigated using classical microbiological methods [22, 43]. Antimicrobial susceptibility was performed using disk diffusion method on the Muller-Hinton agar according to EUCAST.

Bacterial DNA of *E. faecalis* was isolated using DNA-express kit (Lytech, Russia). The testing of enterococci (n = 31) pathogenicity genes was made by polymerase chain reaction (PCR), using previously developed primer sets (**Table 1**) [5, 11, 32, 34], synthesized by Eurogen (Russia), on TProfessional 96 (Biometra, Germany). The amplification products were analyzed in a 1% agarose gel containing 1 μg/ml ethidium bromide in ultraviolet light using BioDocAnalyze (Biometra, Germany).

The obtained data were processed using the parametric analysis method. From the indicators of descriptive statistics, the relative values (P, %), their errors (m_p, %) were calculated. To evaluate the degree of interrelation, the Pearson correlation analysis (R) was performed with calculation of correlation coefficient (r) and reliability of correlation (p). At statistical processing of the received materials, the software package Statistica 10.0 is used in the operating environment Windows 2010.

The research was approved by Interdisciplinary Committee for Ethics of the Federal State Budgetary Educational Institution of Higher Education "Pacific State Medical University" of the Ministry of Healthcare of the Russian Federation (protocol no. 4, 26.12.2016).

3. Results

The most common uropathogen in children with UTI, who were treated at the multispecialty regional children's hospital, is *Escherichia coli*, for which specific gravity equals from 33.92 ± 1.7 to 62.96 ± 1.2%. Most frequently, it was pointed out in outpatients (in 62.96 ± 1.2% cases); less frequently, it was found at departments for newborns (33.92 ± 1.7%). The second significant

etiological factor of UTI in children was *E. faecalis*, for which specific gravity ranged from 16.14 to 32.5% [22]. At the same time, *E. faecalis* was the most important factor in development of UTIs at departments for newborns: it was found in 57.2% of cases (**Figure 1**). Frequency of identifying *E. faecalis* in newborns diagnosed with UTI ranged from 30.8 to 74.5% of cases over 9 years.

The present study analyzed the features of phenotypic manifestations of biological properties, including antibiotic resistance of *E. faecalis* (n = 71) isolated from urine of children with UTI. All analyzed uropathogenic *E. faecalis* had typical properties—morphology (cocci or oval Gram-positive bacteria), biochemical activity against mannitol, methylene blue, its absence in rhamnose fermentation, variability in glucose, lactose, sucrose and 2,3,5-triphenyltetrazolium chloride (TTC), and lack of mobility and catalase (**Table 2**).

Most *E. faecalis* isolated from the urine of children with UTI had *in vitro* enzymatic activity associated with pathogenicity: hemolytic, proteolytic, lipolytic, and lecithinase. A capsule was found in 45.6 ± 6.6% of uropathogenic enterococci. An inverse relationship was established between the presence of a capsule in *E. faecalis* with α-type hemolysis ($r = 0.3$, $p = 0.0001$), fermentation of milk ($r = 0.31$, $p = 0.00$), and a positive correlation with the microorganism titer in the urine ($r = 0.33$, $p = 0.0332$).

A direct correlation was established among the lecithinase and DNAase activity of *E. faecalis* ($r = 0.31$, $p = 0.0438$), the manifestation of hemolytic activity (α- or β-type) in uropathogenic enterococci, and the presence of the gene *gel*E ($r = 0.49$; $p<0.05$).

The capsule ($p_{1-2}<0.001$; $p_{1-3}<0.01$), milk fermentation ($p_{1-2}<0.001$; $p_{1-3}<0.01$), gelatinous ($p_{1-2}<0.001$, $p_{1-3}<0.05$), and lipolytic activity with respect to the tween 60 ($p_{1-2}<0.01$, $p_{1-3}<0.01$) was determined more often in *E. faecalis*, isolated from the urine of newborn children (group 1) with UTI, than in other age groups.

E. faecalis, isolated from children under 1 year (group 2), have hemolytic activity more often than other age groups ($p_{2-1}<0.05$, $p_{2-3}<0.05$), but expressed less gelatinous activity ($p_{1-2}<0.001$;

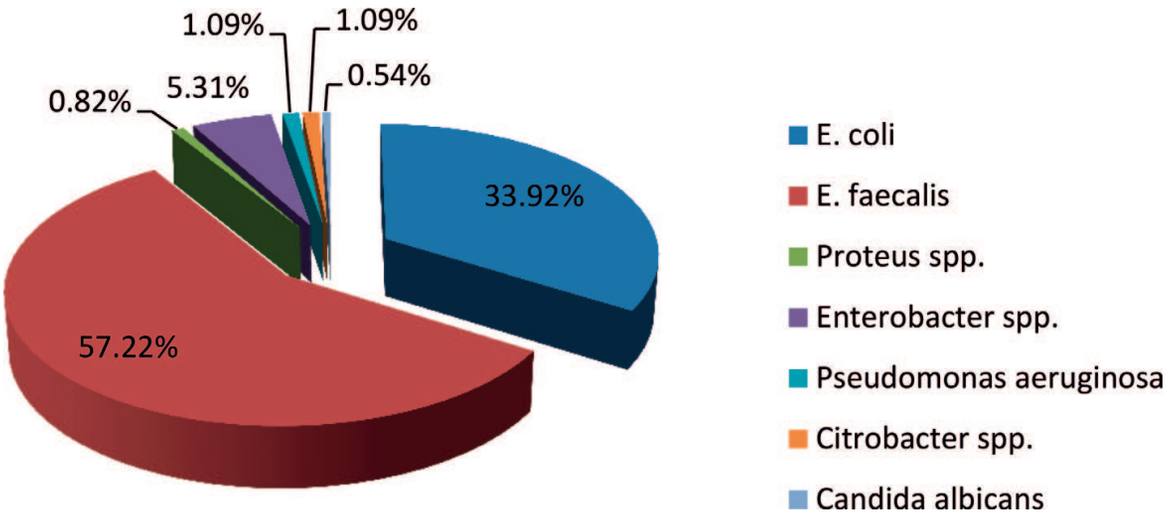

Figure 1. The etiological structure of UTI in newborn.

$p_{2–3}$<0.001). In this group, lipolytic activity against Tween 60 was less pronounced, but the differences were significant only between groups 1 and 2 (p<0.01).

E. faecalis isolated from patients older than 1 year (group 3) showed more lipolytic activity against Tween 80, compared to other age groups ($p_{1–3}$<0.001; $p_{2–3}$<0.01), gelatin's fermentation was determined more often than in enterococci of the group 2 (p<0.001). The hemolytic activity of enterococci in the group 3 differed little compared to the group of newborn children. The other biochemical properties associated with pathogenicity factors of *E. faecalis* strains of this age group were similar to enterococci isolated from children under 1 year of age (**Table 2**).

Assessment of antibiotics resistance revealed that all studied cultures of uropathogenic enterococci (n = 71) are sensitive to vancomycin and nitrofurantoin. We found that enterococci are

Biological properties	**Enzymatic activity of *E. faecalis***					
	Newborns (n = 21)		**Children from 29 days to 1 year (n = 18)**		**Children over 1 year old (n = 21)**	
	Number of examined cultures, n	**Abs. (P ± m_p, %)**	**Number of examined cultures, n**	**Abs. (P ± m_p, %)**	**Number of examined cultures, n**	**Abs. (P ± m_p, %)**
Reduction of TTC *■	19	18 (94.7 ± 5.1)	14	11 (78.6 ± 11.0)	21	20 (95.2 ± 4.7)
Reduction of methylene blue	19	19 (100)	14	14 (100)	21	21 (100)
Biochemical activity in relation to:						
Mannitol	20	20 (100)	13	13 (100)	19	19 (100)
Glucose ▲	20	19 (95.0 ± 4.9)	13	12 (92.3 ± 7.4)	19	19 (100)
Lactose ▲	20	19 (95.0 ± 4.9)	13	12 (92.3 ± 7.4)	19	16 (84.2 ± 8.4)
Rhamnose	21	0	13	0	19	0
Sucrose	20	17 (85.0 ± 8.0)	13	11 (84.6 ± 10.0)	19	16 (84.2 ± 8.4)
Presence of a capsule *▲	21	12 (57.1 ± 10.8)	17	6 (35.3 ± 11.6)	19	8 (42.1 ± 11.3)
Proteolytic activity in relation to:						
Milk *▲	21	18 (85.7 ± 7.6)	18	9 (50.0 ± 11.8)	21	10 (47.6 ± 10.9)
Gelatin *▲■	20	9 (45.0 ± 11.1)	17	2 (11.8 ± 7.8)	21	7 (33.3 ± 10.3)
Lecithinase activity	19	5 (26.3 ± 10.1)	15	4 (26.7 ± 11.4)	21	6 (28.6 ± 9.9)
Lipolytic activity in relation to:						
Tween 20	17	14 (82.4 ± 9.2)	12	11 (91.7 ± 7.9)	18	17 (94.4 ± 5.4)
Tween 60 *▲	8	4 (50.0 ± 17.2)	6	1 (16.7 ± 15.2)	11	3 (27.3 ± 13.4)
Tween 80 ▲■	21	14 (66.7 ± 10.3)	17	12 (70.6 ± 11.0)	19	16 (84.2 ± 8.4)

Note: *, p < 0.05 between 1 and 2 groups; ▲, p < 0.05 between 1 and 3 groups; ■, p < 0.05 between groups 2 and 3; Abs., absolute.

Table 2. Peculiarities of phenotypic manifestations biological properties of *E. faecalis* depending on the patient's age.

Id	ST	Isolate	Genotype						Total number of genes
			aggA	*esp*	*cylA*	*efaA*	*eep*	*gelE*	
1787	ST116	PR042	+	+	+	+	+	+	6
1789	ST179	PRV 052	+	+	+	+	+	+	6
1791	ST179	PRV100	+	+	+	+	+	+	6
1790	ST179	PRV105	+	+	+	+	+	+	6
	nd	PRV086	+	+	+	+	+	+	6
	nd	PR 230	+	+	+	+	+	+	6
1788	ST179	PRU047	+	—	+	+	+	+	5
	nd	PR 181	+	+	+	+	+	—	5
1781	ST16	PR050	+	+	+	+	+	—	5
1786	ST41	PRV049	+	+	—	+	+	+	5
	nd	PRL079	+	+	—	+	+	+	5
	nd	PRV080	+	+	—	+	+	+	5
1793	ST774	PRAs81	+	+	—	+	+	+	5
	nd	PR 198	+	—	—	+	+	+	4
	nd	PR 158	+	—	—	+	+	+	4
1780	ST6	PRV054	+	—	—	+	+	+	4
1779	ST6	PRN030	+	—	—	+	+	+	4
1795	ST774	PRA029	+	—	—	+	+	+	4
1792	ST774	PR51	+	—	—	+	+	+	4
1784	ST40	PR055	—	+	—	+	+	+	4
1785	ST40	PRA038	—	+	—	+	+	+	4
	nd	PR 228	+	—	+	+	+	—	4
1782	ST16	PRV092	+	—	+	+	+	—	4
	nd	PR 215	+	+	—	+	+	—	4
	nd	PR 223	+	+	—	+	+	—	4
1794	ST774	PR040	+	+	—	+	+	—	4
	nd	NCTC 12697	—	—	+	+	+	+	4
	nd	PR 97	—	—	+	+	+	+	4
	nd	PR 161	—	—	—	+	+	+	3
	nd	PR 206	—	—	—	+	+	+	3
1783	ST21	PRV082	—	—	—	+	+	—	2

Note: nd, not determined.

Table 3. Genotypes of uropathogenic *E. faecalis* in Primorsky region.

highly resistant to erythromycin (77.1 ± 5.02%), tetracycline (73.2 ± 5.3%), fluoroquinolones of the II and the III generations (ciprofloxacin (55.1 ± 5.9%), norfloxacin (48.6 ± 9.9%), and levofloxacin (46.5 ± 5.9%)). Were identified *E. faecalis* cultures, which are resistant (20.1 ± 4.9%) and mid-resistant (8.9 ± 3.5%) to the reserve drug linezolid. More than half (59.2 ± 5.8%) of the studied cultures of uropathogenic enterococci were resistant to several antibiotics (four or more antimicrobial agents).

It was found that enterococci, which are sensitive to penicillins, were characterized with lipolytic, lecithinase, and hemolytic (β-type) activity *in vitro*. Enterococci cultures which are resistant to fluoroquinolones, fermented sucrose, had proteolytic activity, and did not break down lactose. *E. faecalis*, which are resistant to gentamicin and erythromycin, had a capsule. Furthermore, *E. faecalis*, which are resistant to linezolid and chloramphenicol, had gelatinase, lecithinase, and lipolytic activities, as compared to other cultures (these data were not published).

In that way, analysis of biological properties of *E. faecalis*, isolated from urine of children with UTI in Primorsky region, showed that overwhelming majority of cultures had typical properties.

Id	Isolate	ST	Phenotype of antibiotic resistance	Genotype
1785	PRA038	40	TET-ERT-AmG-CHL-LZD	*esp-efaA-eep-gelE*
1784	PR055		TET-ERT-AmG-CHL	
1780	PRV054	6	TET-ERT-AmG-CHL-FLQ	*aggA-efaA-eep-gelE*
1779	PRN030		TET-ERT-AmG-CHL-FLQ	
1788	PRU047	179	TET-ERT-AmG-LZD	*aggA-cylA-efaA-eep-gelE*
1789	PRV052		TET-ERT-AmG	*aggA-esp-cylA-efaA-eep-gelE*
1791	PRV100		TET-ERT-AmG-CHL	
1790	PRV105		ERT-AmG-CHL-FLQ	
1787	PR042	116	TET-ERT-AmG-CHL-LZD	
1781	PR050	16	TET-ERT	*aggA-esp-cylA-efaA-eep*
1782	PRV092		TET-ERT-AmG	*aggA-cylA-efaA-eep*
1794	PR040	774	AmG-FX	*aggA-esp-efaA-eep*
1793	PRAs081		TET-ERT-PEN-AmG-FLQ	*aggA-esp-efaA-eep-gelE*
1795	PRA029		TET-ERT-PEN-FLQ	*aggA-efaA-eep-gelE*
1792	PR051		TET-ERT-AmG-FLQ	
1786	PRV049	41	TET	*aggA-esp-efaA-eep-gelE*
1783	PRV082	21	FLQ	*efaA-eep*

Note: TET, tetracycline; ERT, erythromycin; AmG, aminoglycosides; FLQ, fluoroquinolones; CHL, chloramphenicol; LZD, linezolid; PEN, penicillins.

Table 4. Antibiotic resistance of uropathogenic *E. faecalis* depending on the sequence type.

The mentioned variability of biochemical and fermentation activities of pathogenicity factors and resistance to antibiotics suggested a certain phenotypic heterogeneity of *E. faecalis*.

3.1. Molecular genetics typing of *E. faecalis*

E. faecalis was tested for genes, coding various pathogenicity factors, using PCR method. It was found that clinical strains (n = 30) of enterococci isolated from urine of children with UTI contained two and more studied pathogenicity genes. In this context, 27 out of 30 uropathogenic *E. faecalis* had four and more of the studied genes (**Table 3**).

Eleven variants of genes combinations, which code pathogenicity factors of *E. faecalis,* were identified. The most common variants are (*agg*A-*esp*-*cyl*A-*efa*A-*eep*-*gel*E) and (*agg*A, *efa*A, *eep*, *gel*E).

Multilocus sequence typing (MLST) divided 17 *E. faecalis* strains into eight (ST6, ST16, ST21, ST40, ST41, ST116, ST179, and ST774) sequence types (The results have not been published.) It was noticed that uropathogenic *E. faecalis* strains attributed to ST6, ST40, ST179, ST774, and ST116 are resistant to four and more groups of antimicrobial agents (**Table 4**).

The results demonstrate broad variability of the range of genes, which code pathogenicity factors and reveal sequence types with multiple resistances to antimicrobial agents among uropathogenic *E. faecalis* isolated from children with UTI in Primorsky region.

4. Discussion

Urinary tract infection is an inflammatory process in the organs of the urinary system without specifying the level of damage or growth of microorganisms in the urinary tract with possible development of local inflammatory changes. UTI refers to the factors that initiate the development of chronic kidney disease and depends on the age of the children [27]. Etiological multifactority is peculiarity of these infections. For a long time, commonly recognized pathogens of uroinfections are Gram-negative enterobacteria, among which *Escherichia coli* is prevalent [25, 26, 29, 31, 40, 44].

Interest to studying enterococci as participants of infectious diseases has increased in recent years. Our research has shown that *E. faecalis* are a common pathogen, which causes UTI in children, most often in newborns (from 30.8 up to 75% of cases). Perhaps this is not accidental, as according to some authors, this microorganism is detected in children from the first days of life and its amount exceeds the content of *E. coli* in the newborn period [19]. The reason for this microbial composition of urine appears to be functional immunodeficiency in this category of patients.

However, until now, the true etiological significance of these microorganisms in the development of the infectious process and unfavorable outcomes remains uncertain due to the ever-changing properties of *E. faecalis*. It is known that the ability of bacteria to affect the kidneys and urinary tract is determined not by one but by a complex of properties necessary for this process, that at the different stages of the infectious process in the urinary system organs from the microorganism, priority expression of certain pathogenetically significant traits and/or their combinations is required [6].

http://dx.doi.org/10.5772/intechopen.80485

All analyzed uropathogenic *E. faecalis* had typical properties—morphology, biochemical activity against mannitol, methylene blue, its absence in rhamnose fermentation, variability in glucose, lactose, sucrose and 2,3,5-TTC, and lack of mobility and catalase.

At the same time, weak and delayed acid formation from lactose was in three cultures (only on the third day), and in 12 isolates during fermentation of sucrose (by day 10). The results obtained with respect to a number of carbohydrates differ from the literature data, as it is known that *E. faecalis* ferment lactose and sucrose, and in relation to other sugars can be variable (Berdzhi). Possibly, this is associated with the spread of certain *E. faecalis* biovars in the territory of Primorsky Krai or within a single multispecialty hospital.

According to results of research conducted in recent years, it was determined that enterococci produce many virulence factors, which conduce to development of the infectious process (hemolysin, gelatinase, enterococcal surface protein, aggregation substance, serine protease, capsule, etc.). The greatest number of virulence factors was found in *E. faecalis* isolated from urine. High proteolytic activity of *E. faecalis* (hydrolysis of gelatinase, casein, and collagen) causes toxic damage to tissues and conduce to cicatricial changes in kidney [6, 19, 43].

Moreover, the change in the properties of the microorganisms, causing the urinary tract infection, such as the development of resistance factors to antimicrobial drugs and the biofilm formation, makes it difficult to manage patients, especially with chronic persistent and often recurrent infection.

Our research confirmed high virulence properties of *E. faecalis* isolated from urine of patients with and their manifestations depending on the patient's age. For example, there is a negative correlation between the proteolytic activity of *E. faecalis* and the age of the children ($r = 0.28$, $p = 0.002$). Most *E. faecalis* isolated from the urine of children with UTI had *in vitro* enzymatic activity associated with pathogenicity: hemolytic, proteolytic, lipolytic, and lecithinase.

Currently, lipase is referred to understudied factors of enterococcal persistence, although it is known that lipase may be a potential virulence factor of *E. faecalis* [12]. Among the studied enterococci isolated from children with UTI, lipolytic activity was determined in $85.0 \pm 8.2\%$ of the cultures (more often with respect to Tween 20 and Tween 80). Enterococcus cultures showed heterogeneity in proteolytic and hemolytic activity. A reliable direct correlation between the phenotypic manifestation of β-type hemolytic activity with hydrolysis of gelatin ($r = 0.58$, $p = 0.0001$) and lecithinase activity ($r = 0.52$, $p = 0.0004$) of this uropathogen has been established. This confirms the combined effect of these pathogenic factors at a certain stage of the inflammatory process. A relationship was established between the phenotypic manifestation of pathogenicity factors and the age of patients.

The most common properties of *E. faecalis* isolated from urine of newborn children were a capsule, proteolytic, and lipolytic (in relation to Tween 60) activity. Enterococci isolated from 1-year-old children with UTI most frequently were characterized with hemolytic activity. Lipolytic (in relation to Tween 80) activity was most frequently found in cultures isolated from patients older than 1 year. The prevalence of these virulence factors suggests that they are associated with virulence of this species in UTI. Such features of the manifestation of biological properties *in vitro* indicate the selection of etiologically significant *E. faecalis* at the level of the macroorganism.

Furthermore, connection between sensitivity to antimicrobial drugs of *E. faecalis* and its biological properties was identified. It was found that uropathogenic enterococci characterized with proteolytic activity are resistant to antibacterial agents with different action mechanisms. In the work, it was found that *E. faecalis*, resistant to linezolid and chloramphenicol drugs that suppress protein synthesis at the level of the 50S subunit of the bacterial ribosome, possess a high pathogenic potential. These results require further research in this direction.

Interesting data were obtained with regard to the sensitivity of uropathogenic *E. faecalis* to the reserve drug linezolid, recommended for treatment of infections caused by strains, which are resistant to vancomycin, aminoglycosides, and betalactams. In Primorsky Krai, *E. faecalis* cultures were found resistant and intermediate sensitivity to linezolid. However, in Russia in the period from 2005 to 2013, there were isolated single enterococcal strains with reduced sensitivity to linezolid.

This way, the mentioned variability of biochemical and fermentation activity of factors related to pathogenicity demonstrated phenotypic heterogeneity of enterococci and might have a certain diagnostic significance.

Pathogenicity factors of bacteria are genetically determined by properties which are localized in genome of microorganisms in the form of "pathogenicity islands" [17]. These genetic elements can contain various sets of virulence genes, which are important for the development of the enterococcal infectious process, including genes of antibiotics resistance [17, 24]. At the present stage, the association of antibiotic resistance of *E. faecalis* with pathogenic factors is actively studied. It is known that strains of enterococci resistant to ampicillin, ciprofloxacin, and gentamicin, but sensitive to vancomycin and nitrofurantoin have more pathogenicity factors (hemolysin, gelatinase, hyaluronidase, form biofilms) than vancomycin resistant [2, 28]. *E. faecalis* having the *asa*1 gene are more resistant to fluoroquinolones (norfloxacin, ciprofloxacin, and levofloxacin) than isolates lacking this gene. Resistance to ciprofloxacin is significantly higher in *E. faecalis* having the genes *cyl*L and *cyl*S than in strains with their absence [23, 28]. *esp* gene-positive *E. faecalis* are more resistant to doxycycline than *esp* gene-negative cultures [3]. Among the strains with multidrug resistance, a high prevalence of genes *asa*1 and *esp was* observed [23, 36].

The research implemented using PCR method enabled to characterize in greater detail the structure of *E. faecalis* population isolated from children with UTI from Primorsky region. In our research, significant variability in occurrence frequency of the studied genes was found. Two of them—*efa*A (coding the surface antigen A (EfaA), which initiates the infectious process) and *eep* (coding Eep protein, which conduces to formation of a biofilm, making it resistant to various biological stress factors) were found in all studied uropathogenic *E. faecalis*, which proves their involvement in certain stages of the infectious process.

MLST analysis conducted earlier revealed eight sequence types, five of which were characterized by multidrug resistant.

This way, clinically significant *E. faecalis* strains have a complex of virulent properties, which allow the bacteria to materialize their pathogenic potential on all stages of the inflammation process in urinary system. This makes further research of the listed factors in clinical *E. faecalis* necessary to estimate objectively the contribution of these properties of the agent into pathophysiologic mechanism of infectious and inflammatory diseases.

http://dx.doi.org/10.5772/intechopen.80485

5. Conclusions

1. Important role in the etiology of UTI is played not only by Gram-negative bacteria of the *Enterobacteriaceae* family, but also by gram-positive *E. faecalis*, which are of paramount importance in the development of UTI in newborns.
2. *E. faecalis*, isolated from the urine of children with UTI, have a complex of pathogenicity factors necessary for the development of the inflammatory process and their prolonged persistence in the urinary tract. The relationship between *E. faecalis* pathogenicity factors and the age of patients was determined.
3. Uropathogenic *E. faecalis* possess a polyantibiotic resistance, which is associated with its biological properties and belonging to a particular sequence type.
4. A set of phenotypic manifestations of the biological properties of *E. faecalis* (the presence/absence of hemolytic, gelatinase, lecithinase, lipolytic activities) established in the study may determine its clinical significance and serve as an *in vitro* diagnostic marker of resistance of the studied uropathogen to certain groups of antibacterial drugs.

Acknowledgements

The research was supported with an internal grant from the university (registration No. AAAA-A18-118031390014-9).

Conflict of interest

The authors declare that there is no conflict of interests.

Author details

Zaitseva Elena Aleksandrovna[1]*, Komenkova Tatiana Sergeevna[1], Melnikova Elena Aleksandrovna[1,2], Shadrin Andrey Mikhailovich[3,4] and Luchaninova Valentina Nikolaevna[5]

*Address all correspondence to: elza200707@mail.ru

1 Pacific State Medical University, Vladivostok, Russia

2 Regional Children's Clinical Hospital № 1, Vladivostok, Russia

3 G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, Russia

4 Pushchino State Institute of Life Sciences, Pushchino, Moscow Region, Russia

5 North-Western State Medical University named after I.I. Mechnikov, Saint-Petersburg, Russia

References

[1] Alberici I, Bayazit AK, Drozdz D, et al. Pathogens causing urinary tract infections in infants: A European overview by the ESCAPE study group. European Journal of Pediatrics. 2015;**174**(6):783-790. DOI: 10.1007/s00431-014-2459-3

[2] Banerjee T, Anupurba S. Prevalence of virulence factors and drug resistance in clinical isolates of *Enterococci*: A study from North India. Journal of Pathogens. 2015:7. ID 692612. DOI: 10.1155/2015/692612

[3] Baylan O, Nazik H, Bektöre B, Citil BE. The relationship between antibiotic resistance and virulence factors in urinary *Enterococcus* isolates. Mikrobiyoloji Bülteni. 2011;**45**(3):430-445

[4] Beganovic M, Luther MK, Rice LB, Arias CA, Rybak MJ, et al. A review of combination antimicrobial therapy for *Enterococcus faecalis* bloodstream infections and infective endocarditis. Clinical Infectious Diseases. 2018. DOI: 10.1093/cid/ciy064. [Epub ahead of print]

[5] Bittencourt ME, Suzart S. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. Journal of Medical Microbiology. 2004;**53**:1069-1073

[6] Bukharin OV, Gritsenko VA, Vyalkova AA. Factors of bacterial urotogenicity: A role in pathogenesis and importance in the diagnosis of pyelonephritis. Nephrology and Dialysis. 2001;**3**(4):469-475

[7] Chang SL, Shortliffe LD. Pediatric urinary tract infections. Pediatric Clinics of North America. 2006;**53**(3):379-400, vi. DOI: 10.1016/j.pcl.2006.02.011

[8] Chaschina IL, Tatochenko VK, Barkadze MK. The place of cephalosporins in the treatment of urinary tract infections in children. Current Pediatrics. 2012;**1**(1):158-161

[9] Chugunova OL, Shumikhina MV, Dumova SV. Current overview of the urinary system infection in newborns and infants. Effective Pharmacotherapy. 2013;**42**:38-47

[10] Diederich A-K, Wobser D, Spiess M, Sava IG, Huebner J, et al. Role of glycolipids in the pathogenesis of *Enterococcus faecalis* urinary tract infection. PLoS One. 2014;**9**(5):e96295. DOI: 10.1371/journal.pone.0096295

[11] Eaton TJ, Gasson MJ. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates applied and environmental microbiology. 2001;**67**(4):1628-1635. DOI: 10.1128/AEM.67.4.1628-1635.2001

[12] Elsner H, Sobottka I, Mack D, Wirth R. Virulence factors of *Enterococcus faecalis* and *E. faecium* blood culture isolates. European Journal of Clinical Microbiology & Infectious Diseases. 2000;**19**:39-42

[13] Esther H, Bonten MJ, Willems Rob JL. Enterococcal surface protein Esp is important for biofilm formation of *Enterococcus faecium* E1162. Journal of Bacteriology. 2007;**189**(22):8233-8240. DOI: 10.1128/JB.01205-07

[14] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nature Reviews. Microbiology. 2015;**13**(5):269-284. DOI: 10.1038/nrmicro3432

[15] Forster CS, Shaikh N, Hoberman A, Jackson E. Uropathogens and pyuria in children with neurogenic bladders. Pediatrics. 2018;**141**(5). DOI: 10.1542/peds.2017-3006

[16] Frank KL, Vergidis P, Brinkman CL, et al. Evaluation of the *Enterococcus faecalis* biofilm-associated virulence factors AhrC and Eep in rat foreign body osteomyelitis and in vitro biofilm-associated antimicrobial resistance. PLoS One. 2015;**10**(6):e0130187. DOI: 10.1371/journal.pone.0130187

[17] Gilmore MS, Clewell DB, Yasuyoshi I, Shankar N. Enterococci from commensals to leading causes of drug resistant infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014. 668 pp

[18] Grazioli S, Parvex P, Merlini L. Antenatal and postnatal ultrasound in the evaluation of the risk of vesicoureteral reflux. Pediatric Nephrology. 2010;**25**(9):1687-1692

[19] Haid B, Pichler R, Becker T, Koen M, Berger C, et al. Late renal scarring and loss of kidney function despite successful therapy of vesicoureteral reflux in childhood: Case report and review of literature. International Journal of Case Reports in Medicine. 2015. DOI: 10.5171/2015.737441

[20] Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. Microbiology Spectrum. 2016;**4**(2). DOI: 10.1128/microbiolspec.UTI-0012-2012

[21] Medeiros AW, Pereira RI, Oliveira DV, Martins PD. Molecular detection of virulence factors among food and clinical *Enterococcus faecalis* strains in South Brazil. Brazilian Journal of Microbiology. 2014;**45**(1):327-332. DOI: 10.1590/S1517-83822014005000031

[22] Melnikova EA, Zaitseva EA, Luchaninova VN. Features of urinary tract infection in children associated with *Enterococcus faecalis* in view of its biological properties. Nephrology. 2016;**20**(3):42-47

[23] Mete E, Kaleli I, Cevahir N, Demir M, et al. Evalution of virulence factors in *Enterococcus* species. Mikrobiyoloji Bülteni. 2017;**51**(2):101-114

[24] Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in enterococci. Expert Review of Anti-Infective Therapy. 2014;**12**(10):1221-1236. DOI: 10.1586/14787210.2014.956092

[25] Naboka YL, Rymashevsky AN, Sviraeva EG, Bragina LE. Formation of the microflora of the digestive tract of newborns in dynamics. Journal of Microbiology, Epidemiology and Immunology. 2012;**3**:65-70

[26] Taneja N, Chatterjee SS, Singh M, Sharma M. Pediatric urinary tract infections in tertiary care center from north India. Indian Journal of Medical Research. 2010;**131**:101-105

[27] Osipovich SA. Immunological disorders, course and prognosis of recurrent infections of the urinary system in children. Pediatrics. Eastern Europe. 2013;**2**(2):42-55

[28] Padilla C, Lobos O. Virulence, bacteriocin genes and antibacterial susceptibility in *Enterococcus faecalis* strains isolated from water wells for human consuptision. Springerplus. 2013;**2**(1):43

[29] Petrosyan EK, Gavrilova VA, Reznikov AY. Treatment and prevention of recurrent urinary tract infection in children. Russian Herald of Perinatology and Pediatrics. 2010;**1**:85-88

[30] Preethee T, Kandaswamy D, Hannah R. Molecular identification of an *Enterococcus faecalis* endocarditis antigen *efa*A in root canals of therapy-resistant endodontic infections. Journal of Conservative Dentistry. 2012;**15**(4):319-322. DOI: 10.4103/0972-0707.101886

[31] Stein R, Dogan HS, Hoebeke P, Kocvara R, Nijman RJM, Radmayr C, Tekgul S. Urinary tract infections in children: EAU/ESPU Guidelines. European Urology. 2015;**67**:546-558

[32] Shankar V, Baghdayan AS, Huycke MM, et al. Infection-derived Enterococcus faecalis strains are enriched in esp, a gene encoding a novel surface protein. Infection and immunity. 1999;**67**(1):193-200

[33] Seno Y, Kariyama R, Mistuhata R, Monden K, Kumon H. Clinical implications of biofilm formation by *Enterococcus faecalis* in the urinary tract. Acta Medica Okayama. 2005;**59**(3): 79-87

[34] Soares RO, Fedi AC, Reiter KC, Caierão J, d'Azevedo PA. Correlation between biofilm formation and *gelE*, *esp*, and *agg* genes in *Enterococcus* spp. clinical isolates. Virulence. 2014;**5**(5):634-637. DOI: 10.4161/viru.28998

[35] Shankar N, Lockatell CV, Arto SB, Drachenberg C, Gilmore MS, Johnson DE. Role of *Enterococcus faecalis* surface protein Esp in the pathogenesis of ascending urinary tract infection. Infection and Immunity. 2001;**69**(7):4366-4372. DOI: 10.1128/IAI.69.7.4366-4372.2001

[36] Shahraki S, Nezhad Mousavi MR. Determination of virulence factors in clinical multidrug resistance *Enterococci* isolates at Southeast of Iran. Jundishapur Journal of Microbiology. 2017. DOI: 10.58.12/jim.45514

[37] Skepyan EN, Vasilevski IV, Toptun PD. Analysis of the spectrum of infections of the urinary tract and the characteristic their sensitivity to antimicrobial drugs the children's outpatient. Medical Panorama. 2013;**8**:35-38

[38] Stereva T, Atanasova D, Savov E, Petrova G, Mitov I. Incidence of virulence determinants in clinical *Enterococcus faecalis* and *Enterococcus faecium* isolates collected in Bulgaria. The Brazilian Journal of Infectious Diseases. 2016;**20**(2):127-133. DOI: 10.1016/j.bjid.2015.11.011

[39] Strezelecki Y, Hryniewicz W, Sadowy E. Gelatinase-associated phenotypes and genotypes among clinical isolates of *Enterococcus faecalis* in Poland. Polish Journal of Microbiology. 2011;**60**(4):287-292

[40] Vasilyeva LI, Kogan MI, Gudima IA, Naboka YL. Etiological structure and antibiotic susceptibility of pathogens of acute non-obstructive pyelonephritis in children. Attending Physician. 2009;**8**:8-11

[41] Upadhyaya PM, Ravikumar KL, Umapathy BL. Review of virulence factors of enterococci: An emerging nosocomial pathogen. Indian Journal of Medical Microbiology. 2009;**27**(4): 301-305. DOI: 10.4103/0255-0857.55437

[42] Upadhyaya PM, Umapathy BL, Ravikumar KL. Comparative study for the presence of enterococcal virulence factors gelatinase, hemolysin and biofilm among clinical and commensal isolates of *Enterococcus faecalis*. Journal of Laboratory Physicians. 2010;**2**(2):100-104. DOI: 10.4103/0974-2727.72159

[43] Zaitseva EA, Krukovich EV, Melnikova EA, Luchaninova VN, Komenkova TS, Vaysero NS. The role of pathogenicity factors of *Enterococcus faecalis* in the development of pyelonephritis in children. Pacific Medical Journal. 2017;**2**(68):58-61

[44] Zorc JJ, Kidoo DA, Shaw KN. Diagnosis and menagment of pediatric urinary tract infections. Clinical Microbiology Reviews. 2005;**18**:417-422

IntechOpen