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Antimicrobial Resistance of Uropathogens in Moscow Center for Children's Health

Abstract: Urinary tract infections (UTI) are the second most common children's infections. The prevalence in Russian Federation is 18 cases in 1000. Also, spreading of antimicrobials resistance among children's UTI poses a high epidemiological threat. Retrospective analysis was performed with 163 sequentially collected midstream portion of urine. Samples were collected from patients of FSAI of the Ministry of Health of the Russian Federation «NMR Center for Children's Health» during 2019. Species and antimicrobial susceptibility tests were performed using microbiological methods. Detection of antimicrobial resistance gene determinants was performed using quantitative real-time PCR assays. Species of uropathogens were identified in 69 samples while others being classified as «gut flora» (n=14), «coccus flora» (n=10) or «mixed flora» (n=70). The most prevalent uropathogens were *Escherichia coli* (24.7%) and *Enterococcus faecalice* (20.3%). Genes of CTX-M-like group, including *bla*CTX-M-1-like, *bla*CTX-M-2-like, *bla*CTX-M-8-like, and *bla*CTX-M-9-like, were determined in 33%, *bla*VIM in 6%, *bla*IMP in 1%, *bla*NMD in 4%, and *bla*OXA-48-like in 3% of studied samples. No *bla*KPC genes were identified. In all 69 samples with identified species, antimicrobial resistance profile, determined by microbiological methods, including resistance to penicillins, cephalosporins, carbapenems, and monobactams, was in accordance with found gene determinants. It is of great importance to introduce antimicrobial resistance genes determinants testing in clinical practice. It would provide the opportunity to determine correct in-time treatment for antimicrobials resistant bacteria infections.

Keywords: antimicrobials resistance genetic determinants; UTI; uropathogens; children's urine; β-lactamase genes

INTRODUCTION

Urinary tract infections (UTI) are the second most common children's infections [1] with prevalence varying up to 8% worldwide [2]. Prevalence of children's UTI in Russian Federation is 18 cases in 1000, with being responsible for 10-15% cases of critically ill infants and young children with fever [3]. UTI are the cause of urination problems in children in 7.8% (95% CI: 6.6-8.9) cases [6]. The most common pathogen causing UTI in children are *Escherichia coli* (79,7% cases) [3,4], *Klebsiella* spp., *Serratia* spp., *Pseudomonas* spp., and *Streptococcus* spp. [5].

Furthermore, rapidly increasing prevalence of antimicrobials resistant bacteria poses a high epidemiological threat [6]. According to Mahony et al. [7] review extended spectrum β-lactamase (ESBL) producing bacteria percentage causing children UTI may vary up to 48.8% while prevalence of multidrug resistant (MDR) *E. coli* may reach up to 90% in some parts of the World. While there are studies [8–12] dedicated to patterns of antimicrobials resistance prevalence performed with microbiological tests, there is a lack of studies in terms of resistance genetic determinants. So, in Thänert et al. [13] study it was shown that 79.8% children UTI causing bacteria harbored ESBL-encoding genes, like *bla*CTX-M-15 (45.9%) and *bla*OXA-1 (44%).

CTX-M-type β-lactamases are widespread in many countries [14], causing resistance to 2nd-, 3rd- and 4th-generation cephalosporins, penicillins, and monobactams [15]. OXA-type of β-lactamases able to confer resistance to penicillins and cephalosporin [16]. Spreading of other types of lactamases is also of great concern. So, *Klebsiella pneumoniae* carbapenemase (KPC) provides resistance to penicillins, cephalosporins, aztreonam, and carbapenems [17]. Metallo-β-lactamases may provide

resistance to penicillins, cephalosporins, and carbapenems with the most prevalent families being IMP (inactivate imipenem), VIM (Verona Integron-encoded Metallo-β-lactamase), and NDM (New Delhi Metallo-β-lactamase) [18].

In our study we analyzed sequentially collected 163 samples collected in 2019 from patients of the urological department of the National Medical Research Center of Children's Health. All samples were tested on harboring antimicrobials resistance genetic determinants like *bla*CTX-M group, *bla*KPC, *bla*OXA, and various metallo-β-lactamase genes. Phenotypic manifestation of antimicrobials resistance was validated using microbiological tests.

RESULTS

Sample collection

It is important to highlight that in our study we only included samples with significant bacteriuria. According to Reaffirmation of AAP Clinical Practice Guideline: The Diagnosis and Management of the Initial Urinary Tract Infection threshold for patients with symptomatic UTI is 10^4 CFU/ml, and for patients without symptoms – 10^5 CFU/ml. Since it was not known of patients' UTI symptoms, threshold of 10^4 CFU/ml was chosen.

Of all collected samples species of uropathogens were identified in 69 samples with others being classified as «gut flora» (n=14), «coccus flora» (n=10) or «mixed flora» (n=70). Prevalence of all identified species in urine samples is described in Table 1.

Table 1. Prevalence of uropathogens.

Pathogen species	Percentage of patients (including infections caused by multiple pathogens), %
E. coli	24.6
E. faecalis	20.3
K. pneumoniae	20.2
P. aeruginosa	20.2
E. faecium	5.8
Acinetobacter spp.	5.8
Enterobacter spp.	5.8
P. mirabilis	5.8
M. morganii	1.4
Candida spp.	4.3
Klebsiella spp.	2.9
Staphylococcus haemolyticus	2.9
Providencia stuartii	1.4
Chryseobacterium indologenes	1.4

In studied samples with bacteriuria average meaning of leucocytes was shown to be 834,21±770,82 cells/μl.

Phenotypical resistance

Resistance profile of all 69 samples with identified pathogens was determined using corresponding bacteriological methods. Testing was performed according to European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 9.0 [19], valid at the time of the analysis. Data on percentage of antimicrobials susceptibility is presented in Supplement Materials (Table 1).

Resistance genetic determinants

Using real-time based PCR assays, previously developed in our laboratory, 163 samples were tested on the presence of resistance genes determinants. Genes of CTX-M-like group, including *bla*CTX-M-1-like, *bla*CTX-M-2-like, *bla*CTX-M-8-like, and *bla*CTX-M-9-like, were determined in 33% samples. Genes of *bla*VIM were determined in 6% samples, *bla*IMP in 1%, *bla*NMD in 4%, and *bla*OXA-

48-like − in 3% of studied samples. No *bla*KPC, *bla*OXA-23-like, *bla*OXA-58-like, *bla*OXA-40-like, *van*A, and *van*B genes were identified.

Of all studies samples 26.3% contained only CTX-M-like genes, 3% only VIM genes, 1.8% only NDM genes, and 0.6% − only OXA-48-like genes. It was shown that 6.7% of strains contained more than one type of resistance genes with the most prevalent combination being CTX-M-like and OXA-48-like genes.

Antimicrobial resistance profile of 69 samples with identified species, determined by microbiological methods was in accordance with found resistance-associated genes Supplenet Materials (Table 2).

DISCUSSION

In our study we analyzed 163 samples of urine, obtained from urological department with reproductology and transplantology groups FSAI of the Ministry of Health of the Russian Federation «NMR Center for Children's Health». The most prevalent uropathogens were *E. coli* (24.7%) and *E. faecalis* (20.3%). For *E. coli* the lowest levels of susceptibility were observed to trimethoprim/sulfamethoxazole (68.8%), cefepime (62.5%), ampicillin (31.3%), ceftazidime (62.5%), amoxicillin/clavulanic acid (62.5%), and aztreonam (62.5%). For *E. faecalis* lower susceptibility was shown only for ciprofloxacin (87.5%) and levofloxacin (87.5%). Overall susceptibility of all tested strains was shown to be the lowest for cefepime (70.5%), ampicillin (68.8%), ceftazidime (66.3%), amoxicillin/clavulanic acid (60.4%), and aztreonam (67.1%).

In the study of Vazouras et al. it was shown that the most prevalent was *E. coli* (79.2%) with high resistance rates to ampicillin (42.0%), trimethoprim/sulfamethoxazole (26.5%), and amoxicillin/clavulanic acid (12.2%) [11]. Prevalence of the main pathogens causing bacteriuria was shown to be in accordance with other published results. In study of Lutter [20] et al. among children's UTI the most common pathogen also was shown to be *E. coli*. It is also of great interest, that in abovementioned study overall resistance to cefotaxime sodium and aminoglycoside antibiotics varied dramatically from patients receiving no prophylactic antibiotics (3% and 1%, respectively) to patients receiving prophylaxis (27% and 5%, respectively). In the study of Wang et al. [12] the most common isolated pathogens were shown to be *Enterococcus* spp. (35.2%) and *E. coli* (22.3%) with low susceptibility to linezolid (3.5%), vancomycin (0.9%), imipenem (5.7%), and amikacin (3.2%). In the study of Mirsoleymani et al. [21] antimicrobial susceptibility of *E. coli* were shown to be to amikacin (79.7%), ofloxacin (78.3%), gentamicin (71.6%), ceftriaxone (41.8), cefotaxime (41.4%), and cefixime (27.8%). Bryce et al. in their study shown that *E. coli* strains had the highest resistance to amoxicillin (49.37% pathogenic versus 37.32% contaminant, $P = 0.04$), trimethoprim (27.85% versus 16.52%, $P = 0.01$) and co-amoxiclav (16.46% versus 21.48%, $P = 0.30$), with multidrug resistance being present in 17.07% of pathogens and 30.13% of contaminants ($P = 0.04$) [22].

Considering all studies mentioned earlier, authors suggest, that results obtained are in accordance with previously published studies.

It is important to highlight that now bacteriological methods now are required for antimicrobial resistance determination [23], although development of new resistance detection methods is on [24]. It is of concern that in accordance with CLSI/EUCAST [25] interpretation criteria are of compromising nature. Although the «golden standard» assay for colistin MIC determination is broth microdilution method [26], it was shown to have high error rate [27]. Alas, the authors suggest that microdilution in polystyrene plates as a standard method requires reconsideration.

Authors suggest that using PCR analysis on resistance-associated genes in clinical practice is a promising alternative method for bacteriological diagnostics. Application of this method allows not only to speed up the analysis, but also to determine correct antimicrobial treatment. The growing threat of antibiotic resistance is the reason not only for the search for new antimicrobial drugs, but also for the development of new systems for diagnosing the presence of resistance determinants in bacterial infectious agents. The advent of highly accurate and easy-to-use diagnostic systems will help control the spread of pathogenic antibiotic-resistant strains, as well as determine the appropriate course of antimicrobial treatment for the patient at an early stage.

MATERIALS AND METHODS

Sample collection

Retrospective analysis was performed with sequentially collected urine samples obtained from patients of 4 weeks to 17 years in 2019 in FSAI of the Ministry of Health of the Russian Federation «NMR Center for Children's Health». All samples were collected from patient of urologic department with groups of transplantology and reproductology. Samples obtained included midstream portion of urine, urine from ureter or stoma. Mean age of patients was 3,54±3,29 years. Leukocyturia was shown in 15.3% studied samples. Among samples obtained from patients with bacteriuria and established diagnosis the most common were urethral stricture (31.6%), hydronephrosis (18.4%), and urolithiasis disease (15.8%).

Bacteriological studies

For all studies samples bacteriological tests were performed using biomaterial seeding on URISELECT™ (Bio-Rad Laboratories, USA) media with further incubation on 37°C through the course of 24 – 48 h. Seeding was performed by non-sectorial assay with a 10 μm loop, bacterial load was determined according to clinical guidelines [31]. Species in studies samples were determined by MALDI-TOF MS (Bruker Daltonics, Germany) and using bacteriological analysator Vitek 2 (BioMerieux, France).

Antimicrobials susceptibility

For phenotypical resistance determination three methods were used: disc-diffusion (Bio-Rad, USA), E-tests (BioMerieux, France) and bacteriological analyzer VITEK 2 Compact (BioMerieux, France). For disc-diffusion method and E-tests Muller-Hinton agar was used (Bio-Rad, USA). E-tests were used for determination of imipenem, meropenem, and vancomycin resistance. Colistin resistance determination was conducted by using microdilutions in plate (Lyofilchem, Italy). Results were interpreted according to Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing Position Statements on Polymyxin B and Colistin Clinical Breakpoints [25].

Amplification assay

All samples were transported in batches in molecular laboratory strictly in accordance with cold chain. Before the extraction all samples were stored at − 70°C. DNA extraction was performed using «RIBO-prep» (AmpliSens, Russia). Range of species of each sample was detected using real-time quantitative PCR-based assay «AmpliSens® UTI-screen-monitor» (Central Research Institute of Epidemiology, Russia). Analysis was performed using quantitative real-time PCR assays «AmpliSens® MDR MBL-FL», «AmpliSens® MDR KPC-OXA-48-FL», «AmpliSens® ESBL CTX-M», «AmpliSens® MDR VRE-FL», and «AmpliSens® MDR A.b.-OXA-FL » (all Central Research Institute of Epidemiology, Russia) (Table 2).

Table 2. Resistance genes determinants in used assays.

Reagent kit	Antimicrobials resistance gene region detection
AmpliSens® MDR MBL-FL	*bla*VIM, *bla*IMP, *bla*NMD
AmpliSens® MDR KPC-OXA-48-FL	*bla*OXA-48-like, *bla*KPC
AmpliSens® ESBL CTX-M	*bla*CTX-M-1-like, *bla*CTX-M-2-like, *bla*CTX-M-8-like, *bla*CTX-M-9-like
AmpliSens® MDR A.b.-OXA-FL	*bla*OXA-23-like, *bla*OXA-58-like, *bla*OXA-40-like
AmpliSens® MDR VRE-FL	*van*A, *van*B

All assays were performed using RotorGene® 6000 amplifier.

Amplification data and statistical analysis

Data obtained with all used reagent kits was analyzed using RotorGene 6000 v 1.8 software according to manufacturer's instructions.

CONCLUSION

In our study we estimated the prevalence of UTI children's pathogens with corresponding antimicrobials resistance profile. Also, the performance of real-time PCR based assays previously developed in our laboratory was evaluated. The accordance of microbiological methods of resistance detection was shown to be in accordance with PCR-based assays. Thus, authors suggest that newly developed methods can be used both for epidemiological monitoring of antimicrobials resistance and estimation of resistance-associated genes distribution. Prevalence of main children's uropathogens and antimicrobials susceptibility profiles were shown to be in accordance with previously published results. Authors suggest that introduction in clinical practice of PCR-based methods of resistance-associated genetic determinants may be of great importance for both decreasing the time of antimicrobials susceptibility analysis and aid in determination of suitable treatment.

AUTHOR CONTRIBUTIONS

Conceptualization, A.V. and G. E.; methodology, S. E. and L. A.; experiments performance, S. E and L. A.; writing—original draft preparation, S. E.; writing—review and editing, L.A., A. V., and G. E.. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTAL MATERIALS

Table 1. Percentage of susceptible strains amongst tested species, %

Species	Antimicrobials

	imipenem	meropenem	ciprofloxacin	amikacin	gentamycin	netilmicin	tobramycin	trimethoprim/sulfamethoxazole	colistin	cefepime	ampicillin	nitrofurantoin	ceftazidime	amoxicillin/clavulanic acid	aztreonam	tigecycline	lynezolid	vancomycin	levofloxacin	triclocarban	piperazine citrate/tazobactam	oxacillin	clindamycin
Acinetobacter spp.	100	100	100	100	100	100	100	100	100	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Chryseobacterium indologenes	0	0	0	0	0	0	0	0	0	100	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Enterobacter spp	100	100	100	100	100	100	nt	100	100	50	0	100	50	0	50	nt	nt	nt	nt	nt	nt	nt	nt
E. faecalis	100	nt	87.5	nt	nt	nt	nt	nt	nt	nt	100	100	nt	nt	nt	100	100	100	87.5	nt	nt	nt	nt
E. faecium	0	nt	0	nt	nt	nt	nt	nt	nt	nt	0	75	nt	nt	nt	100	100	100	0	nt	nt	nt	nt
Escherichia coli	100	100	75	93.8	87.5	87.5	nt	68.8	100	62.5	31.3	93.8	62.5	62.5	62.5	nt	nt	nt	nt	nt	nt	nt	nt
Klebsiella spp	87.5	87.5	56.3	93.8	75	81.3	nt	62.5	100	56.3	0	68.8	56.3	56.3	56.3	nt	nt	nt	nt	nt	nt	nt	nt
Morganella morganii	100	100	100	100	100	100	nt	100	0*	66.7	0	66.7	66.7	33.3	66.7	nt	nt	nt	nt	nt	nt	nt	nt
Proteus	100	100	75	100	100	100	nt	100	0*	50	75	50	50	50	50	nt	nt	nt	nt	nt	nt	nt	nt

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Providencia stuartii	100	100	100	100	0	0	nt	100	0	100	0	0	100	100	100	nt	nt	nt	nt	nt	nt	nt
P. aeruginosa	78.6	78.6	78.6	71.4	78.6	78.6	71.4	nt	100	78.6	nt	nt	78.6	nt	nt	nt	nt	nt	nt	78.6	78.6	nt	nt
Serratia marcescens	100	100	100	0	0	100	nt	0	0	0	0	0	0	0	0	nt	nt	nt	nt	nt	nt	nt	nt
Staphylococcus haemolyticus	nt	nt	nt	nt	100	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	100	100	100	nt	nt	nt	50	100

* – intrinsic resistance

nt – not tested

Table 2. Percentage of resistant strains among bacteria, containing resistance-associated genes, %

Resistance genes	Antimicrobials																					
	imipenem	meropenem	ciprofloxacin	amikacin	gentamycin	netilmicin	tobramycin	trimethoprim/sulfamethoxazole	colistin	oxacillin	clindamycin	tigecycline	lynezolid	vancomycin	ampicillin	levofloxacin	nitrofurantoin	triclocarban	piperazine citrate/tazobactam	cefepime	ceftazidime	amoxicillin/clavulanic acid	aztreonam
CTX-M-like group	25	25	60	30	50	35	100	63	25	N/A	N/A	N/A	N/A	N/A	94	N/A	19	100	100	95	100	88	100

OXA-48	33	33	67	33	33	0	N/A	33	67	N/A	N/A	N/A	N/A	N/A	100	N/A	100	N/A	N/A	100	100	100	100
VIM	33	33	50	17	50	50	100	40	0	N/A	N/A	N/A	N/A	N/A	100	N/A	20	N/A	N/A	67	67	100	80
IMP	75	50	50	50	50	33	60	33	17	N/A	N/A	N/A	N/A	N/A	33	N/A	N/A	60	60	33	50	33	N/A

N/A – data not availabe