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PEDIATRIC URINARY INFECTIONS, CAUSED BY EXTENDED-SPECTRUM BETA-LACTAMASE - PRODUCING MICROORGANISMS IN VARNA, BULGARIA

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ABSTRACT

Background: Extended-spectrum beta-lactamase (ESBLs) producing bacteria are microorganisms which have the ability to hydrolyze β-lactame ring of a large part of the antibiotics, commonly used to treat bacterial infections including urinary tract infections.

Purpose: The aim of this study is present the epidemiology of childhood urinary tract infections caused by ESBL-producing strains in Varna, Bulgaria.

Material/methods: A total of 3895 urine samples of children patients (aged 0 to 18 years) were examined during the period 2010-2012 for presence of ESBL-producing bacteria.

Results: Six percent of the tested urinary samples were positive for ESBL production. All of the isolates were resistant to ampicillin, piperacillin, cephalothin, cefprozil, cefuroxime, ceftriaxone, ceftazidime, levofloxacin, cefaclor, but were were sensitive to meropenem and imipenem.

Conclusions: Cephalosporins and penicillins are the most used antibiotics in Bulgaria, but they should be very precisely prescribed in medical practice, because otherwise preconditions for maintaining high share of ESBLs are created.

Key words: Extended-spectrum beta-lactamase producing bacteria, urinary tract infections, pediatric infections, antibiotic resistance.

INTRODUCTION

Urinary tract infections (UTIs) are the most common infections in inpatients and outpatients [1, 2] – approximately 150 million people in the world are suffering from UTIs [3, 4]. The major uropathogenic infectious agents are Gram-negative bacteria from Family *Enterobacteriaceae* – *Escherichia coli* (responsible for about 80-90% of infections in outpatients and more than 50% in inpatients [5]), *Klebsiella spp*., and less frequently *Proteus mirabilis* and *Serratia spp*. Leading Gram-positive bacteria causing UTIs are *Staphylococcus aureus* and species from genus *Streptococcus* and genus *Enterococcus*.

The main antibiotics for treatment of UTIs are beta-lactame antibiotics (penicillins, second and third generation cephalosporins) and fluoroquinolone, but *Enterobacteriaceae* can efficiently produce extended-spectrum beta-lactamases (ESBLs) to hydrolyze the β-lactame ring of a significant part of beta-lactames. ESBL-producing bacteria are mainly *Escherichia coli* (considered as major producer) and *Klebsiella spp.* (*Klebsilla pneumoniaå*). These types of multidrug resistant bacteria are usually selected in hospital environment and respectively provoke nosocomial infections. However, strains of ESBL-producing bacteria are progressively isolated in community-acquired infections and currently are a global public health problem [6].

The present study aims to determine the number of urinary tract infections caused by ESBL-producing bacteria in children outpatients in Varna, the third biggest city in Republic of Bulgaria. The main purpose is to assist the therapeutic practice and to stimulate prevention of these infections.

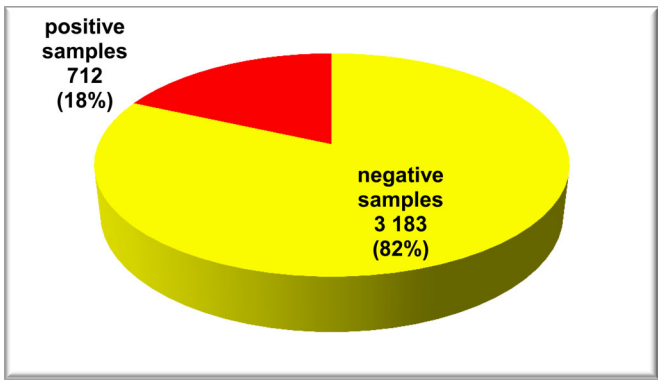
MATERIALS AND METHODS

We performed a retrospective analysis of 3895 urine samples provided by "Laborexpress 2000" - Varna. They include all urine samples of children patients (aged 0 to 18 years), examined during the period 2010-2012. Phenotypic methods detecting ability of ESBL-enzymes to hydrolyze different β-lactame antibiotic groups (mainly cephalosporines) were used for ESBLs screening. Disk diffusion method was used for routine susceptibility testing and double-disk synergy [DDS] method on Mueller-Hinton [MH] agar – for detection of ESBL production.

RESULTS

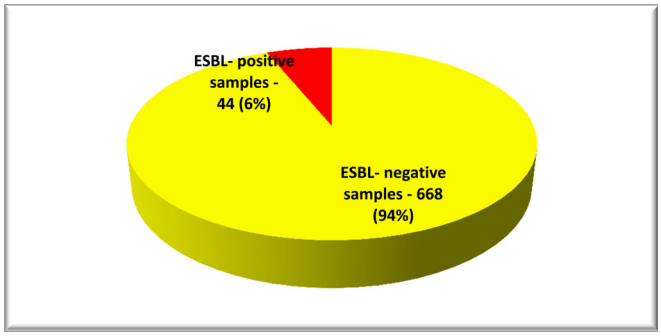
From 3895 samples tested, 712 were positive for pathogenic microorganisms and 3183 were negative (Fig. 1). The isolated strains included: *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Enterobacter spp.*, *Pseudomonas aeruginosa*, *Citrobacter diversus*, *Morganella morgani*, *Staphylococcus spp.*, *Streptococcus agalactiae*, *Enterococcus faecalis* and *Candida spp.*

Fig. 1. Distribution of urinary tract isolates from children outpatients in Varna, 2010-2012 [7]



Out of 712 positive for bacterial growth samples, 460 showed presence of bacterial species previously reported to be ESBL-producers and they were subsequently tested for ESBL-enzymes production. A total of 44 samples were found to be positive for ESBL synthesis (e.i. 6% from all positive for pathogens samples) (Fig. 2).

Fig. 2. Share of ESBL-producing isolates from urinary tract infected children in Varna, 2010-2012 [7].

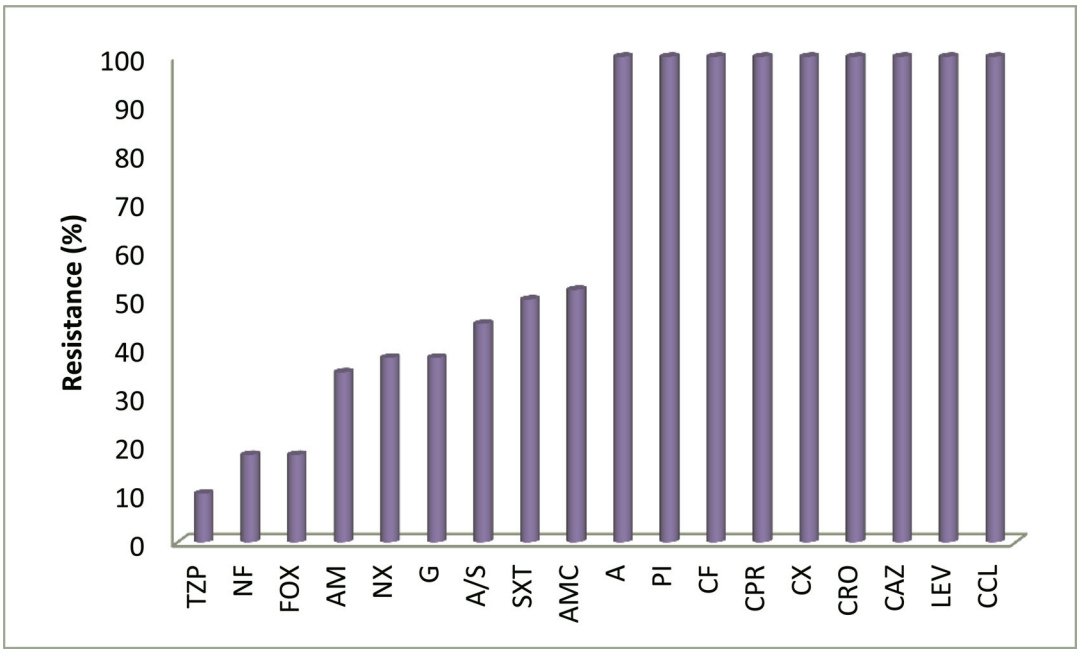


Generally, the share of ESBLs, causing UTIs is around 24-42% [1, 3] in hospitalized patients. In nonhospitalized patients, these values are significantly lower – 5-18% [8]. Compared to the worldwide tendency, the ESBL-proportion in Varna is relatively low, but clearly demonstrates that these types of infection exist as "community-acquired" infections and it is a matter of time to reach more serious values.

According to recent studies ESBL-producing bacteria are resistant to ceftazidime, cefotaxime and ceftriaxone [9] (third generation cephalosporins), monobactams (aztreonam) and others [10]. ESBLs also demonstrate resistance to second generation cephalosporins and penicillins and currently only cephamycins (cefoxitin and cefotetan) and carbapenems (meropenem and imipenem [6]) are effective against them. However, in Turkey and other countries carbapenemase-producing *Klebsiella pneumoniae* [11] was already detected.

Our study found the following antibiotic resistance (in %) among isolated strains (Fig. 3): Piperacillin/tazobactam - 10% < Nitrofurantoin - 18% < Cefoxitin - 18,2% < Amikacin - 34,6% < Nalidixic acid - 38,1% < Gentamicin - 38,2% < Ampicillin/Sulbactam - 45,5% < Trimethoprim/sulfamethoxazole – 50% < Amoxicillin/clavulanic acid – 52% < Ampicillin – 100% < Piperacillin – 100% < Cephalothin – 100% < Cefprozil – 100% < Cefuroxime – 100% < Ceftriaxone – 100% < Ceftazidime – 100% < Levofloxacin – 100% < Cefaclor – 100%. In contrast, all isolated strains were sensitive to Meropenem and Imipenem (data not shown). *E. coli* strains were sensitive to Ampicillin/Sulbactam, while all other ESBL-producers – *K. pneumoniae*, *K. oxytoca*, *E. aerogenes* were resistant. Eight of 13 *E. coli* isolates showed resistance to Nalidixic acid but the other ESBL species – sensitivity [7].

Fig. 3. Antibiotic resistance of isolated ESBLs in urinary samples from children in Varna, 2010-2012 [7].



Legend: **TZP** - Piperacillin/tazobactam; **NF** - Nitrofurantoin; **FOX** - Cefoxitin; **AM** - Amikacin; **NX** – Nalidixic acid; **G** - Gentamicin; **A/S** - Ampicillin Sulbactam; **SXT** - Trimethoprim/sulfamethoxazole; **AMC** - Amoxicillin/clavulanic acid; **A** - Ampicillin; **PI** - Piperacillin; **CF** - Cephalothin; **CPR** - Cefprozil; **CX** - Cefuroxime; **CRO** - Ceftriaxone; **CAZ** - Ceftazidime; **LEV** - Levofloxacin; **CCL** - Cefaclor.

DISCUSSION

Treatment of patients suffering from UTIs should include adequate diagnosis by medical laboratory, because with routine tests, ESBL-strains may remain unnoticed [1, 12]. Unfortunately, many microbiological laboratories in Bulgaria do not use specific methods for ESBL-detection. Modern phenotypic and genotypic tests for ESBL-detection and other poly-resistant strains require significant resources and availability of the relevant equipment. Especially, genotypic methods, which detect specific genes for ESBL-production, are widely inaccessible. These techniques have high rate of sensibility and generate data, which phenotypic methods might miss [2]. Moreover, molecular analyses save time by testing directly the clinical sample, without need of bacterial cultivation [13].

In the treatment of UTIs the physicians should be informed about the high rate of morbidity with ESBLs, because many of the recently effective drugs need to be administered only after detailed laboratory tests. Irrational treatment with arbitrarily applied antibiotics, strongly favors the increase of ESBL-producers share. One of the main strategies in the fight against ESBLs is to reduce the use of antibiotics, which these bacteria are resistant to. Otherwise, more bacterial stains have the opportunity to acquire resistance.

"Summary of the latest data on antibiotic consumption in the European Union" (2014) showed that the the most prescribed antibiotics in Bulgaria were beta-lactame antibiotics, with leading share of penicillins. The high percentage of beta-lactame consumption leads to a selective pressure to the bacteria from *Enterobacteriaceae* family and this stimulates the development of high resistant ESBL-producers [14].

CONCLUSIONS

Medical-diagnostic laboratories have a leading role in the detection and reporting of ESBL-producing bacteria. But still, parts of them are not realizing the importance of using specific phenotypic and genotypic methods for identification of ESBL-producing strains [7].

The following recommendations for control of UTIs, caused by ESBLs could be postulated:

- Physicians should be informed about the nature and the significant number of ESBL-strains in Bulgaria and in Varna, in particular. They should apply methods for specific prevention and treatment of ESBL-infections;
- Penicillins and cephalosporins in medical practice should be very precisely prescribed, because otherwise preconditions for maintaining high share of ESBLs are created;
- Medical laboratories should improve detection of ESBLs, by using appropriate screening tests, equipment, and well trained staff;
- Self-medication of patients with urinary tract infections should be reduced to minimum.

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