Antibiotic resistance in urinary tract infection in children

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Abstract. This retrospective study (November 2021-October 2022) aimed at identifying pathogen agents from positive urine cultures and tested their resistance to antibiotics at Emergency Clinical Hospital for Children “Sf. Ioan” in Galati, Romania.

Included in this study were 167 bacterial strains and both Gram-positive and Gram-negative bacteria have been isolated.

Most bacterial strains were *Escherichia coli* (56.28 %), *Klebsiella pneumonia* (12.57 %), *Proteus mirabilis* (8.38 %) and *Enterococcus faecium* (12.57 %).

Others bacterial strains were: *Pseudomonas aeruginosa* (4.79 %), *Staphylococcus saprophyticus* (1.20 %), *Staphylococcus epidermidis* (0.59 %), *Staphylococcus schleifferi* (0.59 %), *Staphylococcus aureus MSSA* (0.59 %), *Staphylococcus aureus MRSA* (0.59 %), *Streptococcus agalactiae* (0.59 %), *Enterobacter cloacae* (0.59 %), *Acinetobacter baumanii* (0.59 %).

*Escherichia coli* was the most frequent etiologic pathogen, being responsible for half of urinary tract infection (UTI) and the highest resistance in terms of pathogens was found among this bacteria. This research found a high rate of resistance to ampicillin and followed by co-trimoxazole, cephalosporines and aminoglycosides.

Antimicrobial resistance in urinary tract infection in our days is a real threat that requires vigilant monitoring to be done.

Keywords: urinary tract infection, pathogenic agents, antibiotics, antimicrobial resistance.
Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in childhood [Karmazyn et al., 2017; Korbel et al., 2017], encountered by pediatricians. In 30% of children with urinary tract abnormalities, UTI may be the first sign [Sastre et al., 2007]. The infection can affect the upper urinary tract (pyelonephritis) or the lower one (cystitis), being impossible to distinguish between the two conditions based on symptoms and clinical signs, especially in infants and young children [Balighian et al., 2018; Clark et al., 1991]. For both the physician and the parents, the high incidence, propensity for recurrence, associated morbidity, and problems with collecting an uncontaminated urine sample represent significant challenges [Leung, 2011]. About 8.4% of girls and 1.7% of boys by the age of 7 years are diagnosed with a UTI, and 30% of infants and children experience recurrent infections in the first six to 12 months after the initial UTI [Moustafa et al., 2008; Hellström et al., 1991]. The common pathway of UTI is bacterial ascent from the urethra and hematogenous spread of infection. Short-term symptoms of UTI are usually fever, dysuria and flank pain [Larcombe et al., 2010]. In childhood, UTI treatment aims to eradicate the bacterial pathogen, identify abnormalities, and avoid recurrent infections [Neal, 2008]. Detection and identification of the pathogen in urine is the Gold Standard for the diagnosis of UTI. Before testing the urine culture, the doctor empirically administers antibiotics quickly, which can favor the growth of antibiotic resistance of the pathogen [Alós, 2015]. In addition, prompt diagnosis and appropriate treatment are very important to reduce the morbidity associated with this condition [Korbel et al., 2017]. A urine culture test can identify the bacteria or yeasts that cause a urinary tract infection (UTI). The pathogens present in urinary tract infection are: Gram-negative bacilli (Escherichia coli, Klebsiella spp, Enterobacter spp, Citrobacter spp, Proteus spp, Pseudomonas aeruginosa, Providencia stuartii, Morganella morganii, Gardenella vaginalis, Serratia spp), Gram-negative cocci (Neisseria gonorrhea), Gram-positive cocci (Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterococcus spp, Streptococcus group B, Streptococcus group D) and other pathogens, such as Candida spp, Chlamydia, Mycoplasma [Lo et al., 2014; Foxman, 2014; Levison et al., 2013; Fisher et al., 2011; Nielubowicz et al., 2010; Jacobsen et al., 2008; Ronald et al., 2002].

Antimicrobial resistance (AMR) has become a challenge in modern medical practice. The rate at which microbes are becoming resistant to antibiotics is faster than the discovery of new antimicrobial agents. The antibiogram is an essential resource for institutions to track changes in antimicrobial resistance and guide antimicrobial therapy [William et al., 2021]. There is a need to study antimicrobial patterns, and to this end, ward and hospital-level antibiograms should be developed and studied to better guide us in choosing appropriate therapy for pediatric patients. A number of databases are available in the United States of America to monitor antimicrobial resistance nationally [Halstead et al., 2004]. Instead, in Romania we found a lack of information about national guidelines for in vitro antimicrobial susceptibility testing and differences in interpretation between laboratories in different regions.

To overcome these difficulties, our study aimed to determine the susceptibility (resistance) of bacteria isolated from urinary tract infections to the classes of antibiotics used to treat that infection.
Material and method

The study is a retrospective analysis of 167 bacterial strains isolated from urine samples collected from child patients, aged between 0-17 years, hospitalized in the wards of Emergency Clinical Hospital for Children “Sf. Ioan” in Galati and were collected over twelve months between November 2021 to October 2022.

Urine samples were collected from 57 male (34.14 %) and 110 female (65.86 %) patients.

Most of urine samples were collected from patients under 1 year old (31.73 %) followed by 26.34 % (1-4 years old), 17.36 % (5-9 years old), 9.58% (10-14 years old) and 14.97 % (15-17 years old).

Prevalence of UTI in urban area was 51.49 %. The isolation of bacteria from urine samples was performed according to the classical methodology of microbiological diagnosis. Gram-stained smears were made for all the samples and studied in order to determine the inflammatory character of the products and to observe the morphotinctorial characteristics of the germs. The identification of the bacteria genus and species was made based on the biochemical characters observed on these media, and for same species identification was made with the help of the automatic system Vitek 2 COMPACT.

The determination of the spectrum of resistance to antibiotics and chemotherapeutics was assessed by Kirby-Bauer disk diffusion method or using the automatic VITEK 2 COMPACT system, the minimum inhibitory concentration (MIC) method. The production of Extended Spectrum β-Lactamase (ESBL) by Enterobacteriaceae strains was determined by using the test of double diffusion, with the help of cefotaxime in combination with inhibitory clavulanic acid and the Vitek 2 Compact.

Methicillin resistant *Staphylococcus aureus* (MRSA) was tested using the cefoxitin disk diffusion test. Antibiotic sensibility using the Kirby–Bauer disk diffusion-method was interpreted according to CLSI (Clinical and Laboratory Standards Institute) standards which are renewed annually and the interpretation of the results regarding the MIC values is done following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

According to the antimicrobial resistance profile of the strains, they were categorized as resistant to one or two antimicrobial categories or multidrug-resistant (MDR). The definition for MDR was suggested by the European Centre for Disease Prevention and Control (ECDC). (Magiorakos, et al., 2011). The results were collected in hospital databases on regularly monitoring of local antibiotic resistance and were statistically processed by Microsoft XL software.

Results and discussions

From the 167 bacterial strains isolated from urine samples, the following 2 categories of pathogens were isolated: Gram negative bacilli (*Escherichia coli*, *Klebsiella spp*, *Proteus spp*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*) and Gram-positive cocci (*Enterococcus spp*, *Staphylococcus spp*, *Streptococcus agalactiae*). (Figure 1)
Other strains isolated from urine samples (10.17%) were: *Pseudomonas aeruginosa* (4.79%), *Staphylococcus saprophyticus* (1.20%), *Staphylococcus epidermidis* (0.59%), *Staphylococcus schleiferi* (0.59%), *Staphylococcus aureus* MSSA (0.59%), *Staphylococcus aureus* MRSA (0.59%), *Streptococcus agalactiae* (0.59%), *Enterobacter cloacae* (0.59%), *Acinetobacter baumanii* (0.59%). In our study the most frequent pathogens were *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus faecium*, which is in accordance with the data from the specialized literature where *Escherichia coli* is the most common etiological pathogen for urinary tract infections in children, accounting for almost 80% of them [Eldin, et al., 2013]. Other Enterobacterales, such as *Klebsiella spp.*, *Enterobacter spp.*, and *Proteus spp.*, followed. Other Gram-negative germs, such as *Pseudomonas aeruginosa*, can cause UTIs less often. *Enterococcus spp.* and *Staphylococcus saprophyticus* are two Gram-positive uropathogens in youngsters [Khoshnood et al., 2017]. Distribution of patients from whom strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecium* were isolated, according to number (%), sex and environment are presented in Table 1 and the frequency of these pathogens according to age is illustrated by Figure 2.

### Table 1. Distribution of pathogens according to number (%), sex and environment

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>No/(%)</th>
<th>Sex</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male (%)</td>
<td>Female (%)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>94 (56.28%)</td>
<td>26.60 %</td>
<td>73.40 %</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>21 (12.57%)</td>
<td>47.62 %</td>
<td>52.38 %</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>14 (8.38%)</td>
<td>28.57 %</td>
<td>71.43 %</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>21 (12.57%)</td>
<td>40 %</td>
<td>60 %</td>
</tr>
</tbody>
</table>

No= number
Escherichia coli and Klebsiella pneumoniae are the two most common causative organisms of urinary tract infection (UTI) in childhood [Sitthisarunkul N et al., 2019; Moore CE et al., 2016]. Both species frequently produce extended-spectrum beta-lactamase (ESBL) enzymes which confer resistance to beta-lactam antibiotics including third- and fourth-generation cephalosporins, and monobactams [Bradford PA, 2001; Pana ZD et al., 2018].

The increasing isolation of ESBL-producing Escherichia coli and Klebsiella pneumoniae causing UTI in children is of concern worldwide due to the failure of empirical therapy which may result in serious clinical complications such as sepsis, renal scarring and prolonged hospitalisation, compared to infection with non-ESBL strains [Moore CE et al., 2016; Kocak M et al., 2016].

Overall, in our study, from all 94 Escherichia coli isolated strains, 11 strains were positive for Extended Spectrum β-Lactamase producing Escherichia coli and from all 21 Klebsiella pneumonia isolated strains, 9 strains were positive for Extended Spectrum β-Lactamase.

According to the antimicrobial resistance profile the pathogens were categorized as resistant to one or two antimicrobial categories or multidrug-resistant (MDR - multidrug-resistant, non-susceptibility to at least 1 agent in ≥ 3 antimicrobial categories). In literal terms, MDR means „resistant to more than one antimicrobial agent”, but no standardized definitions for MDR have been agreed upon yet by the medical community. Many definitions are being used in order to characterize patterns of multidrug resistance in Gram-positive and Gram-negative organisms [Falagas ME et al., 2006; Cohen AL et al., 2008; Hidron AI et al., 2006-2007; Macgowan AP, 2008; Paterson DL, 2006]. The absence of specific definitions for MDR in clinical study protocols gives rise to data that are difficult to compare.

In our study we focused on identifying antimicrobial resistance to one or two antimicrobial categories or MDR (Figure 3).
Antibiotic resistance is becoming a serious health problem [Boucher, et al., 2009]. Due to the high empiric use of antibiotics for the treatment of UTI, the antibiotic resistance of main UTI pathogens, *Escherichia coli* and *Klebsiella pneumonia*, increased significantly worldwide [Mazzariol et al., 2017]. Drug resistance of *Escherichia coli* to many antibiotics is high with the maximum prevalence of resistance being to drugs that have been used for a long time, such as ampicillin and co-trimoxazole [Saudi J, 2009].

Overall in our study resistance to ampicillin was observed in 76,79 % of *Escherichia coli* isolates and resistance to co-trimoxazole in 63,33 % cases. Resistance to cephalosporines (23,11 %), aminoglycosides (19,30 %) and fluoroquinolones (5,60 %) of *Escherichia coli* isolates, was lower. Over 60 % of *Klebsiella pneumonia* strains were found resistant to chloramphenicol and tetracycline. Between 28 to 76 % of them were resistant to cephalosporines [Archan Singh et al., 2011]. In this study most of *Klebsiella pneumonia* isolates were resistant to ampicillin – sulbactam (29,30 %) and followed by resistance to cephalosporines (17,80 %) and tetracycline (9,8 %).

Antibiotic resistance to *Proteus mirabilis* isolates ranged from 59.1 % for cotrimoxazole to 2.7 % for amoxicillin-clavulanic acid [Mirzaei A et al., 2019]. Overall we observed resistance to *Proteus mirabilis* isolates ranged from 23,33 % about co-trimoxazole and 5,88 % about amoxicillin clavulanic acid. *Enterococcus faecium* strains displayed resistance to ampicillin, imipenem, teicoplanin, vancomycin and high level gentamicin [Yaeghob Sharifi et al., 2013]. We identified the same high level resistance to ampicillin or gentamicin (16,04 %), followed by vancomycin (4,76 %) and teicoplanin (3,58 %). One case of Methicillin-resistant *Staphylococcus aureus* (MRSA) and one Vancomycin-resistant enterococci (VRE) were noticed.

**Conclusions**

Data presented in this study highlights the local situation regarding the incidence of bacterial species in UTIs, but also the antibiotic resistance profile of bacterial strains. In our study, *Escherichia coli* was the most frequent etiologic pathogen, being responsible for half of UTIs and the highest resistance in
terms of pathogens was found among this bacteria. This research found a high rate of resistance to ampicillin and followed by co-trimoxazole, cephalosporines and aminoglycosides. The resistance to antibiotics of bacterial strains is changing continuously all over the world, and for this reason AMR surveillance is very important for antibiotic prescription in the clinics, for updating local protocols, and for establishing an antibiotic management strategy.

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