ANTIBIOTIC RESISTANCE IN URINARY TRACT INFECTIONS IN CHILDREN

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Abstract

Introduction: These days, urinary tract infections (UTIs) represent the third cause of infection in children. Their treatment can be a problem in near future due to increasing antibiotic resistance.

Objectives: The aim of our study was to determine the prevalence of antibiotic resistance amongst pathogens causing UTIs in children admitted in the 1st Pediatric Clinic, Children Emergency Hospital, "Louis Ţurcanu," Timişoara, between January-June 2008.

Materials and methods: Inclusion criteria were clinical symptoms and significant bacteriuria (at least 100,000 colony-forming units/ml urine). The antibiograms were made from the first morning urine sample, based on the principle of dilution in agar, using MicroScan® WalkAway-96 system.

Results: The majority isolates were Escherichia coli (58%), followed by Klebsiella pneumoniae (21%) and Proteus mirabilis (9%). Female: male ratio was 2.45: 1. Pseudomonas aeruginosa and Klebsiella pneumoniae were prevalent in infants, while E. coli was found more often in puberty age girls. The results of the antibiograms showed a high percent of resistance to Ampicillin (86%), Sulfamethoxazole/Trimethoprim (74%) and to Cephalosporins (42-72%). The lowest resistance was identified to Imipenem, Fluoroquinolones and to beta-lactam/beta-lactamase inhibitor combinations. We identified that 25% of strains were beta-lactamases producers (65% Escherichia coli strains and 35% Klebsiella pneumoniae strains), from which 80% were ESBL-producing organisms.

Conclusions: Ampicillin and Sulfamethoxazole/Trimethoprim should be avoided for the use as the empiric treatment of UTIs. The high percent of multidrug resistant bacteria is increasing, so a complex observation program of antibiotics resistance should be imposed.

Key words: children, urinary tract infection, resistance, ESBL-producing strains

Introduction

The urinary tract infections (UTIs) are the most frequent bacterial infections found in children and an important cause of morbidity. Community acquired UTIs cause significant illness in the first 2 years of life. This are considered as common health problem in school and pre-school aged children[1]. In adults, persisting UTIs can lead to arterial hypertension and renal failure. Etiologic agents of UTIs are variable and usually depend on time, geographical location and age of patients. Although UTIs can be caused by any pathogenic organism from the urinary tract, the most frequent are from the Enterobacteriaceae family (Gram-negative bacilli, facultative anaerobic germs)[1].

Antibiotics used in the treatment of UTIs play an important role. Choosing an antibiotic depends on various factors such as the patient past medical records, past hospital reports of other illnesses, their spontaneous cure rates and their antibiotic-resistance charts, the frequently identified etiological agent, its antimicrobial sensitivity testing, its pharmacokinetics and its toxicity, the patients' age etc.

Antibiotic resistance is highly increasing these days. The epidemiology and the resistant patterns show a regional variability and prove to have a continuous change of frequency, due to excessive use of antibiotics. Studies show that the risk factors play an important role, for the emergence of the antibiotics resistance. Some of them are due to the mal-administration of the antibiotics in the past history, renal malformations associated and the frequent use of antibiotics for the prophylaxis of recurrent infections. However, many reports have indicated the presence of multidrug resistance in organisms causing UTIs[1,2]. The Gram-negative bacteria have a plasmid or chromosome resistance mechanism such as the presence of beta lactamses or enzymes that change of antibiotic structure, impermeability of outer membrane, efflux pumps, altered tagged molecules. Extended spectrum beta-lactamases (ESBL) are responsible for the secretion of new enzymes (cephalosporinases), which are capable of hydrolyzing all beta lactams, especially the last generation cephalosporins and Aztreonam. Amp C beta-lactamases are of two types plasmid-mediated and chromosomal or inducible. Chromosomal Amp C enzymes are typically inducible by beta lactam antibiotics such as Cefoxitin and Imipenem, but poorly induced by the 3rd or 4th generation Cephalosporins. The transposition of Amp C gene (responsible for producing novel plasmid-mediated beta lactamas enzymes) in Escherichia coli and Klebsiella pneumoniae strains determine the emergences of antibiotic resistance[1,2,3,4].

Objectives

The objectives of this retrospective study were to determine the prevalence of antimicrobial resistance against urinary tract pathogens and to identify the patterns of resistance in children admitted in 1st Pediatric Clinic, "Louis Turcanu" Children Emergency Hospital, Timişoara, during

January-June 2008. Hence, the result of this study can help direct empirical therapy in UTIs for future.

Materials and methods

The study lot comprised of 356 urocultures from children less than 18 years old admitted in 1st Pediatric Clinic during January-July 2008. The identification of fermentative and non-fermentative Gram-negative bacteria was made from the first morning urine sample, after sowing on agar plate, based on conventional biochemistry and cromogenic tests.

Antibiograms were performed using the MicroScan® WalkAway-96 Dade Behring (Sacramento, California), computerized system that allows automatic determination of minimum inhibitory concentration (MIC) or qualitative susceptibility for the test organism (sensitive S, resistant R or intermediate I). This technique was based on the principle of agar dilution. MicroScan® Dried Gram Negative Panels containing antibiotics in increasing concentrations were used. Some well-isolated colonies from 18-24 hour non-inhibitory agar plate were emulsified with Inoculum water, so the final turbidity should be equivalent to that of a 0.5 McFarland Barium Sulfate turbidity standard. The suspension was subsequently transferred to Mueller Hinton broth enriched with calcium and magnesium. After incubation at 35°C for 16-18 hours in a CO_2 environment, the MIC is determined, by observing the lowest antimicrobial concentration showing inhibition of growth. For quality control, strains of Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Klebsiella pneumonia ATCC 700603 were used. Reading and interpreting the results were made by the current NCCLS standards.

The antibiotics tested were Ampicillin, Cephalothin Cefuroxime, Ceftazidime, Cefepime, Ceftriaxone, Cefazolin, Gentamicin, Amikacin, Tobramycin, Trimethoprim/Sulfamethoxazole, Ampicillin/Sulbactam, Amoxicillin/ Clavulanate, Ticarcillin/Clavulanate, Piperacillin/Tazobactam, Imipenem, Ciprofloxacin, Levofloxacin.

The MIC of Ceftazidime, Cefotaxime, Ceftriaxone, Cefpodoxime and Aztreonam were used to identify beta lactams strains (ESBL and AmpC type) after comparison with the recommended NCCLS standards (Table 1). Cephamycin differentiates between ESBLs and AmpC beta lactams: it's an ESBL strain, if it is sensible to Cephamycin; and if the germ was resistant, it was an Amp C type.

Table 1 NCCLS standard for identification of ESBL and Amp C betalactamses[1]

Beta lactams	Cefpodoxime	Ceftazidime	Ceftriaxone	Cefotaxime	Cefotetan	Cefoxitin
ESBL	≥2µl/ml	≥4 µl/ml	≥4 µl/ml	≥4 µl/ml	≤16 µl/ml	≤16 µl/ml
Amp C	≥2µl/m	≥4 µl/ml	≥4 µl/ml	≥4 µl/ml	>32 µl/ml	>32 µl/ml

Results

From the 356 urocultures made in the hospital laboratory in the first half of 2008, 55 children were diagnosed with urinary tract infections, verified both by the clinical symptoms (fever, dysuria, pain in the abdomen and pelvic area) and the laboratory data (leukocytosis, leukocyturia, significant bacteuria with growth of at least 100.000 colony-forming units/ml of urine).

The most identified germs were Escherichia coli (58%), followed by Klebsiella pneumoniae (21%), Proteus mirabilis (9%) and Pseudomonas aeruginosa (5%). Other rare bacteria found were Kluyvera ascorbata, Enterobacter aerogenes, Enterococcus faecium, Raoultella ornithinolytica**.** UTIs dominated in female children than males (70.90% versus 29.09%). There were 17 infants (30.90%), 13 (23.63%) children with age between 1-10 years and 25 (45.45%) teenagers. Pseudomonas and Klebsiella were seen in 71.42% of infants, while E. coli was found in 77.14% of puberty age girls.

The highest rate of resistance was seen to Ampicillin (86%), Trimethoprim/Sulfamethoxazole (74%), Cephalothin (72%) and Ampicillin/Sulbactam (70%). (Figure 1).

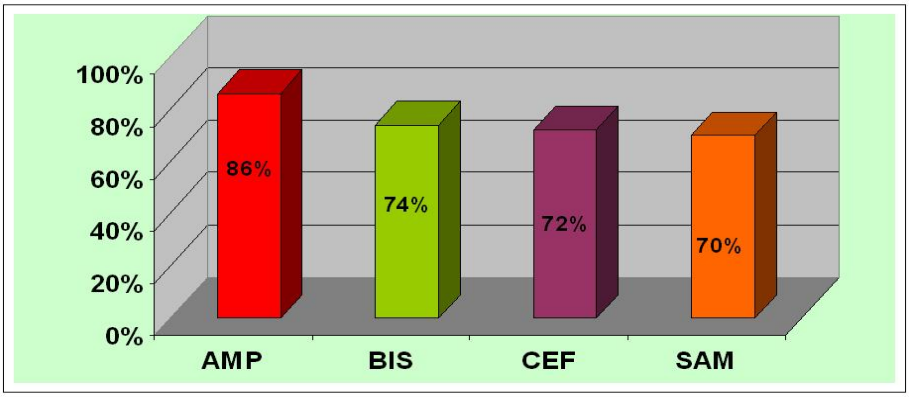

Figure 1 High rates of antibiotics resistance found in our study (AMP- Ampicillin, BIS- Trimethoprim/Sulfamethoxazole, CEF-Cephalothin, SAM-Ampicillin/Sulbactam).

From the Cephalosporins, the 1st generation was found to have more resistance (Cephalothin 72%, Cefazolin 58%). In addition, we observed that there were not significant differences between the Cephalosporins generations concerning resistance, the percentages being between 42-47%. The lowest resistance was identified to Imipenem 5%, followed by Fluoroquinolones (Ciprofloxacin and Levofloxacin) 14%, beta-lactam/beta-lactamase inhibitor combinations (Amoxicillin/Clavulanate, Ticarcillin/Clavulanate and Piperacillin/Tazobactam) 16% and Aminoglycoside (Amikacin) 24% (Figure 2).

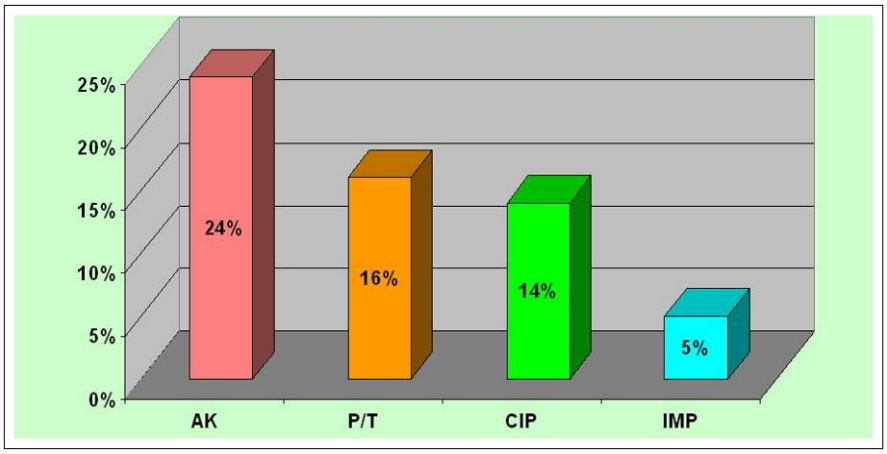

Figure 2 Low rates of antibiotics resistance encountered in our study.
(AK-Amikacin, P/T-Piperacillin/Tazobactam,
CIP- Ciprofloxacin, IMP- Imipenem).

We compared the most frequently used antibiotics in UTIs with the identified bacteria from our study. Klebsiella pneumoniae showed 100% resistance to Ampicillin. In this case, it is a well-known resistance. We did not find any strain of Klebsiella resistant to Ciprofloxacin. E. coli had the lowest resistance to Trimethoprim/Sulfamethoxazole (61%), fact that permits us, for its use in prophylactic treatment of recurrent urinary tract infections. The first line antibiotic therapy for E. coli infections should be a beta-lactam/beta-lactamase inhibitor combinations (Piperacillin/Tazobactam) with a resistance rate of 3%. Proteus was sensitive to Ciprofloxacin and Piperacillin/Tazobactam (81%, 100%, respectively). (Figure 3).

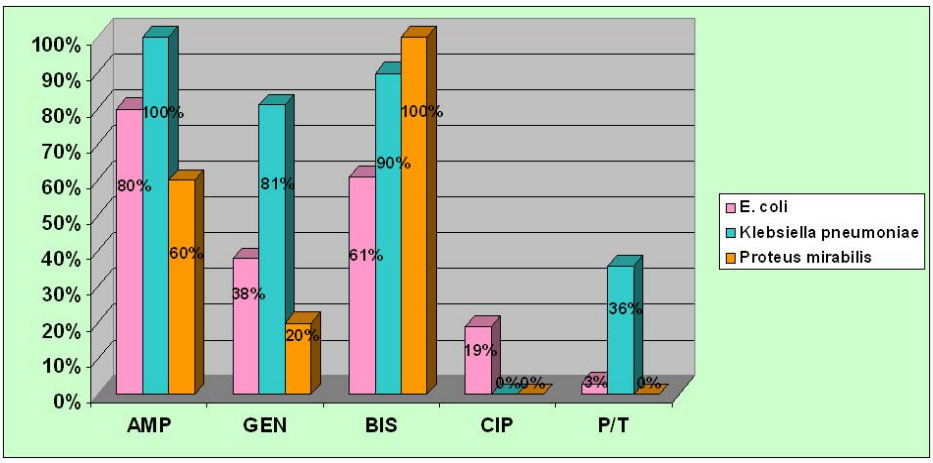

Figure 3 Distribution of antibiotics resistance between Gram-negative bacteria.
(AMP- Ampicillin, GEN-Gentamicin, BIS- Trimethoprim/Sulfamethoxazole,
CIP-Ciprofloxacin, P/T-Piperacillin/Tazobactam)

According to NCCLS standards for identification of beta lactamases (Table 1), 14 strains were identified. 9 strains were E. coli and the rest were Klebsiella pneumoniae. The MIC<16 µl/ml of Cefotetan and Cefoxitin permitted the identification of 11 strains with extended beta-lactamases spectrum (ESBL), from which 7 were E. coli types and the rest were Klebsiella pneumoniae producing high level of SHV-1 beta lactamases[1]. Hence Amp C

producing strains are revealed by CMI>32µl/ml of Cefoxitin and Cefotetan, 3 strains were discovered (two E. coli strains and one Klebsiella pneumoniae). Resistance phenotypes to Tetracycline and Trimethoprim/Sulfamethoxazole were also encountered.

Discussions

Management of children with UTIs implies a proper documentation of diagnosis, identification of the associated anatomical abnormalities, a correct antibiotic therapy according to antibiograms and a follow-up of children with renal malformations.

Enterobacteriaceae strains were the dominant bacterial species isolated from urine cultures that was in agreement with previous works[1,2]. Gram-positive cocci had a comparatively low contribution in causing UTIs. Studies reported that 90% of all acquired community UTIs and more than 30% of nosocomiale acquired UTIs are caused by E. coli. According to the demographic data, females are affected more often than males due to anatomical differences[1]. We found similar results in our study.

Trimethoprim/Sulfamethoxazole, frequently used in the prophylaxis treatment of recurrent UTIs in children for its tolerability, availability, pharmacokinetics and urinary pathogens spectrum, presented a high percentage of resistance (74%). Medical studies made in 3 different hospitals[1] showed an increasing rate of resistance to Ampicillin, Trimethoprim/Sulfamethoxazole and 1st generation Cephalosporins, similar to that documented in our study. Compared to other studies made in Europe, the resistance found to these antibiotics was higher that found in our study (Ampicillin 62%, Trimethoprim/Sulfamethoxazole 46%)[9,1].

Comparing our results with the literature data[1,2] we observed a higher resistance to Cephalosporins, which indicates an excessive use of this class of antibiotic in our hospital. The resistance to Cephalosporins is explained through the enzymatic mechanisms and efflux pumps[5,8]. The resistance rate to Aminoglycoside (Amikacin 24%, Gentamicin 47%) in our study was low, which was similar to some cohort studies made on urine samples of children with UTIs[1].

According to our study, the first line antibiotics to be used for the treatment of UTIs when the causative pathogen is unknown should be beta-lactam/beta-lactamase inhibitor combinations (Ticarcillin/Clavulanate or Piperacillin/Tazobactam). Although they presented a low rate of resistance 13% and 14%, Fluoroquinolones are used, only in some selected cases, in order to prevent the negative effects on growth cartilage. This was proved by experiments made on young animals, which showed it to have adverse effects on bone cartilage and had tendon lesion after using these antibiotics[1]. Imipenem is a broad-spectrum beta lactamase antibiotic, active against all kinds of bacteria (Gram-positive and Gram-negative bacteria, aerobic and anaerobic germs). It is the main anti-pseudomonas antibiotic, reason for which it is used in special cases, which appear to be more severe in order to avoid the emergence of resistance to Carbapenems.

Pediatric urinary tract colonizing bacteria are becoming increasingly resistant to commonly used antibiotics. In our clinic, resistance to 3rd generation Cephalosporins through the acquisition and expression of ESBL among Enterobacteriaceae was frequently seen. ESBL phenotypes have become more complex due to the production of multiple enzymes including inhibitor-resistant TEM enzymes, AmpC, enzyme hyperproduction and porin loss[1,2,3]. The high resistance levels found could be explained though the high frequency of Cephalosporins used for both prophylactic and therapeutic treatment of hospitalized children. This practice may have exerted selective pressures leading to the emergence of multidrug resistant strains, which in turn may have stimulated the acquisition of genes encoding resistance mechanisms via horizontal transfer mechanisms between bacterial strains within the hospital environment[1]. Genotypic methods based on enzyme assays, PCR and others are not suitable for routine clinical testing. The clinical manifestations of ESBLs are extremely serious hence sensitive diagnostic methods are urgently required to guide therapy, then to monitor resistance development and to implement intervention strategies are a must.

The results of this study showed that the rate of resistance to widely used antibiotics was high for Gram-negative bacteria. The most effective antibiotics against Gram-negative bacteria were Carbapenems, Fluoroquinolones and beta-lactam/beta-lactamase inhibitor combinations. Cefuroxime, Trimethoprim/Sulfamethoxazole, Ampicillin/Sulbactam, Gentamicin which is most frequently used for the treatment of community acquiring UTIs was found to be the least sensitive antibiotics for the Gram-negative microorganisms. The most important reason for resistance to antibiotics is the widespread use of antibiotics in hospitals. In order to prevent or decrease resistance to antibiotics, the use of antibiotics should be kept under supervision and always thought thoroughly before initiating, further on if found necessary it should be given in appropriate doses for an appropriate period of time and control programmes for hospital infections should be carried out periodically. A multidisciplinary approach should be used to achieve the above-mentioned goals.

Conclusions

The UTIs represent the third major cause of infections in children (after the respiratory and digestive tract infections), which when treated incorrectly can lead to some irreversible serious complications. The treatment of UTIs must be initiated with a broad-spectrum antibiotic and after obtaining the antibiotic susceptibility test results, treatment should be changed accordingly. Ampicillin and Trimethoprim/Sulfamethoxazole were frequently used for UTIs in the past but are no longer used as the first line antibiotics because of their high rate of resistance. The results of this study leads to the conclusion that empirical therapy in UTIs should consist in beta-lactam/beta-lactamase inhibitor combinations. Imipenem and Fluoroquinolones should be used only in particular cases in order not to loose its sensitivity. The high rate of multidrug

resistant bacteria seen in our study, points out the fact, that frequent and excessive use of antibiotics should be avoided in day-to-day practice, to provide a better healthy future to children of all ages.

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