In Vivo Susceptibility of ESBL Producing *Escherichia Coli* to Ceftriaxone in Children with Acute Pyelonephritis

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SUMMARY

Introduction The choice of empiric therapy of acute pyelonephritis (APN) in children should be based on the knowledge of *Escherichia coli* (E. coli) as the most common uropathogen and its antibiotic sensitivities considering that nowadays ESBL-producing [ESBL (+)] E. coli is on the rise worldwide.

Objective To examine in vivo susceptibility of ESBL (+) E. coli to ceftriaxone (CTX), and to evaluate the options for empiric therapy for APN in children.

Methods Retrospective study of CTX empiric therapy of APN in children treated at the University Children's Hospital in Belgrade from January 2005 to December 2009. ESBL phenotypic confirmatory test with ceftazidime, CTX and cefotaxime was performed for all urine isolates by disc diffusion method on Mueller-Hinton agar plates. In vivo sensitivity of CTX documented by clinical response to empiric CTX therapy was compared between two groups of children: group I with ESBL (+) E. coli and group II with ESBL (-) E. coli APN.

Results Group I with ESBL (+) APN consisted of 94 patients and group II of 120 patients with ESBL (-) APN, respectively. All patients received CTX as empiric therapy at a mean dose of 66.9 mg during 7.2±2.6 days of therapy. Clinical effect of CTX was similar in patients with ESBL (+) compared to those with ESBL (-) APN.

Conclusions In vitro resistance of ESBL E. coli to CTX determined by standard methods is not sufficiently predictive for its in vivo sensitivity. Therefore CTX may be used as empiric therapy for acute pyelonephritis in children.

Keywords: empiric antibacterial therapy; acute pyelonephritis; *Escherichia coli*; extended spectrum β-lactamases; children

INTRODUCTION

Urinary tract infection (UTI) is common in childhood, second only in frequency to that of the respiratory tract [1, 2, 3]. Depending on the localization of the infection (lower or upper urinary tract and renal parenchyma), severity of its clinical presentation and possible acute and long-term complications, UTI may be described as either acute cystitis or as acute pyelonephritis [4-7].

Acute pyelonephritis may result in renal scarring, which can predispose patients to long-term complications including toxaemia during pregnancy, hypertension and chronic renal failure later in life. Prompt treatment of childhood acute pyelonephritis is likely to reduce the risk of permanent scarring [8]. Therefore, if there is a high clinical suspicion of acute pyelonephritis, empiric antibiotic therapy is realistic while awaiting urine culture results. When faced with increased urinary pathogen resistance [9, 10] the first choice of an anti-microbial agent for empiric treatment of paediatric UTI is often uncertain.

Based on anti-microbial resistance data in the literature [11-14] treatment with ceftriaxone (CTX), a third-generation cephalosporin, as preferable empiric therapy has been practiced in children with acute pyelonephritis treated in Serbia from 2005 onwards. However, the emergence of uropathogen strains producing extended spectrum β-lactamases (ESBLs) has threatened the empirical use of third-generation cephalosporins. Despite this, they still appear to be effective in the treatment of UTI. Accordingly, it is not yet clear whether in vitro resistance to CTX determined by standard methods presents an excluding factor for its use as empiric therapy of acute pyelonephritis in children.

OBJECTIVE

The primary objective of this study was to examine in vivo susceptibility of ESBL producing *Escherichia coli* (E. coli) to ceftriaxone, and secondary to evaluate the options of empiric therapy for acute pyelonephritis in children.

METHODS

The medical records from January 2005 to December 2009 of all children treated by CTX as empiric drug at the Nephrology or Neonatology Department of the University

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Children's Hospital in Belgrade for their first UTI were reviewed. The patients who met the following criteria were included in the study: fever higher than 38.5 °C with no other recognized cause, leukocytosis, C reactive protein (CRP) higher than 20 mg/l, positive dipstick for leukocyte esterase and/or piuria (urine specimen with ≥10 white blood cells (WBC)/(high power field) (hpf), isolation of more than 10^5 colony-forming units (CFUs)/ml of E. coli in a urine sample obtained by midstream clean catch or sterile bags, and empiric antibiotic therapy with CTX. Those receiving antibiotics within the previous 7 days, immunosuppressed children and those with history of previous UTI were excluded from the study. Analyzed data included age, gender, UTI symptoms, treatment and outcome as well as a history of infections, antibiotic therapy and hospitalization during the last 3 months and urinary tract imaging were recorded for each patient. Ultrasonography performed within 72 h of admission into hospital was required for all patients, while voiding cysto-urethrography (VCUG) and Tc-99m DMSA scintigraphy (DMSA scan) were optional at the treating physician's request and parents' decision.

All urine samples were obtained in hospital by health care personnel. Contaminated specimens were discarded from the study. Standard methods for isolation and identification of the isolates were used. Anti-bacterial susceptibility testing of the isolates was performed by the standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [13]. ESBL phenotypic confirmatory test with ceftazidime, ceftriaxone and cefotaxime was performed for all isolates by disc diffusion method on Mueller-Hinton agar plates. A ≥5 mm increase in a zone diameter for antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was considered indicative of ESBL production. The methods used did not vary throughout the study period. No tests were performed to further characterize the clonal origin of isolates.

For all patients repeat urine and urine cultures were performed after 48-72 h and blood WBC and CRP were done within 5-7 days of empiric CTX therapy. The clinical effect of CTX was evaluated by analyzing the response of clinical (fever, WBC and CRP) and urine parameters (urine WBC and urine culture) to empiric CTX therapy. In addition, the clinical outcomes of ESBL (+) and ESBL (-) UTI were compared. A successful treatment (in vivo sensitivity) was defined by resolution of fever, sterile control urine cultures at ≤72 hours and decreasing trend in leucocytes, CRP and urine WBC within 5-7 days.

Statistical analysis

SPSS 13 for Microsoft Windows was used for all statistical analyses. Results for continuous variables were presented as mean (±SEM). The Fisher's exact test was used to compare categorical variables. The Mann-Whitney U test was used for continuous variables. P value <0.05 was considered to be statistically significant.

RESULTS

Clinical characteristics

A total of 335 children received CTX as empiric therapy for the first UTI between the 1st of January 2005 and the 31st of December 2009. Of these, 214 fulfilled study inclusion criteria. The patients were divided in two groups based on the ESBL phenotypic characteristics of the isolated E. coli: group I consisted of 94 patients diagnosed with UTI due to ESBL-producing E. coli (ESBL (+) UTI), and group II consisted of 120 patients with E. coli ESBL (-) UTI. Renal ultrasound was performed in all patients, while VCUG and DMSA scan were done in 65.9 % and 31.9 % of the patients from group I and in 65% and 33.3 % of the patients from group II, respectively. Data for evaluating infections, use of antibiotics and hospitalization in the last 3 months were available in 79.8% of the patients from group I and 88.8% of the patients from group II. The percentage of urine specimen-obtaining methods for urine culture, midstream clean catch urine and sterile bags were quite comparable between groups; 64% and 36% in group I and 65% and 35% in group II, respectively.

Clinical characteristics of the patients are shown in Table 1. The patients with ESBL (+) UTI were younger and had higher CRP before therapy than the patients with ESBL (-) UTI. Nevertheless, the groups were well-matched according to gender, dose of CTX and to the available data for underlying risk factors for ESBL (+) UTI including renal ultrasound abnormalities, vesicoureteral reflux, infections in the last 3 months, use of antibiotics in the last 3 months, and hospitalization in the last 3 months. Moreover, the clinical parameters of acute pyelonephritis (Table 2), as well as the percentages of the patients with acute pyelonephritis documented on DMSA scan (Table 1) were similar between the two groups of patients.

Table 1. Clinical characteristic of the patients

Characteristic	Group I (n=94)	Group II (n=120)	p
Gender – male/female (%)	48.9/51.1	44.2/55.8	NS
Age (months)	5.9±0.8	10.5±1.3	<0.01
CRP (mg/L)	86.01±6.4	63.8±4.7	<0.01
Dose of ceftriaxone (mg/kg/24 h)	68.7±14.6	65.4±15.9	NS
Renal ultrasound abnormalities (%)	34.5	28.8	NS
Acute pyelonephritis documented by DMSA scan (%)[a]	31.9	33.3	NS
VUR (%)[b]	27.4	32.4	NS
History of infections in last 3 months (%)[c]	10.7	13.0	NS
Use of antibiotics in last 3 months (%)[c]	8.0	13.4	NS
History of hospitalization in last 3 months (%)[c]	2.7	1.0	NS

CRP – C-reactive protein; NS – not statistically significant
[a] Acute pyelonephritis on DMSA scan was found in all tested patients: 30 patients in group I and 40 patients in group II
[b] Voiding cystourethrography was done in 62 patients from group I and in 78 patients with group II
[c] Data were available in 75 patients from group I and in 97 patients from group II

Table 2. Clinical effect of cefrtiaxone therapy

Characteristic		Group I (n=94)	Group II (n=120)	p
Before therapy	Temperature (°C)	39.0±0.6	39.3±0.6	NS
	WBC in blood (10^3/mm^3)	18.5±0.7	19.0±0.7	NS
	Neutrophils (%)	54.9±2.05	53.7±1.6	NS
	CRP (mg/L)	86.01±6.4	63.8±4.7	<0.01
At ≤72 h of therapy	Resolution of fever (% of patients)	81.6	85.3	NS
	Sterilisation of urine culture with 72 h (%)	87.5	86.0	NS
At 5-7 days of therapy	WBC in blood (10^3/mm^3)	9.9±0.4	10.1±0.6	NS
	Neutrophils (%)	30.6±2.6	34.0±3.03	NS
	CRP (mg/L)	12.7±1.9	13.05±2.1	NS
	Urine ≤15 WBC/ hpf (%)	80.3	83.5	NS

WBC – white blood cells; CRP – C-reactive protein; hpf – high power field on microscopic examination; NS – not statistically significant

Treatment outcome

All patients received parenteral CTX as empiric therapy at a mean dose of 66.9 mg (range 43-100 mg/kg) during 7.2±2.6 days of therapy. Almost all patients with ESBL (+) UTI (87.5 %) responded by sterilization their urine culture during the first 48-72 h. Therefore, most of them (85.4 %) continued under the same drug even if in vitro resistance was recognized. Clinical effect of CTX was similar in the patients with ESBL (+) compared to those with ESBL (-) UTI (Table 2).

DISCUSSION

To start empiric therapy of UTI is important in febrile children as the delay of the antibiotic therapy increases the chance of the acute and long term kidney injury [7, 8]. The choice of empiric therapy should be based on the knowledge of E. coli as the most common uropathogen (72-96%) and its antibiotic sensitivities, considering that nowadays ESBL-producing E. coli is on the rise worldwide [12, 13, 16, 17].

At present, most common options for empiric therapy of UTI in children includes third-generations of cephalosporins [11-13] for which ESBL-E. coli is by definition resistant [18]. In addition, multidrug resistance which is common in ESBL (+) E. coli [17, 19] seriously affects the management of children with UTI. On the other hand, there are restrictions against the routine use of some drugs in paediatric patients due to their side effects, such as are for fluoroquinolones [20], or due to the limited experience, as is the case for fosfomycin sodium [21]. Currently, carbapenem-resistant E. coli are rarely isolated, but these drugs should be used only for severe acute infections. Consequently, paediatricians are remained with limited options for empiric therapy of acute pyelonephritis in children. Fortunately, the clinical response of ESBL (+) E. coli to antibiotics seems to be much better than their in vitro sensitivity [22, 23]. Although very important from the practical side, this topic remains unclear primarily because there have not been prospective studies designed specifically to evaluate clinical outcomes among a statistically meaningful number of patients with ESBL- producing E. coli. According to the existing data from the literature, it is apparent that there is a disagreement with regard to the role of third-generation cephalosporin treatment in outcome [22, 24]. Although ceftazidime treatment was always associated with treatment failure, a favourable response to treatment with a third-generation cephalosporin other than ceftazidime was observed for cases in which the ESBL was identified as TEM-6 or TEM-12; these 2 ESBLs have relatively weaker hydrolytic activity against extended-spectrum cephalosporins [25].

Our study is based only on clinical data from practice. Therefore, it lacks the extensive investigations of the genetic and/or enzyme types of E. coli. We examined microbiological in vitro versus clinical in vivo susceptibility of ESBL E. coli to CTX in children with acute pyelonephritis. Our results demonstrated that the clinical response (in vivo sensitivity of E. coli) to CTX was similar in the children with ESBL (+) UTI compared to those with ESBL (-) UTI. More than 80% of patients in whom ESBL- producing E. coli was identified were successfully treated with this drug. Thus, CTX could be effective for the treatment of UTI even when in vitro susceptibility testing suggests ESBL (+) E. coli. It means that in vitro resistance of E. coli to CTX documented by standard methods was not sufficiently predictive for its in vivo resistance. This may be due to the fact that the drug is concentrated in urine, while susceptibility testing is mostly based on blood concentration determinations. Urinary concentrations of anti-microbial agents enable bactericidal levels to be achieved despite apparent in vitro resistance.

According to our findings, CTX may be the first line therapy of acute pyelonephritis in children, although this premise should be analyzed prospectively.

Our analysis has some limitations. The greatest limitation of this study is its retrospective design; it was not possible to identify underlining risk factors associated with ESBL (+) strains for all patients. Also, sterile bags or midstream clean catch urine is not the method of choice to obtain sterile urine in infants and children. However, the strictly matched both groups of patients makes it easier to balance the confounding factors. Urine samples were obtained in hospital by health care personnel and the collections of data as well as the laboratory methods were the same for both groups of patients. In addition, selection criteria included patients in whom the diagnosis of acute pyelonephritis was made solely on clinical grounds, while confirmative renal DMSA scan was done in only one-third of patients in both groups. Nevertheless, according to the results of CRP before therapy, our patients with ESBL (+) UTI had more severe UTI than the patients with ESBL (-), but the response of therapy was comparable in both groups which carried more evidence for in vivo susceptibility of ESBL E. coli to ceftriaxone. Finally, ESBL-producing E. coli testing in our study did not include identification of its enzyme

specific variations. By all means, our findings warrant a prospective multicentre evaluation.

CONCLUSION

Microbiological in vitro resistance of ESBL E. coli to ceftriaxone determined by standard methods is not sufficiently predictive for its in vivo sensitivity. Therefore it is not an excluding factor for the use of CTX as empiric therapy for acute pyelonephritis in children.

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Ефекат цефтриаксона *in vivo* на бактерију *Escherichia coli* која ствара *ESBL* код деце с акутним пијелонефритисом

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КРАТАК САДРЖАЈ

Увод Избор емпиријске терапије код деце с акутним пијелонефритисом (АПН) треба базирати на чињеници да је бактерија *Escherichia coli* (*E. coli*) најчешћи узрочник и да су њени сојеви који стварају проширени спектар бета-лактамазе [*ESBL(+)*] све распрострањенији у свету.

Циљ рада Циљ истраживања био је да се испита клиничка осетљивост *ESBL(+) E. coli* на цефтриаксон и размотри могућности емпиријске терапије код деце са АПН.

Методе рада Ретроспективно је испитан клинички ефекат цефтриаксона код деце која су лечена од АПН на Универзитетској дечјој клиници у Београду од јануара 2005. до децембра 2009. године. *ESBL(+) E. coli* је дијагностикована помоћу диск-дифузионе методе на Милер–Хинтоновим (*Mueller–Hinton*) подлогама с агаром и антимикробних лекова, цефтазидима, цефтриаксона, цефотаксона и клавулонске киселине. Сензитивност цефтриаксона *in vivo*, документована клиничким одговором на емпиријско лечење овим леком, упоређена је између две групе деце са АПН: једне са *ESBL(+)* и друге са *ESBL(-) E. coli*.

Резултати Прву групу чинила су 94 болесника, док је другу групу чинило 120 деце. Сви болесници су примали цефтриаксон као емпиријску терапију у средњој дози од 66,9 *mg* током 7,2±2,6 дана. Клинички ефекат овога лека се није разликовао међу групама посматраних болесника са АПН.

Закључак Микробиолошка резистенција *ESBL E. coli* у условима *in vitro* на цефтриаксон одређена стандардном методом није довољно предиктивна за његову сензитивност *in vivo*. Према томе, цефтриаксон се може користити као емпиријска терапија код деце са АПН.

Кључне речи: емпиријска антибактеријска терапија; акутни пијелонефритис; *Escherichia coli*; бета-лактамазе проширеног спектра (*ESBL*); деца

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