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BLOODSTREAM INFECTIONS IN CHILDREN CAUSED BY EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE*

DEANA MEDIĆ, VERA GUSMAN, MIRA MIHAJLOVIĆ-UKROPINA, ZORA JELESIĆ and BILJANA MILOSAVLJEVIĆ

Institute of Public Health of Vojvodina, Center for Microbiology, 21000 Novi Sad, Serbia

Abstract - The aim of this study is to determine the prevalence of extended spectrum beta-lactamase (ESBL) producing *Klebsiella pneumoniae* strains isolated from blood in children and their susceptibility to antimicrobial drugs commonly used in the therapy. The study was conducted at the Institute of Public Health of Vojvodina Province, Serbia, in a two-year period, from January 2009 to December 2010. A total of 424 non-duplicate strains were isolated from the blood of pediatric patients hospitalized in various wards in the Institute of Health Care of Children and Youth of Vojvodina Province. Fifty isolates of *Klebsiella pneumoniae* were reported. The frequency of isolation of *Klebsiella pneumoniae* was 27/222 (12.2%) and 23/202 (11.3%) isolates in 2009 and 2010, respectively. There was a high prevalence of ESBL-producing *Klebsiella pneumoniae*, 76% (38/50), and 17 isolates (44.7%) were multidrug resistant (MDR). Further drug resistance surveillance in hospitals and the molecular characterization of ESBL-positive isolates in our country is necessary.

Key words: Klebsiella pneumoniae, bloodstream infection, antimicrobial resistance, extended spectrum beta lactamase (ESBL), children

INTRODUCTION

Symptomatic bacteremia is a common condition in children and requires urgent rational antibiotics therapy. It is also a significant cause of high morbidity and mortality (Ogunleye et al., 2005).

Klebsiella pneumoniae, a gram-negative bacterium, was the most common causative agents in pediatric bloodstream infection (Arnoni et al., 2007). Risk factors for colonization and infection are recent surgery, central venous catheters, tracheotomy, parenteral nutrition, and uncontrolled use of broad-spectrum antibiotics (third generation cephalosporins, fluoroquinolones, carbapenems). Reservoirs of infection are the intestinal and respiratory tracts of hospitalized patients and hospital staff, and the way of transmissions is by the hands of the staff or contaminated medical equipment and fluids (Zakaria et al., 2008).

Due to the irrational use of antibiotics, *K. pneumoniae* isolated from blood culture is mostly multidrug resistant (resistant to three or more antibiotic classes), often producing beta-lactamases (Kang et al., 2004).

Of great concern is the emergence of *K. pneumoniae* strains producing extended spectrum beta-lactamases (ESBLs). ESBL was first identified in Western Europe (France and Germany) in the mid-1980s (Gagliotti et al., 2008) and is capable of hydrolyzing oxyimino-cephalosporins and inhibited by β-lactamase inhibitors. These enzymes are not active against carbapenems (Ullah et al., 2009). Until now, because of mutations, more than 70

ESBLs have been described and most of them are members of the TEM-1, TEM-2 and SHV-1 family of enzymes (Mumtaz et al., 2011). During the past decade, a CTX-M-type of ESBLs has emerged in many countries worldwide, and it is the most commonly found in Enterobacteriaceae (Mirzaee et al., 2009). The presence of ESBL-producing isolates is an indicator of the selective pressure of 3rd generation of cephalosporins, which are extensively used in hospitals.

Bloodstream infections caused by ESBL-producing bacteria have been associated with longer, more costly hospital stays and high mortality rates (Nwadioha et al., 2010; Velaphi et al., 2009).

The plasmid encoding ESBLs frequently also encodes a resistance to other antimicrobial classes, such as fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, leaving few therapeutic options available for treatment (Mumtaz et al., 2011).

Carbapenems, which are stable to most of the prevalent beta-lactamases, including ESBLs, are considered the agents of choice for treatment of serious infections like bloodstream infections caused by multiresistant *K. pneumoniae* (Nadkarni et al., 2009).

A new group of class A beta-lactamases, known as KP-type carbapenemases, has been described in northeastern USA in 1996. They quickly spread and have often appeared in Israel and Greece and other European countries (Marschall et al., 2009; Woodford et al., 2004; Giakkoupi et al., 2009; Samuelsen et al., 2009; Grundmann et al., 2011). These enzymes were serine carbapenemases KPC (*Klebsiella pneumoniae* carbapenemase).

Since 2008, a novel metallo-beta-lactamase encoded by the bla_{NDM-1} gene produced by Enterobacteriaceae was named New Delhi metallo-beta lactamase-1 (NDM-1) (Yong et al., 2009). The isolates producing this enzyme have been imported repeatedly into Europe from the Indian subcontinent, particularly the United Kingdom, but also into Canada, Japan, Africa and Australia (Rolain et al., 2010). Most of them were resistant to all antibiotics, except polymyxins, tigecycline, aztreonam, and occasionally certain aminoglycosides. NDM-1 producers are mainly *K. pneumoniae* strains (Grundmann et al., 2011; Yong et al., 2009).

In order to formulate the policy of empirical therapy in high-risk units where infections due to resistant organisms are much higher, it is necessary to investigate the frequency of ESBL- and KPC-positive strains in hospitals.

This study was designed to investigate the prevalence of ESBL-producing *K. pneumoniae* strains isolated from blood in children and their susceptibility to antimicrobial drugs commonly used in the therapy.

MATERIALS AND METHODS

Study design and specimen types

The study was conducted at the Center for Microbiology of the Institute of Public Health of Vojvodina Province, Serbia. Samples of blood cultures from children younger than 18 years of age hospitalized in various pediatric departments in the Institute of Health Care of Children and Youth of Vojvodina Province, were collected from January 2009 to December 2010. During the two-year study period, 5397 blood samples were processed: 2565 samples in 2009. and 2832 in 2010. A total of 50 non-duplicate strains of *K. pneumoniae* were isolated.

Microbiological methods

All blood samples were routinely cultured in pediatric blood culture bottles (BioMérieux, Marcyl' Etoile, France) using a semi-automated blood-culture system (BacT/Alert, BioMérieux, Marcyl' Etoile, France). The positive samples were inoculated onto blood agar and MacConkey agar plates (HiMedia, India) that were incubated aerobically for 24 h at 37°C. Isolation and identification of the causative organisms were performed by standard microbiological methods.

Antimicrobial susceptibility testing

Susceptibility to antimicrobial agents was tested by standard disk-diffusion on Mueller-Hinton agar (HiMedia, India) using antibiotic discs BioRad, USA. The performance and interpretation were based on the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2009; CLSI, 2010).

The following antimicrobial agents were tested: ampicillin, cefalotin, ceftriaxone, ceftazidime, amoxicillin/clavulanic acid, piperacillin/tazobactam, meropenem, imipenem, ertapenem, gentamicin, amikacin, co-trimoxazole and ciprofloxacin. *Escherichia coli* American Type Culture Collection (ATCC) 25922 and *Staphylococcus aureus* ATCC 25923 were used for quality controls.

Detection of extended spectrum beta lactamases (ESBL) production

The initial screening and phenotypic confirmatory test were recommended by the CLSI (CLSI, 2009; CLSI, 2010). In this test, a disc of amoxicillin/clavulanic acid was placed in center of the Petri dish already inoculated with the test microorganism, while cefotaxime, ceftazidime and ceftriaxone discs were placed at a distance of 20-25 mm (center-to-center) from the amoxicillin/clavulanic acid disc on the same plate. After incubation of 18-24 h at 37°C, zones of inhibition around the 3rd generation cephalosporin discs were observed. The microorganism was labeled as ESBL-positive if the zone of inhibition around one or more cephalosporin discs was extended on the side nearest to the amoxicillin/clavulanic acid.

The double-disk synergy test was used as the phenotypic confirmatory test, where the tested bacteria were inoculated on Mueller-Hinton agar and discs of cefotaxime and ceftazidime separately, and each of these in combination with clavulanic acid were placed on the surface of the plate. The microorganism was considered a ESBL-positive isolate if there was a difference of ≥5mm between the zones of inhibition of a single antibiotic and the disc with combination of antibiotic and clavulanic acid (CLSI, 2009; CLSI, 2010).

Statistical analysis

The results were analyzed using SPSS (Statistical Package for the Social Sciences) for Windows. The chi-square ($\chi 2$) test was used to compare associations between proportions and p values <0.05 were considered significant.

RESULTS

K. pneumoniae was the second leading isolated microorganism, and the first among Gram-negative isolates out of the total 424. The frequency of isolation of *K. pneumoniae* was 12.2% (27 of 222 isolates) in 2009 and 11.3% (23 of 202 isolates) in 2010. There was a high frequency of ESBL-producing *K. pneumoniae* in both years: 70.4% (19 out of 27) and 82.6% (19 out of 23) in 2009 and 2010, respectively. Despite the increase of incidence, the difference was not statistically significant.

The results of our study show that resistance to aminoglycosides (gentamicin and amikacin) and co-trimoxazole decreased in 2010 compared to 2009, while for ciprofloxacin stayed unchanged.

Resistance to piperacillin/tazobactam increased from 21.1% to 78.9% from 2009 to 2010, and the difference was statistically significant ($p<0.05$), and amoxicillin/clavulanic acid resistance also increased from 78.9% to 84.2%, but the difference was not statistically significant ($p>0.05$).

Among the ESBL-producing strains, a total of 17 isolates (44.7%) were multidrug resistant. The most common MDR pattern was resistance to beta-lactams, aminoglycosides and co-trimoxazole.

Resistance to the antibiotics tested, except carbapenems, was higher in the ESBL-producing strains compared to the non-ESBL-producing bacteria.

Table 1. In vitro resistance to antimicrobials of ESBL-positive and ESBL-negative *K. pneumoniae* strains isolated from blood in 2009.

ANTIBIOTIC	ESBL + (No=19)		ESBL - (No=8)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	0 (0)	19 (100)	0 (0)	8 (100)
Cefazolin	0 (0)	19 (100)	3 (37.5)	5 (62.5)
Ceftriaxone	0 (0)	19 (100)	4 (50)	4 (50)
Ceftazidime	0 (0)	19 (100)	4 (50)	4 (50)
Piperacillin/tazobactam	15 (78.9)	4 (21.1)	6 (75)	2 (25)
Amoxicillin/clavulanic acid	4 (21.1)	15 (78.9)	4 (50)	4 (50)
Imipenem	19 (100)	0 (0)	8 (100)	0 (0)
Meropenem	19 (100)	0 (0)	8 (100)	0 (0)
Ertapenem	19 (100)	0 (0)	8 (100)	0 (0)
Gentamicin	0 (0)	19 (100)	4 (50)	4 (50)
Amikacin	9 (47.4)	10 (52.6)	6 (75)	2 (25)
Co-trimoxazole	7 (36.8)	12 (63.2)	4 (50)	4 (50)
Ciprofloxacin	16 (84.2)	3 (15.8)	6 (75)	2 (25)

Table 2. In vitro resistance to antimicrobials of ESBL-positive and ESBL-negative *K. pneumoniae* strains isolated from blood in 2010.

ANTIBIOTIC	ESBL + (No=19)		ESBL - (No=4)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	0 (0)	19 (100)	0 (0)	4(100)
Cefazolin	0 (0)	19 (100)	2 (50)	2 (50)
Ceftriaxone	0 (0)	19 (100)	3 (75)	1 (25)
Ceftazidime	0 (0)	19 (100)	3 (75)	1 (25)
Piperacillin/tazobactam	4 (21.1)	15 (78.9)	3 (75)	1 (25)
Amoxicillin/clavulanic acid	3 (15.8)	16 (84.2)	3 (75)	1 (25)
Imipenem	19 (100)	0 (0)	4 (100)	0 (0)
Meropenem	19 (100)	0 (0)	4 (100)	0 (0)
Ertapenem	19 (100)	0 (0)	4 (100)	0 (0)
Gentamicin	1 (5.3)	18 (94.7)	2 (50)	2 (50)
Amikacin	12 (63.2)	7 (36.8)	3 (75)	1 (25)
Co-trimoxazole	15 (78.9)	4 (21.1)	2 (50)	2 (50)
Ciprofloxacin	16 (84.2)	3 (15.8)	2 (50)	2 (50)

Table 3. ESBL-positive and ESBL-negative *K. pneumoniae* isolates in various pediatric wards

WARD	2009		2010		Total	
	ESBL+ (%)	ESBL-(%)	ESBL+ (%)	ESBL-(%)	ESBL+ (%)	ESBL-(%)
Pediatric Surgery	2 (10.5)	1 (12.5)	2 (10.5)	1 (25)	4 (10.5)	2 (16.7)
Pediatric Intensive Care Unit (PICU)	15 (79)	5 (62.5)	7 (36.8)	2 (50)	22 (57.9)	7 (58.3)
Other	2 (10.5)	2 (25)	10 (52.7)	1 (25)	12 (31.6)	3 (25)
Total	19 (100)	8 (100)	19 (100)	4 (100)	50 (100)	

Table 4. Demographic characteristics of patients with bloodstream infection caused by ESBL producing *Klebsiella pneumoniae* during 2009-2010.

VARIABLES	ESBL + (%)	ESBL – (%)	Total (%)
Sex			
Male	17 (44.7)	8 (66.7)	25 (50)
Female	21 (55.3)	4 (33.3)	25 (50)
Age group (months)			
0-12	35 (92.1)	8 (66.6)	43 (86)
13-24	2 (5.3)	2 (16.7)	4 (8)
25-36	0 (0)	0 (0)	0 (0)
37-48	1 (2.6)	2 (16.7)	3 (6)

All ESBL-producing isolates were sensitive to carbapenems, which included imipenem, meropenem and ertapenem, in both years of investigation. Resistance to antimicrobial drugs during the two-year period is given in Table 1 and Table 2.

ESBL-producing *K. pneumoniae* was most commonly isolated in the pediatric intensive care unit (PICU) of the Institute of Health Care of Children and Youth of Vojvodina Province.

The finding of ESBL-producing isolates in each ward of the Institute is shown in Table 3.

For the purposes of the study, patients were divided into different age groups: group I – patients from 0 to 12 months (n=43, 86%), group II – patients from 13 to 24 months of age (n=4, 8%), group III – patients from 25 to 36 months of age (n=0, 0%) and group IV – patients from 37 to 48 months of age (n=3, 6 %). The majority of ESBL-producing *K. pneumoniae* was found in the first group. A total of 55.3% of the patients were female, but the difference was not statistically significant according to the gender (p value >0.05).

DISCUSSION

According to our results, *K. pneumoniae* was found to be the second most common etiological agent of bloodstream infections in children, and the first leading agent in the group of gram-negative bacteria. The frequency of isolation of *K. pneumoniae* was 12.2% (27 of 222 isolates) in 2009 and 11.3% (23 of 202 isolates) in 2010. The prevalence of the pathogen observed was in accordance with other authors. In the Iranian study by Mamishi, *K. pneumoniae* was the most frequently isolated from blood cultures in children. The author found that the frequency of *K. pneumoniae* was 8.5% (Mamishi et al., 2005). Similar data were reported by Ben Jaballah in Tunisia and Almuneef in Saudi Arabia (Ben Jaballah et al., 2006; Almunnef et al., 2005).

ESBL-producing organisms are becoming increasingly common worldwide, and represent an emerging infection threat (Kader and Kumar, 2005). Recent studies have revealed that patients with septicemia caused by ESBL-producing organisms had significantly a higher fatality rate than those with non-ESBL isolates (Mehrgan and Rahbar, 2008). Our study shows an increase of ESBL-producers. The percentage of ESBL-producing *K. pneumoniae* was high in both years: 70.4% (19 out of 27) and 82.6% (19 out of 23) in 2009 and 2010 respectively. Despite the increase of incidence, this difference was not statistically significant (p value >0.05).

ESBL prevalence varies in different countries. In some European countries, such as France, the frequency of ESBL-producing *K. pneumoniae* is below 5% (Raymond et al., 2007).

Even in pediatric institutions in the USA, ESBL-producing organisms are an emerging problem. The prevalence of ESBL-producing *K. pneumoniae* in pediatric bloodstream infections in the USA was between 18-52.9%. The same results were found in Korea (Blaschke et al., 2009).

Thirty percent of the *K. pneumoniae* were ESBL-producing strains in the Grisaru-Soen study reported from Israel (Grisaru-Soen et al., 2007). Recent studies from Africa have reported a 33.3% prevalence of ESBL-producing *K. pneumoniae* in Ethiopia (Seid and Asrat, 2005).

In our study incidence was much higher than reported in these studies.

Similar findings have been reported from Tunisia by Ben Jaballah, who found that 87.5% *K. pneumoniae* were ESBL-positive, and the rate of 86.6% was found by Malkan Rad in an Iranian hospital (Ben Jabalah et al., 2006; Rad and Momtazmanesh, 2004). Among the ESBL-producing strains in our study, a total of 17 isolates (44.7%) were multidrug resistant (MDR). Our results show a much lower frequency than those of Ben Jaballah, who found that MDR *K. pneumoniae* strains were detected in 85% of the cases (Ben Jaballah et al., 2006).

The most prevalent ESBL-producing *K. pneumoniae* was isolated from pediatric patients in the PICU ward of the Hospital, which is in accordance with Grisaru-Soen and many others studies (Grisaru-Soen et al., 2007).

Resistance to other antimicrobial drugs among ESBL-producing *K. pneumonia* was commonly found.

Gentamicin susceptibility was low among these isolates and this antibiotic should be used with caution when treating empirically. Resistance to gentamicin was higher than to amikacin. Malkan Rad reported similar findings for resistance to amikacin (Rad and Momtazmanesh, 2004).

Resistance to co-trimoxazole significantly decreased in 2010 in compared to 2009 (p value <0.05).

Ciprofloxacin is not routinely recommended for pediatric use, except in special cases where the benefit outweighs the short-term risk of joint toxicity. Ciprofloxacin showed around 80% effectiveness towards all bacterial isolates tested in this study. Similar results have been found in the Nwadioha study from Kano and the Zaoutis study from the USA (Nwadioha et al., 2010; Zaoutis et al., 2005).

The increase in resistance to amikacin and ciprofloxacin in 2010 was not significant (p value>0.05).

In this study, a statistically significant increase of the resistance to piperacillin/tazobactam in 2010 compared to the results in 2009, was found (p value <0.05), while resistance to amoxicillin/clavulanic acid was unchanged, around 80%.

Resistance to carbapenems was not found in this study. KPC-positive bacteria were present as a causative agent for bacteremia in a study by Aneja from Pittsburgh (Aneja et al., 2011). Carbapenem resist-

ance is still rare in most countries. Some authors reported emerging infections with a newly described carbapenem-resistant, so-called NDM-1-producing strains (Sidjabat et al., 2011). The presence of the carbapenem-resistant *K. pneumoniae* NDM-1 in bloodstream infections was also confirmed by Roy from India and Mochon from the USA (Roy et al., 2011; Mochon et al., 2011).

Carbapenems are widely used for the treatment of infections caused by MDR gram-negative bacteria that produce extended-spectrum beta-lactamases (Zaoutis et al., 2005).

Several investigators have concluded that the initial treatment of bloodstream infections caused by ESBL-producing strains with non-carbapenem agents may be associated with higher mortality than treatment with a carbapenem agent (Yun-Kyung et al., 2002).

In conclusion, *K. pneumoniae* is the most common gram-negative bacterial isolate responsible for bloodstream infections in pediatric patients. Our study shows a very high prevalence of ESBL-producing *K. pneumoniae* among hospitalized children with bloodstream infections. According to our two-year surveillance data, it seems likely that the burden of ESBL-producing pathogens in pediatrics will continue to increase. We noted very high rates of resistance to all tested classes of antimicrobial agents, except carbapenems. Our data suggest that amikacin should be considered as a synergistic antibiotic for the treatment of bloodstream infections, together with other antimicrobial drugs. The study also highlights the need for the continual surveillance and understanding of factors associated with infections, which are essential for developing containment strategies to prevent this organism becoming a common problem in pediatric hospitals.

A rational use of antibiotics, especially in this tender age group, in order to achieve a relatively high-level antibiotic activity against offending bacterial organisms, is recommended. Further drug resistance surveillance is necessary in our hospitals, as well as the molecular characterization of ESBL isolates. An accurate and rapid detection of these pathogens is necessary for therapeutic considerations and for the implementation of infection control measures.

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