Prevalence and Risk Factors of Fecal Carriage of Extended-Spectrum β-lactamase (ESBL) -Producing Enterobacteriaceae in Hospitalized and Ambulatory Children

Çocuklarda Genişletilmiş Spektrumlu Beta Laktamaz Üreten Enterobacteriaceae Fekal Taşıyıcılığı Prevalansı ve Risk Faktörleri

Abdurrahman Kiremitçi[1], Ener Çağrı Dinleyici[2], Zeynel Abidin Yargıç[2], Gül Durmaz[1], Neslihan Tekin[3], Aşkın Derya Aybey[1], Mehmet Arif Akşit[3]

[1]Department of Medical Microbiology, Faculty of Medicine, Eskişehir Osmangazi University, *Eskişehir, Turkey*

[2]Department of Pediatrics, Faculty of Medicine, Eskişehir Osmangazi University, *Eskişehir, Turkey*

[3]Department of Pediatrics, Division of Neonatology, Faculty of Medicine, Eskişehir Osmangazi University, *Eskişehir, Turkey*

Abstract

Objective: The isolation of extended-spectrum β-lactamase (ESBL) -producing bacteria has increased worldwide. Fecal colonization with ESBL-producing isolates is considered a prerequisite for infection.

Material and Methods: We prospectively evaluated the prevalence and risk factors for fecal carriage of ESBL-producing *E. coli* and *Klebsiella spp.* in hospitalized and ambulatory children. A total of 464 fecal samples from pediatric patients (270 hospitalized and 194 ambulatory) were collected from a tertiary care center in Turkey during a non-outbreak period. All stool samples were evaluated for ESBL production by the combination disc test and for imipenem susceptibility by the CLSI agar dilution method.

Results: Sixty-six (24% of total) hospitalized children (25 with *Escherichia coli*, 39 with *Klebsiella pneumoniae* and 2 with *Klebsiella oxytoca*) and 14 (7.2% of total) ambulatory children (10 with *E. coli*, 3 with *K. pneumoniae* and 1 with *K. oxytoca*) had a positive fecal sample for ESBL-producing microorganisms. All isolates were susceptible to imipenem; however, the MIC values of strains isolated from hospitalized patients were 3-4 fold higher in dilution than were those of strains isolated from ambulatory children. Univariate analyses showed that intensive care unit stay, urinary catheterization, indwelling catheterization, surgical interventions and prior 3rd-generation cephalosporin usage were associated with ESBL positivity for the hospitalized patients. Recent 2nd- or 3rd-generation cephalosporin use was found to be

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Correspondence Address:
Yazışma Adresi:
Dr. Ener Çağrı Dinleyici
Department of Pediatrics, Faculty of Medicine, Eskişehir Osmangazi University, *Eskisehir, Turkey,*
Phone: +90 222 229 00 64
E-mail: timboothtr@yahoo.com

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Özet

Amaç: Tüm dünyada genişletilmiş spektrumlu beta laktamaz (GSBL) üreten mikroorganizmalar ile oluşan enfeksiyonlarda artış gözlenmektedir. GSBL üreten mikroorganizmaların taşıyıcılığı, ciddi enfeksiyon için zemin hazırlamaktadır.

Gereç ve Yöntemler: Bu çalışmada 3. basamak sağlık hizmeti veren bir hastanede salgın olmayan bir dönemde hastanede yatan ve ya polikliniğe başvuran çocuklarda gayta örneklerinde (464 gayta örneği; 270 hastanede yatan, 194 polikliniklere başvuran çocukta) GSBL üreten *E. coli* ve *Klebsiella spp.* sıklığının ve risk faktörlerinin prospektif olarak değerlendirilmesi planlandı. Tüm gayta örnekleri, GSBL üretimi yönünden disk yöntemi ile değerlendirildi ve imipenem duyarlılığı agar dilüsyon metodu ile CLSI kılavuzuna göre değerlendirildi.

Bulgular: Hastanede yatan çocukların 66'sında (%24; 25 *Escherichia coli*, 39 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*), ayaktan başvuran çocukların ise 14'ünde (%7.2; 10 *E. coli*, 3 *K. pneumoniae*, 1 *K. oxytoca*) gayta örneklerinde GSBL üreten mikroorganizma gösterildi. Elde edilen tüm izolatlar imipenem duyarlı idi. Hastanede yatan hastalardan elde edilen izolatlarda saptanan imipenem MIC değerlerinin, poliklinik hastalarından elde edilenlere göre 3-4 dilüsyon daha yüksek olduğu görüldü. Hastanede yatan hastalarda GSBL üreten mikroorganizmaların fekal taşıyıcılığı ile yoğun bakım ünitesinde kalış, mekanik ventilasyon gereksinimi, idrar sondası, santral kateter takılması, cerrahi girişim uygulanması ve öncesinde tedavide 3. kuşak defalosporinlerin kullanılmasının ilişkili olduğu görüldü. Ayaktan poliklinikle-

associated with ESBL positivity for the ambulatory children.

Conclusion: The increased prevalence and antibiotic resistance of ESBL-producing fecal isolates in asymptomatic hospitalized and ambulatory children highlights the importance of these pathogens for the population. *(J Pediatr Inf 2011; 5: 54-8)*

Key words: Extended-spectrum beta lactamase, ESBL, children, fecal carriage, *E. coli*

re başvuran hastalarda ise öncesinde 2. kuşak ve ya 3. kuşak sefalosporin kullanımının fekal GSBL pozitif etken taşıyıcılığı ile ilişkili olduğu görüldü.

Sonuç: Salgın dışı dönemde hastanede yatan ve ya ayaktan polikliniklere başvuran hastalarda GSBL üreten mikroorganizmaların fekal taşıyıcılığının artmış ve sık olarak saptanması Türkiye'de bu mikroorganizmaların hastalık etkeni olarak önemini indüklenebilir. *(J Pediatr Inf 2011; 5: 54-8)*

Anahtar kelimeler: Genişletilmiş indüklenebilir beta laktamaz, GSBL, çocuk, fekal taşıyıcılık, *E. coli*

Introduction

Extended-spectrum β-lactamases (ESBLs) are a heterogeneous group of plasmid mediated bacterial enzymes that can hydrolyze oxymino-β-lactams and are responsible for bacterial resistance against extended-spectrum β-lactam antibiotics. Production of ESBL was first described in *K. pneumoniae* isolates in 1983. Especially with the excessive worldwide use of cephalosporins, the incidence of multidrug-resistant ESBL-producing *Enterobacteriaceae* is markedly elevated (1,2). These organisms are major nosocomial pathogens, which cause urinary tract infections, bacteremia or intra-abdominal infections. Because of the cross-resistance that they show to other groups of antibiotics, few antimicrobial agents are available as therapeutic options for these infections (3). At the same time, they are important community-acquired urinary pathogens, and their spread in the community is an unique public health problem (2). Use of Carbapenems, which are still effective against ESBL-producing microorganisms, has been associated with a low risk of mortality in cases of serious infections caused by these pathogens (4-6).

Colonization with ESBL-producing strains is considered a prerequisite for infection (1). The aim of this study was to prospectively investigate the prevalence and possible risk factors for fecal carriage of ESBL-producing *E. coli* and *Klebsiella spp*. Hospitalized and ambulatory patients at a tertiary care center in Eskisehir, Turkey, were studied during a non-outbreak period between May and October 2007.

Materials and Methods

This study was performed between May 2007 and October 2007 in a tertiary care hospital in Eskisehir, Turkey. This university hospital serves the city (population approximately 700 000), and surrounding cities. The pediatric and neonatal intensive care units (ICUs) located in this hospital are the only tertiary ICUs for these cities. Nearly 150 new patients were hospitalized in pediatric services (including ICUs) and 2 000 children were admitted to our outpatient clinic and emergency unit every month. We prospectively evaluated our inpatient clinics for fecal carriage of ESBL-producing microorganisms every week. Children seen in the outpatient clinics or emergency department with no history of hospital admission in the preceding month were enrolled in the outpatient group. Patients were excluded if an ESBL-producing microorganism had been previously isolated from sterile sites. This study was approved by the local ethical committee and informed consent was obtained from at least one of the parents of each patient.

For the inpatient group, the following data were recorded: age, gender, primary diagnosis, chronic underlying conditions, presence of co-morbidity, total hospital stay, intensive (pediatric or neonatal) care stay, history of receiving broad-spectrum antibiotics, history of cephalosporin usage (before hospitalization), total parenteral nutrition, indwelling catheter, surgical intervention, urinary catheterization and presence of neutropenia. For outpatients, age, gender, chronic underlying disease (diabetes mellitus, renal or cardiac disease) and history of 3rd or 2nd generation cephalosporin usage in the previous 3 months were recorded. The prevalence of carriage was calculated as the percentage of carriers among participants of each group. To investigate the risk factors associated with fecal carriage within groups, carriers were compared with non-carriers in terms of exposure to the different variables studied.

Fecal carriage was studied either by performing a rectal swab or by immediately sending stool samples to the microbiology unit. Patients with an ESBL-producing *E. coli* isolate on a rectal swab were considered carriers. The stool samples were inoculated onto EMB agar plates without antibiotics for evaluation of aerobic gram-negative flora and with 2 μg/ml cefotaxime for selection of ESBL-producing strains. Inoculated plates were incubated at 35°C for 24 to 48 h under aerobic conditions, and colonies consistent with *E. coli* or *Klebsiella* were subjected to identification with the MicroScan WalkAway-96 SI System (Dade Behring, USA). *E. coli* and

Klebsiella isolates that showed positive results with the screening test were tested for ESBL production using the combination disk test, based on CLSI methodology. All ESBL-producing strains were also evaluated for imipenem susceptibility by the agar dilution method, according to CLSI (7,8). The range of antimicrobial resistance concentrations tested by agar dilution was 0.03 to 32 µg/ml. Antibiotics were supplied as powders of known potency by Merck Sharp & Dohme®. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the reference strains for quality control.

Statistical analyses were performed with SPSS 13.0 for Windows. For independent samples, categorical variables were compared using the Chi square test or Fisher's exact test. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated from 2-by-2 contingency tables. A p-value of <0.05 was considered significant.

Results

Four hundred and sixty-four samples were collected between May and October 2007. Two hundred and seventy (58%) were from hospitalized patients, and 194 (42%) were from ambulatory patients.

Sixty-six (24%) of 270 hospitalized patients (including 70 newborns, 13 of whom were ESBL positive) harbored ESBL-producing bacteria, including 25 (37%) who harbored *Escherichia coli*, 39 (60%) who harbored *Klebsiella pneumoniae* and 2 (3%) who harbored *Klebsiella oxytoca*. Thirty-four (51%) patients in this group were male, and 32 (49%) were female; no significant difference was found between genders (99 male and 105 female p>0.05). Duration of hospital stay was between 3 and 351 days (median 40 days). Intensive care unit stay length varied between 1 and 190 days (median 110 days). Mechanical ventilation duration in ICU patients was between 1 and 290 days (median 79 days). Fifty-three patients (80%) had at least one previously described co-morbidity, while 13 (20%) had none. Nineteen (28%) had taken total parenteral nutrition, 27 (40%) had urinary catheterization, 24 (36%) had indwelling catheterization, 32 (48%) had an ICU stay, 26 (39%) had surgical intervention, 12 (18%) had neutropenia, and 11 (16%) had prior cephalosporin use (10 with ceftriaxone and one with cefepim) before hospitalization. For the hospitalized patients, univariate analysis showed that ICU stay (p<0.001), urinary catheterization (p=0.008), indwelling catheterization (p=0.007), surgical interventions (p<0.001) and prior 3rd-generation cephalosporin usage (p=0.004) were associated with ESBL positivity (Table 1). In this group, imipenem MIC values for *E. coli* ranged from 0.06-0.5 µg/ml: the MIC50 was 0.125 µg/ml, and the MIC90 was also 0.125 µg/ml. For *Klebsiella* spp. against imipenem, MIC values ranged from 0.125-4 µg/ml: the MIC50 was 0.5µg/ml, and MIC90 was 2 µg/ml.

Fourteen isolates from 194 ambulatory patients (7.2%) were identified as ESBL producers, including 10 (72%) *E. coli,* 3 (21%) *K. pneumoniae* and 1 (7%) *Klebsiella oxytoca*. Ten patients had a history of 2nd and 3rd-generation cephalosporin use (5 cefuroxime axetil, 1 cefaclor, 4 ceftriaxone). However, there was no statistically significant difference in gender, age or presence of underlying disease (renal disease, hepatic failure, malignancy, cardiac disease etc.) (p>0.05). Among ambulatory patients positive for ESBL producers, the most remarkable result is the prior 2nd and 3rd-generation cephalosporin usage as an independent risk factor (p=0.003) for fecal carriage (Table 1). In the outpatient group, *E. coli* MIC values for

Table 1. Univariate analysis of factors influencing fecal carriage of ESBL producing Enterobacteriaceae in hospitalized and ambulatory patients

	Hospitalized patients					
	ESBL (+) n=66	**ESBL (-) n=204**	**OR**	**Lower**	**Upper**	***p***
Total parenteral nutrition	19	43	1.51	0.77	2.87	*p=0.196*
Urinary catheterization	27	49	2.19	1.17	4.10	*p=0.008*
Indwelling catheterization	24	41	2.27	1.18	4.35	*p=0.007*
Intensive care unit stay	32	17	10.35	4.92	22.0	*p<0.001*
Surgical intervention	26	37	2.93	1.53	5.64	*p<0.001*
Neutropenia	12	20	2.04	0.88	4.73	*p=0.067*
Prior cephalosporin usage	11	11	3.51	1.33	9.27	*p=0.004*
	Ambulatory patients					
	ESBL (+) n=14	**ESBL (-) n=180**	**OR**	**Lower**	**Upper**	***p***
Presence of chronic illness	8	76	1.82	0.55	6.23	*p=0.278*
Prior 2nd and 3rd generation cephalosporins	10	54	5.83	1.59	23.23	*p=0.003*

imipenem varied between 0.06 and 0.125 µg/ml, with 3 *K. pneumoniae* and 3 *K. oxytoca* isolates at the highest level of 0.125 µg/ml.

Discussion

The rate of fecal carriage of ESBL-producing isolates was 24.4% in hospitalized children, and the rate of occurrence of ESBL-producing isolates among ambulatory children was 7.2%. According to another study including adults and children from our country, fecal carriage rates are 47% in hospitalized patients and 15% in outpatients (9). Valverde et al. (1) reported dramatically increased levels of ESBL-producing isolates in recent years and showed that fecal carriage rates of ESBL-producing *Enterobacteriaceae* in Spain increased from 0.6% in 1991 to 7.0% in 2003. According to previous reports similar to our study, *E. coli* accounts for the vast majority of isolates. Recently, in Spain, Rodiriguez-Bano et al. (10) showed that 7.4% of patients admitted to the emergency department have fecal carriage of ESBL-producing *E. coli*. The prevalence of fecal carriage varies between different geographical areas; in 2003, it was 1.4% in the UK, 2.4% in Lebanon, 7% in India and 15.4% in Saudi Arabia (11-14). A higher prevalence of 4.2% was found in hospitalized neonates upon admission to a neonatal ICU in the United States (15). Ben-Ami et al. (16) found that 10.8% of the patients studied on hospital admission in Israel were fecal carriers. A significant increase in the prevalence of fecal carriage was also observed in healthy children in Bolivia and Peru, from 0.1% in 2001 to 1.7% in 2005 (17). Rates from different geographical regions vary according to different antibiotic policies and whether the study was performed during nosocomial outbreak situations. Our study result of a 7.4% rate of fecal carriage of ESBL-producing isolates among ambulatory children is similar with the recent study from Spain, but higher than other previous reports (except from Saudi Arabia) (10,14).

In our study, pediatric or neonatal ICU stay, urinary catheterization, indwelling catheter, surgical interventions (including tracheotomy) and prior 3rd-generation cephalosporin use were associated with ESBL positivity for hospitalized patients. Demir et al. (18) reported that the strongest independent predictors of ESBL-producing *K. pneumoniae* colonization were mechanical ventilation and hospitalization for longer than 14 days. Celebi et al. (19) defined pediatric risk factors and clinical outcomes associated with Bacteremia from ESBL-producing *Klebsiella* spp. in Bursa, Turkey, between 2003 and 2007. The overall incidence was 4.7 per 1000 admissions; 57% of the isolates were ESBL producers, and malignancy was the common underlying condition according to their study. They described aspects of the previous therapy with broad-spectrum antibiotics, prolonged hospitalization, the presence of a central venous catheter and total parenteral nutrition as risk factors for colonization with ESBL-producing *Klebsiella spp.*

Physicians should be aware that ESBL-producing organisms are not only circulating in hospital environments but in the community as well. This situation can supply the causative agents for both community acquired and nosocomial infections. Our study suggests that 7.2% of outpatients have fecal carriage of ESBL-producing bacteria. Although there were limited patients for statistical analysis (14 patients), the imipenem MIC values for these isolates were considerably lower than those from hospitalized patients. The increase in the proportion of carriers in the community raises the risk that other individuals will become carriers as a consequence of human-to-human transmission of resistant bacteria or through the environment, enriching the resistance gene pool and thus facilitating the acquisition of resistant genes by susceptible bacteria.

One way to decrease ESBL prevalence in an institute is a strict antibiotic policy that reduces the usage of cephalosporins. A history of 3rd generation cephalosporin use is one of the risk factors for fecal carriage of ESBL-producing microorganisms in our hospitalized patients. Interestingly, for non-hospitalized patients, 2nd or 3rd generation cephalosporin use incurs a 2.4-fold increased risk for fecal carriage of ESBL-producing microorganisms. Rodriguez-Bano et al. (10) investigated the risk factors associated with fecal carriage in healthy individuals in non-outbreak situations, and they suggested that home food may be a source of these isolates in patients with community acquired UTI caused by ESBL-producing *E. coli.* Furthermore, Ben-Ami et al. (16) found that poor functional status, current antibiotic use, chronic renal insufficiency, liver disease and use of histamine 2-receptor antagonists were the independent risk factors for fecal carriage; their study included a wide range of ESBL-producing *Enterobacteriaceae*, and one-third of their patients were from a long-term care facility. Colodner et al. (20) reported that 2nd and/or 3rd generation cephalosporins are risk factors for urinary tract infections due to ESBL-producing microorganisms. A history of 3rd generation cephalosporin usage was the only previously known independent risk factor for bloodstream infections with ESBL-producing *E. coli* and *K. pneumoniae* (21).

Our findings for outpatients show that strict antimicrobial agent usage policy, especially restricting extended-spectrum cephalosporins, could help to control the high carriage rates for these organisms. These findings warrant further studies about the consequences of coloniza-

tion with ESBL-producing bacteria, both in the community and the hospital setting.

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Conflict of Interest

No conflict of interest was declared by the authors.

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