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Prevalence of Extended-spectrum β-lactamase Producing *Enterobacteriaceae* Strains in Latvia

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Abstract: *A total of 507 strains of the Enterobacteriaceae family were tested for the production of ESBL using mini API, ATB Expert system as a screening method, as well as the double disk method and E-test for confirmation. The prevalence of ESBL producing E. coli is 5.95%, Klebsiella spp. strains 37.7%. All ESBL- producing isolates are susceptible to imipenem and clavulanate. The susceptibility to other antimicrobials varies from 36 to 92%.*

Keywords: *Extended spectrum β-lactamases, Resistance, Antimicrobials.*

Introduction

The introduction of antimicrobial agents in clinical practice has greatly contributed to improvements in health. Antimicrobial agents have been introduced for decades to treat and prevent infectious diseases and infections. However, their use has been accompanied by an increasing prevalence of microorganisms that have acquired resistance to one or more of these agents, the so-called “antimicrobial resistance”, which has become one of the most urgent problems in medicine nowadays.

There is an association between the growing use of antimicrobial agents and an increase in the prevalence of microorganisms resistant to these agents.

Antimicrobial resistance poses a threat to public health, may prolong the suffering of patients, increase healthcare costs, and has economic implications for society.

To develop strategies for the prevention of infections and containment of resistant pathogens, accurate surveillance systems generating reliable data on incidence, prevalence and modes of spread of resistant microorganisms must be established [1, 4, 8, 10].

Microorganisms develop resistance through different mechanisms.

One of the most widespread resistance mechanisms in microorganisms is their ability to produce destroying or modifying enzymes. The most known of them are β-lactamases.

β-lactamases comprise the major defence of gram-negative bacteria against β-lactam antibiotics. They hydrolyse the β-lactam ring of penicillins, cephalosporins and related drugs. There are dozens of β-lactamases, which vary in the substrate specificity and host range.
These enzymes have evolved over decades of the β-lactam use and have developed the capability of targeting the growing number of antimicrobial compounds.

After the introduction of broad-spectrum penicillins and early cephalosporins in 1960-1978, plasmids determining β-lactamases (e.g. TEM-1) disseminated among gram-negative bacilli. As a result, gram-negative bacilli acquired the capability to produce broad-spectrum β-lactamases.

The clinical use of cephamycins, oxyimunocephalosporins, monobactams, carbapenems, β-lactamase inhibitors, led to the production of extended spectrum β-lactamases (ESBL) with an increased affinity for 3rd generation cephalosporins and monobactams. It was the result of mutations in the plasmid genes TEM, SHV, OXA. Now there are approximately 50 different TEM mutants and 20 SHV β-lactamase mutants. ESBL are most prevalent in *Klebsiella* spp, but have also been described in many other *Enterobacteriaceae* [2, 4, 9, 11].

The aim of the present study was to elucidate the frequency of ESBL producing strains among the representatives of the *Enterobacteraceae* family, isolated from hospitalised patients, and to evaluate their antimicrobial susceptibility.

Materials and methods

The study had been carried out in two hospitals in Riga – Infectology Centre of Latvia and children hospital "Gailezers" during 2005-2006. Identification of the isolated strains to the species level was performed in the mini API system; for antimicrobial susceptibility testing, we used the mini API system, agar disk diffusion test (BBL) according to CLSI standards and E-test (AB Biodisk) [5, 6, 12].

In order to detect ESBL production, we have systematically screened all *Enterobacteriaceae* strains isolated in our hospitals. We used the mini API ATB Expert system for screening. For confirmation, the following methods were applied:

1) a double-disk synergy test as suggested for *Enterobacteriaceae* [3]. We used cephtazidime disk 30 μg (CAZ), cephtazidime / clavulanate 30 / 10 μg (CAZ/CL) and cephotaxime 30 μg (CTX), cephotaxime / clavulanate 30 / 10 μg (CTX/CL).

2) E-test. For E-test, cephtazidime / cephtazidime + clavulanate and cephotaxime / cehotaxime + clavulanate (AB Biodisk).

Results and discussion

A total of 507 strains, representatives of 8 genera of the *Enterobacteraceae* family, were isolated and tested for the production of ESBL. Positive results were registered in representatives of 3 genera – *Escherichia*, *Klebsiella* and *Enterobacter*.

The most active ESBL production was documented in the *Klebsiella* genus: among *K. pneumoniae* strains, there were 36.65% ESBL producers, in *K. oxytoca* strains 38.8% (on the average, 37.7% for the genus).

Among the isolated *E. coli* strains, on the average, 5.95% were ESBL-producers (Table 1).

The double-disk method confirmed 89.3% of ESBL production, from them CTX, CTX / CL – 96.0%, CAZ, CAZ / CL – 56%.

Using the E-test, 82.1% of positive results were confirmed, from them CT / CTL – 95.7%, TZ / TZL – 47.8%.

Table 1 Production of ESBL among the representatives of the *Enterobacteriaceae* family during 1985-1986 in Latvia

Microorganisms	1985			1986		
	No. of cultures	**ESBL producers**		**No. of cultures**	**ESBL producers**	
		Abs.	**%**		**Abs.**	**%**
E. coli	160	5	3.1	159	14	8.8
K. pneumoniae	23	7	30.4	28	12	42.9
K. oxytoca	17	7	41.2	11	4	36.4
K. ornithinolytica	1	-	-	-	-	-
E. cloacae	21	6	28.6	25	1	4.0
E. aerogenes	1	-	-	6	-	-
E. amniogenes	3	-	-	4	-	-
E. sakazakii	1	-	-	4	-	-
C. freundii	5	-	-		-	-
S. marcescens	3	-	-	6	-	-
S. liquefaciens	1	-	-	1	-	-
P. mirabilis	9	3	33.3	7	-	-
P. vulgaris	2	-	-		-	-
M. morganii	3	-	-	3	-	-
Salmonella spp.	-	-	-	3	-	-
In total:	**250**	**28**		**257**	**31**	

The antimicrobial susceptibility of the isolated strains was tested using the panel of antimicrobials.

ESBL producing strains had the following characteristics: all strains of *E. coli*, *Klebsiella* and *Proteus* were susceptible to imipenem and clavulanate, *E. coli* to amikacin 100%, nitrofurantoin 92.3%, norfloxacin and ciprofloxacin 53.8%, cotrimoxazole 69.2%, gentamicin 53.8% (Table 2).

Table 2 Susceptibility of broad-spectrum and extended-spectrum β-lactamases (ESBL) producing *E. coli* strains to antimicrobials

Antimicrobials	Susceptibility of *E. coli* strains (%)	
	Production of broad spectrum β-lactamases	Producers of ESBL
Imipenem	100	100
Clavulanate	100	100
3rd generation cephalosporins	100	-
Amikacin	100	100
Gentamicin	100	53.8
Nitrofurantoin	96.3	92.3
Norfloxacin	90.7	53.8
Ciprofloxacin	91.5	53.8
Cotrimoxazole	51.7	69.2

Klebsiella strains were susceptible to amikacin 81.8%, norfloxacin and ciprofloxacin 54.5%, cotrimoxazole 45.5%, gentamicin 36.4%.

In the present study, these data were compared with the results of investigating the susceptibilities of the strains, producing broad-spectrum β-lactamases.

In this group, the results were different.

All broad-spectrum β-lactamases producing *E. coli*, *Klebsiella* and *Proteus* strains were susceptible to imipenem, clavulanate, 3rd generation cephalosporins, amikacin; *E. coli* - to gentamicin 100%, nitrofurantoin 96.3%, norfloxacin 90.7%, ciprofloxacin 91.5%, cotrimoxazole 51.7% (Table 2), *Klebsiella* to gentamicin, norfloxacin and ciprofloxacin 94.4%, cotrimaxazole 77.8%, nitrofurantoin 72.2%.

According to the polyresistance of ESBL-producing microbial strains, they are of crucial interest nowadays [1, 7, 8, 10, 11].

Extended-spectrum β-lactamases (ESBL) constitute a growing class of β-lactamases, which are often plasmid-mediated and are most commonly expressed in enterobacterial species. The majority of ESBLs are point mutant derivatives of the narrow-spectrum β-lactamases TEM-1, TEM-2 or SHV-1. They are Ambler class A β-lactamases, hydrolysing to different extents oxyiminocephalosporins, such as ceftriaxone, cefotaxime and ceftazidime, and monobactams such as aztreonam. The activity of these penicillinases remains inhibited by clavulanic acid.

So, ESBL producing *Enterobacteriaceae* strains have acquired the resistance to all cephalosporins, penicillins and aztreonam. It is of great importance to implement ESBL detecting methods in clinical practice because *Enterobacteriaceae* may be incorrectly interpreted as susceptible to 3rd and 4th generation cephalosporins. The existing conventional susceptibility testing methods do not reveal all strains, producing ESBL.

Conclusions

1. The prevalence of ESBL producing *E. coli* is rather low – 5.95% and that of *Klebsiella* is high – 37.7%.
2. All ESBL producing strains are susceptible to imitenem and clavulanate, most of them to amikacin. The susceptibility to other antimicrobials varies from 36 to 92%.
3. For effective treatment of patients with 3rd and 4th generation cephalosporins, isolated *Enterobacteriaceae* strains should be tested for ESBL production.

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