RAPID COUNTRY-WIDE DISSEMINATION OF NOSOCOMIAL CTX-M-14-β-LACTAMASE-PRODUCING STRAINS IN RUSSIA

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INTRODUCTION AND OBJECTIVES

Over the last years, the CTX-M-type extended-spectrum β-lactamases (ESBLs) have become widespread in many parts of the world. B-Lactamases of the CTX-M-9 genetic cluster, especially the CTX-M-9 and CTX-M-14, have been reported as one of the prevailing ESBL types in Spain [4], Korea [5], China [1] and Canada [6], and have been identified in many other countries.

During the late 1990's, CTX-M ESBLs were already common among nosocomial *Enterobacteriaceae* in Russia. However, only the members of the CTX-M-1 and CTX-M-2 clusters were detected in the national survey at that time [2]. The more recent epidemiological surveillance confirmed the predominance of the CTX-M-1-cluster ESBLs, namely the CTX-M-3 and CTX-M-15, and also revealed for the first time the production of CTX-M-9-related enzymes in nosocomial strains from various cities of Russia [3]. This study was focused on the molecular analysis of the latter group of strains.

MATERIALS AND METHODS

Bacterial strains. During the period of November 2002 through June 2003, fourteen *Escherichia coli* and one *Klebsiella pneumoniae* nosocomial strains producing the CTX-M-9-related β-lactamases were isolated from ICU patients in 6 hospitals of 5 geographically distant cities: Krasnodar, Moscow, Novosibirsk, St. Petersburg and Tyumen (Fig 1). Collection and characterization of the isolates were performed as a part of the nation-wide surveillance study RESORT.

Detection and subtyping of bla_{CTX-M} genes. The $bla_{CTX-M-9}$-related genes were detected by PCR-RFLP analysis as previously described [2, 7]. Amplification products were directly sequenced on both strands using the primers CTX-M/F': 5'-TTTGCGATGTGCAGTACCAGTAA-3' and CTX-M/R1: 5'-CTCCGCTGCCGGTTTTATC-3', an ABI PRISM Big Dye Terminator V3.1 cycle sequencing kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Stafford, TX).

Susceptibility testing. MICs of cefotaxime (CTX), cefotaxime-clavulanate (CTX-C, 4 mg/L fixed clavulanate concentration), ceftazidime (CAZ), ceftazidime-clavulanate (CAZ-C, 4 mg/L fixed clavulanate concentration), cefepime (FEP), gentamicin (GM), amikacin (AN), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (1:19) were determined using agar dilution method according to the NCCLS (2005) guidelines.

Resistance transfer by conjugation. The *K. pneumoniae* 2100 and six *E. coli* strains: 333, 390, 1242, 2097, 2307 and 2679 (one strain per each hospital) were mated in broth with *E. coli* AB1456 (Rif^R) to determine transferability of CTX-M-coding plasmids. The transconjugants were selected on plates containing rifampin (100 mg/L) and cefotaxime (1 mg/L).

Molecular typing. Molecular typing was performed by ERIC-PCR (primer ERIC1) and RAPD (primer AP7: 5'-GTGGATGCGA-3') as described previously [2]. Cluster analysis of genomic fingerprints was done with GelCompar v. 4.1 software (Applied Maths, Sint-Martens-Letem, Belgium) using the Pearson correlation coefficient and unweighted pair group method using arithmetic averages (UPGMA) algorithm.

RESULTS AND DISCUSSION

Sequences of the $bla_{CTX-M-9}$-related genes in all the isolates studied were identical to that of the $bla_{CTX-M-14}$ (GenBank acc. #AF252622).

The CTX-M-14-producing strains were isolated from patients with different types of nosocomial infections (Tab. 1).

All the strains were resistant to CTX (MICs 16-256 mg/L) and had elevated MICs of CAZ. The *E. coli* strain Spb390 was highly resistant to CAZ due to co-production of CTX-M-14 and SHV-5 ESBLs (data not shown). Ten (66,7%), 6 (40,0%), 13 (86,7%) and 12 (80,0%) isolates were also resistant to GM, AN, CIP and SXT, respectively.

In mating experiments, the resistance to CTX was transferred from 4 strains (333, 2097, 2100, and 2679) to the recipient *E. coli* at high frequencies (7E-3 - 3E-2).

Cluster analysis of ERIC-PCR and RAPD profiles demonstrated high level of genetic diversity among the CTX-M-14 producers (Fig. 2). The *E. coli* isolates were divided into 8 distinct clonal types according to ERIC-PCR and RAPD analysis. Two clones included, respectively, 4 isolates from one hospital in Moscow and 4 isolates from one hospital in St. Petersburg. Two isolates from Novosibirsk had common RAPD but dissimilar ERIC profiles, and were therefore clustered separately. The remaining strains from sporadic cases in Krasnodar and Tyumen were apparently unrelated.

To our knowledge, this study for the first time reports the production of a CTX-M-9-related β-lactamase in Russian nosocomial isolates. The fact that the CTX-M-14-producing strains were genetically divergent and were isolated in geographically distant areas of Russia suggests that the CTX-M-14 could have been present in the population of nosocomial strains before the 2000's but was overlooked in the earlier study [2].

Figure 1. Geographic locations of the hospitals surveyed in RESORT study. The cities where the CTX-M-14-producing strains were isolated are marked by red flags.

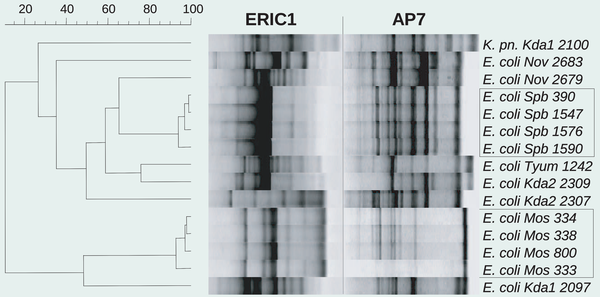

Figure 2. UPGMA clustering of combined ERIC-PCR and RAPD profiles of CTX-M-14-producing strains. Two groups of clonally related isolates are shown in boxes.

Table 1. Characteristics of *E. coli* clinical isolates.

Strain	Center	Date of isolation	Specimen	Infection	MICs, mg/L *								
					CTX	CTX-C	CAZ	CAZ-C	FEP	GM	AN	CIP	SXT
K. pn. 2100	Krasnodar H1	16.04.03	soft tissues	infected wound	**256**	0.06	**32**	0.5	**32**	**256**	**512**	0.03	**256**
E. coli 2097	Krasnodar H1	21.03.03	blood	bacteraemia	**256**	0.25	8	0.5	**256**	**256**	**512**	0.03	**128**
E. coli 2307	Krasnodar H2	10.04.03	abdominal wound discharge	infected wound	**128**	0.125	2	0.25	8	1	0.5	**128**	1
E. coli 2309	Krasnodar H2	04.06.03	abdominal wound discharge	infected wound	**256**	0.25		1	**32**	**256**	**512**	**128**	**256**
E. coli 333	Moscow	20.11.02	BAL	pneumonia	**64**	0.125	1	0.25	4	**128**	4	**64**	**128**
E. coli 334	Moscow	21.11.02	BAL	pneumonia	**256**	0.125	4	0.25	**32**	**128**	4	**64**	**128**
E. coli 338	Moscow	05.11.02	BAL	pneumonia	**128**	0.125	1	0.25	8	**128**	4	**64**	0.125
E. coli 800	Moscow	22.11.02	BAL	pneumonia	**256**	0.125	2	0.25	**16**	**256**	8	**64**	**128**
E. coli 2679	Novosibirsk	12.05.03	soft tissues	infected burn	**16**	0.06	1	0.25	2	1	1	**32**	**128**
E. coli 2683	Novosibirsk	12.05.03	soft tissues	infected burn	**16**	0.06	1	0.25	2	1	1	**32**	**128**
E. coli 390	St. Petersburg	19.11.02	BAL	pneumonia	**256**	0.25	**128**	0.5	**64**	**32**	**16**	**128**	**128**
E. coli 1547	St. Petersburg	22.11.02	blood	pneumonia	**256**	0.25	8	0.25	**16**	**16**	**64**	**128**	**256**
E. coli 1576	St. Petersburg	06.02.03	BAL	bacteraemia	**256**	0.25	2	0.5	**16**	1	4	**128**	**256**
E. coli 1590	St. Petersburg	26.02.03	BAL	pneumonia	**256**	0.25	4	0.25	**64**	2	**64**	**128**	**128**
E. coli 1242	Tyumen	06.02.03	blood	pneumonia	**256**	4	8	1	**256**	**256**	**32**	**128**	2

* Boldface data indicate nonsusceptibility ("I" and "R" categories as defined by standard NCCLS breakpoints)

CONCLUSION

The results of this study evidence the rapid emergence and broad dissemination of CTX-M-14-producing strains in Russia facilitated by both vertical and horizontal gene transfer.

REFERENCES

1. Chanawong A., et al., Antimicrob Agents Chemother. 2002; 46(3):630-7.
2. Edelstein M., et al, Antimicrob Agents Chemother. 2003; 47(12):3724-32.
3. Edelstein M., et al., 44th ICAAC, 2004, Washington, DC; P. C2-1331.
4. Hernandez J.R., et al. Antimicrob Agents Chemother. 2005; 49(5):2122-5.
5. Kim J., et al., Antimicrob Agents Chemother. 2005; 49(4):1572-5.
6. Mulvey M.R., et al. Antimicrob Agents Chemother. 2004; 48(4):1204-14.
7. Pimkin M., et al., 15th ECCMID, 2005, Copenhagen, Denmark; P. 649.

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