FIRST REPORT OF THE *Escherichia coli* CO-PRODUCING CTX-M-15 ESBL AND VIM-4 MBL IN RUSSIA

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ABSTRACT

Background: In this study, we describe a resistance phenotype and β-lactamase context of the *E. coli* strain 55/1389 with decreased susceptibility to carbapenems that was isolated from a 26-year old female neurosurgery ICU patient with catheter-associated UTI in November 2006 in Moscow.

Methods: Susceptibility testing was performed by agar dilution method according to CLSI guidelines. Double-disk synergy tests with clavulanic acid and EDTA were used for detection of ESBL and MBL phenotypes, respectively. Transferability of β-lactam resistance was tested in mating experiments with the *E. coli* AB1456, Rif^R recipient. The β-lactamase genes and their environments were identified by PCR, PCR-mapping, and sequencing.

Results: The *E. coli* 55/1389 exhibited high-level resistance to ampicillin, piperacillin, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, cefoxitin, aztreonam (MICs, >=256 mg/L) and border-line susceptibility to imipenem and ertapenem (MICs, 2 and 4 mg/L). It was also resistant to all non-β-lactam agents tested: ciprofloxacin, gentamicin, amikacin, netilmicin, and co-trimoxasole, except fosfomycin. Phenotypic tests for ESBL and MBL were positive.PCR and sequencing identified the presence of IS*Ecp1* element upstream of the $bla_{CTX-M-15}$ gene and a class 1 integron with the bla_{VIM-4} gene cassette in the first position. No transfer of these resistance determinants to the recipient strain was observed.

Conclusions: To our knowledge, this is the first report of the co-production of ESBL and MBL in a clinical strain of *Enterobacteriaceae* in Russia. While CTX-M-15 is the most common ESBL found in Russian *E. coli* strains, the production of VIM-4 MBL has never been reported in any species in Russia.

INTRODUCTION

Resistance of Gram-negative bacteria to carbapenems due to production of acquired metallo-β-lactamases (MBLs) is an increasing international public health problem [3,15]. The problem of MBL producing strains in Russia was originally confined to *Pseudomonas aeruginosa*. The first nosocomial outbreak caused by *P. aeruginosa* harboring MBL of the VIM-2 type occurred in Omsk in 2003 [13]. Since than, production of MBLs, mostly VIM-2, was identified in *P. aeruginosa* isolates from various geographic regions of Russia [10]. The dissemination of MBLs was attributed to the clonal spread of *P. aeruginosa* of the two sequence types: ST234 and ST235 [9].

Over the past few years, several reports from Europe, primarily, Greece, Italy, France and some other countries noted the dissemination of VIM-type MBLs in *Enterobacteriaceae* [4,6,7,14]. Pertinent to this study, two reports from Italy and Tunisia described the local outbreaks of infections due to *Klebsiella pneumoniae* and *Enterobacter cloacae* co-producing VIM-4 and CMY-4 cephalosporinase [5,7] encoded by a single plasmid [2]. Interestingly, the Tunisian *K. pneumoniae* isolates have been also found to express the CTX-M-15 ESBL encoded by additional conjugative plasmid.

In this study we report for the first time the emergence of the *Escherichia coli* strain that co-produces VIM-4 and CTX-M-15 β-lactamases in Burdenko Neurosurgery Institute, Moscow, Russia.

MATERIALS AND METHODS

Source of multidrug-resistant E. coli isolate (55/1389). On 26 September 2006, a 26-year-old female patient was admitted to the ICU of Burdenko Neurosurgery Institute (Moscow, Russia) with a diagnosis of severe head trauma (diffuse axonal injury, intracerebral and subdural hematoma). Patient was on mechanical ventilation and had an indwelling urinary catheter. On 31 October 2006 she was transferred to the neurotrauma unit with the same diagnosis complicated by VAP and ca-UTI. *E. coli* was isolated from BAL-fluid and urine. After a 4-day therapy with amoxicillin-clavulanic acid and ceftazidime the symptoms of VAP and UTI were relieved and the patient was extubated. On 8 November 2006, however, the patient developed repeated signs of UTI with high fever, leucocytosis, pyuria, and increased CRP level. The multiple drug-resistant isolate of *E. coli* (55/1389) was recovered from urine. A subsequent treatment with uroantiseptics (nitroxoline for one week and furazidin for two weeks) was successful and the patient was discharged.

Susceptibility testing. Susceptibility testing of *E. coli* 55/1389 was performed by agar dilution method according to CLSI guidelines.

Determination of inoculum size effect. The effect of inoculum size on susceptibilities of *E. coli* 55/1389 to doripenem, ertapenem, imipenem, and meropenem was determined by broth microdilution method in MH Broth using standard ($5x10^5$ CFU/ml) and 100-fold-higher inoculum ($5x10^7$ CFU/ml). A 2,3,5-triphenyltetrazolium chloride (TTC) was added to the medium at 0.005% final concentration to assist in colorimetric detection of bacterial growth [8].

Phenotypic tests for β-lactamases. Double-disk synergy tests with clavulanic acid and EDTA were used for detection of ESBL and MBL phenotypes, respectively.

Molecular techniques. The presence of bla_{IMP} and bla_{VIM} genes was investigated by multiplex real-time PCR (Table 1) [10]. The isolate was also tested for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes encoding most common molecular class A β-lactamases by real-time PCRs [11], and for the genes of CMY-4-like class C β-lactamases using conventional PCR and electrophoresis [2]. The association of bla_{CTX-M} gene with IS*Ecp1* insertion sequence and the structure of $bla_{VIM-gene}$ carrying integron were studied by PCR mapping and direct sequencing [12]. PCR-based replicon typing of plasmids was performed as described previously [1].

Table 1. *Primers used for detection and characterization of MBL gene and its environment*

Primer	Target	Sequence, 5'-3'	Application
VIM-Fa	bla_{VIM}	GTTTGGTCGCATATCGCC	Real-time PCR detection
VIM-Ra	bla_{VIM}	TCGTCATGAAAGTGCGT	
IMP-F	bla_{IMP}	GCTAAAGATACTGAAAAATTAGT	
IMP-R	bla_{IMP}	TCATTTGTTAATTCAGATGCATA	
INT/5CS	*intI1*	CTTCTAGAAAACCGAGGATGC	PCR-mapping and sequencing
3-CS QAC-EXT	*qacE_1*	AATGCGGATGTTGCGATTAC	
ISPa21-rev	*tnpA of ISPa21*	TGATCGAGCGTCCGGAAGTCTG	
SMR-rev	*smr*	ATGCCCGTCCAAACAGCGTAGG	
dhfr-fw	*dhfr1*	AATGGAGTTATCGGGAATGG	
dhfr-rev	*dhfr1*	GTCTTGCGTCCAACCAACAGCC	
aadA1-fw	*aadA1*	CGATGAGCGAAATGTAGTGC	
aadA1-rev	*aadA1*	GAAAGGCGAGATCACCAAGG	

RESULTS

Resistance profile of E. coli 55/1389. In vitro susceptibility tests showed that the *E. coli* 55/1389 exhibited high-level resistance to most of the β-lactams: ampicillin, piperacillin, piperacillin-tazobactam, cefotaxime, ceftazidime, cefepime, cefoxitin, aztreonam (MICs,≥256 mg/L). Most notably, it exhibited reduced susceptibility to carbapenems, although MICs of imipenem (4 mg/L), ertapenem (2 mg/L), and meropenem (0.5 mg/L), as measured by agar dilution method, were still in the range of susceptibility according to CLSI guidelines (Table 2). Nevertheless, a strong inoculum effect was observed in broth microdilution tests (Figure. 1). The MICs of all carbapenems increased up to 64 mg/L (16 to 128 times) with a 100-fold increase in the inoculum size.

The isolate was also resistant to ciprofloxacin, gentamicin, amikacin, netilmicin, and co-trimoxasole, but susceptible to fosfomycin.

An enhancement of oxyimino-cephalosporins activity in the presence of clavulanic acid indicated ESBL production (Table 2, Figure 2a), and a synergy between carbapenems and EDTA indicated MBL production (Figure 2b).

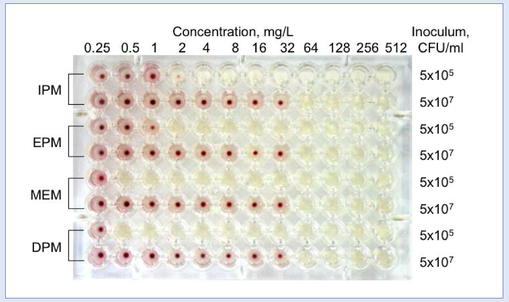

Figure 1. *A broth microdilution test showing the effect of inoculum size on MICs of ertapenem (EPM), imipenem (IPM), doripenem (DPM) and meropenem (MEM) in E. coli 55/1389 .*

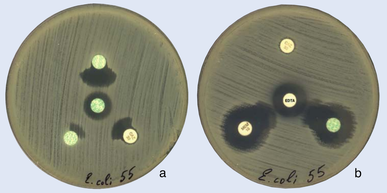

Figure 2. *Detection of ESBL (a) and MBL (b) phenotypes in E. coli 55/1389 by double-disk synergy tests.*

Table 2. *Susceptibilities of E. coli 55/1389 to various antimicrobial agents*

Antimicrobial agent	MIC, mg/L
Ampicillin	>256
Amoxicillin-clavulanic acid (2:1)	>32/16
Piperacillin	>256
Piperacillin-tazobactam (4 mg/L)	>256/4
Cefoperazone	256
Cefoperazone-sulbactam (1:1)	64/64
Ceftazidime	256
Ceftazidime-clavulanic acid (4 mg/L)	32/4
Cefotaxime	>256
Cefotaxime-clavulanic acid (4 mg/L)	128/4
Cefepime	128
Cefepime-clavulanic acid (4 mg/L)	4/4
Aztreonam	>256
Cefoxitin	>256
Doripenem	0.25
Imipenem	4
Meropenem	0.5
Ertapenem	2
Gentamicin	>256
Amikacin	64
Netilmicin	>256
Ciprofloxacin	>128
Trimethoprim-sulfamethaxazole (1:19)	128/2432
Fosfomycin	1

Characterization of β-lactamase genes and their genetic context. PCR and direct sequencing identified the presence of $bla_{CTX-M-15}$ gene downstream of the IS*Ecp1* element. No bla_{TEM} and bla_{SHV} genes were detected.

The MBL-encoding gene was identified as bla_{VIM-4}. It was found as the first gene cassette of a class 1 integron, and was followed by *aacA7*, *dhfr1-aadA1* fused gene cassette, and smr gene cassette. The integron lacked a typical 3'-CS region and instead contained an IS*Pa21*-like element (Figure 3).

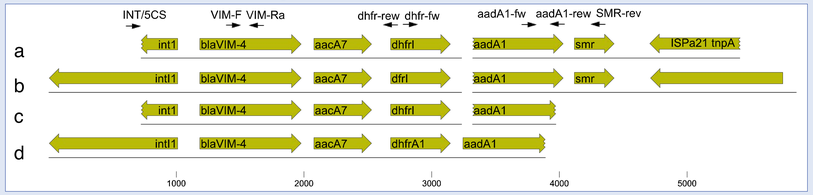

Figure 3. *Structure of class 1 integrons carrying bla_{VIM-4} gene cassette in E. coli 55/1389 and other enterobacteria isolated in Europe. a, E. coli 55/1389 (this study); b, In416 of K. pneumoniae (AJ704863, Italy); c, K. pneumoniae (AM181293, Tunisia); d, E. cloacae (EF467306, Greece).*

The composition of gene cassette array was identical to those of VIM-4-encoding integrons found in *E. cloacae* and *K. pneumoniae* from Italy (In416, GB Acc.# AJ704863) and *K. pneumoniae* from Tunisia (GB Acc.# AM181293).

Because the highly similar integron, In416, was found on the IncA/C plasmid (pCC416) that also carried the bla_{CMY-4} gene, we performed analysis of plasmid replicons and detection of bla_{CMY-4} gene in *E. coli* 55/1389 by means of PCR. Five plasmid replicons were detected: I1, FIA, FIB, W, and F (data not shown). However, no A/C replicon or bla_{CMY} gene were found, suggesting a possible translocation of bla_{VIM-4}-containing integron.

CONCLUSIONS

To our knowledge, this is the first report of the co-production of ESBL and MBL in a clinical strain of *Enterobacteriaceae* in Russia and the first report of VIM-4 expression in *E. coli*.

While CTX-M-15 is the most common ESBL found in Russian *E. coli* strains, the production of VIM-4 MBL has never been reported in any species in Russia.

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