ASSESSMENT OF CARBAPENEM RECISTANCE AND PRESENCE OF ACQUIRED CARBAPENEMASES IN RUSSIAN NOSOCOMIAN ISOLATES OF *ACINETOBACTER* SPP.

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ABSTRACT

Objectives: To determine resistance rates to imipenem (IPM) and meropenem (MER) and occurrence of acquired metallo-beta-lactamases (MBLs) and class D (OXA) carbapenemases in nosocomial Acinetobacter spp. from Russian ICUs.

Materials and methods: A total of 464 consecutive nosocomial isolates collected as part of the national surveillance study RESORT in 30 ICUs of 20 Russian cities in 2002-2004 were studied. The susceptibilities to IPM and MER were determined by agar dilution method and interpreted according to CLSI guidelines. A double-disk synergy test with EDTA was used for phenotypic detection of MBL production. The presence of the genes for VIM- and IMP-type MBLs and the genes for acquired class D carbapenemases of three groups: OXA-23-like, OXA-40-like and OXA-58, was examined by PCR.

Results: A total of 11(2.4%) and 17(3.7%) isolates were nonsusceptible to IPM (MIC range: 8 – 128 µg/ml) and MER (MIC range: 8 – 64 µg/ml), respectively. MBL production was not detected among carbapenem-nonsusceptible isolates by either phenotypic or molecular tests. The genes for acquired OXA-type carbapenemases were found in 11 (2.4%) isolates of *A. baumannii* exhibiting variable MICs of IPM (4 – 32 µg/ml) and MER (8-32 µg/ml). Eight of these isolates from two hospitals of Moscow and Novosibirsk harboured the genes for OXA-58 and the remaining three isolates from Moscow and Irkutsk carried the genes for OXA-23-like enzymes. No acquired carbapenemase genes were detected in the other six isolates exhibiting increased resistance to IPM or MER (MICs: 8 – 128 µg/ml).

Conclusions: Resistance to carbapenems remains rare among nosocomial Acinetobacters in Russia. However, the identification of acquired OXA-type carbapenemases in isolates from geographically distant regions of Russia poses the risk of future dissemination of resistance.

INTRODUCTION AND PURPOSE

Carbapenems currently remain the most effective agents for the treatment of infections caused by gram-negative bacteria. However, during the last decade, resistance to carbapenems has emerged worldwide among non-fermenting bacteria. In *Acinetobacter* spp., resistance is related to different mechanisms among which the production of acquired carbapenemases of molecular classes B (metallo-β-lactamases, MBLs) and D (OXA enzymes) is one of the most clinically and epidemiologically significant. The former class of enzymes typically include VIM and IMP types and the latter is comprised of the three genetic subgroups epitomized by OXA-23, OXA-40 and OXA-58.

This study was performed to determine resistance rates to carbapenems and occurrence of acquired carbapenemases in nosocomial strains of *Acinetobacter* spp. isolated in Russian ICUs.

MATERIALS AND METHODS

- A total of 464 consecutive non-duplicate nosocomial isolates collected as part of the national surveillance study RESORT in 30 ICUs of 20 Russian cities in 2002-2004 were studied. The geographic origin of the strains is shown in Figure 1.

Figure 1. *Geographic origin of the strains studied.*

- The susceptibilities to imipenem (IPM), meropenem (MER), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), cefoperazone (CFP), cefoperazone-sulbactem (CFP-S; 1:1), trimethoprim-sulfamethoxazole (SXT; 1:19), gentamicin (GM), amikacin (AN), and ciprofloxacin (CIP) were determined by agar dilution method and interpreted according to CLSI guidelines.
- A double-disk synergy test with EDTA was used for phenotypic detection of MBL production.
- Isolates exhibiting decreased susceptibilities (MICs 4µg/ml) to either of the two carbapenems where screened for production of acquired carbapenemases.
- A double-disk synergy test with IPM, MER, CAZ and EDTA was used for phenotypic detection of MBLs.
- The presence of the genes for VIM- and IMP-type MBLs was examined by multiplex real-time PCR as described previously [Shevchenko et al., 2007].
- The genes for OXA-23-like, OXA-40-like and OXA-58 b-lactamases were detected by multiplex PCR with three pairs of primers, respectively (Table 1).

Table 1. PCR primers used in this study

Primer	Target	Sequence, 5'-3'
VIM-Fa VIM-Ra	bla_{VIM}	GTTTGGTCGCATATCGC TCGTCATGAAAGTGCGT
IMP1-F IMP1-R	bla_{IMP}	GCTAAAGATACTGAAAAATTAGT TCATTTGTTAATTCAGATGCATA
OXA-23-F OXA-23-R	$bla_{OXA\text{-}23\text{-}like}$	TTTCTTTCTGGTTGTACGGTTCA CATTTCTGACCGCATTTCCA
OXA-40-F OXA-40-R	$bla_{OXA\text{-}40\text{-}like}$	GATGAAGCTCAAACACAGGGTG TTTCCATTAGCTTGCTCCACC
OXA-58-F OXA-58-R	$bla_{OXA\text{-}58}$	GGGCTTGTGCTGAGCATAGT CGTAGAGCAATATCATCACCAGC

- The primers were designed based on the on the known bla_{OXA} gene sequences encoding acquired carbapenemases of Acinetobacter spp.: OXA-23 (GB Acc. # AJ132105), OXA-24 (GB Acc. # AJ239129), OXA-25 (GB Acc. # AF201826), OXA-26 (GB Acc. # AF201827), OXA-27 (GB Acc. # AF201828), OXA-40 (GB Acc. # AF509241), OXA-49 (GB Acc. # AY288523), and OXA-58 (GB Acc. # DQ385607).
- 25-µl PCR mixtures contained: 0.6 µM of each primer, 200µM of each dNTP, 1.5 mM MgCl2, 1.5U of Taq-F DNA-polymerase (AmpliSens, Russia), 0.5 µl of SYBR Green I (SBG I; 1:1000 dilution in DMSO) and 2 µl of template DNA prepared by boiling of 3-5 colonies in TE buffer.
- Thermal cycling was performed on a Rotor-Gene 2000 System (Corbett Research, Australia) and included initial 15-min denaturation at 95°C, and 30 cycles of 20-sec denaturation at 95°C, 20-sec annealing at 61°C, and 30-sec elongation at 72°C.

RESULTS

Figure 2 shows the frequency distribution of MICs of IPM and MER in the studied isolates. A total of 11(2.4%) and 17(3.7%) isolates were nonsusceptible, respectively, to IPM (MIC range: 8 – 128 µg/ml) and MER (MIC range: 8 – 64 µg/ml). The clinical, phenotypic and genotypic characteristics of these isolates are summarised in the Table 2.

Table 2. Characteristics of carbapenem-non-susceptible isolates of *Acinetobacter* spp.

Species, Strain #	City, Hospital #	City, Hospital #	Date of isolation	MIC, µg/ml										PCR for acquired OXAs
				IPM	MER	CTX	CAZ	FEP	CFP	CFP-S	SXT	GM	AN	CIP	
A. baumannii 548	Irkutsk, 1	02-Dec-2002	Pneumonia	4	8	≥256	8	≥256	≥256	32	0.25/4.75	16	64	≥128	OXA-23-like
A. baumannii 547	Irkutsk, 1	02-Dec-2002	Pneumonia	4	8	≥256	8	≥256	≥256	32	0.25/4.75	16	64	≥128	OXA-23-like
A. baumannii 3577	Moscow, 1	09-Mar-2004	Pneumonia	8	8	16	4	4	≥256	1	2/38	64	4	8	OXA-23-like
A. baumannii 831	Moscow, 2	04-Dec-2002	Pneumonia	16	16	32	8	16	≥256	4	≥128/2432	8	128	8	OXA-58
A. baumannii 816	Moscow, 2	24-Dec-2002	Pleuritis	32	16	32	8	32	≥256	8	≥128/2432	8	128	8	OXA-58
A. baumannii 801	Moscow, 2	02-Dec-2002	Cystitis	32	16	≥256	64	128	≥256	64	8/152	≥256	256	64	OXA-58
A. baumannii 797	Moscow, 2	18-Dec-2002	Pyelonephritis	32	16	≥256	64	128	≥256	32	8/152	≥256	≥512	≥128	OXA-58
A. baumannii 1357	Moscow, 2	11-Feb-2003	Pneumonia	16	8	64	8	32	64	8	≥128/2432	8	128	8	OXA-58
A. baumannii 2650	Novosibirsk, 1	08-Aug-2003	Pneumonia	32	32	≥256	16	≥256	≥256	16	16/304	64	128	≥128	OXA-58
A. baumannii 2281	Novosibirsk, 1	13-May-2003	Infected wound	32	16	≥256	64	32	≥256	8	0.25/4.75	≥256	≥512	32	OXA-58
A. baumannii 2280	Novosibirsk, 1	28-May-2003	Pneumonia	32	16	16	8	32	≥256	8	32/608	≥256	≥512	≥128	OXA-58
A. baumannii 1957	Novosibirsk, 1	14-Apr-2003	Burn sepsis	4	8	≥256	32	8	≥256	4	32/608	≥256	256	≥128	negative
A. baumannii 523	Smolensk, 1	27-Jan-2003	Infected burn	4	8	≥256	32	16	≥256	4	4/76	256	128	32	negative
A. baumannii 3827	Smolensk, 1	14-May-2004	Infected burn	1	32	128	8	16	≥256	2	16/304	128	4	0.5	negative
A. baumannii 4203	Yakutsk, 1	24-May-2004	Sepsis	1	16	128	4	8	≥256	4	8/152	32	1	4	negative
A. baumannii 3546	Yakutsk, 1	05-Mar-2004	Intra-abdominal	16	32	8	8	4	≥256	1	0.125/2.375	≥256	256	32	negative
A. lwoffii 3944	Ekaterinburg, 1	24-Oct-2003	Pneumonia	128	64	≥256	≥256	128	64	32	0.5/9.5	16	256	0.5	negative

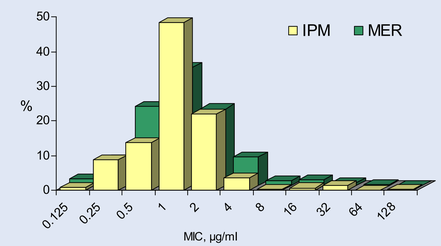

Figure 2. *Frequency distribution of carbapenem MICs in nosocomial isolates of Acinetobacter spp. from Russian ICUs.*

Production of MBLs was not detected among carbapenem-nonsusceptible isolates by either phenotypic or molecular tests.

The genes for acquired OXA-type carbapenemases were found by PCR in 11 (2.4%) isolates of *A. baumannii* exhibiting variable levels of resistance to IPM (MICs 4 – 32 µg/ml) and MER (MICs 8-32 µg/ml). Eight of these isolates from two hospitals of Moscow and Novosibirsk harboured the genes for OXA-58 and the remaining three isolates from Moscow and Irkutsk carried the genes for OXA-23-like enzymes. The molecular weights of the PCR products obtained were identical with those of the corresponding predicted products (Fig. 3).

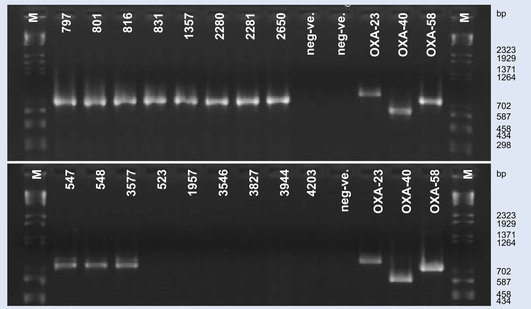

Figure 3. *Electrophoresis of PCR-products of bla_{OXA} genes from selected clinical isolates and control stras.*

No acquired carbapenemase genes were detected in the remaining six isolates exhibiting increased MICs (8 – 128 µg/ml) of IPM or MER suggesting the involvement of alternative mechanisms in resistance. Notably, the majority of carbapenem non-susceptible strains were also resistant to non-β-lactam agents: 17 (100%) to GM, 14 (82.4%) to AN, 15 (88.2%) to CIP, and 11 (64.7%) to SXT.

CONCLUSIONS

Resistance to carbapenems remains rare among nosocomial Acinetobacters in Russia. However, the identification of acquired OXA-type carbapenemases in isolates from geographically distant regions of Russia poses the risk of future dissemination of resistance.