FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 46, No. 2, 2008
pp. 137-142

Review article

Metallo-β-lactamases of *Pseudomonas aeruginosa* - a novel mechanism resistance to β-lactam antibiotics

Paweł Sacha[1], Piotr Wieczorek[1], Tomasz Hauschild[2], Marcin Żórawski[3], Dorota Olszańska[4], Elżbieta Tryniszewska[1]

[1]Department of Microbiological Diagnostics, Medical University of Bialystok
[2]Department of Microbiology, Institute of Biology, University of Bialystok
[3]Department of Pharmacology, Medical University of Bialystok
[4]Department of Microbiological Diagnostics, University Hospital of Bialystok

Abstract: Since about twenty years, following the introduction into therapeutic of news β-lactam antibiotics (broad-spectrum cephalosporins, monobactams and carbapenems), a very significant number of new β-lactamases appeared. These enzymes confer to the bacteria which put them, the means of resisting new molecules. The genetic events involved in this evolution are of two types: evolution of old enzymes by mutation and especially appearance of new genes coming for some, from bacteria of the environment. Numerous mechanisms of enzymatic resistance to the carbapenems have been described in *Pseudomonas aeruginosa*. The important mechanism of inactivation carbapenems is production variety of b-lactam hydrolysing enzymes associated to carbapenemases. The metallo-β-enzymes (IMP, VIM, SPM, GIM types) are the most clinically significant carbapenemases. *P. aeruginosa* posses MBLs and seem to have acquired them through transmissible genetic elements (plasmids or transposons associated with integron) and can be transmission to other bacteria. They have reported worldwide but mostly from South East Asia and Europe. The enzymes, belonging to the molecular class B family, are the most worrisome of all β-lactamases because they confer resistance to carbapenems and all the β-lactams (with the exception of aztreonam) and usually to aminoglycosides and quinolones. The dissemination of MBLs genes is thought to be driven by regional consumption of extended -spectrum antibiotics (*e.g.* cephalosporins and carbapenems), and therefore care must be taken that these drugs are not used unnecessarily.

Keywords: *Pseudomonas aeruginosa* - Class B β-lactamases - Metallo-β-lactamases.

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen associated with a range of nosocomial infections. Strains of *P. aeruginosa* cause disease in hospitalized patients, predominantly pneumonia, urinary tract infections, as well as, skin and soft-tissue infections [1]. The increased involvement of this ubiquitous organism in infections is due to a number of factors, including the growing numbers of invasive procedures and immunocompromised patients together with the increased use of antibiotics, which has promoted the selection of resistant organisms. The rise in involvement has been documented by several groups [2]. Patients in intensive care units, oncology departments, burn units and surgery wards frequently show multiresistant isolates, which contributes to high morbidity and mortality [3].

The spread of this organism in healthcare settings is often difficult to control, due to the presence of multiple intrinsic and acquired mechanisms of antimicrobial resistance. The dissemination of genes encoding metallo-β-lactamases is thought to be driven by regional consumption of extended-spectrum cephalosporins and/or carbapenems [4,5,6].

Classification of β-lactamases

The categorization of β-lactamase enzymes involves the use of two classification schemes (Table 1). The enzymes fall into four classes on the basis of their sequence homology, or on the basis their substrate

Correspondence: P. Sacha, Dept. of Microbiological Diagnostics, Medical University of Bialystok, Waszyngtona 15A, 15-274 Bialystok; tel.: (+4885) 7468571, fax.: (+4885) 7468571, e-mail: sachpt@amb.edu.pl

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2008:46(2): 137 (137-142)
doi: 10.2478/v10042-008-0020-9

Table 1. Classification of β-lactamases.

Functional mechanism	Ambler class	Bush (Groups)	Examples	Substrates
Serine-β-lactamases	Class A-penicillinases	(2a,2b,2c)	Broad-spectrum β-lactamases: TEM-1, TEM-2, SHV-1	Benzylpenicillin (penicillin), aminopenicillins (amoxicillin, ampicillin), carboxypenicillins (carboxypenicillin, ticarcillin), narrow-spectrum cephalosporins (cefzolin, cefuroxim and others)
		(2be)	Expanded-spectrum-β-lactamases (ESBL):TEM family and SHV-family	Substrates of the broad- spectrum group β-lactamases plus cloxacillin, methicillin and oxacillin
			Others: BES-1,GES/IBC family, PER-1,PER-2, SFO-1, TLA-1, VEB-1/2	Same as for TEM and SHV family
		(2br)	TEM family (TEM-30, TEM-31) IRTs*	Same as for TEM and SHV family and * inhibitors resistant
		(2e)	CTX-family	Substrates of the expanded-spectrum-β-lactamases group, for some enzymes, cefepime
		(2f)	Carbapenemases: (KPC-1, KPC-2 and KPC-3; GES-1, GES-2)	Substrates of the expanded-spectrum-β-lactamases group plus cephamycins and carbapenems (ertapenem, meropenem, imipenem)
Metallo-β-lactamases	Class B-metallo-β-lactamases (zinc)	(3a,3b,3c)	Carbapenemases: IMP family, VIM-family, SPM-1, SPM-2, GIM-1, and L1, CcrA	Same as for carbapenemases class A
Serine-β-lactamases	Class C-cephalosporinases	(1)	AmpC-type: AAC-1, ACT-1, CFE-1, CMY-family, DHA-1, DHA-2, FOX-family, LAT-family, MIR-1, MOX-1, and MOX-2	Substrates of the expanded-spectrum-β-lactamases group plus cephamycins
Serine-β-lactamases	Class D-cloxacillin-hydrolyzing enzymes (OXA)	(2d)	Most of OXA family	Substrates of the broad- spectrum group plus cloxacillin, methicillin and oxacillin
			Other OXA: OXA-23 → OXA-27,and OXA-40, OXA-48	Same as for IMP family, VIM-family, SPM-1, SPM-2 and GIM-1
Unknown		(4)	AVS-1	Miscellaneous or unsequenced/ uncharacterized enzymes that do not fit into any function or molecular group

spectrum and responses to inhibitors into a larger number of functional groups [7,8]. In the Ambler classification [7], class A, C and D enzymes employ serine (Fig. 1) as the reactive site to attack the β-lactam bond of penicillins, cephalosporins and carbapenems [9,10]. These enzymes cleave the amide bond of the β-lactam ring thus inactivating the antibiotic, while class B (metallo-β-lactamases) requiring zinc ions for their activity. Metallo-β-lactamases (MBLs) catalyze the identical (as serine enzymes) chemical reaction, using one or two divalent cations ($Zn^{2+)}$ coordinated to two water molecules as the reactive nucleophiles (Fig. 2).

MBLs are produced by bacteria as extracellular or periplasmatic enzymes. All known representatives' posses conserved metal binding sites and require zinc ions as enzymatic cofactors. These enzymes can degrade all of β-lactam antibiotics except monobactams and are special constant and efficient carbapenemases activity. Moreover, metallo-β-lactamases are not susceptible to therapeutic β-lactamase inhibitors [11].

©Polish Histochemical et Cytochemical Society

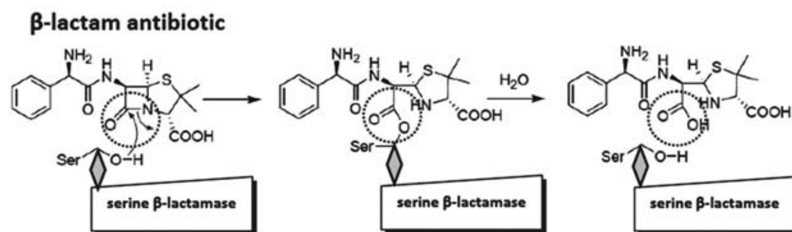

Fig. 1. Mechanism of the hydrolysis of β-lactam antibiotics through serine-β-lactamases.

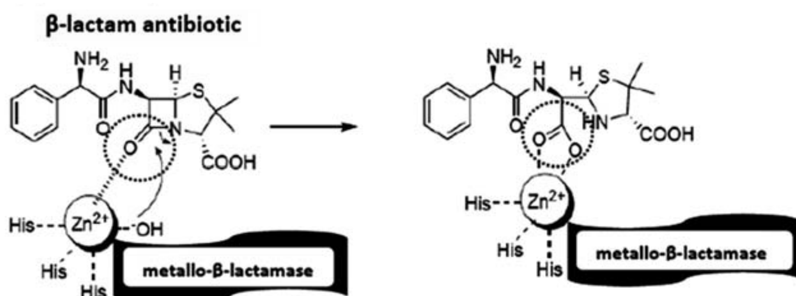

Fig. 2. Mechanism of the hydrolysis of β-lactam antibiotics through metallo-β-lactamases.

Their increasing emergence in pathogenic bacterial strains (particularly *P. aeruginosa* and Enterobacteriaceae) due to a rapid dissemination by horizontal gene transfer induced a growing interest in this enzyme family because of the lack of efficient therapies to treat infected patients [11,12,13].

Biochemical and genetic characteristic of enzymes class B

The class B of β-lactamases are completely distinct from the serine β-lactamases in terms of sequence, fold and resistance mechanisms. All are metalloenzymes. Only class B, the β-lactamases require zinc ions for catalytic activity [14]. These enzymes constitute a group of heterogeneous proteins which divided into three subclasses 'B1', 'B2' and 'B3' [15,16,17]. Subclass B1 exhibits a broad substrate spectrum profile and is characterized by zinc binding site 1 composed of three His residues (His-116, His-118, His-196) and zinc binding site 2, composed of one His-263, one Cys-221 and one Asp-120. In subclass B2 the zinc ligands on site 2 are conserved whereas His-116 in site 1 is replaced by Asp. Representatives of subclass B2 efficiently hydrolyze only carbapenems [18] and are active as mono-zinc enzymes whereas binding of a second zinc ion causes non-competitive inhibition [19]. Subclass B3 has the same ligands in zinc binding site 1 as subclass B1, but the cysteine ligand of subclasses B1 and B2 is binding site 2 is replaced by histidine. These enzymes exhibit a broad-spectrum activity profile with a putative preference for cephalosporins and carbapenems (only subclasses B2) [20].

The genes encoding β-lactamases can be located on the bacterial chromosome, on plasmids, or transposons. The genetic environment of β-lactamase (*bla*) gene dictates whether the β-lactamases are produced in constitutive or inducible manner.

Some enzymes of subclass B1 (metallo-β-lactamases) have been found on plasmids and part of transmissible genetic elements called integrons [4]. An integron is a specialized group of gene cassettes each of which encode an antibiotics resistance gene. Each gene cassette is composed of a resistance gene bounded at the 5' end by ribosomal binding site and downstream by a 59-base element that is a recombination site common to the cassette. The integron normally encodes its own integrase (*int*) that facilitates insertion of the gene cassette into integrations site (*attI*) of the integron (Fig. 3).

Many studies characterizing of MBLs *bla* genes have found them inserted into common class I integron [21,22,23,24,25]. These integrons are responsible for transfer of *bla* gene among divergent species of Gram-negative bacteria. Recently, an increasing number of *bla* genes are being discovered on integrons [26]. Mobile genetic elements that contain integrons are important source for spread of *bla* genes and for the dissemination of other determinants. Integrons are not mobile but their location in mobile genetic elements (plasmids, transposons) enables their movement [27]. Genes of β-lactamases located on integrons are often accompanied by genes encoding resistance to unrelated antibiotics [4]. The transferable metallo-β-lactamases are commonly encoded by genes carried by type 1 or type 3 integrons. These integrons could be carried by large plasmids or be located on the chromosome [11,23].

While the majority of MBL genes (IMP-type or VIM-type) are mobilised by integrons and/or transposons, a minority appear to be mobilised with mobile common regions (CR) that have also been associated

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2008:46(2): 139 (137-142)
doi: 10.2478/v10042-008-0020-9

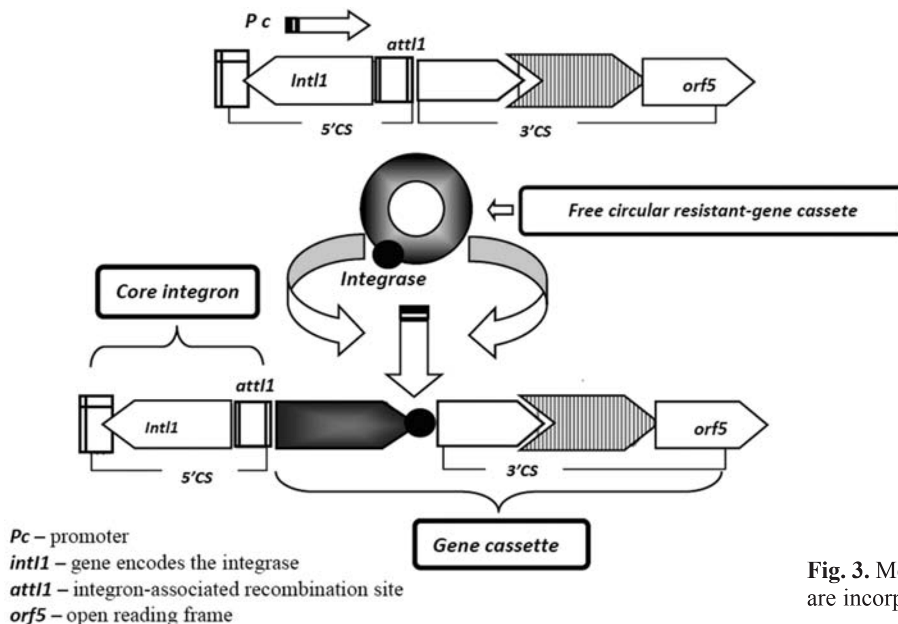

Fig. 3. Mechanism by which resistance-gene cassettes are incorporated into capture elements as integron.

with other mobile elements called SXT regions. The gene encoding SPM-1 enzyme is associated with two different types of CR (ISCR - IS Common Region) element [28,29]. The gene bla_{SPM-1} is not part of a gene cassette, nor is it found in the vicinity of class 1 integron as found other metallo-β-lactamases genes. The gene is located besides the ISCR variant ISCR4 [29].

ISCR, a new type of genetic element, was recently identified as being closely associated with spread of many antibiotic resistance genes. They can be divided into two groups: ISCRs1 - form complex class 1 integrons and ISCRs2 to 13 are those associated with other type integrons. Toleman *et al.* [28] has detected ISCR elements in several strains of *P. aeruginosa*. ISCR2 was discovered in a *P. aeruginosa* isolate that harboured bla_{VIM-1} and ISCR3 was discovered in two of *P. aeruginosa* strains that have bla_{VIM-1}.

Epidemiology of MBLs in clinical isolates of *Pseudomonas aeruginosa*

Most of metallo-β-lactamases (class B1) were found in *Pseudomonas aeruginosa* strains (Table 2).

The first 'mobile' MBL of *P. aeruginosa* characterised was IMP-1(active on IMiPenem), discovered in Japan 1988 [30,31,32]. Japan has become a major reservoir for IMP-type metallo-β-lactamases, which now include many variants of enzymes, and these have spread to a number of strains *Pseudomonas spp.*, *Acinetobacter spp., and Enterobacteriaceae*. IMP-type MBLs have now been found in many other countries, also in Poland [33,34,35,36,37,38]. The most recent IMP type enzyme (IMP-18) was found in a *P. aeruginosa* isolate from Las Cruces, United States of America [39]. Currently have been described 23 variants of enzymes from IMP family.

VIM family of metallo-β-lactamases are the second dominant group, and currently 18 known derivatives [40]. The 'European MBL' likely to be that VIM rather than IMP groups, and its global spread is rapid and worrisome. VIM type enzymes demonstrates little amino acid similarity to IMP (< 32%).VIM-1 (Verona IMipenemase) was first characterized from a *P. aeruginosa* strain isolated in 1997 in Italy [41]. Although VIM-1 shares less than 30% amino acid identity with the IMP enzymes, it possesses the same broad-spectrum profile activity. VIM-2 (VIM-1 variant) was identified from a *P. aeruginosa* strain from neutropenic patient in 1996 (France) [22] and very recently, VIM-2 and novel variant of the VIM series, VIM-3, have been identified in the same species of bacteria, in Taiwan [42]. Since 1995, bla_{VIM} positive *P. aeruginosa* strains were isolated in many countries of the world [25]. It has been found also in Poland [43,44,45].

The third type of acquired MBL in *P. aeruginosa* is SPM-1 (Sao Paulo Metallo-β-lactamase). First *P. aeruginosa* with bla_{SPM-1} gene was isolate in 1999 in Brazil. The sequence of SPM-1 possesses moderate identity with that of IMP-1 (35.5%) but is differently from VIM types [29].

The new type of MBL is GIM-1 (German IMipenemase) was recovered from five *P. aeruginosa* isolates in 2002 from Dusseldorf (Germany). The GIM-enzyme is encoded by genes carried by a class 1 integron on a plasmid. The amino-acid sequence of GIM-1 displayed most identity with other MBLs variants (42.1% with IMP-1, 29% similarity with VIM-1 and 28% similarity with SPM-1) [46]. At this time, it has not been reported elsewhere in the world.

©Polish Histochemical et Cytochemical Society
Folia Histochem Cytobiol. 2008:46(2): 140 (137-142)
doi: 10.2478/v10042-008-0020-9

Table 2. Metallo-β-lactamases (class B1) of *Pseudomonas aeruginosa*.

Enzyme *	Nucleotide *	Gene location*	Reference
IMP-type			
IMP-1	S71932	Chromosome	Zhi, ZQ *et al.*** [24,30]
IMP-5	AF290912	Plasmid	[21]
IMP-7	AF318077	ND	[35]
IMP-9	AY033653	ND	[37]
IMP-10	AB074433	Plasmid	[48]
IMP-11	BAB72073	ND	Iyobe S, *et al.***
IMP-13	AJ550807	ND	[36]
IMP-16	AJ584652	Chromosome	[49]
IMP-18	AY780674	ND	[39]
VIM-type			
VIM-1	Y18050	Chromosome	[41]
VIM-2	AF191564	Plasmid	[22]
VIM-3	AF300454	Chromosome	[42]
VIM-4	AY135661	ND	[23]
VIM-7	AJ536835	Plasmid	[43]
VIM-8	AY524987	ND	[50]
VIM-9	AY524988	ND	Woodford N, *et al.***
VIM-10	AY524989	ND	Woodford N, *et al.***
VIM-11	AY605049	Chromosome	[51]
SPM-type			
SPM-1	AJ492820	Plasmid	[29]
GIM-type			
GIM-1	AJ620678	Plasmid	[46]

* [40]; **- not published; ND - not determined.

Concluding remarks

Pseudomonas aeruginosa MBLs-producing strains (generally multidrug resistant) cause infections that are difficult to threat and have high mortality rates. An increase in both to extend and diversity of metallo-β-lactamases in *Pseudomonas aeruginosa* severely limits treatment options at least 3 continents (Asia, Europe, and South America), resulting in use of combinations of antimicrobial agents, in an attempt to achieve synergy between drugs that are otherwise ineffective [4,25,47]. Metallo-β-lactamase - producing isolates resistant to many used antibiotics, signalling to need for the development of new, potent therapeutic agents with novel modes of action. Alternative, older, more toxic drugs, such as polymyxin B and colistin, are being using.

References

[1] Giamarellou H. Prescribing guidelines for serve *Pseudomonas* infections. *J Antimicrob Chemother.* 2002;49:229-233.

[2] Cristino JM. Correlation between consumption of antimicrobials in humans and development of resistance in bacteria. *J Antimicrob Agents.* 1999;12:199-202.

[3] Giamarellos-Bourboulis EJ, Papadimitriou E, Galanakis N, *et al.* Multidrug resistance to antimicrobials as a predominant factor influencing patient survival. *Int J Antimicrob Agents.* 2006;27:476-481.

[4] Boucher Y, Labbate M, Koening JE, Stokes HW. Integrons: mobilizable platforms that promote genetic diversity in bacteria. *Trends Microbiol.* 2007;15:301-309.

[5] Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*; our worst nightmare? *Clin Infect Dis.* 2002;34,634-660.

[6] Lombardi G, Luzzaro F, Docquier JD, *et al.* Nosocomial infections caused by multidrug-resistant isolates of *Pseudomonas* putida producing VIM-1 metallo-β-lactamase. *J Clin Microbiol.* 2002;40:4051-4055.

[7] Ambler RP. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci. 1980;289:321-31.

[8] Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother.* 1995;39:1211-1233.

[9] Fisher JF, Meroueh SO, Mobashery S. Bacterial resistance to beta-lactam antibiotics: compelling opportunism, compelling opportunity. *Chem Rev.* 2005;105:395-424.

[10] Helfand MS, Bonomo RA. Beta-lactamases: a survey of protein diversity. *Curr Drug Targets Infect Disord*. 2003;3:9-23.

[11] Walsh TR, Tolemenn MA, Poirel L, Nordman P. Metallo-β-lactamases; the quiet before the storm? *Clin Microbiol Rev.* 2005;18:306-325.

[12] Nordmann P, Poirel L. Emerging carbapenemases in gram-negative aerobes. *Clin Microbiol Infect.* 2002;8:321-331.

[13] Senda K, Arakawa Y, Ichiyama S, *et al.* PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. *J Clin Microbiol.* 1996;34:2909-2913.

[14] Heinz U, Adolph HE. Metallo-β-lactamases: two binding sites for one catalic metal ion. *Cell Mol Life Sci.* 2004;61: 2827-2839.

[15] Garau G, García-Sáez I, Bebrone C, *et al.* Update of the standard numbering scheme for class B β-lactamases. *Antimicrob Agents Chemother.* 2004;48:2347-2349.

[16] Hall BG, Salipante SJ, Barlow M. Independent origins of subgroup Bl + B2 and subgroup B3 metallo-beta-lactamases. *J Mol Evol*. 2004;59:133-41.

[17] Wommer S, Rival S, Heinz U, *et al.* Substrate-activated zinc binding of metallo-β-lactamases. Physiological importance of the mononuclear enzymes. *J Biol Chem.* 2002;277:24142-24147.

[18] Galleni M, Lamotte-Brasseur J, Rossolini GM, Spencer J, Dideberg O, Frere JM. Standard Numbering Scheme for Class B β-Lactamases. *Antimicrob Agents Chemother.* 2001;45:660-663.

[19] Hernandez Valladares M, Felici A, Weber G, *et al.* Zn(II) dependence of the Aeromonas hydrophila AE036 metallo-beta-lactamase activity and stability. *Biochemistry.* 1997;36: 11534-41.

©Polish Histochemical et Cytochemical Society

doi: 10.2478/v10042-008-0020-9

[20] Boschi L, Mercuri PS, Riccio ML, *et al.* The Legionella (Fluoribacter) gormanii metallo-β-lactamase: a new member of the highly divergent lineage of molecular-subclass B3 β-lactamases. *Antimicrob Agents Chemother.* 2000;44:1538-1543.
[21] Brizio A, Conceiçao, Pimentel M, Da Silva G, Duarte A. High-level expression of IMP-5 carbapenemase owing to point mutation in the promoter region of class 1 integron among *Pseudomonas aeruginosa* clinical isolates. *Int J Antimicrob Agents.* 2006;27:27-31.
[22] Poirel L, Naas T, Nicolas D. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother*. 2000;44:891-897.
[23] Pournaras S, Tsakris A, Maniati M, Tzouvelekis LS, Maniatis AN. Novel variant (bla_{VIM-4}) of the metallo-β-lactamase gene bla_{VIM-1} in a clinical strain of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2002;46:4026-4028.
[24] Shibata N, Doi Y, Yamane K, *et al.* PCR typing of genetic determinats for metallo-β-lactamases and integrases carried by gram-negative bacteria isolated in Japan with focus on the class 3 integron. *J Clin Microbiol.* 2003;41:5407-5413.
[25] Walsh TR, Toleman MA, Hryniewicz W, Benett PM, Jones RN. Evolution of an integron carrying bla_{VIM-2} in Eastern Europe: report from the SENTRY Antimicrobial Surveillance Program. *J Antimicrob Chemother.* 2003;52:116-119.
[26] Weldhagen GF. Integrons and beta-lactamases - a novel perspective on resistance. *Int J Antimicrob Agents.* 2004;23:556-562.
[27] Bennett PM. Integrons and gene cassettes: a genetic construction kit for bacteria. *J Antimicrob Chemother.* 1999;43:1-4.
[28] Toleman MA, Bennett PM, Walsh TR. ISCR Elements: novel gene-capturing systems of the 21st century? *Microbiol Mol Biol Rev.* 2006;70: 296-316.
[29] Toleman MA, Simm AM, Murphy TA, *et al.* Molecular characterization of SPM-1, a novel metallo-β-lactamase isolated in Latin America: report from the SENTRY Antimicrobial Surveillance Program. *J Antimicrob Chemother.* 2002;50: 673-679.
[30] Hirakata Y, Izumikawa K, Yamaguchi T, *et al.* Rapid detection and evaluation of clinical characteristics of emerging multiple-drug-resistant gram-negative rods carrying the metallo-β-lactamase gene blaimp. *Antimicrob Agents Chemother.* 1998; 42:2006-2011.
[31] Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 1991;35:147-151.
[32] Senda K, Arakawa Y, Nakashima K, *et al.* Multifocal outbreaks of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta-lactams, including carbapenems. *Antimicrob Agents Chemothe*r. 1996;40: 349-353.
[33] Sacha P, Żórawski M, Hauschild T, *et al.* The presence of blaIMP genes on plasmids DNA isolated from multidrug - resistant *Pseudomonas aeruginosa* strains at University Hospital in Bialystok (Poland) - first report. *Folia Histochem Cytobiol.* 2007; 45:405 - 408.
[34] Yan JJ, Ko WC, Wu JJ. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-β-lactamase, IMP-8, from a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother.* 2001;45:2368-2371.
[35] Gibb AP, Tribuddharat C, Moore RA, *et al.* Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new blaIMP allele, blaIMP-7. *Antimicrob Agents Chemother.* 2002;46:255-258.
[36] Toleman MA, Biedenbach D, Bennett D, Jones RN, Walsh TR. Genetic characterization of a novel metallo-β lactamase gene, blaIMP-13, harboured by a novel Tn5051-type transposon disseminating carbapenemase genes in Europe: report from the SENTRY worldwide antimicrobial surveillance program. *J Antimicrob Chemother.* 2003;52:583-590.
[37] Xiong J, Hynes MF, Ye H, *et al.* blaimp-9 and its association with large plasmids carried by *Pseudomonas aeruginosa* isolates from the People's Republic of China. *Antimicrob Agents Chemother.* 2006;50:355-358.
[38] Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y, and Korean Nationwide Surveillance of Antimicrobial Resistance Group. VIM- and IMP-type metallo-beta-lactamase-producing *Pseudomonas spp.* and *Acinetobacter spp.* in Korean hospitals. *Emerg Infect Dis.* 2003;9:868-71.
[39] Hanson ND, Hossain A, Buck L, Moland ES, Thomson KS. First occurrence of a *Pseudomonas aeruginosa* isolate in the United States producing an IMP metallo-β-lactamase, IMP-18. *Antimicrob Agents Chemother.* 2006;50:2272-2273.
[40] Lahey Clinic. IMP-type β-lactamases and VIM-type β-lactamases. Available at: http://www.lahey.org/studies/other.htm. Accessed November 6, 2007.
[41] Lauretti L, Riccio ML, Mazzariol A, *et al.* Cloning and characterization of blaVIM, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother.* 1999;43:1584-1590.
[42] Yan JJ, Hsueh PR, Ko WC, *et al.* Metallo-β-Lactamases in clinical *Pseudomonas* isolates in Taiwan and identification of VIM-3, a novel variant of the VIM-2 enzyme. *Antimicrob Agents Chemother.* 2001;45:2224-2228.
[43] Toleman MA, Rolston K, Jones RN, Walsh TR. blaVIM-7, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. *Antimicrob Agents Chemother.* 2004;48:329-332.
[44] Fiett J, Baraniak A, Mrówka A, *et al.* Molecular epidemiology of acquired-metallo-ß-lactamase-producing bacteria in Poland. *Antimicrob Agents Chemother.* 2006;50:880-886.
[45] Patzer J, Toleman MA, Desphande LM, *et al. Pseudomonas aeruginosa* strains harbouring an unusual bla_{VIM-4} gene cassette isolated from hospitalized children in Poland (1998-2001). *J Antimicrob Chemother.* 2004;53:451-456.
[46] Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a β-lactamase gene, bla_{GIM-1}, encoding a new subclass of metallo-β lactamase. *Antimicrob Agents Chemother*. 2004;48:4654-4661.
[47] Sader HS, Castannheira M, Mendes RE, Walsh TR, Jones RN. Dissemination and diversity of metallo-β-lactamases in Latin America: report from the SENTRY Antimicrobial Surveillance Programme. *Int J Antimicrob Agents.* 2005;25:57-61.
[48] Iyobe S, Kusadokoro H, Takahashi A, *et al.* Detection of a variant metallo-β-lactamase, IMP-10, from two unrelated strains of *Pseudomonas aeruginosa* and an *Alcaligenes xylosoxidans* strain. *Antimicrob Agents Chemother*. 2002;46: 2014-2016.
[49] Mendes RE, Toleman M A, Ribeiro J, Sader HS, Jones RN, Walsh T R, *et al.* Integron carrying a novel metallo-β -lactamase gene, blaIMP-16, and a fused form of aminoglycoside-resistant gene *aac(6')-30/aac(6')-Ib'*: Report from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother.* 2004;48:4693-4702.
[50] Crespo MP, Woodford N, Sinclair A, *et al.* Outbreak of carbapenem-resistant *Pseudomonas aeruginosa* producing VIM-8, a novel metallo-β-lactamase, in a tertiary care center in Cali, Colombia. *J Clin Microbiol.* 2004;42:5094-5101.
[51] Pasteran F, Faccone D, Petroni A, *et al.* Novel variant (blaVIM-11) of the metallo-ß-lactamase blaVIM family in a GES-1 extended-spectrum-β-lactamase-producing *Pseudomonas aeruginosa* clinical isolate in Argentina. *Antimicrob Agents Chemother.* 2005;49:474-475.

Submitted: 22 November, 2007
Accepted after reviews: 27 January, 2008

©Polish Histochemical et Cytochemical Society