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 contribute 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
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 (Zn2+) coordinated to two water molecules as the reactive nucleophiles (Fig. 2).

MBLs are produced by bacteria as extracellular or periplasmatic enzymes. All known representatives' possess 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].

Table 1. Classification of β-lactamases.

<table>
<thead>
<tr>
<th>Functional mechanism</th>
<th>Ambler class</th>
<th>Bush (Groups)</th>
<th>Examples</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine-β-lactamases</td>
<td>Class A- penicillinas</td>
<td>(2a,2b,2c)</td>
<td>Broad-spectrum β-lactamases: TEM-1, TEM-2, SHV-1</td>
<td>Benzylpenicillin (penicillin), amoxicillin, ampicillin, carboxy-β-lactamases (carboxy-β-lactamases, ticarcillin), narrow-spectrum cefalosporins (cefazolin, cefuroxim and others)</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>(2bc)</td>
<td>Expanded-spectrum-β-lactamases (ESBL): TEM family and SHV-family</td>
<td>Substrates of the broad-spectrum group β-lactamases plus clavulanic, methicillin and oxacillin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Others: BES-1, GES/IBC family, PER-1, PER-2, SFO-1, TLA-1, VEB-1/2</td>
<td>Same as for TEM and SHV family</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2br)</td>
<td>TEM family (TEM-30, TEM-31) IRTs*</td>
<td>Same as for TEM and SHV family and inhibitors resistant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2e)</td>
<td>CTX-family</td>
<td>Substrates of the expanded-spectrum-β-lactamases group, for some enzymes, cefepime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2f)</td>
<td>Carbapenemases: (KPC-1, KPC-2 and KPC-3, GES-1, GES-2)</td>
<td>Substrates of the expanded-spectrum-β-lactamases group plus cefapemycins and carbapenem (ertapenem, meropenem, imipenem)</td>
</tr>
<tr>
<td>Metallo-β-lactamases</td>
<td>Class B-metallo-β-lactamases (zinc)</td>
<td>(3a,3b,3c)</td>
<td>Carbapenemases: IMP family, VIM-family, SPM-1, SPM-2, GIM-1, and L1, CcrA</td>
<td>Same as for carbapenemases class A</td>
</tr>
<tr>
<td>Serine-β-lactamases</td>
<td>Class C- cephalosporinas</td>
<td>(1)</td>
<td>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</td>
<td>Substrates of the expanded-spectrum-β-lactamases group plus cephamycins</td>
</tr>
<tr>
<td>Serine-β-lactamases</td>
<td>Class D-clavulanic hydrolyzing enzymes (OXA)</td>
<td>(2d)</td>
<td>Most of OXA family</td>
<td>Substrates of the broad-spectrum group plus clavulonic, methicillin and oxacillin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Other OXA: OXA-23 → OXA-27, and OXA-40, OXA-48</td>
<td>Same as for IMP family, VIM-family, SPM-1, SPM-2 and GIM-1</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>(4)</td>
<td>AVS-1</td>
<td>Miscellaneous or unsequenced/uncharacterized enzymes that do not fit into any function or molecular group</td>
</tr>
</tbody>
</table>
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 (*attl*) 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.
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<sub>SPM-1</sub> 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 <em>et al.</em> [28] has detected ISCR elements in several strains of <em>P. aeruginosa</em>. ISCR2 was discovered in a <em>P. aeruginosa</em> isolate that harboured bla<sub>VIM</sub><sub>1</sub> and ISCR3 was discovered in two of <em>P. aeruginosa</em> strains that have bla<sub>VIM</sub><sub>1</sub>.

**Epidemiology of MBLs in clinical isolates of <em>Pseudomonas aeruginosa</em>**

Most of metallo-β-lactamases (class B1) were found in <em>Pseudomonas aeruginosa</em> strains (Table 2).

The first 'mobile' MBL of <em>P. aeruginosa</em> 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 <em>Pseudomonas</em> spp., <em>Acinetobacter</em> spp., and <em>Enterobacteriaceae</em>. 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 <em>P. aeruginosa</em> 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 <em>P. aeruginosa</em> 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 <em>P. aeruginosa</em> 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<sub>VIM</sub> positive <em>P. aeruginosa</em> 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 <em>P. aeruginosa</em> is SPM-1 (Sao Paulo Metallo-β-lactamase). First <em>P. aeruginosa</em> with bla<sub>SPM-1</sub> 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 <em>P. aeruginosa</em> 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.
Pseudomonas aeruginosa

MBLs-producing strains (generally multidrug resistant) cause infections that are difficult to treat and have high mortality rates. An increase in both the extent 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 a 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 used.

References


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 used.


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