



## *Metallo- $\beta$ -lactamases*

### **Introduction**

The use of antibiotics has been a double-edged sword. On one hand they have provided successful treatments of infectious diseases for many years, but on the other hand their use has resulted in the development of antimicrobial resistance. When first used, the antibiotic penicillin was active against many different bacterial species, but the rapid development of penicillin resistance followed the widespread use. The ability of bacteria to rapidly change and adapt to environmental pressures caused by high use of antibiotics soon became apparent and remains an issue today.

Bacteria can develop antimicrobial resistance in a variety of ways: by acquiring new genetic material such as plasmids, mobile genetic elements such as insertion sequences or transposons, or by changing binding sites via mutations. The earliest form of penicillin resistance was caused by the  $\beta$ -lactamase. The enzyme became mobile and was transferred to other bacterial microorganisms on plasmids. The transfer of these beta-lactamase genes allowed bacteria to produce an enzyme to destroy the structure of penicillin and thereby to cause the loss of function of the antibiotic. These  $\beta$ -lactamases have since become widespread and have evolved by mutation. As

a result, we now see enzymes affecting the extended activity to newer generation cephalosporin antibiotics through TEM and SHV genes or the carbapenemases such as metallo- $\beta$ -lactamases (MBL).

### **Metallo- $\beta$ -lactamases**

The metallo- $\beta$ -lactamases are members of the molecular class B enzymes that require the presence of heavy metal such as zinc for functionality. Out of all the carbapenemases, MBLs are the most molecularly diverse and of great clinical concern.

The genes encoding MBLs are part of the bacterial chromosome producing intrinsic resistance of some gram positive and gram negative organisms. Transferable MBLs are encoded by *bla* genes. The *bla* genes are typically found as gene cassettes in class 1 integrons and move between organisms mainly on plasmids but also on transposons, locations that facilitate their horizontal spread. In these locations, *bla* genes are frequently found in association with aac- or aad-type genes that confer resistance to aminoglycosides or with extended spectrum  $\beta$ -lactamases (ESBLs) of the OXA-, TEM- and CTX-types.

There are five types of acquired MBLs identified to date: IMP, VIM, SPM-1, GIM-1 and SIM-1. IMP and VIM types have a wide

geographic distribution. There are 18 imp gene types (imp1 -18) and 12 vim types (vim1-12) distributed around the world. Each gene type of MBL has different levels of  $\beta$ -lactam hydrolysis for example vim-1 hydrolyses all  $\beta$ -lactam antibiotics except aztreonam.

The *bla* imp-1 gene (initially found in Japan) is the most widespread type for this group and is found in *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Providencia rettgeri*, *Morganella morganii* and *Alcaligenes sp.*

The vim-1 gene was initially found in Greece and Italy. The *bla* vim-2 is a common vim type of MBL and is found in *P. aeruginosa* but has also been detected in *P. putida*, *P. fluorescens*, *A. baumannii*, *K. pneumoniae*, *K. oxytoca*, *E. cloacae*, *Proteus mirabilis* and *E. coli*.

The other MBL types are less widespread among bacterial species and seen mainly in *P. aeruginosa*.

SPM-1 was initially identified in Brazil in 2001 in a multi-drug-resistant (MDR) *P. aeruginosa* isolate (susceptible to polymyxin B only).

GIM-1 has been detected in five MDR *P. aeruginosa* isolates from different patients hospitalised during 2002 in a medical centre in

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# Metallo- $\beta$ -lactamases

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Düsseldorf, Germany. SIM-1 has been detected in seven clinical *A. baumannii* isolates from a tertiary hospital in Seoul, South Korea.

## Laboratory Testing

Currently, there are no Clinical Laboratory Standards Institute (CLSI) guidelines for screening bacterial isolates for acquired MBL production. Several phenotypic tests have been developed for MBL detection, such as the MBL Etest, double-disk synergy tests, combined disk (CD) assay, microdilution, and the Hodge test. All of these tests are based upon the ability of chelating agents, EDTA and thiol-based compounds, to inhibit the MBL activity.

## Pilot Study

32 non-duplicate *Pseudomonas aeruginosa* isolates from respiratory and blood culture samples were selected for this study. They were non-susceptible to imipenem (IPM) and/or meropenem (MEM). The isolates were screened with IPM-ethylenediaminetetraacetic acid (EDTA) and MEM-EDTA combined disk test (CDT), IPM-dipicolinic acid (DPA) combined disk test and Modified Hodge Test (MHT). Two MBL-producing positive control strains, IMP-4 producing *E. coli*, and VIM-4 producing *P. aeruginosa* were included. PCR for universal *bla*<sub>-IMP</sub> and *bla*<sub>-VIM</sub> was performed for confirmation of phenotypic tests.

The combined disk test with IPM-EDTA detected 29 out of 32 strains of IPM or MEM-non-susceptible *P. aeruginosa*. Only 7 strains were positive with IPM-DPA combined disk test and only one isolate was MHT positive. The only PCR confirmed MBL producer was positive by all 3 phenotypic screen tests and carried *bla*<sub>-IMP</sub> gene. DNA sequencing result showed it produced *bla*<sub>-IMP-7</sub>.

The low specificity of the EDTA test can be attributed to its inherent antimicrobial activity and antibiotic lysis properties. DPA does not have any detectable antimicrobial activity and shows better specificity than EDTA. The MHT was the most specific of the phenotypic based assays but is not specific for MBL as it can detect other carbapenemases such as KPC. The imipenem/meropenem resistance amongst the MBL negative *P. aeruginosa* isolates could be due to efflux and impermeability mechanisms.

IMP-7 was first seen in 2002, and has been reported from Canada, Japan, Malaysia and Slovakia. The patient with IMP-7-producing *P. aeruginosa* isolate in our study was a haematology patient with Burkitt-like lymphoma who was a resident initially treated in China and Hong Kong. She had subsequently returned to New Zealand for chemotherapy. It is likely that this isolate was imported from overseas.

## Clinical Treatment

*P. aeruginosa* can harbour many antibiotic resistant mechanisms such as loss of porin function, aminoglycoside co-resistance, ESBL and AmpC enzymes that limit the treatment choices to imipenem. The acquisition of MBL genes further limits treatment options to colistin and tigecycline only. The control of this resistance mechanism is therefore important especially for patients in intensive care and in the cystic fibrosis group.

## Recommendations

These results, although numbers are small, highlight the difficulties in the detection of Gram negative  $\beta$ -lactamases by phenotypic tests. It is recommended to screen isolates for MBL using the MHT and the IMP-DPA tests and confirm positives using the IMP and VIM PCR. Active monitoring should be performed for *Pseudomonas spp* isolates, and if this mechanism is detected infection control measures should be initiated to prevent nosocomial outbreaks and further spread of resistance genes to other organisms.

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