

Detection of Metallo- β -lactamases in CDS Testing

A number of Gram negative species including *Pseudomonas aeruginosa* and members of Enterobacteriaceae are capable of hydrolysing carbapenems and all other β -lactams with the exception of aztreonam by expression of metallo- β -lactamases (MBL). These strains have been reported in Victoria and, more recently, they have been detected by laboratories in Sydney.

Screening for MBL in the Initial Disc Test

Ps. aeruginosa

MBL producing *P. aeruginosa* are presumptively identified by their resistance to imipenem and meropenem and all other β -lactams except aztreonam.

Enterobacteriaceae

MBL producing Enterobacteriaceae may appear susceptible to both imipenem and meropenem and hence the expression of the enzyme may not be apparent. The MBLs of Enterobacteriaceae hydrolyse other β -lactams more efficiently than they hydrolyse the carbapenems. We take advantage of this characteristic by loading a cefepime 10 μ g disc next to the Augmentin 60 μ g disc in the disc dispenser. Resistance to cefepime and the absence of clear zone of enzyme antagonism by clavulanic acid between the adjacent cefepime and Augmentin discs is strong presumptive evidence of the presence of an MBL.

Confirmation

MBL expression can be confirmed by using one of two methods that demonstrate EDTA chelation of zinc ions required for MBL activity. The first uses parallel testing of a carbapenem disc and an EDTA supplemented carbapenem disc, as described in the fourth edition of the CDS Manual. A larger zone around the EDTA supplemented disc confirms the presence of an MBL. With *Pseudomonas* in particular the concentration of the EDTA in the supplemented disc is critical as EDTA itself may show antibacterial activity. This aspect of the test and the need for a control disc of EDTA alone are discussed in section 11.2.2.1 (appendix) of the CDS Manual.

An alternate technique involves positioning a disc loaded with EDTA 415 μ g next to a cefepime 10 μ g or Imipenem 10 μ g disc. When the EDTA and the antibiotic discs are positioned about 10 mm apart, edge to edge, a zone of inhibition of growth between the two discs indicates the presence of an MBL.

Co-expression of MBL and ESBL

It is not uncommon for some organisms to elaborate both an ESBL and an MBL. In these cases expression of an ESBL cannot be detected in the usual way. In Enterobacteriaceae co-expression of both an MBL and an ESBL can be detected by a disc approximation tests using cefepime, aztreonam and Augmentin. The disc cartridge is loaded with cefepime 10 μ g and aztreonam 30 μ g either side of the Augmentin 60 μ g disc. Aztreonam is hydrolysed by the ESBL but not by the MBL. However, the clavulanic acid from the Augmentin disc blocks the activity of the ESBL resulting in a “keyhole” deformity of the aztreonam zone or an elliptical zone

of inhibition of bacterial growth. On the other hand the MBL hydrolyses cefepime and the enzyme activity is not affected by clavulanic acid.

Note : These recommendations are provisional as they are based on reports in the literature and at this stage the results of testing a small number of strains in the CDS laboratory. CDS users are asked to send to the CDS laboratory those strains they suspect are elaborating MBL not only for confirmation but also so that we can build up a greater experience with these strains.