DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE

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Introduction

It is recognised that detection of carbapenemase producing Enterobacteriaceae may be problematic using disc methods of antimicrobial susceptibility. However, laboratories using the CDS disc method do not appear to have the same difficulty. There are a number of reasons for this, The first is that the CDS method employs a higher inoculum and lower potency antibiotic discs which facilitates the detection of enzyme-mediated resistance. Secondly users are encouraged to strategically position antibiotic discs on the susceptibility plate then examine inhibitory zone morphology and the interaction of inhibitory zones of adjacent discs. CDS users have ready access to a wealth of information via the CDS Manual and the CDS website with diagrammatic illustrations of the methodology and process. Finally laboratories using the CDS method have a unique and immediate access to the CDS Reference Laboratory for advice and confirmatory testing.

A full description of the detection of carbapenemases can be accessed in the on-line version of the CDS Manual at "Gram negatives 5.5.8 - 5.5.9", supporting photographs (plates 11.15 – 11.16) also can be viewed and enlarged simply by clicking on the image. Also available on the website is a number of Powerpoint slides from the presentation given by Jeanette Pham PhD at the CDS Workshop in 2012. The slides are freely available for viewing or downloading.

This report summarises the information that is available to CDS users and includes a number of plates to illustrate the phenomena. CDS methodology detects three main classes of carbapenemases:

I. Metallo-β-lactamases or MBLs (Bush group 3, Ambler class B)

The MBLs of the Enterobacteriaceae hydrolyse carbapenems less efficiently than they hydrolyse other β-lactams consequently isolates that express an MBL may still appear susceptible to both imipenem and meropenem. However, their more efficient hydrolysis of other β-lactams and their non-inhibition by clavulanic acid can be used to screen for their presence.

Screening for an MBL

Resistance observed with a cefepime 10 µg disc and the absence of a synergistic zone of inhibition between this disc and an adjacent Augmentin 60 µg disc (containing clavulanic acid) is suggestive of the presence of an MBL,

see Fig 1.

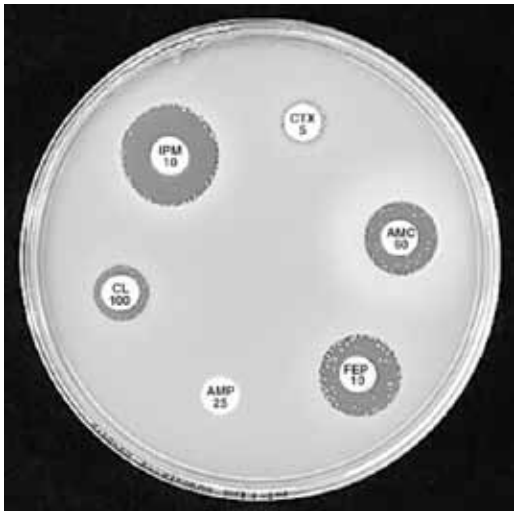

Figure 1. *K. pneumoniae* producing an MBL on routine CDS test.

Resistant to ampicillin (AMP), Augmentin (AMC), cefotaxime (CTX), cephalexin (CL), cefepime (FEP) and colonies at the edge of imipenem (IPM) zone ≥ 6 mm.

Confirmation of an MBL

The expression of an MBL can be confirmed by demonstrating the loss of β-lactamase activity following chelation of zinc ions. When a disc loaded with EDTA 415 µg is positioned 10 mm, edge to edge, from an imipenem or an ertapenem 10 µg disc, there is a deformity of the inhibitory zone towards the EDTA disc indicating inhibition of the MBL by EDTA, see Fig 2.

Expression of both MBL and ESBL

It is not uncommon for Enterobacteriaceae isolates to express both an extended spectrum β -lactamase (ESBL) and an MBL. In these cases, expression of an ESBL cannot be detected in the usual way. An aztreonam 30 µg disc placed in the centre of the plate will show the typical "key hole" with an Augmentin 60 µg disc. The presence of the MBL should be confirmed as described above, see Fig 2.

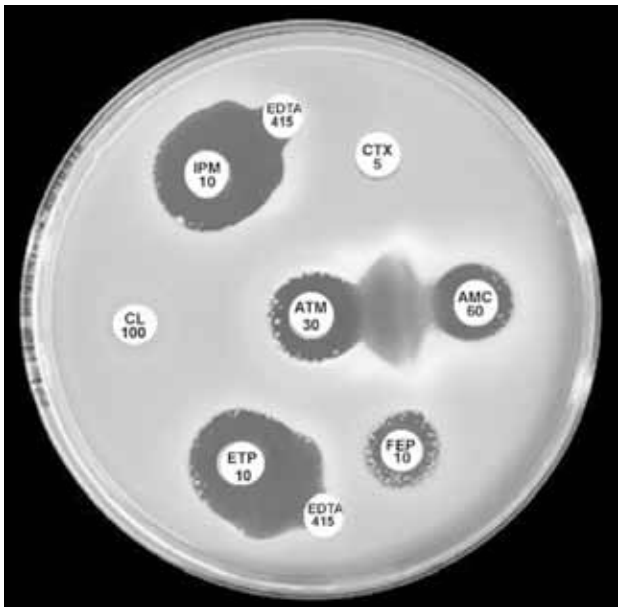

Figure 2. *K. pneumoniae* producing an MBL and an ESBL - confirmation.
MBL presence indicated by deformity of imipenem (IPM) and ertapenem (ETP) zones caused by inactivation of MBL by adjacent EDTA disc. ESBL indicated by "key hole" between aztreonam (ATM) and Augmentin (AMC).

II. *Klebsiella pneumoniae* carbapenemases or KPCs (Bush group 2f, Ambler Class A)

KPCs are essentially "fully extended spectrum β-lactamases" of Bush group 2 (Ambler class A) plasmid mediated β-lactamases that hydrolyse all β-lactam antibiotics including the carbapenems. Although inhibited to some extent by clavulanic acid and tazobactam, the enzyme is very efficient and affects all β-lactams including those containing β-lactamase inhibitors ie Timentin, Augmentin and Tazobactam.

Due to the use low potency discs and a the higher inoculum used in the CDS test, KPC producers are readily recognised as resistant to all β-lactam antibiotics tested including the carbapenems, see Fig 3.

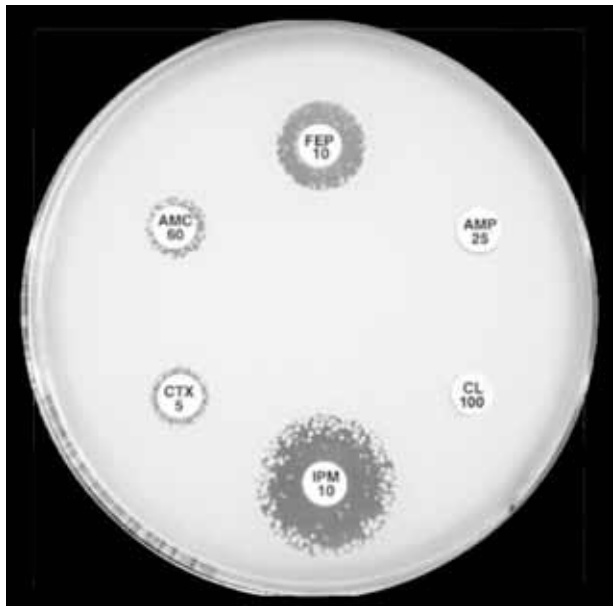

Figure 3. *K.pneumoniae* producing carbapenemase (KPC)
Obvious resistance to all agents tested including imipenem (IPM) and cefepime (FEP)

Confirmation of a KPC.

The presence of a KPC can be confirmed by positioning an Augmentin disc next to the imipenem disc. The presence of a KPC is indicated by a deformity of the imipenem zone towards the Augmentin disc indicating inhibition of the KPC by Augmentin, see Fig 4.

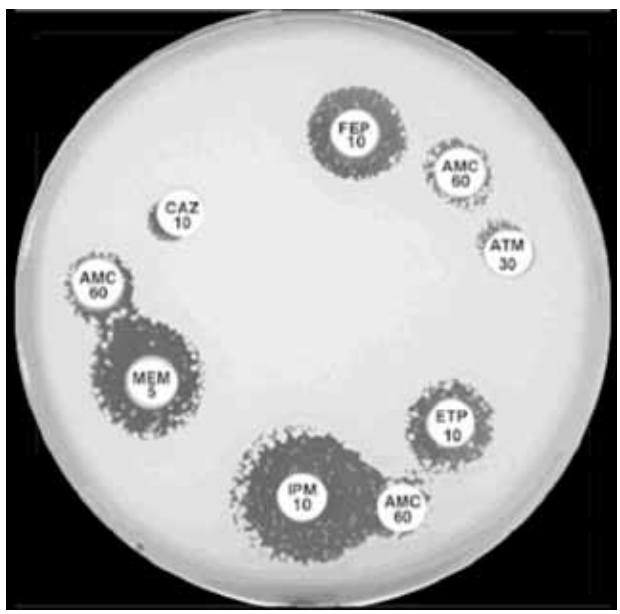

Figure 4. *K. pneumoniae* producing carbapenemases (KPC) – confirmation.
KPC presence indicated by deformity of imipenem (IPM) zone caused by inactivation of carbapenemase by Augmentin (AMC).

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III. Oxa-48 and Oxa-181 carbapenemases (Bush group 2d, Ambler Class D)

Oxa-48 and Oxa-181 carbapenemases cannot be detected using phenotypic detection technique and are rarely seen in Australia. Strains harbouring theses genes are highly resistant to all carbapenems and will be recognised as resistant to the carbapenems in CDS routine testing. The Oxa-48 and Oxa-181 genes can be detected by PCR.

Conclusion

Provided the methodological process is followed closely, laboratories using the CDS method should have no problem presumptively identifying those strains which elaborate a carbapenemase. Where they need assistance it is no further than a phone call to the CDS Reference laboratory away. If they are still not sure, CDS users can send these isolates to the CDS laboratory for confirmation of identity by MALDI-TOF and the detection by PCR of carbapenemase genes including bla_{IMP}, $bla_{\text{NDM-1}}$, bla_{VIM}, bla_{SPM}, $bla_{\text{AIM-1}}$, $bla_{\text{GIM-1}}$, bla_{KPC} and bla_{Oxa} genes.