BREAK POINT

2013 - ISSUE 05

WELCOME TO ANOTHER EDITION OF BREAKPOINT

Welcome to another, more-leisurely, August issue of Breakpoint. As a follow up to the article on the detection of carbapenemases (June Breakpoint) where phenotypic and molecular methods are discussed, Professor Syd Bell and Ms. Jeanette Pham, from the Microbiology Department at the Prince of Wales Hospital, Sydney extend the perspective by discussing the ins and outs of using the CDS method for the screening for, and detection of, carbapenemase producing Enterobactericeae. Readers can access the CDS website and are directed particularly to the illustrations in this issue.

A picture quiz is provided by a colleague, Koh Tse Hsien from Singapore (and the answer is given at the end of the news-sheet!). A conference calendar follows.

The Committee as always, welcomes feedback.

Sharon Chen
ASA Newsletter Editor

Antimicrobials 2014

Thurs 20th - Sat 22nd February 2014 Melbourne Convention Exhibition Centre. Melbourne, Victoria
www.antimicrobials2014.com

PLENARY SPEAKERS

Resistance Amplification by Cross Transmission. Susan Huang. University of California, USA
Epidemiology and Susceptibility Testing of Fungal Infections. Maiken Arendrup. Statens Serum Institute, Denmark
Antibiotic Dosing in ICU: Moving towards Individualised Therapy? Jason Roberts. University of Queensland, Australia

KEYNOTE SPEAKERS

Targeted versus Universal Decolonization to Prevent ICU Infection. Susan Huang. University of California, USA
BSAC Outpatient Parenteral Antimicrobial Therapy Guidelines. Andrew Seaton. Gartnavel General Hospital, UK

SYMPOSIUM

Clostridium difficile Still Very Difficult	MDR – Many Different Responses	Investing in Fungal Futures	Therapeutic Drug Monitoring – Peaks and Troughs in the Real World	Bug Time Stories
Epidemiology: "Where the Wild things Are? (Tom Riley)	Modelling a Response to MDR (Emma McBryde)	Australian Perspective: Antifungal Susceptibility (Sarah Kidd)	β-lactam TDM in Clinical Practice (Jason Roberts)	*Streptococcus pneumoniae*: the Attributable Disease Burden Due to Resistance (Susan Huang)
Hypervirulence or Just Hype? (Allen Cheng)	Antibiotics in Agriculture – Is there an Ethics Dilemma (Peter Collignon)	Non-Culture Based Diagnostics in Mycology (Catriona Halliday)	Aminoglycoside Dosing – Current Controversies (Evan Begg)	Staphylococcal Bacteraemia: New Knowledge on Optimum Treatment (Natasha Holmes)
Infection Control Issues (Rhonda Stuart)	How can Whole Genome Sequencing Enhance our Understanding – Information Overload (Ben Howden)	Treatment of Candidaemia: What's New (Maiken Arendrup)	Practical Challenges (John Turnidge)	Multi resistance Plasmids in Enterobacteriaceae (Sally Partridge)
Establishing a Faecal Matter Unit (Patrick Charles)				

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IN THE NEWS

In perfect timing with the ASID Antibiotic Resistance Summit, just held, the September 1 issue of *Clinical Infectious Diseases* features a Review article in which Professor Peter Collignon, amongst others has played a major role.

Entitled "The scourge of antibiotic resistance: the important role of the environment", it reminds us to take step in that in mitigating the further mergence and dissemination of antibiotic-resistant organisms. This requires us as a community to not only focus on antibiotic stewardship but to also reduce human activity that enhances resistance in the environment – including antimicrobial practice in agriculture and aquaculture. Water is an underappreciated route for the dissemination of antibiotic resistance.

DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE

Sydney M. Bell and Jeanette Pham
Microbiology, SEALS, Sydney, Australia.

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 given 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.

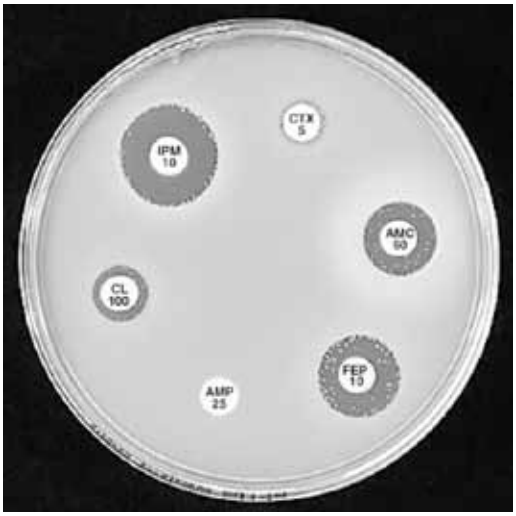

Figure 1. *K. pneumoniae* producing an MBL on routine CDS test.

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

DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE CONT'D

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Microbiology, SEALS, Sydney, Australia.

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.

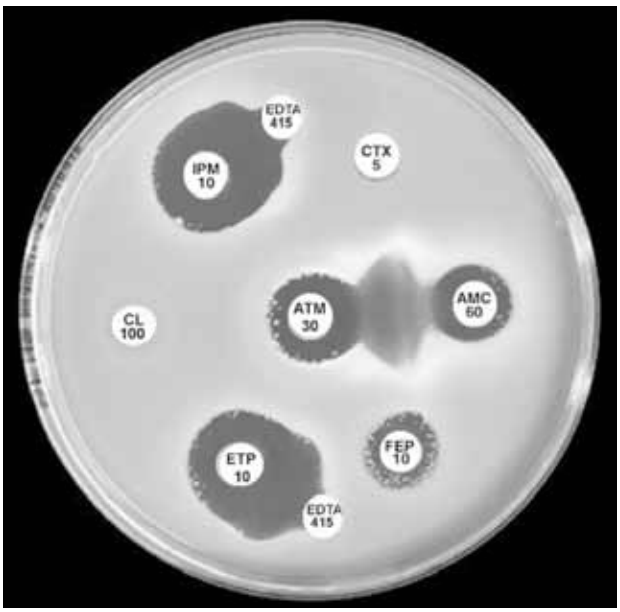

Figure 2. *K. pneumoniae* producing an MBL and an ESBL - confirmation.
MBL presence indicated by deformity of imipenem (IPM) and ertapenem (ETP) zones caused by inactivation of MBL by adjacent EDTA disc. ESBL indicated by "key hole" between aztreonam (ATM) and Augmentin (AMC).

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.

DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE CONT'D

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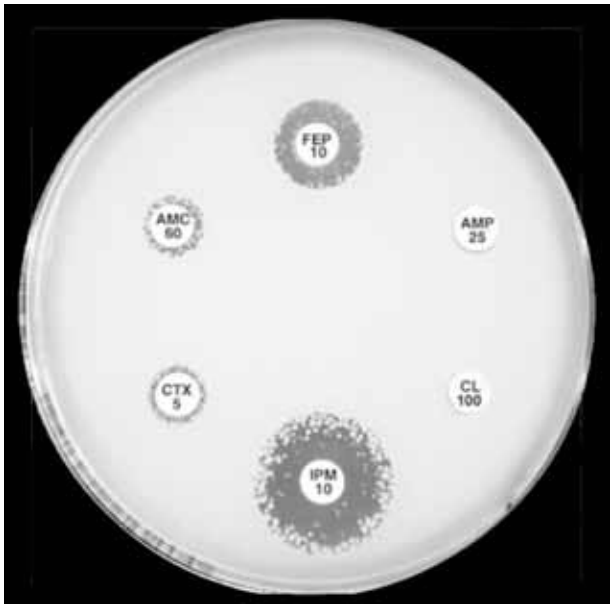

Figure 3. *K.pneumoniae* producing carbapenemase (KPC)
Obvious resistance to all agents tested including imipenem (IPM) and cefepime (FEP)

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.

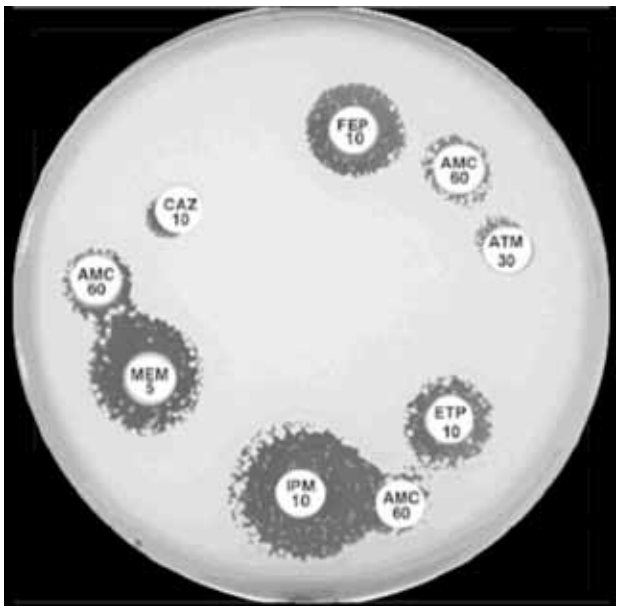

Figure 4. *K. pneumoniae* producing carbapenemases (KPC) – confirmation.
KPC presence indicated by deformity of imipenem (IPM) zone caused by inactivation of carbapenemase by Augmentin (AMC).

DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE CONT'D

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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_{NDM-1}, bla_{VIM}, bla_{SPM}, bla_{AIM-1}, bla_{GIM-1}, bla_{KPC} and bla_{Oxa} genes.

ASA RESEARCH GRANTS

Annual grants of up to $25,000 to support original research have been made available by the Australian Society of Antimicrobials (ASA). Grants may/may not be awarded annually at the discretion of the ASA Executive Committee. The following conditions apply:

- Funding is limited to ASA financial members.
- The Principal Investigator (PI) must have been an ASA member for at least twelve months
- The successful applicant will present their work at an ASA annual scientific meeting
- If applicable the PI will submit an article for the ASA newsletter and website
- ASA will be acknowledged on all resulting publications and presentations
- 12 and 24 month progress reports must be submitted to the ASA committee

The application must be performed on line via the ASA website **Research Grant Application Form** and must include:

- A copy of the PI's CV.
- Head of Department confirmation of the application and the Department has the resources required to undertake the project.

The grant application and CV will be assessed by a panel of at least six independent reviewers using a standardised scoring system. Applicants will be informed of the decision one week before the ASA annual scientific meeting "Antimicrobials 2014".

The closing date for 2013 is Friday 25th October.

Click here for the **Research Grant Application Form**.

For further enquiries please contact the ASA secretary on info@asainc.net.au

2013 - 2014 MEETING CALENDAR

2013

52nd Interscience Conference for Antimicrobial Agents and Chemotherapy
Sept 10-13, Denver, Colorado
Website: www.icaac.org/

Australian College of Infection Prevention and Control
Sept 30-Oct 2, Gold Coast, Queensland
Website: www.ashm.org.au/conferences

ID week (IDSA, SHEA, HIVMA, PIDS)
Oct 2-6, San Francisco, California
Website: www.icaac.org/

10th International Meeting on Microbial Epidemiological Markers
Oct 2-4, Paris, France
Website: www.immem-10.org/

8th world congress of Pediatric Infectious diseases
November 19-22, Cape Town, South Africa
Website: www2.kenes.com/wspid/

Mycology Master Class VI
Oct 31 - Nov 2, Kingscliffe, NSW
Website: www.asid.net.au

ESCMID Conference on *Escherichia coli*
Nov 20-22, Barcelona, Spain
Website: http://www.escmid.org/

2014

Australian Society for Antimicrobials Annual Meeting
Feb 20-22, Melbourne, Vic
www.antimicrobials2014.com

Royal College of Pathologists of Australasia Update Meeting 2014
February 21-23, Melbourne, Vic
Website: www.rcpa.edu.au

16th International Congress on Infectious Diseases (ICID)
April 2-5, Cape Town, South Africa
Website: www.isid.org/org/icid

SHEA, Society for Healthcare Epidemiology of America
April 3-6, Denver. USA
Website: www.shea-online.org

114th American Society for Microbiology Annual Meeting
May 17-20, Boston, USA
Website: www.asm.org

Australian Society for Microbiology Annual Scientific Meeting
July 6-9, Melbourne
Website: www.theasm.org.au

16th International Symposium on Staphylococci and Staphylococcal Infections - ISSSI 2014
August 26-29, Chicago, USA
Website: www.isssi2014.com

54th Interscience Conference for Antimicrobial Agents and Chemotherapy
Sept 6-9, Washington D.C. USA
Website: www.asm.org

15th Asia Pacific congress of Clinical Microbiology and Infection
Nov 26-29, Kuala Lumpur
Website: www.apccmi2014.org/

ASA QUIZ

An *Enterobacter cloacae* was isolated on chromID CARBA agar from the stool of a healthy nursing home resident in Singapore during the course of a surveillance study. The MICs for imipenem, meropenem and ertapenem were all > 32 mg/L by Etest. The results of the other antimicrobial susceptibility tests are shown in Figures 1-4.

What is the likely mechanism of carbepenem resistance?

Figure 1. Antimicrobials clockwide from top right; ampicillin, cephalothin, cefoxitin, ceftriaxone, amoxicillin-clavulanate, aztreonam, imipenem (center).

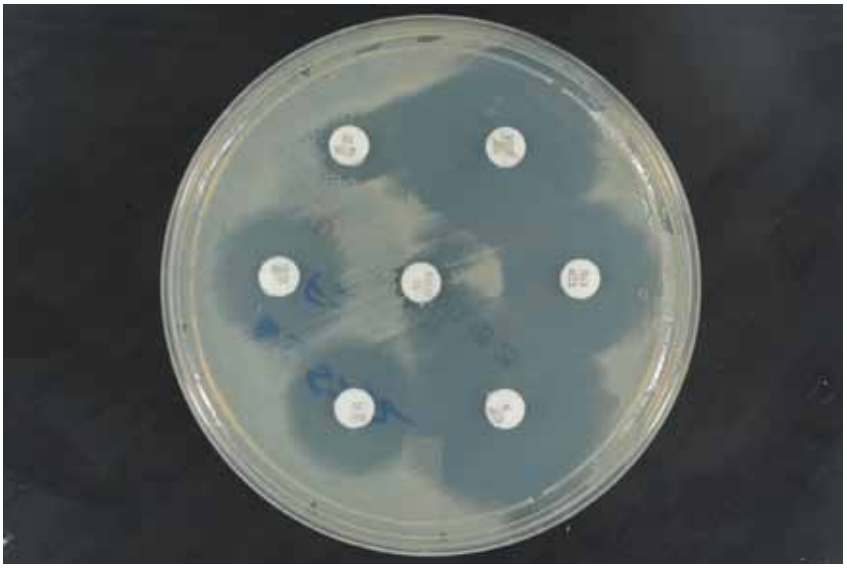

Figure 2. Antimicrobials clockwide from top right; cefepime, piperacillin-tazobactam, ciprofloxacin, amikacin, gentamicin, ertapenem, meropenem (center).

ASA QUIZ CONT'D

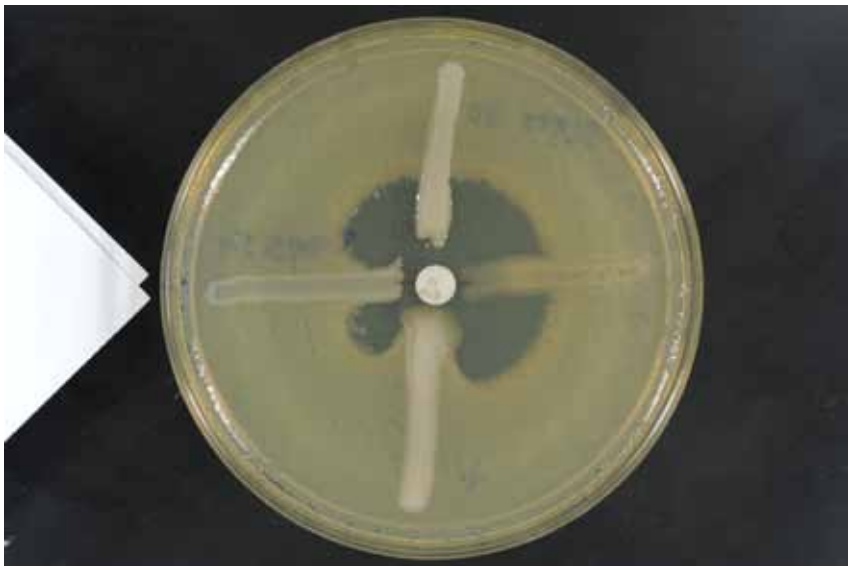

Figure 3. Modified Hodge test with arrow pointing at test isolate.

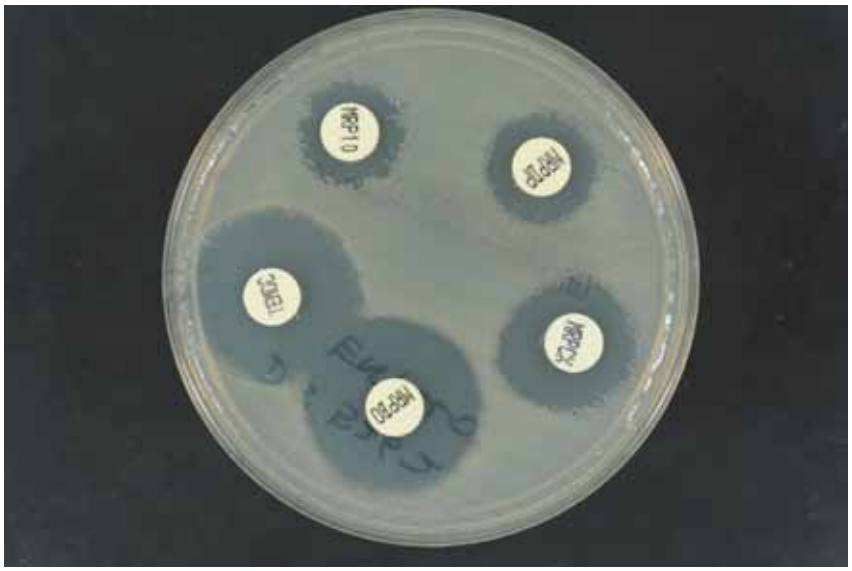

Figure 4. Rosco KPC+MBL kit. Clockwise from top left; meropenem, meropenem and dipicolinic acid, meropenem and cloxacillin, meropenem and boronic acid, temocillin.

ASA QUIZ CONT'D

Answer to Quiz

The isolate was PCR positive for the IMI-1 gene.

IMI-producing *E. cloacae* were first isolated in the United States in 1984, a year before imipenem was approved for clinical use. IMI-1 belongs to a group of class A serine carbapenemases that include Sme-1 and NmcA. Isolates that produce IMI-1 characteristically are resistant to penicillins, early cephalosporins and carbapenems but remain susceptible to oxyimino-cephalosporins. This antibiotic phenotype is similar to that of an OXA-48 producer. However an IMI-1 producer is not resistant to temocillin (Figure 4).

Despite being one of the first carbapenemases to be described in Enterobacteriaceae, IMI-1 remains very rare. Surprisingly, this is not the first IMI-1 producing *E. cloacae* isolated in Singapore as JW Teo *et al* recently described a similar isolate.

Because the IMI-1 gene is chromosomal and oxyimino-cephalosporins like ceftriaxone may still be used for treatment, the implications for clinical management and infection control are less critical than those for Enterobactericaeae with acquired carbapenemases like NDM-1, OXA-48 and KPC-2.

Rasmussen BA, Bush K, Keeney D, Yang Y, Hare R, O'Gara C, Medeiros AA.
Characterization of IMI-1 beta-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob Agents Chemother. 1996 Sep;40(9):2080-6.

Naas T, Cattoen C, Bernusset S, Cuzon G, Nordmann P. First identification of *bla* $_{IMI-1}$ in an *Enterobacter cloacae* clinical isolate from France. Antimicrob Agents Chemother. 2012 Mar;56(3):1664-5.

Teo JW, La MV, Krishnan P, Ang B, Jureen R, Lin RT. *Enterobacter cloacae* producing an uncommon class A carbapenemase, IMI-1, from Singapore. J Med Microbiol. 2013 Jul;62(Pt 7):1086-8.

HOWARD FLOREY ORATION

The Howard Florey Oration is delivered each year during the Society's annual scientific meeting and will be presented by a Scientist who has made a significant contribution to a greater understanding of antimicrobials and their appropriate use.

The Australian Society for Antimicrobials would like to thank The Florey Institute of Neuroscience and Mental Health, University of Melbourne, for allowing the Society to use the Howard Florey name for what we believe will become one of the prestigious scientific presentations on the Australian scientific meeting calendar.

The 2014 Howard Florey Oration will be presented by:

Professor Roger Nation
Faculty of Pharmacy and Pharmaceutical Sciences
Monash University, Australia

"My Polymyxin Life"

Date: Thursday 20th February 2014

Time: 1800 - 1845

Venue: Melbourne Convention Centre