BREAK POINT

2013 - ISSUE 03

WELCOME TO ANOTHER EDITION OF BREAKPOINT

A welcome pharmaceutical addition to help us control *Clostridium difficile* infections is now TGA approved. Salmonella enterica, although affecting largely animals, has again reared its head in terms of acquiring more mechanisms of defeating antibiotic therapy, this time being multi-resistant *(see In the News)*. Peter Collignon comments on superbugs in food in general.

Andrew Ginn and colleagues follow with their article on the Detection of Carbapenemases in Enterobacteriaceae" with the aims of providing busy laboratory scientists with a guide on identifying these increasingly problematic organisms.

The conference calendar continues, with tibits of news relevant to ASA.

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

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

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) Hypervirulence or Just Hype? (Allen Cheng) Infection Control Issues (Rhonda Stuart) Establishing a Faecal Matter Unit (Patrick Charles)	Modelling a Response to MDR (Emma McBryde) Antibiotics in Agriculture – Is there an Ethics Dilemma (Peter Collignon) How can Whole Genome Sequencing Enhance our Understanding – Information Overload (Ben Howden)	Australian Perspective: Antifungal Susceptibility (Sarah Kidd) Non-Culture Based Diagnostics in Mycology (Catriona Halliday) Treatment of Candidaemia: What's New (Maiken Arendrup)	β-lactam TDM in Clinical Practice (Jason Roberts) Aminoglycoside Dosing – Current Controversies (Evan Begg) Practical Challenges (John Turnidge)	*Streptococcus pneumoniae*: the Attributable Disease Burden Due to Resistance (Susan Huang) Staphylococcal Bacteraemia: New Knowledge on Optimum Treatment (Natasha Holmes) Multi resistance Plasmids in Enterobacteriaceae (Sally Partridge)

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IN THE NEWS

Fidaxomicin (Dificid®) is now ARTG listed and TGA approved, as of April 24th 2013. The position of this addition to our drug armamentarium in the management of patients with *Clostridium difficile* infection is awaited with anticipation. Readers of "Breakpoint" are referred to the short article by Professor Peter Collignon in Lancet Infectious Diseases discussing "Superbugs in food: a severe public health concern". See "Articles in Press" May 28th 2013.

Read also the related article by Le Hello et al. (www.thelancet.com/infection, May 28) discussing the increasingly prevalence of ciprofloxacin-resistant *Salmonella enterica* serotype Kentucky stains. The not so good news is that these strains have acquired CTX-M extended spectrum β-lactamase, CMY-2 cephamycinase, VIM-2 and OXA-48 carbapenemases encoding resistance to (i) extended spectrum cephalosporin and (ii) carbapenems. *S. enterica* is a major global food –borne pathogen. The findings have implications for the control of Salmonella in food-producing animals.

DETECTION OF CARBAPENEMASE ENZYMES IN THE ENTEROBACTERIACEAE

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Background

Antibiotic resistance in the Enterobacteriaceae is increasing rapidly with the spread of resistance genes typically found on self-transmissible plasmids. The presence of carbapenem-hydrolysing β-lactamases is of particular concern since these often confer resistance to most β-lactam antibiotics. Carbapenemases produced by Enterobactericaeae are generally of the KPC (Ambler Class A carbapenemase), IMP, VIM, NDM (Class B metallo-β-lactamases; MBL) or OXA-48/181 (Class D oxacillinase) types (14), although other less-common enzymes are also observed; further, these enzymes are found in other Gram-negative bacteria.

Detection of carbapenemases produced by Enterobacteriaceae has traditionally required the use of an antibiotic disk (e.g. modified Hodge test) or disks plus/minus an inhibitor acting on a selected class of enzyme (e.g. EDTA for MBLs). Recent work has however seen the introduction of media capable of supporting the growth of organisms harbouring multiple enzyme classes. Modern methods such as mass spectrometry and PCR-based analysis now also enable rapid detection and discrimination of carbapenemases or the genes that encode them (1, 12). This article briefly summarises the above approaches that are amenable to a diagnostic laboratory.

Methods

1. CLSI and EUCAST methods

Current guidelines for susceptibility testing of Enterobacteriaceae according to both CLSI and EUCAST documents have reduced the breakpoints for carbapenems from previous versions for pharmacodynamic reasons, with the added benefit of increasing the sensitivity of routine methods to detect the presence of carbapenemases (3, 11). However, these breakpoints are not always reliable in the context of carbapenemase-producing Enterobacteriaceae, where the MIC of carbapenems are also dependent on permeability factors (e.g. MICs as low as 1-2 µg/ml have been seen in Enterobacteriaceae producing IMP-4 in Australia (6)). Adequate screening to enable the detection of carbapenemases-producing isolates with low carbapenem MICs is a priority since treatment failure has been observed in carbapenemase-producing isolates with MICs classed as “susceptible” (17).

2. Screening for carbapenemases

Screening media, such as CHROMagar ESBL (CHROMagar, USA), Brilliance CRE (Oxoid, UK) and SUPERCARBA (12), can assist in the detection of carbapenemase-producing Enterobacteriaceae although none has 100% sensitivity or specificity. Comparisons of these media have shown limited detection of OXA-48/181 producers by CHROMagar ESBL, failure of Brilliance CRE to detect KPC isolates and growth of non-carbapenemase-producing isolates (including ESBL producers and Acinetobacter/Pseudomonas spp.) on CHROMagar ESBL or SUPERCARBA (7, 12). A combination of CHROMagar ESBL and Brilliance CRE or use of SUPERCARBA may provide near 100% sensitivity, although specificity is lacking.

3. Confirmation of carbapenemases

Confirmation of carbapenemase-producing isolates (only recommended by the CLSI for the purposes of infection control (3)) can be performed using the modified Hodge test, based on detection of diffusible carbapenemases. KPC, OXA-48/181 and most MBL producers give positive results but NDM producers may not (2); AmpC or ESBL producers with reduced permeability may also give positive results (4). Detection of NDM enzymes can be improved by the addition of ZnSO4 to the media (9). Inhibitors specific to Ambler class A or B enzymes can be useful for the limited number of isolates that produce a single β-lactamase, where masking of the carbapenemase phenotype does not occur, but are unable to detect OXA-48/181 producers. KPC producers can be detected using meropenem/imipenem and boronic acid (15), though isolates producing AmpC enzymes (chromosomal or plasmid-mediated), especially those with reduced permeability, can also give a positive result (4). Cloxacillin, which inhibits Class A

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enzymes, can be used to discriminate between KPC and these AmpC producers (10). EDTA or dipicolinic acid can be used with either meropenem or imipenem (either as combination disks or Etest strips; bioMérieux, France) to inhibit MBL enzymes (4). However, imipenem-inhibitor Etests, but not meropenem-inhibitor Etests, may fail to detect MBLs with low imipenem MICs (13).

The recent development of a biochemical assay (known as the Carba NP test II) provides a more rapid method for confirmation of carbapenemases and also the identification of the Ambler class, based on acidification resulting from imipenem hydrolysis. The Carba NP test II can distinguish carbapenemase-producing isolates from those without a carbapenemase that are resistant to carbapenems, while differentiating Ambler classes is based on the inhibition of KPC and MBLs by tazobactam and EDTA, respectively, and growth of OXA-48/181 producers under both conditions (5). Hydrolysis of carbapenems can also be detected by mass spectrometry (via MALDI-TOF), which is becoming a routine approach in diagnostic laboratories for bacterial identification. This method is able to detect KPC and MBL enzymes but has not been not evaluated for the detection of OXA-48/181 (1).

The gold standard for detection of carbapenemase-producing Enterobacteriaceae remains nucleic acid techniques, mostly PCR-based, capable of identifying the type of carbapenemase gene rather than simply the presence/production of the carbapenemase, and may be performed as multiple target assays to improve detection (16). Sequencing can be used to further identify gene variants, though in many cases is not essential outside the purposes of epidemiology. A disadvantage is that the specificity of PCR (nearly 100%) means that novel carbapenemase genes may not be detected. Regular surveys of local epidemiology can improve knowledge of currently important or novel genes, and it has been suggested that limited targets may be locally important (8). Other disadvantages of PCR are the high costs (both setup and cost per specimen) and need for highly trained technicians.

Conclusions

The detection of carbapenemase-producing Enterobacteriaceae is of the utmost importance in public health. High cost techniques are the single most effective method for confirmation of the presence of these enzymes but these costs may be a barrier to their use. For most purposes, detection of carbapenemase production is sufficient, providing that screening methods are sensitive. Current guidelines to screen only isolates not susceptible to carbapenems can result in some carbapenemases being missed and ultimately in treatment failure. The use of the following scheme may assist in efficient detection of carbapenemase-producing Enterobacteriaceae:

1. Screening using either
   a. CHROMagar ESBL and Brilliance CRE; or
   b. SUPERCARBA medium
2. Confirmation of carbapenem hydrolysis using
   a. Carba NP Test version II; or
   b. MALDI-TOF
3. PCR-based identification of genes (if epidemiologically important)

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2013 - 2014 MEETING CALENDAR

2013

Australian Society for Microbiology Annual Scientific Meeting
7-10 July, Adelaide, South Australia
Website: www.theasm.org.au

ASID Gram-negative Superbug Meeting
2-3 Aug, Gold Cost, Queensland
Website: www.asid.net.au/gramnegative

53rd 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/

ID week (IDSA, SHEA, HIVMA, PIDS)
Oct 2-6, San Francisco, California
Website: www.idweek.org/idweek2013

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

2014

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

Royal College of Pathologists Update Meeting
14-16 March, Sydney, NSW
Website: www.rcpa.edu.au

16th International congress on Infectious diseases (ICID)
2-5 April, 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
17-20 May, Boston, USA
Website: www.asm.org

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

54th Interscience Conference for Antimicrobial Agents and Chemotherapy
6-9 Sept, 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/