BREAK POINT

2014 - ISSUE 09

FROM THE NEWSLETTER EDITOR'S DESK

Welcome to another edition of *Breakpoint*. In this issue, we are blessed with a view into "**Surveillance of CMY, ESBL and Carbapenemase-Producing Enterobacteriaceae in Queensland"** by Hanna Sidjabat from the University of Queensland, Brisbane. David Mitchell (Westead Hospital, Sydney) then describes selected items of interest from the 2014 ECCMID meeting in Barcelona, May 9-13, amidst the latest (Including permanently incomplete) architectural feats of the city. Notices and a conference calendar follow. As always, feedback is warmly welcome.

Sharon Chen

ASA Breakpoint Editor,

On behalf of the ASA committee

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IN THE NEWS

For those readers that have access to "UpToDate", my more recent experience has supported the inclination to recommend articles in this resource as "must read".

1. Healthcare-associated infections in the United States

A study retrospectively reviewed records of over 11,000 randomly selected patients admitted to general or pediatric acute care hospitals in the US and found that 4 percent had a healthcare-associated infection. Pneumonia and surgical-site infections were the most common, each accounting for 22 percent of all infections. Based on these results and data on national hospital admissions rates, an estimated 648,000 patients in the US experienced a healthcare-associated infection in 2011. These findings highlight the substantial burden of such infections and ***the importance of infection control measures*** designed to prevent them. (See Magill SS et al. New Engl J Med 2014; 370: 1198)

2. Decreased mortality from sepsis - positive news for the antibiotic

Using an internationally-accepted, standardized definition of sepsis, a 12-year multicenter study of over 100,000 patients in **Australia and New Zealand** with severe sepsis and septic shock reported a 50 percent risk reduction (35 to 18 percent) in in-hospital mortality between 2000 and 2012. This study, together with reports of improved compliance with treatment guidelines, suggests that the reduction in mortality is authentic and possibly due to improved therapeutic strategies for sepsis management. Read: Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. JAMA. 2014; 311:1308.

Australian Government
Department of Health
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ANNUAL SCIENTIFIC MEETING OF THE 24TH ECCMID (EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES)

Dr David Mitchell, Senior Staff Specialist, Microbiology and Infectious Diseases, Westmead Hospital

This meeting appears to be growing each year with approximately 10,000 delegates at the 2014 meeting. These numbers were problematic for popular sessions, with late comers not getting into the room let alone getting a seat. The meeting is always popular with Australians as it combines a nice mix of clinical infectious diseases and laboratory microbiology, the benefit of a European perspective on things and, of course, is held in Europe!, this time Barcelona. The meeting, for those into music, was about 2 weeks shy of a major jazz, pop and light festival which was spectacular.

A noticeable trend in the Industry Display for these conferences is the growth in both number and size of displays for laboratory equipment suppliers with an emphasis on automation, while the number and size of pharmaceutical company displays shrinks, reflecting the dearth of new antimicrobial agents. On this topic, there seems to be little reason for optimism in dealing with the ever increasing antibiotic resistance crisis, especially for multi-resistant Gram negative bacteria. A variety of newer ß-lactam/ ß-lactamase combination agents are in phase 2/3 trials which have activity against some, but not all, carbapenemase producing strains. There was an emphasis on the need for individualising treatment regimens for such isolates, demonstrating the importance of rapid susceptibility testing, both phenotypic and genotypic.

Other personal highlight sessions at ECCMID included the regular EUCAST Workshop, featuring a great talk from John Turnidge comparing susceptibility test methods around the world; the now obligatory MALDI-TOF symposium, highlighting the versatility of this “revolutionary” technology and it’s utility in areas apart from bacterial identification, such as susceptibility testing and identification of viruses and multicellular organisms such as arthropods; an update on the treatment of leishmaniasis by highly experienced South American clinicians; and a symposium on the management of staphylococcal infections. Concerning the latter, I was pleased to hear that a clinical trial looking at the role of rifampicin combination therapy for staphylococcal bacteraemia is currently underway in the UK.

David Mitchell

SURVEILLANCE OF CMY, ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN QUEENSLAND

Hanna Evelina Sidjabat University of Queensland Centre for Clinical Research, Brisbane, Australia

Resistance to β-lactam antibiotics in Enterobacteriaceae is usually caused by the production of β-lactamases. There are four classes of β-lactamases by Ambler classification [1]. Class A, C and D β-lactamases are active-serine β-lactamases. Class B is a metallo-β-lactamases (MBL). Concerning Class A, the production of TEM-1 and TEM-2 causes resistance to narrow spectrum β-lactam antibiotics; other variants of TEM cause resistance to broad-spectrum β-lactams including cephalosporins [2]. β-lactamases which have broad-spectrum activity against the cephalosporins are designated as extended spectrum β-lactamases (ESBLs) [3]. ESBLs remain susceptible to carbapenems. TEM, SHV and CTX-M are the most clinically significant ESBLs [2]. Initially, TEM and SHV were the prevalent enzymes within the ESBL group. However, since 2006, CTX-M has been increasingly prevalent [4]. CTX-Ms are currently the most prevalent β-lactamases worldwide.

Class C or AmpC β-lactamases have activity mostly to cephamycin; however, they also hydrolyse third generation cephalosporins [5]. The chromosomal gene encoding AmpC is poorly expressed and usually present in *Enterobacter* spp. and other species within the Enterobacteriaceae family. Plasmid-mediated AmpC is commonly described in *E. coli* and *Salmonella* spp [6]. Thus far, plasmid-mediated CMY-2 like genes are the most commonly reported AmpC β-lactamases [6, 7]. Enzymes that confer resistance to carbapenems can be found within class A, B and D β-lactamases. The following examples of carbapenemases are relatively common in Enterobacteriaceae: KPC (class A β-lactamase); IMP, NDM and VIM (MBL); OXA-48 (class D β-lactamase). In addition OXA-23 (class D β-lactamase) has been the most prevalent carbapenemase amongst *Acinetobacter baumannii*.

At the University of Queensland Centre for Clinical Research (UQCCR), we began the molecular characterisation of cephalosporin and/or carbapenem resistant Enterobacteriaceae and carbapenem resistant *Acinetobacter baumannii* in 2009. We perform phenotypic characterisation of the antibiotic susceptibility using disk susceptibility tests following CLSI or EUCAST standard interpretation. Phenotypic characterisation includes detection for ESBL by combination disks (third generation cephalosporin alone and combination with clavulanic acid), AmpC using boronic acid, KPC using boronic acid and MBL using EDTA. PCR and sequencing have been used to characterise the antibiotic resistance mechanisms and to determine the gene variants. The focus of the antibiotic characterisation includes the characterisation of β-lactamases, aminoglycoside resistance and quinolone resistance.

In addition to characterisation of the antibiotic resistance mechanisms, we characterise the clonal relationship of the bacteria. We have been mainly using semi-automated rep-PCR or conventional rep-PCR for clonal analysis. For highly clonal strains, we occasionally use pulsed-field gel electrophoresis. Molecular epidemiology analysis of the plasmids is more complicated than the clonal analysis of the bacteria. The clonality of the plasmids is usually determined by PCR-based replicon typing, plasmid transfer experiments and profiling the digested plasmids on agarose gel [7-10]. Understanding the clonality of the bacteria and the plasmids will help to determine the mechanisms of spread of antibiotic resistance. Significant numbers of our publications include the plasmid characterisation, the genetic environment of the antibiotic resistance genes in addition to the clonal analysis of the bacteria and the characterisation of antibiotic resistance mechanisms.

We have performed the following studies at the UQCCR: characterisation of CMY-2 producing *E. coli* and plasmids harbouring CMY-2 gene [10]; characterisation of ESBL producing *E. coli* [11, 12]; IMP producing Enterobacteriaceae; NDM producing Enterobacteriaceae [13-16]; [6] OXA-48 producing Enterobacteriaceae [17, 18] and OXA-23 producing *A. baumannii* [19]. Other than isolates from South East Queensland, we collaborate with institutes in Thailand, Singapore, Pakistan, Turkey and the Middle East to characterise NDM and/or OXA-48 producing Enterobacteriaceae and OXA-23 producing *A. baumannii* [13, 14, 20].

SURVEILLANCE OF CMY, ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN QUEENSLAND (CONT'D)

Hanna Evelina Sidjabat University of Queensland Centre for Clinical Research, Brisbane, Australia

In our CMY-2 study, we included CMY-2 producing *E. coli* from three major hospitals in South East Queensland [10]. We showed that the horizontal transfer of IncI1 plasmids harbouring CMY-2 gene was the main mechanism of spread of CMY-2 genes in South East Queensland. We also found *E. coli* ST131 or pandemic *E. coli* in 8% of CMY-2 producing *E. coli* [10]. Further, an initial description of *E. coli* ST131 from Queensland showed that this pandemic clone produced CTX-M-15 and TEM [12]. A more recent extensive characterisation of *E. coli* from Australia and New Zealand by Rogers and colleagues showed the high prevalence of *E. coli* ST131 among cephalosporin resistant *E. coli* [11]. This is an important finding for the understanding of the predominance of *E. coli* ST131. Generally, there are more infections caused by ESBL than by AmpC producing Enterobacteriaceae, especially in *E. coli* in Queensland. A snapshot to determine the ratio between ESBL and AmpC cases in a single major hospital in Queensland over 9 months, showed that of the 1872 *E. coli* isolates identified, there were 38 and 25 isolates with ESBL and AmpC phenotypes, respectively [10]. The prevalence of cephalosporin resistant Enterobacteriaceae has reached nearly 4,000 cases in 2013 (unpublished data). In contrast, there were less than 30 cases by CPE in 2013; however, the CPE prevalence also has an increasing trend (Sidjabat et al, manuscript in preparation). The increasing prevalence of IMP producers has caused real threat for the treatment of infections by multidrug resistant Gram-negative bacteria. Unfortunately, the production of IMP has not always been detected phenotypically. Other antibiotic resistance mechanism, such as porin mutation and modification in the outer membrane protein can cause carbapenem resistance which complicates the detection of carbapenemases. Modified Hodge test (MHT), Carba NP test, carbapenemase phenotypic test using EDTA and boronic acid have been used regularly to determine the production of carbapenemases. High MIC to meropenem on Vitek2 (bioMerieux) has been a good predictor of the carbapenem resistance. IMP-4 has been the most prevalent carbapenemase not only in Queensland, but also in Australia [21-23]. We found IMP-4 producing *Enterobacter cloacae* more frequently than in other species of Enterobacteriaceae (~60%) [24]. Other CPE cases by NDM producers were usually associated with the importation of the isolates through patient transfer from overseas or patients with travel history to high incidence countries, such as Indian sub-continent and South East Asia. In 2013, we identified OXA-48 and OXA-181 producers in Queensland; however, we have not found any KPC producer yet.

In term of the molecular characterisation of carbapenemase producing *A. baumannii*, no specific carbapenemase phenotypic test is used in-house for to detect the production of OXA. Disc susceptibility testing, PCR and sequencing variant of OXAs carbapenemases have been utilised to determine the OXAs. Recently, a species-specific PCR for OXA genes of *Acinetobacter* spp. was developed [25]. In our studies, OXA-23 has been the predominant carbapenemase in *A. baumannii* isolates from Australia, Thailand, Singapore, Kenya, Turkey and Middle East ([19], Huber et al 2014, Kamolvit et al, manuscript in preparation, Zowawi et al, manuscript in preparation). We use MLST to determine the sequence type and clonal complex (pubmlst.org/abaumannii). We also use semi-automated rep-PCR (bioMerieux) to characterise the clonal relationship of *A. baumannii*. Generally OXA-23 enzymes have been associated with *A. baumannii* clonal complex 92 [19]. Amongst *A. baumannii* from Australia, Thailand, Singapore and Thailand, OXA-23 genes are located in the Tn*2006* (Kamolvit et al, manuscript in preparation). OXA-23 producing CC92 has been the most predominant carbapenem resistant *A. baumannii* worldwide [26].

In conclusion, characterisation of antibiotic resistance mechanisms, clonal analysis of the bacteria and plasmids have provided a comprehensive insight into the mechanisms of spread of antibiotic resistance bacteria. These data will help to understand the molecular epidemiology and predict further spread of antibiotic resistance bacteria.

SURVEILLANCE OF CMY, ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN QUEENSLAND (CONT'D)

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SURVEILLANCE OF CMY, ESBL AND CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE IN QUEENSLAND (CONT'D)

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2014 - 2015 MEETING CALENDAR

2014

20th IEA World Congress of Epidemiology
Aug 17-21, Anchorage, Alaska
Website: http://ieaweb.org/

International Union of Microbiological Societies (IUMS) 2014 Congress
27 July to 1 August, Montreal, Canada
website: www.montrealiums2014.org/

16th International Symposium on Staphylococci and Staphylococcal infections
26-29 August, Chicago, USA
Website: http://isssi2014.com/

54th ICAAC
6-9 Sept, Washington D.C. USA
Website: http://www.asm.org

International Meeting on Emerging Diseases and Surveillance
Oct 3- Nov 3, Vienna, Austria
Website: http://imed.isid.org

ID Week 2014: IDSA
8-12 Oct, Philadelphia, USA
Website: www.idsociety.org

International Meeting on Emerging Diseases and Surveillance
31 Oct – Nov 3, Vienna, Austria
website: http://imed.isid.org

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

2015

ASA 16th Annual Meeting
February 26-28, Brisbane, Queensland
Website: www.asainc.net.au

Pathology Update 2015
Feb 27 – Mar 1, Melbourne, Victoria
Website: www.rcpa.edu.au

ASID Annual Meeting
Mar 18-21, Auckland, New Zealand
Website: www.asid.net.au

The 2015 TB Summit
March 24-26, London, UK
Website: https://www.regonline.co.uk/

7th international Congress of the Asia Pacific Society for Infection Control
26-29 March 2015, Taipei, Taiwan
website: www.apsic2015.org

25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015)
25 - 28 April 2015, Copenhagen, Denmark
Website: http://escmid.org/dates_events/

International Conference in Prevention and Infection Control
16-19 June, Geneva, Switzerland
Website: http://www.icpic2015.com

55th ICAAC
18-21 Sept, San Diego, USA
Website: http://www.asm.org

2016
26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015)
9-12 April 2016, Instanbul, Turkey
Website: http://escmid.org/dates_events/