

be taken when using automatic methods, which may produce false susceptibility results for CPE.

Metallo- $\beta$ -lactamases (MBL, Class B) do not hydrolyze monobactams, while OXA-48-like (O48L, Class D) enzymes cause resistance to temocillin and piperacillin-tazobactam and are poorly active against expanded-spectrum cephalosporins. Importantly, CPE may also produce other  $\beta$ -lactamases (i.e., extended-spectrum  $\beta$ -lactamases, ESBL), which determine very complex phenotypic patterns. KPC (Class A) carbapenemases are inhibited by boronic acid derivatives, while MBL are inhibited by EDTA, 1,10-phenanthroline, thiol compounds and dipicolinic acid. Comparison of zones around discs of carbapenems (usually meropenem) alone or with the indicated inhibitors will suggest the presence of a certain carbapenemase class, and help to exclude other mechanisms of carbapenem resistance (i.e. porin loss plus AmpC/ESBL production). Other smart approaches have also been designed based on the inhibition activity of the indicated compounds.

Detection of carbapenemases can be made demonstrating their hydrolytic activity against carbapenems using bioassays (several variants of the modified Hodge test or the so-called carbapenem inhibition method), colorimetric assays (Carba NP and Carba Blue), MALDI-Tof or spectrophotometry. Immunochromatography assays (including commercial versions for some enzymes) have been designed for IMP-, OXA- and KPC-type carbapenemases. Recently, an electrochemical device (BYG carba test) has been designed for rapid carbapenemase detection.

Carbapenemase-producing Enterobacteria can be selectively cultured using carbapenem-containing media. Clinical samples can be pre-incubated in a liquid medium with a commercial carbapenem disk, then subcultured on MacConkey agar with imipenem or on any of the multiple version of the commercially available chromogenic media (ChromID variants, Supercarba, CHROMagar KPC, HardyCHROM Carbapenemase, Brilliance CRE. . .). Once bacteria have grown, carbapenemase can be detected using phenotypic or molecular methods.

Every laboratory should decide about the more convenient algorithm for detecting CPE, taking into account the incubation time needed for methods based on culture (including bioassays and disc approaches), the usefulness of rapid methods (such as colorimetric assays, MALDI-Tof or immunochromatography) and the need of molecular assays for definitive identification of concrete enzymes.

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#### Type: Invited Presentation

Final Abstract Number: 04.002

Session: *Diagnosis and Treatment of Carbapenem-resistant Enterobacteriaceae*

Date: Thursday, March 3, 2016

Time: 10:15-12:15

Room: Hall 5

#### Molecular diagnosis of carbapenemase producing Enterobacteriaceae infection



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**Abstract:** The gold standard for diagnostics of infectious diseases and detection of antibiotic resistant organisms are culture and antibiotic susceptibility testing respectively. However, culture is sometimes unsuccessful because patients have been treated with

antibiotics or are infected with unculturable or difficult to culture microorganisms.

Antigen detection using an immunochromatographical technique is a simple and rapid method that has been used for the diagnosis of infectious diseases such as Legionnaires' disease, pneumococcal infection, and Mycoplasma pneumonia. We have already constructed system for detecting carbapenemases in Acinetobacter spp. such as the OXA-23 group, OXA-24/40 group, OXA-51 group and OXA-58 group. However, this system is not able to detect carbapenemases directly from clinical specimens.

Multiplex PCR is a useful technique for active surveillance or targeted surveillance of carbapenemase encoding genes in Enterobacteriaceae. However, novel or mutated carbapenemase genes cannot detect by PCR.

On the other hand, next generation sequencing (NGS) is a powerful tool for the diagnosis of infectious diseases and enables a comprehensive search for antibiotic resistant genes. Whole genome sequence (WGS) data or Meta genome sequence (MGS) data is provided from bacterial colony or clinical specimens, respectively.

In this presentation, I will discuss novel techniques for the diagnosis of infectious diseases causing by carbapenemase producing infections.

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#### Control of carbapenem-resistant Enterobacteriaceae



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**Abstract:** Given that treatment options for carbapenem-resistant enterics is limited, we should concentrate our efforts on prevention methods. These preventative interventions are usually combined into a bundle of interventions and include: increased hand hygiene compliance, active surveillance cultures, contact precautions, cohorting patients and hospital personnel, heightened environmental disinfection, limiting communal objects, and antibiotic stewardship. During this session we will review the most relevant papers on this topic and the most recent literature.

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