O041

2-hour Oral Session

Detection of carbapenemases

Detection and characterization of carbapenemases by flow cytometry

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Objectives

Carbapenemase-producing pathogens are responsible for an increasing number of serious infections with high mortality rates. Carbapenemases are produced by multidrug resistant pathogens such as *Pseudomonas* spp, *Acinetobacter* spp and *Enterobacteriaceae*. The classic method recommend by EUCAST is a disk diffusion assay, which combines meropenem with different carbapenemase inhibitors. Besides cumbersome and time consuming, this test depends of bacteria growth, increasing the time to result. Rapid reliable carbapenemase detection is a pre-requisite for successful infection control, and therapeutic management. We present here a novel protocol for fast detection of carbapenemases based on flow cytometry.

Methods

A total of 100 isolates (*Pseudomonas* spp, *Acinetobacter* spp and *Enterobacteriaceae*) phenotypically and molecularly well characterized were assayed. Half of the strains are carbapenemase producers: 20 KPC, 15 MBL, 15 OXA. ATCC, NCTC and CCUG type strains recommended by EUCAST were also included. The remaining strains do not produce carbapenemase and/or produce an AmpC or ESBL associated with porin loss. Firstly, in order to confirm the enzymatic nature of the resistance mechanism, a screening test was performed. Briefly, bacteria cells, at initial exponential growth phase, were incubated with four concentrations of meropenem; afterwards, the supernatant was incubated with *E. coli* ATCC 25922 for 1h and stained with a potential membrane fluorescence probe; adapted from Hodge test. The effect was measured by flow cytometry (FACSCalibur cytometer) and compared with the effect that the same concentration of meropenem incubated for 1h causes in the ATCC strain. A reduced cellular lesion indicates an enzymatic mechanism of resistance. In that case, bacterial cells were incubated for 1h with meropenem at the same concentration in the presence and absence of the following inhibitors (according to EUCAST): EDTA (for MBL), aminophenylboronic acid (for KPC), both EDTA and aminophenylboronic acid (for MBL plus KPC) and cloxacillin (for AmpC); and stained as previously described and analyzed by flow cytometry. Cut-off values and specificities and sensitivities associated to each drug concentration were defined using SPSS version 22.0.

Results

The screening test detected all carbapenemase-producing bacteria, giving accurate negative results for the other strains. The type of carbapenemase was correctly identified in all the strains, including AmpC positive strains, exhibiting 100% of sensitivity and 100% of specificity.

Conclusion

A new and promising cytometric tool is now available for carbapenemase detection in useful time being crucial for patient treatment and control of hospital infection.

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The present technology is undergoing patent protection (W0212164547A), thus all the disclosed information is confidential until publication in the congress abstract book or the presentation delivery.