P1301

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Analysing and overcoming carbapenemase-mediated resistance

Association of carbapenems: a therapeutic solution for carbapenemase-producing bacteria

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Objectives

Therapeutic options for carbapenemase-producing bacteria are evidently limited and the availability of new antimicrobials or the “reinvention” of old ones are far from resolving this problem. Such organisms are responsible for severe infections, reported mortality rate ranging from 24% to 70%, representing a real human threat. Carbapenemases are a heterogeneous group of enzymes that hydrolyse most ß-lactams including carbapenems with no known universal inhibitor. Ertapenem is the carbapenem most sensitive to detect carbapenemase used on the old and cumbersome Hodge test. Our goal was to study the ability of ertapenem to inhibit those enzymes allowing, intact meropenem or imipenem, to produce its effect on carbapenemase-producing strains.

Methods

Sixty strains of carbapenemase-producing bacteria genetically characterized were used: 35 KPC, 20 metallo-beta-carbapenemases (IMP, VIM, SPM, GIM) and 2 OXA-48 and 3 OXA-23. The strains were incubated with ertapenem at concentrations ranging between 0.5-2 µg/ml during 30 minutes followed by ertapenem associated to meropenem or imipenem during 1 h, at different concentrations (ranging from 0.03-16 µg/ml). Time-kill assays as well as a flow cytometric assay using a membrane potential probe were performed using a FACSCalibur Cytometer (BD Biosciences); an increase in the intensity of fluorescence will indicate depolarization i.e. cell lesion. Statistic analysis using SPSS version 22.0 were used to compare the results obtained with and without pre-exposure and ertapenem association.

Results

No reduction on CFU counts or increase in fluorescence was found on carbapenemase producing strains after carbapenem incubation without pre-incubation and ertapenem association. Conversely after pre-incubation and ertapenem association an increase of the bactericidal effect of meropenem and imipenem was found on KPC and OXA carbapenemases producing strains. This effect was evident by a significant reduction of the number of viable cells and an increase on the intensity of the fluorescence of the cells on flow cytometric analysis, implying cell lesion. Regarding metallo-beta-carbapenemases, such finding was only registered with imipenem. The effect of ertapenem was dose-dependent being higher with 2 µg/ml; the bactericidal concentration of the other carbapenem decrease in 4 to 8 dilutions turning the strains susceptible according to the most recent EUCAST protocol.

Conclusion

The pre-incubation and ertapenem association with a carbapenem showed to be synergic. In KPC and OXA strains, synergistic effect was observed with both carbapenems. Regarding metallo-beta-carbapenemases, only the association with imipenem makes those strains susceptible. Ertapenem could be used as a carbapenemase inhibitor, allowing other carbapenem free to be active against carbapenemase producing strains. A new therapeutically perspective for treatment of such critical infections is now ready for *in vivo* trials.