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Abstract (poster session)

Carbapenemase identification by matrix assisted laser desorption/ionisation time-of-flight mass spectrometry

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Objectives: Resistance of Gram-negative rods to carbapenems has been an increasing problem all over the world. This resistance is caused by an alteration in the outer membrane of the cell wall, by an overexpression of the efflux pumps or by carbapenemase production. Carbapenemases can be detected by methods based on the ability of some compounds to inhibit these enzymes, molecular genetic techniques and by direct visualization of carbapenem hydrolysis. We describe here the use of MALDI-TOF mass spectrometry to detect a carbapenem antibiotic and its degradation by carbapenemases. Methods: Buffered meropenem solution (0.1 mM Tris-HCl, pH 6.8) was mixed with an overnight culture of bacteria. After three hours incubation, the reaction mixture was centrifuged and supernatant was analysed by MALDI-TOF MS using 2,5-dihydroxybenzoic acid as a matrix. The presence or absence of peaks representing meropenem and its sodium salts was crucial. Inhibitor-based methods have also been tested for the identification of carbapenemase type. The samples containingng inhibitors (e.g. EDTA, dipicolinic acid, phenylboronic acid) cannot be directly measured by MALDI-TOF MS due to the interference of inhibitors with matrix. Therefore, some procedures for a partial purification of the sample were tested. Results: The sensitivity of this method, validated on 145 strains, including 41 carbapenemase-producing strains, is higher than 97%, with a specificity of a similar value. The strains used for the study produced different KPC enzymes (KPC-2 and KPC-3), VIM, IMP and NDM-1 metallo-beta-lactamases. Positive results were obtained also in NDM-1-producing Acinetobacter baumannii. The inhibitors can be optimally removed from the sample using ZipTip Pipette Tips (Millipore). Validation of the inhibitor-based method for identification of carbapenemase type is under the evaluation Conclusion: MALDI-TOF MS assay for carbapenemase detection was introduced to the routine laboratory praxis in the author’s laboratories. The results are comparable with the reference spectrophotometric imipenem hydrolysis assay using crude bacterial extract. We believe that this method can become a standard technique for a quick carbapenemase identification in routine diagnostic laboratories. This work has been supported by the research project grant NT11032-6/2010.