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BATTERIOLOGIA

ANAEROBES IDENTIFICATION: COMPARISON BETWEEN MALDI-TOF MS AND 16S RRNA GENE SEQUENCING

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INTRODUZIONE

In the last years there has been an increasing interest in the study of anaerobic bacteria that cause human infections. Anaerobic infections are the more underestimated of all infections, especially when anaerobic bacteria grown in mixed culture together with aerobes/anaerobes. Furthermore, antibiotic resistance has been detected among different anaerobe isolates for the drugs of choice to treat anaerobes infections. undelyning the necessity to perform antimicrobial susceptibilities test Many new genera and species have been described using 16S rRNA gene sequencing of clinical isolates Beside the classical, time-consuming identification methods the use of matrix-assisted laser desorption/ionization time of-flight mass spectrometry has revolutionized identification of even unusual and slow-growing anaerobes directly from culture plates, providing the possibility of providing timely information about anaerobic infections.

The aim of this work was to compare the results obtained from identification by Maldi-tof obtain during clinical samples analysis with the Maldi-tof of stored culture of clinical isolates and 16S rRNA gene sequencing that is still the gold standard for anaerobes identification.

METODI

After to be isolated from clinical samples, 100 anaerobes bacteria (55 Bacteroides fragilis and 45 of different species of anaerobes) were stored on glycerol at – 80°C. Stored bacteria were then used to compare different techniques of identification. DNA extraction was performed for all strains under study in order to perform 16S rRNA amplification and sequencing. The identification of well isolated colonies was done Maldi-Tof assay. Bacteroides spp carbapenem resistance was investigated to phenotypic test and CfiA PCR reaction.

RISULTATI

Comparison of identification results reveal a very critical step in the Maldi-tof performed directly from plates were clinical samples were streaked out. Indeed, comparison between Maldi-tof from pure culture and 16S rRNA sequencing showed a higher agreement of the identification results. Furthemore, there is no problem for B. fragilis while there is less agreement for other species.

Five strains of B. fragilis and one strain of Bacteroides thetaiotamicron used in the study showed also a carbapenem resistance. These strains were investigated to understand the mechanism that underling the resistance. The resistance was confirmed by agar dilutions susceptibilities test. Three strains out of 6 confirmed the presence of a carbapenemase, by hydrolysis assay. PCR reactions was performed for the most common carbapenemase present in B. fragilis, the CfiA: Strain 302 resulted positive to CfiA gene.

CONCLUSIONI

The direct identification from clinical samples plates must be improved to let a better clinical results in order to have good epidemiological data regarding infections and a better correspondence between species identification and susceptibility test results.