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Paper Poster Session
Recent studies of non-culture techniques for detection of resistance

Carbapenem-resistance in B. fragilis: a MALDI affair

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Background: Carbapenem-resistance in *Bacteroides fragilis*, associated with *cfiA*-encoded class B beta-lactamase, is an emerging problem. Different genic expression levels result in a broad spectrum of resistance/reduced susceptibility, nevertheless treatment with carbapenem can promote a selective pressure that may lead to therapeutic failure also in phenotypically susceptible strains.
Classical and molecular methods for antimicrobial susceptibility testing of anaerobes present several key issues, as they are either laborious and slow or expensive.
New applications of MALDI-TOF MS allow fast and complete approach to carbapenem-resistance in *B. fragilis*, as a single instrument can work on different analytical "dimensions". Aim of this study was to evaluate a collection of *B. fragilis* strains using MALDI-TOF MS, in terms of identification of *cfiA*-positive strains and carbapenemase-activity confirmation.

Material/methods: From July 2014 to June 2015, 96 non-duplicated clinical isolates of *B. fragilis* were collected.
Identification at species level was performed by MALDI Biotyper (Bruker).
Antibiotic-susceptibility was evaluated following EUCAST criteria; among carbapenems, meropenem was routinely analysed by e-test, while imipenem and ertapenem were tested only for strains with meropenem MIC value ≥ 2 mg/L.
Carbapenemase-production was evaluated phenotypically by disc-diffusion synergy test (DDST-ROSCO).
Detection of *cfiA* gene was performed by MALDI-TOF MS subtyping (Bruker), as described in literature.
Carbapenem-hydrolysis was evaluated analyzing specific peaks corresponding to intact and hydrolyzed ertapenem (Invanz®) with MALDI-TOF MS (Bruker).

Results: 11/96 strains (11.45%) showed resistance/reduced susceptibility to meropenem (MIC ≥ 2 mg/L); they resulted resistant to ertapenem (MIC ≥ 32 mg/ml), while MIC for imipenem resulted between 0.12 and ≥ 32 mg/ml. With DDST, 10/11 resulted positive for MBL-production, 1 as non-interpretable.
MALDI-TOF MS subtyping identified all the strains with reduced susceptibility to carbapenems (11/11) as *cfiA*-positive, and all the remaining strains (85/85) as *cfiA*-negative.

Ertapenem hydrolysis assay resulted positive for all *cfiA*-positive strains (11/11), after an incubation time ranging between 30 minutes and 3 hours (in correlation with MIC values), and negative for *cfiA*-negative strains (85/85).

Conclusions: This study shows, in our epidemiological context, a high frequence of reduced susceptibility to carbapenems in *B. fragilis* (11.45% globally, 21.43% among blood cultures). MALDI-TOF MS proved an extremely versatile method to detect this resistance. Beyond species identification, it provides a complete result, made up of both genetic and functional data. Overcoming limits and issues of classical and molecular methods, this “multi-dimensional” MALDI-TOF MS approach leads to shortened turnaround time by at least 24-48 h, with important clinical implications, enabling an earlier set up of the appropriate antibiotic therapy.
Moreover, strong correlation between ertapenem hydrolysis rate and imipenem/meropenem MICs suggests further investigations.