BACKGROUND – AIM

Bacteroides fragilis is the most common and the most antibiot-resistant anaerobic pathogen, causing severe and life-threatening infections. Carbapenem-resistance in Bacteroides fragilis is an emerging issue, associated with the cfiA-encoded class B carbapenemase. cfIA can be silent or expressed at different levels, resulting in a broad spectrum of MIC values of carbapenems. Nevertheless this is a dynamic process, responsive to selective pressure. A treatment with carbapenems can promote cfIA expression, and consequently an increased carbapenemase-production, so therapeutic failure can occur also with phenotypically susceptible strains.

Traditional and molecular methods for anaerobes present some limitations, either laborious and slow or expensive and not suitable for routine work. Furthermore, optimal laboratory tests performances are necessary, as inadequate identification and resistance detection affects directly clinical outcome. The novel applications of MALDI-TOF MS allow an innovative approach to detect carbapenem-resistance in B. fragilis, as a single instrument can act on different analytical "dimensions", improving analytical accuracy, and significantly shortening reporting times, in order to enable an earlier set up of the most appropriate antibiotic therapy.

Aim of this study was to evaluate a one-year collection of B. fragilis strains using the different new applications of MALDI-TOF MS, in terms of identification of cfIA-positive strains by subtyping in II/II division, and confirmation of carbapenemase-activity by ertapenem and imipenem hydrolysis assay. Moreover, the rate of carbapenem-hydrolysis was investigated to evaluate correlation with resistance level, for a characterization of strains in terms of genetic expression levels.

RESULTS

From July 2014 to June 2015, 96 non-duplicated clinical isolates of B. fragilis were collected. Identification at species level was performed by MALDI-TOF MS (MALDI Biotyper, Bruker Daltonik, Germany).

Antibiotic susceptibilities were evaluated by E-test (Oxoid), following EUCAST criteria. For strains with meropenem (MEM) reduced susceptibility (MIC≥2 mg/ml), also imipenem (IPM) and ertapenem (ETP) were tested.

Detection of cfIA gene was performed by MALDI-TOF MS subtyping of strains in division I or II, based on the detection of slight proteomic differences in mass spectrum profiles of B. fragilis strains, as described by Nagy et al. [1]

Carbapenemase-activity was evaluated by MALDI-TOF MS hydrolysis assay, as described by Sparbier et al. [2], following MBT STAR-BL protocol. All strains were tested with ETP (Invanz®, Merck); the cfIA-positive strains plus an equal number of negative strains were also tested with IPM (Sigma).

Briefly, the assay is based on the incubation of a carbapenem solution with the test strains, followed by an automated analysis of its mass spectrum, to detect peaks corresponding to intact and hydrolyzed forms of the antibiotic. (Video 1).

Furthermore, an automated software analysis of spectra, based on their logRQ score (log[sum hydrolyzed peak intensities]/[sum non-hydrolyzed peak intensities]) was performed, in order to evaluate hydrolysis rate after a prolonged incubation, and to compare it with resistance level in terms of MIC values.


DISCUSSION

This study shows, in our area, a high frequency of reduced susceptibility to carbapenems in Bacteroides fragilis (11.45% globally/21.43% among blood cultures).

MALDI-TOF MS subtyping of B. fragilis in division III has proved to be an innovative method to detect the carbapenemase-encoding gene cfIA in strains with reduced susceptibility to carbapenems, included phenotypically susceptible ones, making possible a correct evaluation of their prevalence.

MALDI Biotyper STAR-BL hydrolysis assay proved to be able to reveal the carbapenemase activity by a simple investigation of mass spectra of carbapenems after a short incubation with the test strains. It showed absolute sensitivity, specificity, negative and positive predictive values, regardless of the carbapenem used.

Moreover, the total correlation found between imipenem hydrolysis rate and its MIC values suggests the plausibility to successfully use this method to characterize B. fragilis strains (but not only) in terms of resistance level.

MALDI-TOF mass spectrometry proved to be an extremely versatile and reliable technology to detect carbapenem resistance in Bacteroides fragilis. Beyond species identification, its new applications allow a “multi-dimensional” approach, that provide a complete result in terms of genetic and functional data in a very short time, and result applicable also to the primary culture.

Overcoming limits and issues of classical and molecular methods, this innovative MALDI-TOF MS approach may have important clinical implications, shortening turnaround time by at least 24-48 h, and enabling an earlier set up of the appropriate antibiotic therapy, that is becoming a concerning issue also for infections caused by anaerobic bacteria.