P 0687

Evaluation of *cfiA* gene positive and negative *Bacteroides* species with Carba NP test

Işın Akyar[1, 2], Meltem Kaya[2], Onur Karatuna[1, 2]

[1]*Acibadem University School of Medicine, Department of Medical Microbiology, Istanbul, Turkey*

[2]*Acibadem Labmed Medical Laboratories, Istanbul, Turke*

Background and Objectives

Bacteroides spp. are the predominant components of the human intestinal flora and can cause serious infections. Carbapenems are widely used for the treatment of anaerobic infections, however production of carbapenemases by *Bacteroides* spp. renders these antimicrobials ineffective. The detection of carbapenemase activity in *Bacteroides* spp. by microbiology laboratories might be helpful to guide treatment regimens. In this study, our aim was to investigate carbapenemase-production in clinical *Bacteroides* isolates both by phenotypic and genotypic methods**.**

Material and Methods

Media and growth conditions

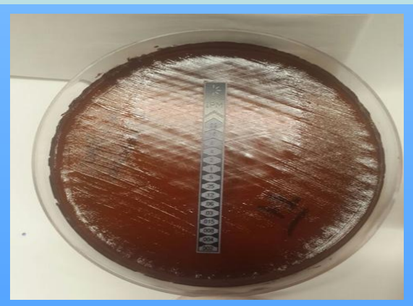
A total of 24 *Bacteroides* species (*B. fragilis* (17), *B. thetaiotaomicron* (3), *B. caccae* (2), *B. vulgatus* (2) collected over a three-year period between 2012 and 2015 were included in this study. The specimen type distribution of these isolates was as follows: blood (n=10), abscess (n=5), periton (n=2), other (n=7). The *Bacteroides* strains were cultured on Schaedler agar (Mediavital, Istanbul, Turkey) and incubated at 37°C in anaerobe pouch system (GasPak™ EZ Anaerobe Pouch System, Becton Dickinson, USA).

Minimum inhibitory concentration (MIC) determination

The susceptibility of the *Bacteroides* strains to imipenem (IMP) was performed with gradient strips (Oxoid, UK) according to the manufacturer's recommendations. In the current study, bacterial suspension of 1 McFarland was prepared and inoculated onto *Brucella* agar with 5% sheep blood, hemin and vitamin K1 (Salubris, Istanbul, Turkey) and afterwards imipenem gradient strip was placed for each strain inoculated. The plates were incubated in anaerobe pouch system at 35-37°C with 5% CO_2 for 24-48 hours. ATCC 25285 *B. fragilis* was used as a reference strain. The standard breakpoints for clinical resistance were applied according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (document M11-A7).

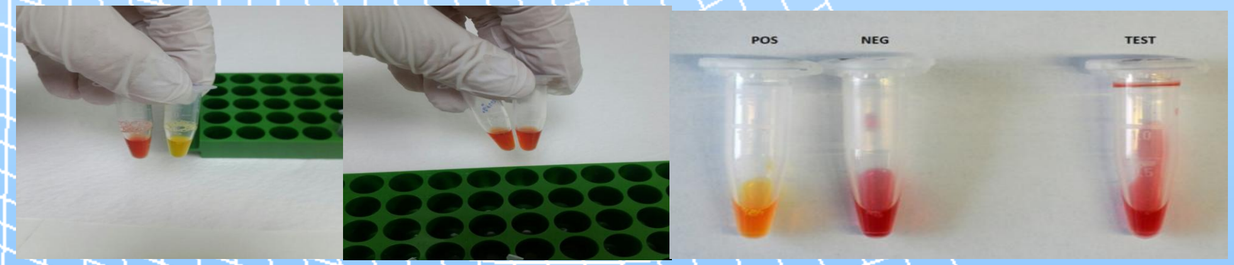
Polymerase chain reaction (PCR) techniques

Bacterial DNA was extracted by a commercial kit (High Pure PCR Template Preparation Kit, Roche, Germany). The carbapenemase coding gene *cfi*A was amplified by polymerase chain reaction (PCR) with GBI-1, GBI-2 and *cfiA*-3 primers. The PCR cycles were as follows: 2 minutes at 94°C, 35 cycles (45 seconds at 94°C, 45 seconds at 51°C, 45 seconds at 72°C) and 2 minutes at 72°C. 2% agarose gel was used for the visualisation of PCR products which were stained with SYBR gold and monitored using ORTE device (Salubris Technica, Istanbul, Turkey). As a control strain a *Bacteroides fragilis* strain with documented *cfi*A positivity was used.



Carba NP test

Carba NP test was performed according to CLSI recommendations. For every strain 2 (a and b) microcentrifuge tubes (1.5 mL) were used. The bacterial colonies grown on Schaedler agar were collected after 24 hours and 10 µL bacteria were added into 100 µL of bacterial protein extraction reagent (B-PERII, Thermo Scientific, Pierce) and stirred for 5 seconds. 100 µL of the solution A (containing zinc sulphate and phenol red, pH: 7.8±0.1) and B (containing solution A and 6 mg/mL imipenem) were added to a and b tubes, respectively, and incubated at 35±2°C for up to two hours. The results were evaluted according to the color changes of the tubes. TAL 2480 *B. fragilis* was used as positive control and *B. fragilis* ATCC 25285 was used as negative control. The microcentrifuge tube which contains only bacterial protein extraction reagent was prepared as reagent control. The prepared microcentrifuge tubes were incubated for 2 hours at 35±2°C. When the Carba NP test was used, the color of the tubes turned from red to orange or yellow for all tested strains that were producing carbapenemases, whereas tubes corresponding to bacterial extracts of isolates that did not produce carbapenemase remained red, whatever their level of carbapenem susceptibility.



Results

Imipenem MIC results were found to be between 0.03 and ≥32 µg/mL. 21 out of 24 strains (87.5%) were found susceptible to imipenem (MIC ≤ 2µg/mL), one strain showed intermediate resistance (MIC=4 µg/mL) and two of the strains were found resistant to imipenem (MIC ≥16 µg/mL). The *cfi*A gene was detected in three *B. fragilis* blood isolates with imipenem MIC ≥4 µg/mL. A correlation between imipenem MIC being ≥4 µg/mL and *cfi*A gene positivity was observed for the tested *Bacteroides* isolates. Carba NP test results were achieved in 2 hours in clinical isolates and in 30 minutes for the reference strain. The two clinical strains with imipenem MIC ≥32 µg/mL and the reference *cfi*A positive strain were tested positive in the Carba NP test, however the imipenem intermediate (MIC=4 µg/mL), *cfi*A positive strain was found negative.

Conclusion

Among our study *Bacteroides* spp. isolates we determined the rate of imipenem nonsusceptibility as 12.5%. Detection of *cfi*A gene by PCR showed a good correlation with imipenem MICs ≥4 µg/mL. For the detection of carbapenemase-production with Carba NP test, however, only isolates with imipenem MICs ≥32 µg/mL gave positive results. Although further investigation is needed, with our preliminary results, we can suggest that Carba NP test can be used as a rapid carbapenemase detection test for *Bacteroides* species.