

# NordicAST *Bacteroides* AST Study 2018

## Background

*Bacteroides fragilis* is the most common anaerobic species causing invasive infections (1). In addition to possessing intrinsic resistance mechanisms against penicillins and cephalosporins (1, 2), *B. fragilis*, as well as other species in the *Bacteroides fragilis* group, have been shown to harbour a large number of acquired antibiotic resistance mechanisms, with resistance to tetracycline, clindamycin and  $\beta$ -lactam/ $\beta$ -lactamase inhibitor combinations being especially high and increasing (3, 4). Resistance to clinically important antimicrobials that are usually considered highly active against anaerobic bacteria, such as carbapenems and metronidazole, is also being reported (4, 5).

The current recommendation from NordicAST for antimicrobial susceptibility testing of anaerobic bacteria, including *Bacteroides*, is to carry out MIC determination, without any particular method being specified (6). One commonly used method is agar gradient diffusion using MIC gradient strips; however, results using this method have shown poor agreement with reference methods (7), and piperacillin/tazobactam MIC gradient strips are reported by EUCAST to be unreliable (8). A disk diffusion method for susceptibility testing of anaerobes is currently being developed by the EUCAST Development Laboratory.

Resistance to carbapenems in *Bacteroides fragilis* is usually mediated by the production of a metallo- $\beta$ -lactamase (MBL) coded for by the *cfiA* gene (9). Carbapenem-resistant strains with other and unknown mechanisms have also been sporadically described in *B. fragilis* (10) and in other species belonging to the *B. fragilis* group (11). The *cfiA* gene is chromosomally present in division II of *Bacteroides fragilis* (12), but its expression depends on the promoter strength of the area upstream of the gene (9). The most common mechanism resulting in high expression of *cfiA*, and consequently high level resistance to carbapenems, is the presence of certain insertion sequence (IS) elements (9, 10, 13). However, strains with MBL activity without the presence IS elements upstream of *cfiA* have also been described. One study found that the region upstream of *cfiA* in such strains differed from a susceptible reference strain in a manner that theoretically results in higher promoter activity (13).

Current guidelines for antimicrobial susceptibility testing of *Bacteroides* spp. do not emphasize detection of MBL production. As the CfiA MBL will also degrade piperacillin and is only poorly inhibited by tazobactam (14), clinical resistance to piperacillin/tazobactam is the expected phenotype in strains with *cfiA*-mediated resistance to meropenem. However, *in vitro* susceptibility to piperacillin/tazobactam in meropenem-resistant, *cfiA*-positive strains is frequently observed with gradient tests (unpublished data). Whether this reflects poor reliability of gradient tests or an *in vitro* phenomenon of inhibition of the *cfiA* MBL by tazobactam is not clear, neither is the clinical significance of *in vitro* susceptibility to piperacillin/tazobactam in MBL-producing strains. Phenotypic tests for MBL production that have shown promise in *Bacteroides* include the EDTA double gradient test (15) and the Carba NP test (16).

## Purpose

We hereby invite Nordic (Denmark, Finland, Iceland, Norway and Sweden) laboratories to examine a collection of *Bacteroides* spp. with varying degrees of resistance to meropenem,

piperacillin/tazobactam, clindamycin and metronidazole, as well as varying degrees of MBL production, in a multi-centre, blinded format.

The aims of this study are:

1. To examine the performance of the new EUCAST disk diffusion method for susceptibility testing of *Bacteroides* spp., compared with reference methodology;
2. More specifically, to examine the reliability of disk diffusion and gradient diffusion in carbapenem-nonsusceptible strains, and the suitability of these methods for routine susceptibility testing and carbapenemase screening;
3. To assess the suitability for routine laboratory use of phenotypic tests for MBL production.

This multi-centre evaluation of the new EUCAST disk diffusion method will clarify the suitability of this method for susceptibility testing of *Bacteroides* spp., and will hopefully lead to reduced costs and workload in routine microbiological laboratories. Further, the results may lead to new recommendations, screening algorithms and/or expert rules for detection of carbapenem and piperacillin/tazobactam resistance as well as MBL production in *Bacteroides* spp.

## Study design

**Timeline.** Invitation to participate in this study will be sent by May 2018. Confirmation of participation (*confirmed participation form*) must be sent by June 20<sup>th</sup>, 2018 to the study coordinating group, also naming the local study supervisor (contact person) for the study and contact information. A blinded panel of strains ( $n=30$ ) will be sent to the participating laboratories during September 2018. The deadline for submitting results will be November 15<sup>th</sup>, 2018.

**Bacterial strains.** The strain panel consists of 30 strains of *Bacteroides* spp. obtained from human specimens during the period 2008-2017, both MBL producers and nonproducers, with a wide range of MICs against meropenem, imipenem and piperacillin/tazobactam, as well as varying resistance mechanisms towards clindamycin and metronidazole. The strains have been characterized by a reference analysis comprising the following methods:

- Identification by MALDI-TOF MS
- Reference MIC determination for meropenem, imipenem, piperacillin/tazobactam, clindamycin and metronidazole using agar dilution/broth microdilution as recommended by CLSI
- Determination of carbapenemase activity by carbapenem hydrolysis assay
- Detection of the *cfiA* gene by PCR, and analysis of its transcriptional activity by mRNA quantification assay

## Methods.

### I. Identification

- a. Required: All laboratories must perform bacterial identification at species level and record the methods used.

## II. **Antimicrobial susceptibility testing**

- a. **Required:** All laboratories must perform antimicrobial susceptibility testing for meropenem, piperacillin/tazobactam, clindamycin and metronidazole using the disk diffusion method on Fastidious Anaerobe Agar, with the following disk strengths: Piperacillin-tazobactam 30-6 µg; meropenem 10 µg; clindamycin 10 µg; metronidazole 5 µg. A Technical Manual / Reading Manual will be provided as a reference for participants.
- b. **Optional:** In addition, laboratories may optionally perform antimicrobial susceptibility testing, including MIC determination, for meropenem, piperacillin/tazobactam, clindamycin and metronidazole using their current routine method for anaerobic susceptibility testing. Results should be interpreted according to EUCAST/NordicAST breakpoints.

## III. **Detection of carbapenemase activity**

- a. Tests for carbapenemase activity are to be carried out on a specified subset of specimens only. In order to avoid biasing the AST results, information about whether a specimen is part of this subset or not will only be provided after the laboratory has submitted AST results for the specimen in a web-based submission tool.
- b. **Required:** All laboratories must test the specified subset of isolates for carbapenemase activity using the meropenem/EDTA double gradient test on Fastidious Anaerobe Agar.
- c. **Required:** All laboratories must test the specified subset of isolates for carbapenemase activity using the β-CARBA test, according to the Technical Manual that will be provided.

## IV. **Procedural information/ Reporting of results**

- a. Specimens may be tested simultaneously or in smaller batches of a size that is found manageable by the laboratory. The date for testing of each specimen must be reported.
- b. All laboratories must report the incubation temperature, and method used for obtaining an anaerobic incubation atmosphere, including manufacturer names and lot numbers of any disposable equipment such as gas packs.
- c. All laboratories must report the manufacturer and lot number of antimicrobial susceptibility testing material, including agar, broths, MIC gradient strips and disks.
- d. Results are to be submitted through a web-based submission tool to which the participating laboratories will be given access with a unique ID.
- e. The results from the reference analysis will be provided to all participants after the submission deadline.

**Organization and publication.** The study originates from and is scientifically approved by NordicAST. We will form a study group (Nordic *Bacteroides* AST study group) consisting of one person from each participating laboratory (the local laboratory supervisor) and a project group. The project group

consists of Dagfinn Skaare and Tore Taksdal Stubhaug (Vestfold Hospital Trust), Ellen Josefsen, Ørjan Samuelsen and Arnfinn Sundsfjord (Norwegian National Advisory Unit on Detection of Antimicrobial Resistance), Ulrik Justesen (Odense University Hospital), Christian Giske (Karolinska University Hospital), Gunnar Kahlmeter and Erika Matuschek (EUCAST Development Laboratory). The project group reports to NordicAST as the reference group for the study. Any publication originating from this study is obliged to follow the Vancouver protocol for determining authorship. All local study supervisors (contact persons) will be included and acknowledged by name as partners in the Nordic *Bacteroides* AST study group.

## References

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