Background
Antimicrobial resistance in anaerobic bacteria is increasing. Penicillin and clindamycin resistance is very common among Gram-negative anaerobic bacteria belonging to the *Bacteroides* spp. group. Members of the *Bacteroides fragilis* group are almost without exception penicillin resistant. Carbapenem resistance, especially meropenem resistance, is increasingly reported in *B. fragilis* and is strongly linked to isolates belonging to division II carrying the *cfiA* gene chromosomally (1). Metronidazole resistance is still relatively rare in general in anaerobic bacteria, but has been reported in *Clostridium ramosum* (proposed new nomenclature *Thomasclavelia ramosa*), the *B. fragilis* group, *Prevotella* spp.
and *Veillonella* spp. In *Propionibacterium* spp., *Cutibacterium* spp., *Lactobacillus* spp. and *Actinomyces* spp. metronidazole resistance is the expected phenotype.

Resistance mechanisms
Non carbapenem beta-lactams: Resistance is usually due to beta-lactamase production for penicillins and cephalosporins. In addition to the *Bacteroides fragilis* group division I, this is also very common in *Prevotella* spp., but not in *Fusobacterium* spp. Penicillinase and cephalosporinase activity in anaerobes are mostly due to Ambler class A beta-lactamases and they are generally inhibited by clavulanic acid and tazobactam.

Monobactams have reduced affinity to penicillin-binding proteins of anaerobes and are considered ineffective treatment.

Carbapenems: The most common resistance mechanism is carbapenem-hydrolyzing metallo-beta-lactamases in *B. fragilis* division II mediated by the chromosomal *cfiA* gene (2). The presence of the gene is not enough to confer resistance, and expression seems to be dependent on the presence and localization of insertion sequences, which function as promoters. Although direct transfer between the *cfi*A genes in a clinical setting has only been described very rarely, the *cfi*A gene is often located within regions of genomic plasticity allowing for the potential horizontal transfer of the gene.

Clindamycin: Resistance is usually constitutive, but inducible clindamycin resistance has been described in the *B. fragilis* group and in *Peptostreptococcus* spp. (1,3-5). Resistance to clindamycin in Gram-negatives is usually mediated through expression of rRNA methylases encoded by *erm*-genes. Data on the resistance mechanisms in Gram-positives is more limited but suggests that *erm*-genes are involved here as well. The presence of one of these genes alone does not correspond very well to phenotypical resistance, and a combination of genes might be needed to develop resistance (6-7).

Metronidazole: The resistance mechanism is not fully elucidated but is usually associated with *nim* genes. It has been suggested that the enzyme encoded by the *nim* gene converts metronidazole to an inactive metabolite by acting as a nitroreductase. The gene is usually located on mobile genetic elements which makes horizontal transfer of the gene possible.

Testing methods
EUCAST disk diffusion methodology for the rapidly growing anaerobic bacteria, *Bacteroides* spp., *Prevotella* spp., *Fusobacterium necrophorum*, *Clostridium perfringens* and *Cutibacterium acnes*, using fastidious anaerobe agar supplemented with 5% defibrinated horse blood (FAA-HB) with breakpoints for common anti-anaerobic antimicrobial agents, was published in January 2022 (8). EUCAST continues to develop this methodology for more species and antimicrobial agents. They have also developed a reference agar dilution method based on FAA-HB, which can also be used for other anaerobic bacteria (9).
Commercial assays are available, but these are often based on CLSI methodology, and studies to support these methods are scarce. Gradient strips is an easy but expensive method, and problems with performance (very major errors) have been reported (9,10).

SUPPLEMENTAL TESTING
- Beta-lactam antibiotics: it is possible to detect beta-lactamase production with the nitrocefin/cefinase test. The test is usually positive within 5-10 minutes (30 minutes in some cases) (11).

- Carbapenems: metallo-beta-lactamase production can be demonstrated by using a double ended gradient strip with meropenem ± EDTA (2). The *cfiA* gene can be indirectly demonstrated by MALDI-TOF, because *B. fragilis* can be divided in division I (subtype I)(*cfiA*-negative) and division II (subtype II) (*cfiA*-positive based on mass spectrometry (12).

- PCR or whole genome sequencing (WGS) can be used to detect a diversity of resistance genes in anaerobic bacteria by using *e.g.* ResFinder (https://genepi.food.dtu.dk/resfinder)

Interpretation
SIR-categorization can be determined from MICs or zone diameters with the EUCAST breakpoint tables for several species,
e.g. *Bacteroides* spp., *Prevotella* spp., *Fusobacterium necrophorum*, *Clostridium perfringens* and *Cutibacterium acnes.* For some species ECOFFs may be available (e.g. *C. difficile*). For the remaining anaerobic bacteria, refer to the EUCAST Guidance document “When there are no breakpoints in breakpoint tables” (https://www.eucast.org/eucastguidancedocuments)

- Penicillins: Isolates in the *B. fragilis* group can be reported as resistant to penicillins without a β-lactamase inhibitor without further testing.

Other anaerobic species
If the isolate is β-lactamase positive, it should be reported as resistant to penicillins without a β-lactamase inhibitor.
If the isolate is β-lactamase negative, penicillin susceptibility should be reported according to the appropriate clinical breakpoints.

β-lactamase testing
β-lactamase testing is not necessary if MIC testing is used as the primary susceptibility method.

- Carbapenems: *B. fragilis* isolates with an MIC above the susceptible breakpoint are not exceptional. Some isolates with an MIC of 1 mg/L may harbour the *cfiA* gene. The clinical significance in these cases is unknown. Supplemental testing can be performed as described above (2).

- Clindamycin: the clinical significance of inducible clindamycin resistance is unknown, and testing should be avoided in anaerobes unless otherwise stated by EUCAST.

- Metronidazole: isolates with an MIC above the susceptible breakpoint are exceptional phenotypes and identification and antimicrobial susceptibility testing should be repeated (3). When metronidazole resistance is observed in the laboratory, it can be due to pseudo-resistance caused by insufficient anaerobic conditions (small amounts of oxygen in the atmosphere). EUCAST recommends that the anaerobic environment is tested with the aerotolerant *Clostridium*

perfringens DSM 25589 strain and a metronidazole 5 µg disk. (13-14).

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Responsible for this document

NordicAST representatives, subgroup for fastidious bacteria, see http://www.nordicast.org

Changes

2012-04-01	Nytt dokument
2014-08-27	Uppdatering
2018-08-31	Revision and translation
2022-09-14	Uppdatering
2025-09-22	Updates to the background and resistance mechanisms section. Clarification of recommendations to clindamycin susceptibility testing. Updated recommendations for unexpected metronidazole resistance.
2025-12-12	Penicillin interpretation section clarified.