

Challenge M171-4

May 2017

Blood: *Bacteroides fragilis*

HISTORY

A simulated blood culture sample collected from a 57 year old male with bowel obstruction was sent to category A laboratories.

Participants were expected to isolate and identify *Bacteroides fragilis* group. No susceptibility testing was required.

CMPT QA/QC/STATISTICS

All simulated blood samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Bacteroides fragilis*.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for at least 18 days.

Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

MAIN EDUCATIONAL POINTS from M171-4

1. Laboratory reports that communicate a presumptive identification of *Bacteroides fragilis* group are important to clinicians, because these organisms possess resistance to more antimicrobial agents than most other anaerobic organisms.¹
2. By performing inexpensive and relatively simple testing – vancomycin, kanamycin, colistin, and bile disks, and aerotolerance – a presumptive identification of *Bacteroides fragilis* group could be provided to the physician while the reference laboratory continues workup.
3. Members of *Bacteroides fragilis* group are the most frequently isolated anaerobic pathogens from bloodstream infections¹, and of anaerobic gram-negative bacilli, possess more virulence and antimicrobial resistance genes than most.

formed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: 13/13 (100%) laboratories reported *Bacteroides fragilis* ± group.

Participants (Table 1): results obtained by all participants; description of results obtained and grades assigned.

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Grading

Maximum grade: 4

Reporting *Bacteroides fragilis*/B. *fragilis* group was graded 4.

Reporting *Bacteroides* species was graded 4.

Reporting anaerobic gram negative bacilli/coccobacilli with referral was graded 4.

Reporting anaerobic gram negative bacilli without referring was graded 3.

Reporting gram negative rods was graded 1.

Reporting *Staphylococcus aureus* was graded 0.

Table 1. Identification results

| Reported results | Total | Grade |
|---|-----------|----------|
| <i>Bacteroides fragilis</i> ± group ± refer ± presumptive | 44 | 4 |
| <i>Bacteroides</i> species, refer ± presumptive | 2 | 4 |
| anaerobic isolate resembling <i>Bacteroides</i> , refer | 1 | 4 |
| anaerobic gram negative bacilli, refer | 8 | 4 |
| anaerobic gram negative coccobacilli, presumptive, refer | 1 | 4 |
| anaerobic gram negative bacilli | 1 | 3 |
| gram negative rods, refer | 1 | 1 |
| <i>Staphylococcus aureus</i> | 1 | 0 |
| snp | 5 | ungraded |
| Total | 64 | |

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

COMMENTS ON RESULTS

97% of participants that process this type of sample correctly identified an anaerobic gram-negative isolate was present.

78% of participants correctly reported to the genus level with 74% of participants reporting correctly to the species level (*Bacteroides fragilis* ± group), which is consistent with the results from the last *B. fragilis* group survey in 2016 (72%).

One participant reported “gram negative rods, refer” with no indication that the isolate was an anaerobe. This does not communicate to the physician that the isolate is anaerobic, possibly delaying appropriate treatment. Also, the isolate may not be viable if sent to the reference laboratory using aerobic transport media. Determination of anaerobicity, even in the absence of specific identification, is an important function of the laboratory.

One participant reported “anaerobic gram negative bacilli” with no indication that the isolate would be referred for further testing. Being from a blood culture, further identification of this isolate is required, and should be referred to a reference laboratory if the participant did not have the means to complete full or presumptive identification to *Bacteroides fragilis* group or beyond.

One participant reported *Staphylococcus aureus* (which was not present in the sample) and was given a grade of 0 since this would result in a different and potentially harmful clinical course.

ISOLATION and IDENTIFICATION

The discussion of the genus *Bacteroides* here is limited to the species within the *Bacteroides fragilis* group as they are the most relevant in human infections.

Bacteroides species appear as pale-staining pleomorphic gram negative bacilli or coccobacilli.⁶ The *B. fragilis* group are obligate anaerobes that grow as grey colonies on both Brucella blood agar and kanamycin-vancomycin-laked blood agar and black colonies on Bacteroides

bile esculin agar where they (except for *B. vulgare*) hydrolyze esculin.¹

B. fragilis group are typically resistant to all three of the high potency disks (5 µg vancomycin, 1000 µg kanamycin, 10 µg colistin), as well as bile, and can be reported as presumptive *B. fragilis* group with the appropriate Gram stain, colonial morphology and aerotolerance testing. MALDI-TOF MS has also proven to be an efficient and reliable method for identification of *B. fragilis* group.²

ANTIMICROBIAL SUSCEPTIBILITY

Routine susceptibility testing is not generally required as most of the strains remain susceptible to metronidazole. However, reference laboratories should provide periodic information on local susceptibility patterns of anaerobic species within the clinically important taxa as there is increasing rates of resistance to various antimicrobials.^{1,3,4}

All strains of *B. fragilis* and many other members of the *B. fragilis* group produce a β-lactamase, imparting resistance to penicillins and third generation cephalosporins. The organism, however, responds well to combinations including β-lactamase-inhibitors, such as amoxicillin-clavulanic acid, or piperacillin-tazobactam.

Carbapenems retain a high degree of activity against *Bacteroides* species, despite recent reports of resistant isolates. Clindamycin, once a first-line agent, has seen a dramatic increase in resistance, such that it is no longer recommended empirically.^{8,9} Moxifloxacin remains an alternative option, but susceptibility rates can vary depending on locale, and antimicrobial susceptibility results should guide treatment.⁹

CLINICAL RELEVANCE

Most anaerobic infections originate from the indigenous microbiota and are acquired when the integrity of the colonized mucosa or lumen is breached by trauma, by underlying disease, or during surgery.

The gastrointestinal tract and the oropharynx are the most common sources for anaerobic bacteremias, with gastrointestinal surgery and underlying malignancies being the major predisposing factors.¹

Members of the *Bacteroides fragilis* group are the most common isolates recovered from patients with anaerobic bacteremia.⁵ *B. fragilis*

has been proved to be the most frequent *Bacteroides* species found in specimens from blood, ulcers, abscesses, bronchial secretions, bone, intra-abdominal infections, inflamed appendixes, and the head.¹

REFERENCES

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