



M063 –5 Vitreous fluid (deep wound) *Bacillus cereus*

HISTORY A simulated deep wound vitreous fluid sample from a 35-year old construction worker with a foreign body in his eye was sent to category A laboratories to culture and report as per their usual laboratory protocol.

CMPT QA Internal validation showed this sample contained a pure growth of 4+ *Bacillus cereus*, viable for 15 days. The isolate was beta-lactamase positive.

GRADING (maximum grade = 4) The identification component of this challenge was acceptable for grading as all reference laboratories reported either *Bacillus cereus* (11) or *Bacillus* species (4).

IDENTIFICATION (grade = 4) Most participants (69/71, 97%) had little difficulty correctly identifying this isolate to genus level, but only 58% (41/71) were able to provide the correct species. However, in a previous challenge with *B. cereus* (M22-2 August 2000) while 93% of category A laboratory participants reported *Bacillus* species, only 34% reported *B. cereus*. Results received and grades assigned are shown in Table 1.

Most participants reported using a combination of classical tests including one or more of the following: catalase, motility, lecithinase, oxidase, nitrate, gelatin, esculin, bile esculin, pigment, citrate, urea, ornithine, TSI, H₂S, indole, VP, OF sugars, casein, starch, and spore stain. PCR was also performed by 1 laboratory.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (ungraded) It was anticipated that participants would report susceptibility results for penicillin, vancomycin, and a fluoroquinolone. However, there was no consensus between reference laboratories in either their reporting scheme or results. One reference laboratory reported a positive beta-lactamase result, 5 reported penicillin resistant by Kirby Bauer, E-test or MicroScan, 3 laboratories reported penicillin resistant by a positive beta-lactamase result and by Kirby Bauer, E-test or MicroScan; 1 reported penicillin sensitive, 1 referred the organism for beta-lactamase

GRADING –Maximum grade = 4

Identification: 97% (69/71) of category A, laboratories received a grade of 4/4 or 3/4.

NOTES

1. When a *Bacillus* species is isolated in pure, predominant, or large numbers, or upon repeated isolation, the laboratory must consider whether to report the isolate and steps must be taken to confirm or rule out *B. anthracis*. In the case of eye injury, any *Bacillus* species should be reported immediately.
2. *B. cereus* is considered the most destructive bacteria that can infect the eye, leading to vision loss and/or loss of the eye.

testing, and 4 did not submit a beta-lactamase or penicillin result. Overall, 10 reference laboratories reported vancomycin as sensitive, including 2 that only reported this antimicrobial; 7 also reported ciprofloxacin sensitive.

Results from the laboratories reporting susceptibility results also varied. Five laboratories issued a comment that there are no standards for susceptibility testing. There are no CSLI standards for susceptibility testing of *B. cereus*; only for *B. anthracis*. Beta-lactamase testing is not reliable with *B. cereus*. One laboratory reported a comment recommending treatment with a fluoroquinolone, imipenem, and clindamycin.

TAXONOMY *Bacillus* species are mostly saprophytic and widely distributed in nature. They are found in all types of soil, in all geographical climates and are found in fresh and salt water. Most members of the *Bacillus* genus are non-pathogenic and are often considered a laboratory contaminant. Some produce antibiotics and vitamins (*B. licheniformis*, *B. subtilis*, *B. polymyxa*, *B. megaterium*). The *Bacillus cereus* group consisting of *B. cereus*, *B. anthracis*, *B. thuringiensis* and *B. mycoides* are considered pathovars of a single species. *B. cereus* has been described as an opportunistic pathogen, while *B. anthracis* is an obligate pathogen of humans and animals¹.

Table 1. M063-5 Identification results received and grades assigned to category A laboratories.

Identification	Total (% out of 71)	Grade
<i>Bacillus cereus</i> (group n=10), with or without presumptive, refer or not <i>B. anthracis</i> ; <i>Bacillus cereus</i> / <i>B. thuringiensis</i> (1)	41 (58%)	4
<i>Bacillus</i> species, not <i>B. anthracis</i> (5 [2 refer]); <i>Bacillus</i> species (22 [19 presumptive, refer]); <i>Bacillus</i> species, strain 1 and 2 (1 refer)	28 (39%)	3
Gram-positive bacillus, refer	1	1
Aerobic spore-forming bacillus (did not refer)	1	1
Do not process/referred	3	ungraded
Total	74	

IDENTIFICATION *Bacillus* species are facultative anaerobic or aerobic gram-positive spore-forming bacilli. They form large 2-7 mm circular to irregular colonies, which are matted or granular, smooth or moist in texture on 5% sheep blood agar. They are rapid growers and can grow in temperatures of 15-45°C, 37°C being optimal. Aerotolerance testing should be performed when identifying isolates, to rule out clostridia. Most *Bacillus* species are catalase positive, therefore this test can be used to differentiate *Bacillus* from aerotolerant clostridia. Members of the *B. cereus* group are all lecithinase positive and facultative anaerobes. *B. cereus* may be dif-

(Continued on page 2)

ferentiated from *B. anthracis* by motility at 35°C, beta-hemolysis and penicillin resistance^{1,2}.

CLINICAL SIGNIFICANCE *B. cereus* is the second most common organism isolated from post-traumatic endophthalmitis after *Staphylococcus epidermidis*. It is considered the most destructive bacteria that can infect the eye, leading to vision loss and/or loss of the eye. *B. cereus* produces exotoxins that cause marked inflammation, cellular death, and tissue necrosis. The organism may infect the eye by means of trauma or be blood-borne. Delay in primary repair to an eye injury, trauma, or dirty wounds increase the incidence of endophthalmitis. When patients present with trauma or foreign body penetration to the eye, anterior chamber or vitreous aspirate samples are collected. Prophylactic intravitreal antibiotics may be injected in an attempt to stem off infection^{1,3,4,5}. *B. cereus* has also been implicated in one case of post-operative infection following cataract surgery⁶.

Bacillus cereus can cause necrosis and gangrene in post-surgical wounds or trauma (traffic/road accidents), burns, drug injection, gunshot or nail bomb injuries. Because of its opportunistic nature, *B. cereus* has been implicated in causing infection in patients with neoplastic disease, immunosuppression, or alcohol or drug abuse, causing septicemia, bacteremia, pneumonia, fulminant sepsis with hemolysis, meningitis, endocarditis, pleurisy, brain abscess, ventricular shunt, osteomyelitis, urinary tract infection, and primary cutaneous infection. Reports of neonatal umbilical stump infections due to *B. cereus* have also been reported¹.

Bacillus cereus also causes food-borne illness – a) diarrhea, with abdominal pain and diarrhea 8-16 hours after ingestion of a variety of foods, or b) emetic disease, with nausea and/or vomiting 1 – 5 hours after eating. In the emetic phase, spores survive normal cooking temperatures, and if the food is stored improperly, the spores germinate; the cells multiply and elicit an exotoxin which causes the illness^{1,2}.

When a *Bacillus* species is isolated in pure, predominant, or large numbers, or upon repeated isolation, the laboratory must consider whether to report the isolate and steps must be taken to confirm or rule out *B. anthracis*. In the case of eye injury, any *Bacillus* species should be reported immediately.

TREATMENT *B. cereus* produces a broad spectrum beta-lactamase that confers resistance to penicillin, ampicillin, and all cephalosporins. It is resistant to trimethoprim and up to 22% of isolates may also be resistant to clindamycin⁴. *B. cereus* is usually susceptible to erythromycin, vancomycin, fluoroquinolones, meropenem, and aminoglycosides. Treatment for ocular infections may include a combination of vancomycin or clindamycin and an aminoglycoside intravitreally. Fluoroquinolones have also been used to successfully treat endophthalmitis, by either IV or oral route, with patients discharged home on oral ciprofloxacin^{1,3,4,5,6}.

SAFETY Laboratorians are reminded to adhere to good safe handling practices when *Bacillus* species is isolated. Use the biological safety cabinet for any manipulation that may create aerosols. Visit the Public Health Agency of Canada website for copies of the *Laboratory Biosafety Guidelines*, 3rd edition (2004)⁷ and information on Bioterrorism and Emergency Preparedness⁸.

REFERENCES

1. Logan NA, Turnbull PCB. 2003. *Bacillus* and other aerobic endospore-forming bacteria. p.445-460 In PR Murray et al. (eds.) *Manual of Clinical Microbiology*, 8th ed., American Society for Microbiology, Washington DC.
2. CMPT Critique M22-2: Wound (burn) swab: *Bacillus cereus*. August 2000.
3. Essex RW, Charles PG, Allen PJ. 2004. Three cases of post-traumatic endophthalmitis caused by unusual bacteria. *Clin Experiment Ophthalmol.* 32(4):445-7.
4. Foster RE, Martinez JA, Murray TG, et al. 1996. Useful visual outcomes after treatment of *Bacillus cereus* endophthalmitis. *Ophthalmology.* 103(3):390-7.
5. Chan WM, Liu DT, Chan CK, et al. 2003. Infective endophthalmitis caused by *Bacillus cereus* after cataract extraction surgery. *Clin Infect Dis.* Aug 1;37(3):e31-4. Epub. 2003 Jul 17.
6. Callegan MC, Ramiriz R, Kane ST, et al. 2003. Antibacterial activity of the fourth-generation fluoroquinolones gatifloxacin and moxifloxacin against ocular pathogens. *Adv Ther.* Sep-Oct;20(5):246-52.
7. Laboratory Biosafety Guidelines 3rd Edition – 2004 <http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/index.html>
8. Bioterrorism and Emergency Preparedness. http://www.phac-aspc.gc.ca/ep-mu/bioem_e.html

Further reading: *CMPT Connections* 3:3 Fall 1999.

Case Presentation: *Bacillus cereus* endophthalmitis (link to [CMPT Connections](#))