

## Challenge M092-4

August 2009

Blood: *Fusobacterium nucleatum*

### HISTORY

This sample was a simulated blood culture sample reportedly from a 18 year old patient with septic shock.

The sample was sent to category A laboratories which were requested to process and report as per their usual protocol. Participants were expected to process the sample, identify isolated microorganism as *Fusobacterium nucleatum*, and perform susceptibility testing.

### CMPT QA

The sample yielded 4+ *Fusobacterium nucleatum* (white colonies on BAP—brucella base sheep blood agar plate) under anaerobic incubation, pure and viable for 5 days.

### SURVEY RESULTS

Identification (see Table 1)

**Reference Labs:** 7 labs reported *F. nucleatum*, 2 labs reported *Fusobacterium* species, 1 lab reported *F. varium*, refer, 4 labs reported no growth, 1 lab reported *Neisseria* species, re-

sembling *N. weaveri*, refer.

Consensus was not achieved by the reference laboratories thus the identification challenge was **ungraded**.

A considerable number of laboratories (53%) was able to identify the microorganism as belonging to the genus *Fusobacterium*, 71% correctly identified the isolate as *Fusobacterium nucleatum*. *Fusobacterium necrophorum* and *Fusobacterium varium* were reported by one laboratory each.

Two laboratories reported *Bacteroides* species and 4 labs reported anaerobic gram negative bacilli. Thirty percent of laboratories did not obtain any growth from the sample and thus could not identify the microorganism.

Four laboratories reported that they do not normally process this kind of sample.

#### Antimicrobial Susceptibility Testing

#### **Reference laboratories:**

Penicillin: 7 labs did not report, 7 labs reported penicillin S, 1 lab reported penicillin R.

Clindamycin: 7 labs did not report, 8 labs reported clindamycin S

#### Grading

Consensus was not achieved by the reference laboratories thus the challenge was **ungraded**.

One laboratory may have mistakenly mixed up the identification of challenge M092-4 (*F.nucleatum*) and M092-5 (*N.weaveri*).

The committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or

**Table –1:** Reported results for M092-4 -Identification component – Challenge ungraded.

| Reported  | No of labs | %            |
|---|------------|--------------|
| <b><i>Fusobacterium</i></b>   | <b>41</b>  | <b>52.6</b>  |
| <i>Fusobacterium nucleatum</i> , +/- presumptive, snnp, refer           | 28         | 35.9         |
| <i>Fusobacterium nucleatum</i> , <i>Staphylococcus epidermidis</i>      | 1          | 1.3          |
| <i>Fusobacterium</i> species, +/- refer +/- presumptive, snnp           | 10         | 12.8         |
| <i>Fusobacterium necrophorum</i> , refer                                | 1          | 1.3          |
| <i>Fusobacterium varium</i> , refer                                     | 1          | 1.3          |
| <b><i>Bacteroides</i></b>   | <b>2</b>   | <b>2.6</b>   |
| <i>Bacteroides</i> species, refer                                       | 1          | 1.3          |
| <i>Bacteroides ureolyticus</i> group, presumptive, refer                | 1          | 1.3          |
| anaerobic gram negative bacilli, refer +/- probable, unable to identify | 4          | 5.1          |
| gram negative bacilli, refer  | 1          | 1.3          |
| <i>Neisseria</i> species, resembling <i>N. weaveri</i>                  | 1          | 1.3          |
| <i>Staphylococcus epidermidis</i>                                       | 2          | 2.6          |
| no growth +/- after 2, 5, 7, 10 days, +/- refer                         | 23         | 29.5         |
| snnp, refer   | 4          | 5.1          |
| <b>Total</b>  | <b>78</b>  | <b>100.0</b> |

snnp: sample not normally processed

Metronidazole: 8 labs did not report, 7 labs reported metronidazole S

Beta-lactamase testing: 8 labs did not report, 7 labs reported beta-lactamase negative

Imipenem: 13 labs did not report, 2 labs reported imipenem S

Meropenem: 14 labs did not report, 1 lab reported meropenem S

Consensus was not achieved by the reference laboratories thus the susceptibility challenge was **ungraded**.

As very few laboratories performed susceptibility testing, results are not presented in this critique.

As this anaerobe was isolated from blood, the CMPT committee expected laboratories to report or refer the isolate for susceptibility testing and/or provide a comment indicating local sensitivity patterns of resistance.

## ISOLATION

The use of selective media along with nonselective media for the isolation of anaerobes increases the yield and saves time in terms of recognition of colonies. The minimum recommended medium setup includes:

- non-selective, enriched, brucella base sheep blood agar plate supplemented with vitamin K1, and hemin (BAP)
- kanamycin-vancomycin laked sheep blood agar (KVLB) for the selection of *Bacteroides* and *Prevotella* species

For specimens from sites/sources below the diaphragm:

- *Bacteroides* bile-esculin agar plate (BBE) for the selection and presumptive identification of the *Bacteroides fragilis* group and *Bilophila* species

When fusobacteria are clinically suspected:

- *Fusobacterium* neomycin-vancomycin agar or *Fusobacterium* selective agar <sup>1</sup>.

## IDENTIFICATION

Due to considerable phenotypic heterogeneity, colony morphology is not a consistent parameter of the fusobacteria and is not sufficient for species identification<sup>1,2</sup>.

Special-potency antibiotic disk pattern can be used to separate the gram-negative rods into

several groups. *Fusobacterium* species, *Bacteroides ureolyticus*, *Campylobacter* species, *Bilophila* species, *Sutterella* species, and *Leptotrichia* species are resistant to vancomycin, but sensitive to both colistin and kanamycin. Most commonly encountered fusobacteria, *F. nucleatum* and *F. necrophorum*, are indole positive and nitrate negative <sup>1</sup>.

Under microscopic inspection, fusobacteria are usually larger than other bacteria with the same identification profile. The typical gram stain of *F. nucleatum* shows slender gram-negative rods; most cells are 5 to 10 µm long and have sharp, pointed ends <sup>2,3</sup>. The microscopic morphology of *F. mortiferum* reveals filaments containing swollen areas with large, round bodies and irregular staining. *F. necrophorum* has a similar morphology to *F. mortiferum* but usually fewer round bodies <sup>1,3,4</sup>.

## ANTIMICROBIAL SUSCEPTIBILITY

Antimicrobial susceptibility should be performed on isolates from normally sterile body sites (e.g. endocarditis, osteomyelitis, joint infection, and bacteremia). Some species of *Bacteroides*, *Prevotella*, *Fusobacterium*, *Clostridium*, *Bilophila*, and *Sutterella* should be considered for antimicrobial testing even though susceptibility to these agents can be unpredictable. It is recommended that the resistance patterns are monitored annually at individual hospitals.

As a result of the standardization and correlation studies, either of two methods is recommended for testing: Agar dilution or Broth microdilution. Broth microdilution method is only recommended by CLSI for *B. fragilis* group organisms since more fastidious anaerobes give inconsistent and unreliable results due to the poor growth of the strains. There are commercial broth microdilution panels that are FDA-approved for testing of all anaerobes, and may work satisfactorily for certain non-*B. fragilis* group species <sup>5</sup>.

E-test (AB BIODISK, Solna, Sweden) has been used in recent years because of its convenience and several studies indicate that results correlate with the CLSI reference method. <sup>6</sup>

β-lactamase activity may be detected using a chromogenic, cephalosporin-based method; some anaerobes may take up to 30 minutes to give a positive test. A negative β-lactamase test,

### *Fusobacterium* susceptibility

Among the oral commensal flora, both β-lactamase-producing and -non-producing variants of *F. nucleatum* can be present simultaneously. Oral colonization with β-lactamase-producing fusobacteria has been shown to increase steadily from 2% at 2 months of age to 49% at 24 months of age <sup>7</sup>.

The incidence of antimicrobial resistance among periodontal gram-negative isolates was studied and the susceptibility profile of *F. nucleatum* and *Prevotella* species were determined. The authors suggest that the high percentage (69%) of resistance to clarithromycin observed among *F. nucleatum* strains might be related to the high consumption of macrolides. All the strains of *Prevotella* species and *F. nucleatum* were susceptible to metronidazole <sup>8</sup>.

however, does not necessarily assure susceptibility to this drug class since some anaerobes are resistant to  $\beta$ -lactam antimicrobial agents by mechanisms other than  $\beta$ -lactamase production.

CLSI recommends clindamycin and metronidazole be tested against all gram negative anaerobes; ampicillin and penicillin should be added to those that are  $\beta$ -lactamase negative; a combination of  $\beta$ -lactam plus  $\beta$ -lactamase inhibitor and carbapenems should be added to the susceptibility profile of isolates that are  $\beta$ -lactamase positive<sup>5</sup> (e.g. a susceptibility panel including clindamycin, metronidazole, penicillin, piperacillin-tazobactam and meropenem).

## CLINICAL RELEVANCE

*Fusobacterium* species are nonspore-forming gram-negative anaerobic bacilli. The most common species isolated from clinical specimens worldwide is *F. nucleatum*.

*F. nucleatum* forms part of the normal oral, gastrointestinal, urogenital and upper respiratory tract flora<sup>2</sup>. It is most commonly associated with periodontal disease; and less frequently with obstetric infections, brain abscess complicated periodontal disease and bacteraemia during prolonged neutropenia<sup>3,9-11</sup>.

*Fusobacterium* species have been reported to be the third most common anaerobe isolated from blood following *Bacteroides* species and *Clostridium* species. Infections involving *Fusobacterium* species are frequently monomicrobial whereas infections caused by other anaerobes are polymicrobial<sup>12</sup>.

During a 14-year period study of wounds and abscesses, fusobacteria were least commonly isolated among the five anaerobes after *Bacteroides* species, *Clostridium* species, *Peptostreptococcus* species and *Prevotella* species. Overall, most wounds and abscesses were polymicrobial and monomicrobial anaerobic isolates were infrequently found (11%)<sup>12</sup>.

Lemierre's syndrome refers to thrombophlebitis of the internal jugular vein due to anaerobic infection first described by Andre Lemierre (1875-1956) in 1936<sup>13</sup>. The disease usually affects young adults and incidence rates of one per million people a year have been reported. Oral anaerobes, particularly *Fusobacterium* species are the most frequently isolated and invasion is usually from intra-oral disease, such

as bacterial tonsillitis, Epstein-Barr virus infection or dental disease. Metastatic infection, particularly pleuropulmonary, is common and a major cause of morbidity. Once almost uniformly fatal, Lemierre's syndrome still carries a mortality rate as high as 20%. The diagnosis is suggested by fever, pain, and swelling along the angle of the jaw and sternocleidomastoid muscle<sup>3,4,10,14</sup>.

The syndrome was rarely reported during the 1960s and 1970s, when penicillin was frequently used to treat pharyngeal infections. Today, antibiotic-resistant organisms are a large clinical concern, thus governing more prudent and judicious prescribing of antibiotics. Subsequent reemergence of this forgotten disease may become much more common in the clinical setting<sup>15</sup>.

## TREATMENT

Treatment of infections by *Fusobacterium* species typically includes penicillin, metronidazole or clindamycin<sup>15</sup>.

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