The fusobacteria: underrated anaerobic pathogens

The subject of anaerobic microbiology returns this month with a look at *Fusobacterium* species. Here, Mike Wren provides an overview of these elusive Gram-negative bacilli.

In a previous issue of *The Biomedical Scientist* Val Hall gave an overview of the significance of anaerobic bacteriology ('Anaerobes in Clinical Microbiology' March, page 174). In that article the potential seriousness of infection with the fusobacteria (especially *Fusobacterium necrophorum*) was highlighted. The present article takes the discussion of fusobacteria a little further and outlines the clinical importance of this genus, and what the diagnostic laboratory can do to ensure recovery of members of this genus from clinical specimens to ensure identification to, at least, the genus level.

NATURAL HABITAT

Some *Fusobacterium* species are normal residents of the mouth, upper respiratory tract, the genitourinary tract and the gastrointestinal tract of humans and animals, while others have not been shown convincingly to be part of the normal flora.

F. nucleatum is found as a common member of the human mouth flora and consequently is the most common species recovered from human clinical material, especially in specimens from the upper and lower respiratory tracts and the brain. *F. mortiferum* and *F. varium* are associated with the gastrointestinal tract and may give rise to infections such as abdominal abscesses and infected abdominal surgery wounds.

F. necrophorum is a highly pathogenic species and although the literature often quotes this organism as being a member of the upper respiratory tract flora, the hard evidence is scant and unconvincing (see reference 4 for discussion).

ISOLATION FROM CLINICAL MATERIAL

The fusobacteria are strictly anaerobic Gram-negative bacilli with variable microscopical and colonial morphology that depend on the species. They are fastidious in their growth requirements, do not grow on all selective media commonly used for anaerobes and, under certain circumstances, may be highly pathogenic.[1]

Fusobacteria do not survive well in air and so care should be taken to ensure that specimens are taken into a good transport medium (for swabs) or, preferably, frank pus should be requested. Pus is always better than swabs, especially for the more oxygen-sensitive species of anaerobe.[2] Whenever possible, there should be the minimum of delay between setting up cultures and placement in an anaerobic atmosphere.

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Furthermore, the use of the correct selective medium is important. Media containing kanamycin as the selective agent should be avoided at all cost, as the fusobacteria are sensitive to this aminoglycoside. Neomycin- or nalidixic acid-containing media must be used, together with a rich agar base developed for the growth of anaerobes (Fastidious Anaerobe Agar [Bioconnections] or Anaerobe Basal Agar [Oxoid] are recommended).

Fusobacteria benefit from prolonged incubation, and culture plates from normally sterile sites should be examined after 48 hours of incubation and again after five days before being discarded as negative.

EXAMINATION OF CULTURES

The genus contains various species that give different colonial appearances on agar media. *F. nucleatum* and *F. necrophorum* are the most commonly isolated species from human clinical material.

F. nucleatum produces three types of colony, 1–2 mm in diameter, that are white and breadcrumb-like; grey, irregular edged with internal flecking; and opaque, grey, with an entire edge. All three types produce a greening effect in the agar around the colony on exposure to the air. Gram stain of *F. nucleatum* reveals the typical javelin forms (Fig 1), which are restricted to this species.

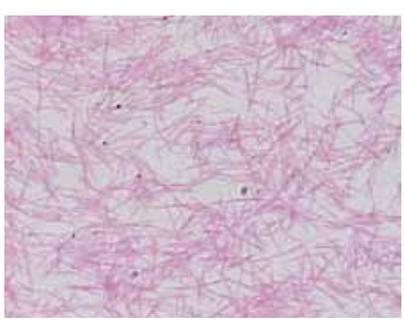

Fig 1. Gram stain of *F. nucleatum* after culture for 48 hours.

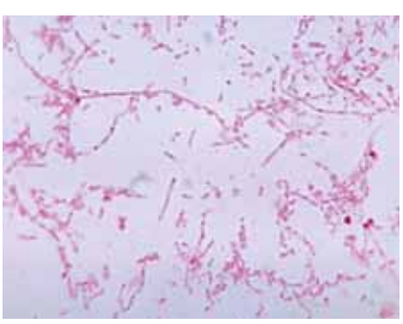

Fig 2. Gram stain of *F. necrophorum* after culture for 48 hours.

F. necrophorum produces grey to yellowish waxy colonies, 3–4 mm in diameter, surrounded by a zone of β-haemolysis. Gram stain yields a highly pleomorphic Gram-negative rod with long and short forms (Fig 2). Stains from five-day cultures show small rounded forms (hence the historic genus name of *Sphaerophorus*). *F. necrophorum* also produces a lipase on egg yolk agar.

Colonial and Gram stain appearances of the other fusobacteria are extremely variable. All cultures of fusobacteria have a smell of boiled cabbage or rancid butter, due to the production of butyric acid (the major end-product of metabolism).

Fusobacteria, when cultured on the media mentioned above, fluoresce yellow-green under long-wavelength ultraviolet light, and many of the species are spot indole-positive.

Identification to the genus level is achieved definitively by gas-liquid chromatography (GLC), but other tests can be employed in laboratories without access to GLC. Gram-negative rods that are sensitive to kanamycin (1 mg disc) and colistin (10 μg disc) but resistant to vancomycin (5 μg disc) are likely to be fusobacteria.

Strains of *Leptotrichia* and *Bacteroides ureolyticus* may give a similar pattern, but they differ morphologically and colonially, are spot indole-negative and do not possess the typical rancid odour.

Species identification depends on the use of biochemical tests, enzyme tests and lipase production on egg yolk agar (made with the agar bases already mentioned).

CLINICAL RELEVANCE

As members of the human normal flora, fusobacteria may be pathogenic when found in sites that are normally considered sterile. This occurs when disruption to the mucosal surfaces on which they reside occurs as a result of surgery, trauma, development of a tumour or the result of chemotherapeutic drugs causing ulceration. Infection arises as a result of the spillage of the normal flora into the adjacent sterile site.

F. nucleatum, therefore, is common in infections of the lung space, sinuses and brain. Blood cultures positive for this organism are the result, most often, of infiltration via a mucosal ulcer – hence the finding of *F. nucleatum* in the blood cultures of leukaemia patients. Severely ill patients may exhibit typical septic shock triggered by fusobacterial lipopolysaccharide (LPS). *F. mortiferum* is found in abdominal abscesses, peritonitis and abdominal wound infections.

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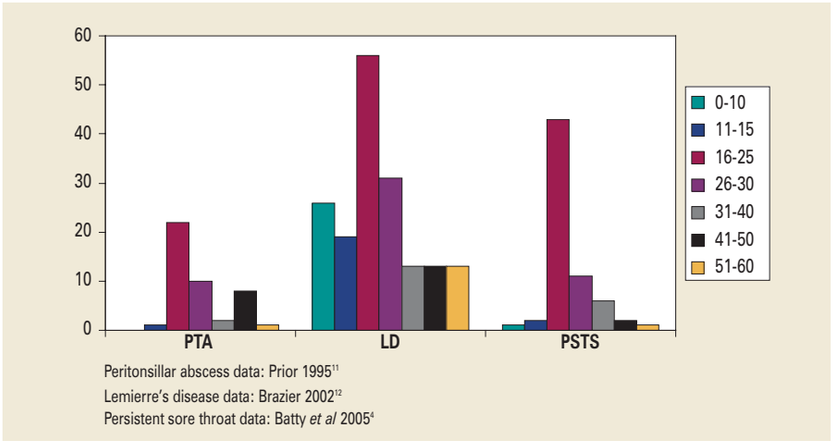

Fig 3. Age distribution of *F. necrophorum* isolates from peritonsillar abscess (PTA), Lemierre's disease (LD) and persistent sore throat syndrome (PSTS).

F. necrophorum is the enigma of the genus. To the author's knowledge, no studies have shown convincingly that this is a member of the normal flora of humans and yet it may be found in infections anywhere in the body, from the brain to bone.

Historically it is associated with Lemierre's disease, an acute infection of the upper respiratory tract leading to pneumonia, lung abscess and septicaemia, and, in some cases, it is the cardinal sign of jugular vein thrombosis. It has a mortality rate of up to 20%, and most cases occur in previously healthy young adults in the 16–30 age range.

More recently, some evidence has been put forward for the role of *F. necrophorum* in persistent or recurrent sore throat syndrome (PSTS).[4] Patients with this clinical manifestation should have cultures for *F. necrophorum* included in the culture regimen for throat swabs. Occasionally, patients may be sufficiently ill to present to the accident and emergency department of their local hospital. Early work suggests that up to 20% of patients with this syndrome may be infected with *F. necrophorum*.

Furthermore, *F. necrophorum* has a similar isolation rate to group A streptococci in patients with sore throat routinely examined by culture methods.[5] Polymerase chain reaction (PCR) studies by one group[6] have shown that 10% of patients with sore throat have *F. necrophorum*, but it was not found in 100 control patients without sore throat; neither was it cultured from the throats of normal controls in the author's laboratory.[4]

The highest rate of infectious episodes in patients with peritonsillar abscess (PTA)[7] and PSTS is associated with *F. necrophorum*. Whether patients with PSTS progress to PTA or to Lemierre's disease is uncertain and a long follow-up study of such patients is required.

Also of interest is the comparable age distribution of patients with PTA, Lemierre's disease and PSTS caused by *F. necrophorum*. The distribution curves for each disease are almost identical (Fig 3). It is not known whether or not these different clinical manifestations are caused by different strain types within the species.

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Of additional importance is the apparent rise in systemic infections due to *F. necrophorum* in recent years. Rarer manisfestations of disease caused by this organism include osteomyelitis, septic arthritis, orbital cellulitis and brain abscess. *F. necrophorum* may also be involved in cancrum oris and *F. ulcerans* in certain types of tropical ulcer. Both are diseases of the tropics and are rarely seen in the UK. For further information the reader is referred to the comprehensive review of *F. necrophorum* by Brazier.[1]

PATHOGENICITY FACTORS

Lipopolysaccharide endotoxin has been found in the fusobacteria and may be a major pathogenicity factor in these organisms. *F. necrophorum* is the only member of the genus studied so far to contain a leucotoxin.[8] This is a high molecular weight extracellular toxin active against leucocytes and is encoded by an *lkt*A gene.

ANTIBIOTIC SENSITIVITY

Most of the common fusobacteria are highly sensitive to antibiotics. In the study by Aldridge *et al*.,[9] all strains tested were sensitive to piperacillin/tazobactam, ampicillin/sulbactam, cefoxitin, imipenem, meropenem and metronidazole. The

quinolones ciprofloxacin and trovafloxacin were effective against 96% of strains and penicillin was effective against 91% of strains. β-lactamase is produced by some penicillin-resistant strains of fusobacteria, but not by all. In a recent study, *F. mortiferum* had higher resistance rates than did other fusobacteria,[10] hence there may be geographical variations in the sensitivity of strains.

OPTIMISED RECOVERY

In conclusion, there can be no doubt that the fusobacteria are human pathogens, given the right circumstances. Some patients may suffer severe life-threatening infection followed by a long period of recovery. Every diagnostic laboratory should ensure that their anaerobic culture methods are optimised for the recovery of these organisms. ■

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