Prevalence of *Fusobacterium necrophorum* in persistent sore throat samples

S. L. PRICE*, S. HARDY†, P. GALE† and G. P. BASTEN*

School of Allied Health Sciences, Faculty of Health and Life Sciences, De Montfort University, Leicester LE1 9BH; and †Microbiology Department, Leicester Royal Infirmary, Leicester, UK

Fusobacterium necrophorum is a non-spore-forming obligate anaerobe and a Gram-negative pleomorphic bacillus. It is thought to spread by human-to-human transmission via close contact, with a peak in the late teens to early-20s.[1] Not surprisingly, it appears to be responsible for 10% of all cases of acute sore throat,[2] 21% of recurring sore throat[3] and 23% of peritonsillar abscess.[4] However, the main pathogenic organism isolated from throat swabs in the UK is group A *Streptococcus*.[5]

This study reports the prevalence of *F. necrophorum* in persistent (PST) and non-persistent (NPT) sore throat samples obtained from a large teaching hospital in the East Midlands, a region in the UK with a heterogeneous population. It also addresses the optimum conditions for sample reception, storage and isolation.

In this study, 112 throat swabs were obtained from samples collected from Leicester Royal Infirmary. The samples, transported in Probact Amies charcoal transport medium, were collected over a 10-week period (December 2008 – February 2009). The anonymised swabs were selected on the basis of the clinical details describing "non-persistent" or "persistent" sore throat (patient age range: 18–65 years).

Primary isolation of *F. necrophorum* was achieved using Wilkins and Chalgrens selective anaerobe agar with a metronidazole disc (Oxoid, UK). The samples were inoculated using the standard plate 'streaking' method. The inoculated plates were then incubated anaerobically at 37°C. The plates were examined at two- and five-day intervals. *Clostridium tetani* (NCTC 540) was used as a control to observe whether or not anaerobic conditions had been achieved. *F. necrophorum* (NCTC 10576) was used as the control organism for identification and as a control for the API rapid ID 32A (bioMérieux, UK).

After 48 h the *F. necrophorum* colonies were approximately 2 mm in diameter, grey/translucent in colour with a waxy appearance and an irregular edge; at five days the colonies were larger and able to produce β-haemolysis. A large zone was observed around the metronidazole disc, indicating sensitivity.

Subcultures were carried out on potential positives using a non-selective blood agar (Oxoid, UK) and incubated for 48 h anaerobically at 37°C. On non-selective blood agar the β-haemolysis was not as apparent and the colonies were whiter in appearance; butyric acid production was still apparent.

A Gram stain was performed on all potentially positive isolates to see if they matched the *F. necrophorum* criteria (ie Gram-negative bacilli with considerable variability in length and width).

A four-hour API rapid ID 32A was performed to confirm the typical profile for *F. necrophorum*. The organism was diluted to make a 5 McFarland standard in 2 mL water (saline is bactericidal for *Fusobacterium*[3]). The organism was found to be positive for alkaline phosphatase and indole but negative for all other biochemical tests.

Correspondence to: Dr G. P. Basten
Email: gbasten@dmu.ac.uk

Table 1. Number of viable colonies counted from sore throat swabs stored at either room temperature (22°C) or 4°C, and at various time points.

Temperature	Number of colonies counted (hours)			
	0	24	48	72
22°C	200	150	81	0
4°C	200	4	0	0

To determine aerotolerance, a fresh culture of the *F. necrophorum* (NCTC 10576) control organism was used to prepare a dilution with 10 mL water to a 0.5 McFarland standard, which is equivalent to $1.5x10^8$ colony-forming units (cfu)/mL. Serial dilutions of the organism were prepared ($x10^4$ to $x10^6$ cfu/mL), and 100 µL of the $x10^4$ dilution was added to each of 60 tubes. The $x10^5$ and $x10^6$ dilutions were added in the same way.

A swab was then added to each tube and left for 10 min; after which the swabs were placed in the transport medium for up to 72 hours at 4°C and 22°C.

The swabs were then inoculated on blood agar plates and incubated anaerobically with a *C. tetani* (NCTC 540) control plate for 48 h at 37°C. The plates were then read and colonies were counted.

Results showed that the time from sample reception to identification of *F. necrophorum* varied between four and 10 days. This was due to the number of colonies present and the time taken to obtain a pure culture. Table 1 shows the aerotolerance at 22°C and 4°C. At room temperature (22°C) an approximate 50% decrease (24 h) in colonial growth of *F. necrophorum* was seen, and at 48 h there was a 98% drop.[6] This was observed from the results with a drop of >200 colonies at 0 h to 150 colonies at 24 h with the $x10^6$ dilution. At 48 h the number of colonies fell to 81 and at 72 h no colonies were observed.

At 4°C there was a greater drop, with just four colonies present at 24 h and none present at 48 h. Therefore, *F. necrophorum* survived better when stored at room temperature, but growth was still observed up to 24 h under both sets of conditions. Other studies using *F. nucleatum* contradict these results and have shown better survival at 4°C compared to room temperature.[7,8] However, *F. nucleatum* is a different species and the results cannot be extrapolated to *F. necrophorum*, but both organisms are of the same genus so their aerotolerance could be similar.

The API rapid ID 32A confirmed initial identification of the *Fusobacterium* species, but API alone was not used for final identification as Gram stain and colonial appearance where also taken into account. *F. necrophorum* only gave a probable ID of 88.8% with the API rapid ID 32A. The organism gave a positive indole (IND) reaction with all positive samples, and showed 95% positivity with the alkaline phosphatase (PAL) test. An initial positive produced a negative reaction with this test but subsequently gave a *F. nucleatum* profile.

F. necrophorum is said to react positively with the glutamic

acid decarboxylase (GDC) test in 25% of cases, but it reacted with three (60%) of the *F. necrophorum* isolates in this study. This suggests that the majority of strains present in the Leicestershire and Rutland area are GDC-positive.

Breakdown of prevalence in cases of persistent sore throat samples were: 4.5% positive for *F. necrophorum*, 83% were negative, and 11% were identified as group A streptococci. Unexpectedly, 4.5% of non-PST samples were *F. necrophorum*. Group C streptococci and *Candida* were identified but in much smaller numbers (<1%) compared to *F. necrophorum* and group A streptococci. One *F. necrophorum* isolate was found to be present with a β-haemolytic group A *Streptococcus*, while another was present with a group C *Streptococcus*.

It was noted in a previous study that *F. necrophorum* has been found in co-infection with groups A and C streptococci.[3] Given that erythromycin (first-line treatment for streptococci) resistance is present in 15% of *F. necrophorum* isolates, patients may have unresolved infections.

The results of this study suggest that immediate inoculation of a swab on an agar plate is necessary to obtain the optimum number of viable colonies. However, it was demonstrated that the sample could be stored (i.e., 24 h at 4°C or 48 h at 22°C) prior to inoculation and still produce a viable colony.

In conclusion, this study found that the prevalence of *F. necrophorum* in samples from the East Midlands is consistent with historical data from the UK and Europe.[3,4] The organism was not found outside the 17–24 age group, which is also consistent with the findings of previous studies.[5,9–12] However, as *F. necrophorum* was found unexpectedly in reported non-persistent sore throat samples, further study or surveillance is warranted to monitor the prevalence of *F. necrophorum* in these cases and in persistent sore throat cases. □

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Misleading hypercalcaemia in a patient with Waldenstrom's macroglobulinaemia

W. H. MAK*, S. P. CHEN† and K. F. LEE*
Kwong Wah Hospital and †Princess Margaret Hospital, Hong Kong

Hypercalcaemia is a common clinical condition and is often associated with primary hyperparathyroidism or malignancy. Waldenstrom's macroglobulinaemia, a rare haematological neoplasm, is characterised by high serum monoclonal IgM, elevated serum viscosity, bone marrow lymphoplasmacytic infiltration and end-organ damage.[1] It is rarely associated with hypercalcaemia. This study reports a case of Waldenstrom's macroglobulinaemia with pseudohypercalcaemia due to calcium assay interference by IgM-κ paraprotein, resulting in unnecessary investigations and treatment.

An 85-year-old Chinese woman presented in August 2008 with poor appetite and significant weight loss over two months. Apart from splenomegaly, physical examinations were unremarkable. Investigations showed normochromic, normocytic anaemia with haemoglobin (Hb) 9–10 g/dL (reference interval 12–15), low albumin (26 g/L, reference interval 32–46), high globulin (58 g/L, reference interval 23–36), and increased erythrocyte sedimentation rate (ESR) to 59 mm/h. On further investigation, serum IgM concentration was grossly elevated to 38.20 g/L (reference range 0.46–3.04) with normal IgG and IgA levels. Serum and urine protein electrophoresis revealed the presence of a paraprotein band in the gamma region, typed as IgM-κ. Bone marrow biopsy revealed no lymphoplasmacytic infiltration but the picture was compatible with

Correspondence to W. H. Mak
Email: makwaihan@yahoo.com