Prevalence of *Fusobacterium necrophorum* and other upper respiratory tract pathogens isolated from throat swabs

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ABSTRACT

Fusobacterium necrophorum, an anaerobic, Gram-negative rod, has been identified recently as a significant cause of persistent sore throat syndrome (PSTS). This disease is characterised by chronic, recurrent or persistent sore throat, which is believed to respond poorly to penicillin *in vivo*. The aim of this study is to examine the prevalence of *F. necrophorum* in all throat swabs received in our diagnostic microbiology department and to compare the results with those for other recognised respiratory pathogens. All throat swabs received in the laboratory over a four-week period were cultured for β-haemolytic streptococcus groups A, C and G, *Corynebacterium diphtheriae*, *Arcanobacterium haemolyticum* and *F. necrophorum*. Latex agglutination techniques, phenotypic reactions and antibiograms are used to identify these organisms. The age of the patient and the clinical details as stated on the request form were noted. Among a total of 248 samples, 27 were positive for β-haemolytic streptococcus group A, two were positive for β-haemolytic streptococcus group C, five were positive for β-haemolytic streptococcus group G and 24 were positive for *F. necrophorum*. The most common isolate in the under 20 age group was β-haemolytic streptococcus group A. In the over 20 age group, *F. necrophorum* was the pathogen most frequently isolated. A clinical diagnosis of 'sore throat' was most likely to be positive for β-haemolytic streptococcus group A, a clinical diagnosis of PSTS was most likely to be positive for *F. necrophorum* and a clinical diagnosis of 'tonsillitis' was equally likely to be caused by β-haemolytic streptococcus group A or *F. necrophorum*. β-haemolytic streptococcus group A was present in 11% of the samples and *F. necrophorum* was present in 10% of the samples. In total, these two pathogens accounted for 18.5% of throat infections in the sampled group. The results show that *F. necrophorum* is as significant a cause of throat infection as is β-haemolytic streptococcus group A. Examination of this provisional data suggests that targeting culture towards these two pathogens may be possible in certain cohorts of patients if more precise clinical data are received from medical staff. However, based on the clinical symptoms routinely provided by clinicians requesting microscopy, culture and sensitivity on throat swabs, *F. necrophorum* culture is required on all throat swabs received in the laboratory.

KEY WORDS: β haemolytic streptococcus group A.
Fusobacterium necrophorum.
Persistent sore throat syndrome.
Pharyngitis.

Introduction

Traditionally, β-haemolytic streptococci and *Corynebacterium diphtheriae* were recognised as the primary causative organisms of bacterial sore throat. However, with the advent of immunisation using diphtheriae toxoid in the late 1800s,[1] *C. diphtheriae* is now rarely seen and many laboratories have ceased to culture for it.[2] Non-toxin producing strains of this organism are capable of causing pharyngitis, which responds to antibiotic therapy. *Arcanobacterium haemolyticum* is a rare cause of bacterial sore throat and is more common in the 10–30 old age group.[3]

It is routine to culture for all three of these pathogens in the authors' laboratory, and to culture for *Fusobacterium necrophorum* when a clinical diagnosis suggests the presence of persistent sore throat syndrome (PSTS), as an isolation rate of 21% for *F. necrophorum* from anaerobic throat swab cultures has been demonstrated in this cohort of patients.[4] However, there is no published evidence to show the prevalence of *F. necrophorum* from anaerobic cultures with respect to age group, clinical diagnosis or other known throat pathogens.

Although previous studies of age and clinical presentation in predicting culture outcome have not been particularly successful, owing to the overlap of symptoms,[5] *F. necrophorum* has been implicated in PSTS in adolescents and young adults.[3] It has been demonstrated that similar strains of *F. necrophorum* are responsible for PSTS and Lemierre's disease (LD),[4] the latter being a rare condition that predominantly effects adolescents and young adults and is characterised by severe sore throat, septicaemia, multiple abcesses and jugular vein thrombosis. An increase in cases of this disease has been noted to coincide with a decrease in the use of antibiotics for upper respiratory tract infections.[6]

The present study aims to compare the prevalence of *F. necrophorum* to other pathogens implicated in bacterial throat infections, and to compare the incidence of the predominant isolates within age bands and clinical presentations to determine if it might be possible to target the diagnostic culture of this organism to a specific at-risk group.

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Materials and methods

Specimens
All throat swabs received during a four-week period were cultured for β-haemolytic streptococcus groups A, C and G, *C. diphtheriae*, *A. haemolyticum* and *F. necrophorum*. Samples were received in Amies's transport medium (Technical Services Consultants, Heywood, Lancs, UK) and cultured as soon as possible after receipt in the laboratory. Patient age and clinical details were recorded. Repeat samples from patients previously positive during the study were not included in the final data analysis.

β-*haemolytic streptococci*
Culture for β-haemolytic streptococci was carried out on staph/strep-selective agar (Columbia agar base) containing 5% sheep blood (Oxoid, www.oxoid.com), incubated anaerobically overnight at 37°C. Suspect colonies were confirmed as streptococci by Gram stain and catalase test. Lancefield streptococcal groups were determined using a latex streptococcal grouping kit, (Oxoid). Colonies of *Streptococcus milleri* suspected of cross reacting were confirmed using Vogues-Proskauer diagnostic tablets (A/S Rosco, 2630 Taastrup, Denmark), following the manufacturer's instructions.

Arcanobacterium haemolyticum
Pinpoint β-haemolytic colonies on staph/strep selective agar were stained to look for typical Gram-positive branching rods. A catalase test was performed and if negative an overnight purity plate was made on Columbia blood agar (BA; Oxoid), incubated at 37°C anaerobically overnight. The following day an API Coryne (bioMérieux; Marcy l'Etoile, France) was performed, following the manufacturer's instructions.

Corynebacterium diphtheriae
Culture for *C. diphtheriae* was carried out on Hoyles medium (Oxoid), incubated at 37°C in air for 48 h. Black or grey colonies were stained and examined for typical Gram-positive rods arranged in 'Chinese lettering' formation. Diphtheroid colonies were inoculated by stabbing Tinsdale medium, prepared in-house using Tinsdale agar base and Tinsdale supplement according to the manufacturer's instructions (Oxoid), and inoculated on a BA purity plate. Both plates were incubated overnight at 37°C in air. An API Coryne (bioMérieux) was performed on colonies showing a brown halo on the Tinsdale medium. All identification results and toxicity testing were confirmed by the reference laboratory (Health Protection Agency, Colindale, London).

Fusobacterium necrophorum
Primary isolation of *F. necrophorum* from clinical samples was carried out on G-N anaerobe medium (GN; Oxoid) incubated anaerobically at 37°C and examined at one, three and five days. Subsequent subcultures were carried out on nonselective anaerobe basal agar (ABA; Oxoid), incubated anaerobically for 24–48 h.

After overnight incubation, matt white colonies less than 1 mm in diameter could be detected if present in large numbers. After three days the colonies were 2 mm in diameter, white-buff in colour, often with a waxy appearance and an irregular edge. At five days they are 3 mm or larger, with a matt yellow, domed 'fried egg' appearance.

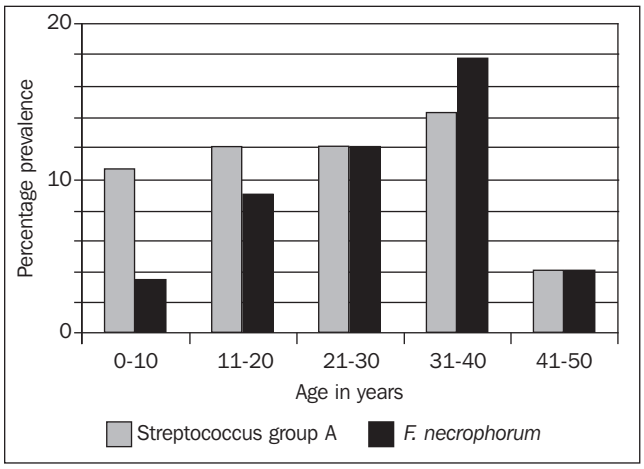

Fig. 1. Prevalence of β-streptococcus group A and *F. necrophorum* from throat swabs.

The colonies' ability to produce β-haemolysis was not always apparent up to 24 h, but subsequently occurred under and in close proximity to the colonies. As a result of their ability to produce butyric acid, cultures give a characteristic foul smell that some workers have compared to the smell of boiled cabbage, but this was only detectable from heavier growths after 48 h.

Suspect colonies from the GN plates were subcultured on a BA (Oxoid) plate, to which was applied a Mast ID (MID8) Identification Mastring (Mast Diagnostics). This detected vancomycin resistance and the kanamycin and colistin sensitivity pattern that is typical of the genus *Fusobacterium*.

The culture was inoculated on a BA plate using a sterile swab to give a semi-confluent growth. Direct inoculum on BA was used instead of a suspension in saline and inoculation on lysed blood agar (as recommended by the manufacturer) because saline was considered bactericidal for *Fusobacterium* spp., and poor growth occurs on lysed blood agar. A Mastring was applied and the plate incubated anaerobically at 37°C overnight.

Zone sizes >15 mm were interpreted as sensitive and ≤ 15 mm as resistant. Organisms fitting the antibiotic profile were further identified using phenotypic tests. At the same time, an ABA plate was streaked for discreet colonies and a 5-µg metronidazole disc (Oxoid) applied. This was used to check isolate purity and to confirm metronidazole sensitivity (typical for all fusobacteria).

A Gram stain was performed to confirm the typical pleomorphic Gram-negative rod appearance. Production of tryptophanase was demonstrated by the detection of indole using p-dimethylaminocinnamaldehyde (spot indole reagent; Pro-Lab Diagnostics, Toronto, Canada), following the manufacturer's instructions. Four-hour rapid API ID32 A (bioMérieux) was used to confirm a typical profile for *F. necrophorum*. Typically, isolates are indole- and alkaline phosphatase-positive but negative for all other tests. Substitution of zym-A and -B reagents in place of fast blue gave more easily readable results.

Instructions with the rapid API ID32 A indicate that 5% of *F. necrophorum* isolates are alkaline phosphatase-negative, which coincides with the profile given for *F. nucleatum* in the rapid API ID32A tests, but the typical Gram-stain

Table 1. Number of isolates of β-haemolytic streptococcus group A from different clinical diagnoses.

Age	Sore throat*	Tonsillitis†	PSTS‡	Misc§	Total
0-10	5	1			6
11-20	3	1			4
21-30	4	4			8
31-40	2	4	1	1	8
41-50			1		1
Total	14	10	2	1	27

* Includes 'sore throat', 'pharyngitis', '? strep', and 'upper respiratory tract infection'.
† Includes 'tonsillitis' and 'quinsy'.
‡ Includes descriptions of 'previous', 'persistent', 'recurrent' and 'chronic' sore throat/ tonsillitis.
§ Any diagnosis not directly related to sore throat (e.g., 'temp', 'pyelonephritis', etc).

Table 2. Number of isolates of *F. necrophorum* from different clinical diagnoses.

Age	Sore throat*	Tonsillitis†	PSTS‡	Misc§	Total
0-10		1	1		2
11-20		2	1		3
21-30	2	2	3	1	8
31-40	6	2	1	1	10
41-50	1				1
Total	9	7	6	2	24

* Includes 'sore throat', 'pharyngitis', '? strep', and 'upper respiratory tract infection'.
† Includes 'tonsillitis' and 'quinsy'.
‡ Includes descriptions of 'previous', 'persistent', 'recurrent' and 'chronic' sore throat/ tonsillitis.
§ Any diagnosis not directly related to sore throat (e.g., 'temp', 'pyelonephritis', etc).

appearance of long spindle-shaped rods demonstrated by *F. nucleatum* are not consistent with the pleomorphic Gram-negative rods seen with *F. necrophorum*. Due to the biochemical inactivity of *F. necrophorum*, care should be taken when using commercial kits and their respective databases, as inaccuracies in identification can occur.

Results

A total of 248 throat swabs requesting microscopy, culture and sensitivity were received from separate patients. Of these, 27 cultures were positive for β-haemolytic streptococcus group A, three were positive for group C, five were positive for group G, and 24 were positive for *F. necrophorum*. There were no isolates of *C. diphtheriae* or *A. haemolyticum*. Of the 24 cases of *F. necrophorum* infection, four were concurrent with four of the β-haemolytic streptococcus group A cases, and a single case was concurrent with one of the cases of β-haemolytic streptococcus group C.

Age distribution for the β-haemolytic streptococcus group A cases ranged from 1 year to 47 years and for the *F. necrophorum* cases from 1 year to 41 years. The results in Figure 1 suggest that β-haemolytic streptococcus group A infection is a more significant cause of throat infection in the under 20 age group and that *F. necrophorum* is a more significant cause of throat infection in the over 20 age group. The prevalence of β-haemolytic streptococcus group A is 11–14% in the 0–40 age group and the graph suggests that prevalence is relatively uniform throughout this group. However, prevalence of *F. necrophorum* is 4–18% in the 0–40 age group and the graph shows a trend that increases with age.

The results in Table 1 suggest that β-haemolytic strepotococcus group A is more likely to be isolated from patients with a clinical diagnosis of 'sore throat' than 'tonsillitis' in the under 20 age group. Table 2 suggests that *F. necrophorum* is less likely to be isolated from patients with a clinical diagnosis of 'sore throat' and more likely to be isolated from patients with a clinical diagnosis of 'tonsillitis' or 'PSTS'. The largest group from which *F. necrophorum* was isolated was the 31–40 age group, whose samples were taken predominantly because of a clinical diagnosis of 'sore throat'.

Table 3. Prevalence of *F. necrophorum* and β-streptococcus group A isolates from different clinical groups.

Clinical diagnosis	Number of samples	*F. necrophorum* (%)	Group A (%)
Sore throat*	112	9 (8)	14 (13)
Tonsillitis†	49	7 (14)	10 (20)
PSTS‡	29	6 (21)	2 (7)

* Includes 'sore throat', 'pharyngitis', '? strep' and 'upper respiratory tract infection'.
† Includes 'tonsillitis' and 'quinsy'.
‡ Includes descriptions of 'previous', 'persistent', 'recurrent' and 'chronic' sore throat/ tonsillitis.

Prevalence rate for each pathogen by age (Fig. 1) relates closely to the total isolation rate for each pathogen by age (Tables 1 and 2), which suggests that there may be a predictable isolation rate for each age group.

Table 3 shows the overall isolation rate for β-streptococcus group A and *F. necrophorum* and suggests that a clinical diagnosis of 'sore throat' or 'tonsillitis' is more likely to be caused by β-haemolytic streptococcus group A, whereas a clinical diagnosis of 'PSTS' is more likely to be caused by *F. necrophorum*.

Overall, the isolation rate for β-haemolytic streptococcus group A was 11% and for *F. necrophorum* was 10% (n=248). If these results are combined and the four concurrent infections are discounted then this shows that β-haemolytic streptococcus group A infections and *F. necrophorum* infections accounted for 18.5% of throat infections in this cohort of patients.

Discussion

The time taken to culture and identify *F. necrophorum* from throat swabs can vary (3–10 days), depending on the number of colonies present and the ease with which a pure

growth is achieved. This can mean a significant and unavoidable delay in reporting to the clinician. In severe cases where the patient has been admitted to the ward and typical colonial and Gram appearances have been obtained, preferably with a positive indole result, the biomedical scientist may be able to alert the clinician to the possibility of *F. necrophorum* infection, but this is not ideal and in most cases full identification should be confirmed before a report is issued. We routinely culture in anaerobic chambers and are able to read plates *in situ*, making judgment prior to removal to air possible. However, smaller laboratories that use anaerobic jars would jeopardise their results if these jars were opened before 48 h.

The predicted isolation rate for β-haemolytic streptococcus group A in throat infections is approximately 10%[5] and the results presented here reflect this. The similar percentage prevalence for β-haemolytic streptococcus group A in the 0–40 age group suggests a standard prevalence throughout the group (Fig. 1). The number of isolates of β-haemolytic streptococcus groups C and G are predictably low and prevent any conclusions being drawn. The isolation rate for *F. necrophorum* in cases of PSTS shown in Table 3 correlates well with previous findings[4] and the total isolation rate of 10% for *F. necrophorum* in this study correlates with recent work[7] that employed a real-time polymerase chain reaction (PCR) technique and produced a detection rate of 10% for *F. necrophorum* from a random sample of throat swabs.

Increased prevalence of *F. necrophorum* with age may be the result of PSTS. Patients who acquire the infection and continue to present with symptoms as previously described[4] would lead to a cumulative effect on the prevalence rate. A more detailed study of patients' clinical histories would determine whether this or other environmental factors were responsible for this trend.

With diphtheria vaccination waning in the adult population, we believe there is an increasing risk of an outbreak of a toxigenic *C. diphtheriae* strain.[2] Our isolation rates for *C. diphtheriae* and *A. haemolyticum* are approximately 17 and six per annum, respectively, so the lack of isolates in this cohort is not unexpected. However, we consider that culture and surveillance of these organisms is important,[8] as we have previously noted a case of *F. necrophorum* isolated from a patient who was also positive for *C. diphtheriae*.

Similarly, the decrease in use of penicillin for upper respiratory tract infections coincides with an increase in serious disease caused by β-haemolytic streptococcus group A[9] and *F. necrophorum*.[6] We believe that treatment with penicillin does not prevent the development of PSTS[4] and that it is important to distinguish between throat infection caused by β-haemolytic streptococcus group A and that caused by *F. necrophorum* so that appropriate antibiotics can be recommended.

Jousimies-Somer *et al*.[10] reported a similar isolation rate for β-haemolytic streptococcus group A and *F. necrophorum* from peritonsillar abscesses (PTA). The similar isolation rates reported in the present study for these pathogens (27 and 24, respectively) suggests that sore throats caused by these organisms may precede the onset of PTA, if left untreated.

In a previous study of PSTS, the peak age for patients with F. necrophorum was in the 15–30 year group.[4] The results of the current study indicate that infants are susceptible to this infection and suggest that patients over 30 years of age rarely present with a clinical diagnosis of 'PSTS'. This could explain their absence from the at-risk group for PSTS (Table 3), despite being the largest *F. necrophorum* group (Fig. 1). This observation, together with the sudden fall in prevalence in the 41–50 age group, suggests that patients may develop resistance over time and thus present with less severe symptoms or succumb to the disease less frequently. Similar factors may play a role in the pathogenicity of β-haemolytic streptococcus group A, which shows the same trend.

Development of a diagnostic serological assay to test for antibody levels to *F. necrophorum* would reduce the time taken to produce a laboratory result and could also be used to provide immunological data to examine these effects in more detail. With the advent of early diagnosis and treatment of *F. necrophorum* throat infections in the younger population, prevalence in the older population may diminish due to the prevention of PSTS.

When reporting positive results for *F. necrophorum* by telephone, it became apparent in some cases that the patient had a history of PSTS, although this was not obvious from the details given on the request form. Furthermore, some clinicians had the habit of writing 'URTI' on every request submitted, which did not permit differentiation between various clinical presentations. More precise information may have reduced the number of isolates in the 'sore throat' group and thus increased the results in the 'tonsillitis' and 'PSTS' groups.

It is possible that the profile of infection markers in serum samples may vary according to whether a β-haemolytic streptococcus group A or *F. necrophorum* infection is responsible for the sore throat. This would be particularly useful in acute-onset cases when combined with precise clinical details indicating a particular type of infection. Further work is needed to investigate this hypothesis.

We have observed an increase in the isolation rate for *F. necrophorum* from patients presenting to the accident and emergency department. This may be the result of early receipt of specimens in the laboratory but may also be due to the acute onset of symptoms that some patients experience as a result of this infection, causing them to seek urgent medical attention. Owing to the potential risk of LD, this group of patients requires careful consideration. Further work is being undertaken to examine this phenomenon.

The two *F. necrophorum* isolates in the 0–10 age group were from siblings. The younger, a 14-month-old infant, was positive on admission to the accident and emergency department with a diagnosis of 'swollen tonsils'. A subsequent request for culture of a throat swab received from the eight-year-old sibling, who was described as suffering from a 'chronic tonsillitis', was also positive for *F. necrophorum*. This lends support to the theory that both close contact to a patient with PSTS and genetic factors may play a role in susceptibility to this infection.[4]

The number of samples included in the present study was small and specific detailed diagnoses were not sought from the clinicians; therefore, only limited conclusions can be drawn. A larger study is required to assess the significance of *F. necrophorum* as a cause of sore throats in the population as a whole and to assess the epidemiological consequences of treating this group of patients with antibiotics better targeted to this anaerobe.

In the meantime, it has been shown that *F. necrophorum* is as significant as β-streptococcus group A in causing sore throats and that the two pathogens were responsible for bacterial infections in 18.5% of patients in this study. Thus, diagnostic laboratories should consider implementing routine culture for *F. necrophorum* so that appropriate antibiotic therapy can be administered in order to prevent the development or continuance of PSTS. □

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References

1 Collier RJ. Understanding the mode of action of diphtheria toxin: a perspective on progress during the 20th century. *Toxicon* 2001; **39**: 1793–803.
2 Lai S, Efstratiou A; European Laboratory Working Group on Diphtheria. Report on the Sixth International Meeting of the European Laboratory Working Group on Diphtheria, Brussels, Belgium. *Eur Surveill* 2002; **7**: (1) 7–12.
3 Janda WM. *Corynebacterium* species and the coryneform bacteria. II. Current status of the CDC coryneform groups. *CMNEEJ* 1998; **20** (7): 53–66.
4 Batty A, Wren MWD, Gal M. *Fusobacterium necrophorum* as the cause of recurrent sore throat: comparison of isolates from persistent sore throat syndrome and Lemierre's disease. *J Infect* 2005. In press.
5 Bourbeau PP. Role of the microbiology laboratory in diagnosis and management of pharyngitis. *J Clin Microbiol* 2003; **41**: 3467–72.
6 Hagelskjaer Kristensen L, Prag J. Lemierre's syndrome: diagnosis and management. *AIDIEX* 2000; **18** (3): 17–24.
7 Aliyu SH, Marriott RK, Curran MD *et al*. Real-time PCR investigation into the importance of *Fusobacterium necrophorum* as a cause of acute pharyngitis in general practice. *J Med Microbiol* 2004; **53**: 1029–35.
8 Wren MWD, Shetty N. Infections with *Corynebacterium diphtheriae*: six years' experience at an inner London teaching hospital. *Br J Biomed Sci* 2005; **62**: 1–4.
9 Wessels MR. Group A streptococcal disease in the 1990s: new clues to pathogenisis. *Int J Infect Dis* 1996; **1**: 107–14.
10 Jousimies-Somer H, Savolainen S, Makitie A, Ylikoski J. Bacteriologic findings in peritonsillar abscesses in young adults. *Clin Infect Dis* 1993; **16** (Suppl 4): S292–S298.