A six-month audit of the isolation of *Fusobacterium necrophorum* from patients with sore throat in a district general hospital

J. A. AMESS, W. O'NEILL, C. NI GIOLLARIABHAIGH and J. K. DYTRYCH

Department of Medical Microbiology, District General Hospital, Eastbourne, East Sussex, UK

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ABSTRACT

Fusobacterium necrophorum is an obligate anaerobe believed to be a member of the normal flora of the human oropharangeal and urogenital tract. It has been associated with deep-seated infections and was first described in 1936 by Lemierre, a French microbiologist. There is now strong evidence to suggest that it is also a cause of recurrent sore throat and persistent sore throat syndrome (PSTS) without leading to full systemic infection. It is considered to be the second most common cause of sore throat after group A β-haemolytic streptococci. This study was performed over a six-month period (October 2004 to March 2005) at the Eastbourne District General Hospital. All throat swabs received in the laboratory are cultured routinely for haemolytic group A streptococci and pathogenic *Corynebacteria* spp. During the study period an extra fastidious anaerobic blood agar plate with neomycin was inoculated, with a 30 µg vancomycin disc placed at the junction of the second and third streaks. This was examined after 48 h for the presence of *F. necrophorum*. A total of 1157 swabs were processed during the study period: 156 were positive for haemolytic group A streptococci, 57 were positive for *F. necrophorum*, 47 for group C haemolytic streptococci, nine for group G haemolytic streptococci, and one was positive for *C. ulcerans*. Patient age ranged from less than a year old to 88. The majority of *F. necrophorum* isolates were from patients in the 11–25 age group, with an isolation rate of 9.48% (44/464). This age group accounted for 40% (464/1157) of the swabs received during the study period and 77% (44/57) of these were positive for *F. necrophorum*. Group A haemolytic streptococci showed an overall isolation rate of 13.5%, with peaks of 23% in the 0–10 and 26–35 age ranges. Together, these two organisms were responsible for 18.4% (213/1157) of all throat infections in this study. The results presented here indicate that *F. necrophorum* is second to group A haemolytic streptococci as a cause of sore throat, especially in the young adult, and introduction of routine culture should be considered.

KEY WORDS: Fusobacterium necrophorum.
Pharyngitis.
Streptococcal infections.

Introduction

Fusobacterium necrophorum is a primary pathogen and has been recognised as a cause of deep-seated systemic infections for many years.[1–10] It was first described in 1936 by Lemierre, a French microbiologist,[1] as a condition affecting young adults, which starts as a sore throat characterised by a pseudomembrane and lymphadenopathy and then progresses to a life-threatening septicaemic illness with metastatic abscesses secondary to a septic thrombophlebitis of the internal jugular vein. The virulence and invasive capability of this organism is due to production of endotoxin, leucocidin, haemolysin, haemagglutinin, lipase and adhesin.[2]

F. necrophorum is an obligate anaerobe and is believed to be a member of the normal flora of the human oropharyngeal, gastrointestinal and urogenital tracts.[9] The species is divided into two subspecies: *F. necrophorum* subspecies *necrophorum* (biovar A) and *F. necrophorum* subspecies *fundiliforme* (biovar B).[2] The latter is the main pathogen in humans, while the former is linked to animal infections (necrobacillosis).

There is now increasing evidence to suggest that *F. necrophorum* is a cause of recurrent sore throat and of persistent sore throat syndrome (PSTS),[2–4] and it is considered by some workers to be the second most common cause of bacterial sore throat after group A β-haemolytic streptococci.[3]

The aim of this study is to investigate the prevalence of *F. necrophorum* in routine samples sent to the microbiology laboratory in Eastbourne.

Materials and methods

All throat swabs received in the laboratory are cultured routinely for *Corynebacterium diptheriae*, *C. ulcerans* and β-haemolytic streptococci. The swabs included in the study were received in Amies transport medium and were processed with routine work. Each swab was inoculated on a blood agar plate anaerobically and on Tinsdale agar aerobically.

F. necrophorum does not grow well on plain blood agar so an additional fastidious anaerobic blood agar (FAA, Bioconnections UK) plate containing neomycin (100 mg/L) was inoculated and a 30 µg vancomycin disc (Mast UK) was

Correspondence to: Mrs. J. A. Amess
Email: fuso@camicro.co.uk

placed at the junction of the second and third streaks. This facilitated the identification of *F. necrophorum* as it suppressed the normal Gram-positive flora and allowed fusobacteria to grow.

Plates were incubated anaerobically at 37°C and examined at 48 h. *F. necrophorum* appears as a 1–2 mm creamy β-haemolytic colony that is resistant to vancomycin. All suspect colonies were examined by Gram film for typical Gram-negative rods with a pleomorphic appearance, and were tested with spot indole reagent (Prolab UK). Indole-positive colonies were subcultured on an FAA blood plate with a 5 µg metronidazole disc, on an FAA blood plate with an anaerobic Mast Id ring (Mast UK) and on a 10% egg yolk emulsion plate (FAA agar base and 10% egg yolk emulsion) to test for lipase production.

The Mast Id ring was read after 24 h for evidence of vancomycin resistance and kanamycin and colistin sensitivity. The lipase reaction was read at 48 h and was demonstrated by a pearly layer produced over the colony.

F. necrophorum produces a characteristic smell, which has been described as like 'boiled cabbage', which is due to the production of butyric acid. The smell is distinctive and is a good aid to identification. *F. necrophorum* also fluoresces a yellow/green colour under ultraviolet (UV) light at 365 nm, which can make identification and selection easier from mixed cultures.

Results

A total of 1157 swabs (451 from male patients and 706 from female patients) were processed during the study period. Patient age ranged from less than a year old to 88 years. *F. necrophorum* was isolated from 57 swabs, group A haemolytic streptococci from 156, group C streptococci from 47, group G streptococci from nine and *C. ulcerans* from one patient.

Twenty-two *F. necrophorum* isolates were obtained from male patients and 35 were obtained from females; however, there was little difference in isolation rates between the two groups (males: 4.87% [22/451]; females: 4.95% [35/706]; overall rate: 4.92% [57/1157]). A difference in isolation rate with age group was observed (Table 1). The majority of isolates were in the 11–25 age group, which showed an isolation rate of 9.48% (44/464), and accounted for 77% (44/57) of all the swabs that were positive for *F. necrophorum*.

Overall, the isolation rate for group A haemolytic streptococci was 13.5% (156/1157), with a wider age distribution. The highest isolation rates were seen in the 0–10 and 26–35 age groups (23% [46/198] and 23.7% [39/163], respectively). Together, *F. necrophorum* and group A haemolytic streptococci accounted for 18.4% (213/1157) of throat infections in this study.

Examination of the results over the six-month study period revealed a fluctuation in isolation rates, with peaks in November and February for *F. necrophorum* and a peak in December for group A haemolytic streptococci (Table 2).

Of the 57 isolates of *F. necrophorum* obtained during the study, three patients had *F. necrophorum* infection on two occasions, three patients also had infections with group A haemolytic streptococci, three also had group G haemolytic streptococci, and six patients also had group C haemolytic streptococci. Three patients had a positive Paul Bunnell test, and two patients were admitted to hospital due to the severity of infection.

Table 1. Percentage prevalence of *F. necrophorum* and haemolytic group A streptococcus across the age ranges during the study period.

Age range (years)	*F. necrophorum*		Group A streptococcus	
0–5	0.9%	(1/110)	21.8 %	(24/110)
6–10	2.2%	(2/88)	25%	(22/88)
11–15	7.69%	(14/182)	12.08%	(22/182)
16–20	12.9%	(23/178)	5.0%	(9/178)
21–25	6.73%	(7/104)	10.5%	(11/104)
26–30	4.34%	(3/69)	20.2%	(14/69)
31–35	2.17%	(2/94)	26.3 %	(25/94)
36–40	4.0%	(3/75)	16.0%	(12/75)
41–45	1.75%	(1/57)	8.0%	(6/57)
46–50	0	(0/43)	9.3%	(4/43)
51–88	0.63%	(1/157)	3.82%	(6/157)

Patients with no age supplied are not included.

Not all patients had been tested for glandular fever; however, there may be a link between Epstein-Barr virus (EBV) infection and *F. necrophorum*,[5] and thus any results were noted.

From the clinical details provided by the requesting clinicians, 24 patients had tonsillitis, seven had recurrent sore throat, three may have had glandular fever, 20 had clinical details of sore throat but no further information was provided, and one patient had quinsy.

Examination of medical records showed that 12 patients previously were thought to have had glandular fever. More detailed investigation revealed that a further 24 patients had suffered recurrent throat problems; however, this information had not been mentioned on request forms received during the course of the study period.

Discussion

Although *F. necrophorum* is considered to be part of the normal oropharangeal flora, a polymerase chain reaction (PCR) study carried out by Aliyu *et al*.[2] failed to identify *F. necrophorum* in a group of 100 healthy individuals who were free of symptoms. However, in 100 individuals with symptoms of sore throat, *F. necrophorum* was detected in 10% of cases.

There is increasing evidence[2–4] to suggest that *F. necrophorum* should be included as part of the routine examination of throat swabs. From the results of the present study, there is a strong indication that *F. necrophorum* can cause severe throat infections.

Early treatment could prevent the distress of recurrent sore throat, especially for those in the younger age groups, as recurrent illness can be disruptive socially and educationally. It may also prevent subsequent more serious systemic infection. The recommended treatment is clindamycin or metronidazole.[4]

Mixed infections of *F. necrophorum* and group A haemolytic

Table 2. Percentage prevalence of *F. necrophorum* and β-haemolytic streptococcus group A over a six-month period.

Month	*F. necrophorum*		Group A streptococcus	
October	2.1%	(2/95)	8.42%	(8/95
November	9.6%	(15/155)	11.6%	(18/155)
December	3.3%	(6/180)	16.11%	(29/180
January	3.3%	(6/178)	12.3%	(22/178)
February	9.36%	(22/235)	12.3%	(29/235)
March	2.4%	(5/207)	16.9%	(35/207)

The study started in mid-September and was completed in mid-April, so isolates in these months are not included.

streptococci or EBV have been documented,[6,7] and it is possible that dual infection renders tissue more permeable[10] and facilitates the deeper tissue penetration of *F. necrophorum*, where conditions are ideal for its growth.

If culture for *F. necrophorum* were to be implemented as part of the routine culture for throat swabs, the time taken to issue a final report would be increased. Group A streptococci can be notified to the clinician by facsimile or telephone after 24 hours; however, *F. necrophorum* requires at least four days, and probably longer, depending on the availability of colonies.

The cost to the laboratory of this extra work would be minimal, as initially only one extra plate is required for screening. Follow-up work on positive cases would require another four or five plates and should take a biomedical scientist five to 10 minutes to perform a spot indole test and inoculate a lipase plate, Id plate and sensitivities.

All isolates obtained during the course of the present study were sent to the Anaerobic Reference Laboratory in Cardiff, where a typing system is being developed. The unit also intends to investigate virulence factors associated with this organism and the infections that it causes.

The authors propose to carry out a follow-up study to investigate the incidence of *F. necrophorum* in non-symptomatic individuals with no history of sore throat or recent antibiotic therapy to assess the rate of carriage in the population. □

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