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Prevalence of *Fusobacterium necrophorum* in Children Presenting with Pharyngitis

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ABSTRACT *Fusobacterium necrophorum*, an obligate anaerobic bacterium, was recently reported to be an important cause of bacterial pharyngitis with a prevalence as high as that of group A *Streptococcus* (GAS) in adolescents and young adults. Importantly, *F. necrophorum* is the primary causative agent of the life-threatening Lemierre's syndrome, and screening of pharyngeal samples may be warranted for its early detection and prevention. The aim of this study was to determine the prevalences of *F. necrophorum* and groups A and C/G streptococci as agents of bacterial pharyngitis in children. Pharyngeal samples ($n = 300$) were collected from pediatric patients presenting to the emergency department with signs and symptoms of pharyngitis. Overall, 10 (3.3%), 79 (26.3%), and 4 (1.3%) patients were PCR positive for *F. necrophorum*, GAS, and group C/G streptococci, respectively. The prevalence of *F. necrophorum* was significantly higher in patients between the ages of 14 and 20 years at 13.5% than in patients aged 14 years and younger (1.9%, $P < 0.001$). All positive patients presented with signs and symptoms similar to GAS pharyngitis. Our data demonstrated a potential role for *F. necrophorum* as a pathogen of pharyngitis among young adults, but suggests that the prevalence of *F. necrophorum* is low in preadolescent patients.

KEYWORDS *Fusobacterium*, children, *necrophorum*, pediatric, pharyngitis

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Fusobacterium necrophorum is a Gram-negative obligate anaerobe commonly associated with peritonsillar abscesses, tonsillitis, and Lemierre's syndrome, a rare life-threatening complication of oropharyngeal infection (1, 2). The classical presentation of invasive *F. necrophorum* infections includes the presence of a sore throat followed by a high fever and rigors, and is accompanied by cervical lymphadenopathy (3, 4) and generally occurs in previously healthy adolescent males (1, 5). *F. necrophorum* was first described in animals and was later divided into two subspecies, *F. necrophorum* subsp. *necrophorum* (biotype A) and subsp. *funduliforme* (biotype B). *F. necrophorum* subsp. *necrophorum* is known as the principle pathogen in animals, while *F. necrophorum* subsp. *funduliforme* is mainly isolated from humans (4). Recent studies have proposed *F. necrophorum* as an important causative agent of pharyngitis in adolescents and young adults (6–9). Contrary to previous reports claiming group A *Streptococcus* (GAS) as the most common bacterial cause of acute pharyngitis (8, 10), these studies reported equivalent or higher prevalence rates of pharyngitis caused by *F. necrophorum* (6, 11–13). It has also been suggested that *F. necrophorum* pharyngitis is associated with increased morbidity and mortality compared with those from GAS pharyngitis in adolescents and young adults (7). Due to increased awareness, routine screening for *F. necrophorum* is becoming a common practice in Europe for the early detection of Lemierre's syndrome (12, 14).

The majority of studies investigated primarily the prevalence of *F. necrophorum* pharyngitis in adolescents and young adults, ranging between 15 and 45 years old,

TABLE 1 Age distribution and positivity rates of *F. necrophorum* for patients enrolled in this study

Age (yr)	No. of patients (%)	*F. necrophorum* positivity[a]	
		No. of isolates (%)	*P* value
<14	263 (87.7)	5 (1.9)[b]	<0.001
14–20	37 (12.3)	5 (13.5)	
Total	300 (100)	10 (3.3)	

[a]Positive for *F. necrophorum* by PCR and culture.
[b]One patient (5 years old) was culture negative but PCR positive.

since this is the age group reported to have the highest rates of peritonsillar abscesses and Lemierre's syndrome (15, 16). The prevalence of *F. necrophorum* among this population is 10% to 48% (7, 9, 11, 12, 17). In contrast, the incidence of Lemierre's syndrome is low in younger children (18, 19). Peritonsillar abscesses often afflict children, and the rates of previous tonsillar or peritonsillar infections and recurrences are higher among those infected with *F. necrophorum* than with GAS (20). To our knowledge, there is a paucity of published data on the prevalence of *F. necrophorum* in preadolescents and younger children.

Anaerobic culture of pharyngeal samples is not currently recommended due to the overwhelming growth of obligate and facultative anaerobes from the oropharynx (21). Thus, most institutions that screen for *F. necrophorum* from pharyngeal samples rely on selective media or molecular detection (12, 17, 22, 23). The use of selective media has been shown to aid in detecting *F. necrophorum* in culture but still requires more than 2 days of incubation (23, 24). Although it is more sensitive, molecular detection comes with its own limitations, including the infrastructure and molecular skillsets required for a laboratory-developed PCR test and the cost associated with molecular tests (14, 23).

To determine the prevalence of *F. necrophorum* in preadolescents, adolescents, and young adults in our patient population, we assessed pediatric patients of all ages presenting to the emergency department (ED) with signs and symptoms of pharyngitis. We prospectively screened for *F. necrophorum* by PCR and culture methods and compared the prevalence to those of GAS and groups C/G streptococci.

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RESULTS

A total of 300 patients were enrolled with ages ranging from 12 months to 20 years (mean age of 7.8 years) from October to December 2015 and from February to March 2016. Sixty-seven percent (201) of the patients were <10 years of age, 23.3% (70) were between 10 and 14 years, and 9.7% (29) were between 15 and 20 years old. Significant comorbidities were absent in 85.0% (255) of all patients enrolled. Approximately 58% (173) of patients presented with two or more of the following symptoms: fever, lymphadenopathy, exudate, sore throat, and absence of a cough. One hundred two (34%) patients presented with coughs.

Bacterial pathogens were recovered from a total of 92 (30.7%) patients using culture and/or molecular methods, including GAS from 79 (26.3%) patients, group C/G streptococci from 4 (1.3%), and *F. necrophorum* from 10 (3.3%) (Table 1). Nine of the 10 *F. necrophorum*-positive patients were identified by culture and PCR, and the tenth positive patient was identified by PCR only (Table 1). This patient identified only by PCR had reproducible positive results, but the result was detected relatively late in the PCR cycling (cycle threshold [C_T] of 39 and 41), suggesting a low bacterial load. All positive culture samples grew on egg yolk agar (EYA), egg yolk agar with kanamycin and vancomycin (EYKV), egg yolk agar with kanamycin, vancomycin, and josamycin (EYKVJ), and *Fusobacterium* selective agar (FSA). The selective media, especially EYKVJ, aided in selecting *F. necrophorum* isolates by suppressing many of the normal flora. Unfortu-

TABLE 2 Symptoms commonly associated with bacterial pharyngitis, by diagnosis

Diagnosis	No. of patients (%) with:				
	Fever	Absence of cough	Lymphadenopathy	Exudate	Sore throat
All (n = 300)	232 (77.3)	194 (64.7)	37 (12.3)	93 (31.0)	220 (73.3)
F. necrophorum PCR positive (n = 10)	8 (80.0)	9 (90.0)	1 (10.0)	5 (50.0)	9 (90.0)
Group A and C/G streptococci PCR positive (n = 83)	68 (81.9)	60 (72.3)	7 (8.4)	20 (24.1)	60 (72.3)
Negative[a] (n = 207)	156 (75.4)	126 (60.9)	29 (14.0)	68 (32.9)	151 (72.9)

[a]Negative for group A and C/G streptococci and *F. necrophorum* by PCR.

nately, hemolysis on the FSA plate was difficult to discern. *Fusobacterium* spp., *Prevotella* spp., and *Veillonella* spp. were the three organisms most commonly encountered on each of the selective media, including EYKVJ. The relevant isolates recovered from each of the cultures demonstrated features concordant with those of *F. necrophorum*, including pleomorphic Gram-negative bacilli, lipase activity, and indole positivity. The growth of the *F. necrophorum* isolates was observed after incubating for 24 h in anaerobic conditions, but colony morphology and lipase activity were more distinctive after 48 h. The identities of all of the isolates were confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), with scores ranging from 2.0 to 2.3.

The age range of the 92 patients with etiologies positive for a bacterial infection was 2 to 19 years (mean, 7.5 years) for those with groups A and C/G streptococci and 5 to 20 years (mean, 12.1 years) for those with *F. necrophorum*. The majority of patients positive for groups A and C/G streptococci were <10 years of age (64/83 [77.1%]), with an overall prevalence of 31.8% (64/201) in this age group. In contrast, a significantly lower prevalence rate of *F. necrophorum* in patients aged 14 years and younger was identified at 1.9% (5/263) compared with 13.5% (5/37) ($P < 0.001$) in patients between 14 and 20 years old (Table 1).

All 92 patients with a positive finding for a bacterial agent by culture or PCR were symptomatic with one or more of the following symptoms: fever, presence of exudate, lymphadenopathy, and absence of a cough. Sixty-three (68.5%) of the 92 patients presented with 2 or more of these symptoms, warranting testing for bacterial pharyngitis based on the Centor scoring system (25). However, 24 (26.1%) patients presented with coughs. The patients positive for *F. necrophorum* presented with signs and symptoms similar to those positive for groups A and C/G streptococci (Table 2). Of the 10 *F. necrophorum*-positive patients, 6 (60.0%) presented with at least 3 of the following symptoms: fever, sore throat, exudate, lymphadenopathy, and the absence of a cough. In total, 8 (80.0%) patients presented with fever, 9 (90.0%) had a sore throat, 9 (90.0%) did not have a cough, 5 (50.0%) had exudate, and 1 (10.0%) had lymphadenopathy (Table 2). One patient was positive for *F. necrophorum* and GAS, a 7-year-old with no comorbidities that presented with a fever, sore throat, headache, abdominal pain, vomiting, the absence of exudate, lymphadenopathy, and a cough.

Based on the codes from the 10th revision of the International Classification of Diseases (ICD-10), 128 (42.7%) and 73 (24.3%) patients were diagnosed with unspecified acute pharyngitis (J02.9) and unspecified viral infection (B34.9), respectively. In addition, 66 (22.0%) of patients were diagnosed with unspecified fever (R50.9). Fifty-one (17.0%) patients were prescribed antibiotics at the time of visit (Table 3), including all 15 patients that were rapid antigen direct test (RADT) positive for GAS in the ED. An additional 29 (14.0%) patients were treated empirically despite negative results (Table 3). Missed treatment opportunities include the 73.5% (61/83) of patients that were positive for group A and C/G streptococci by PCR and 90% (9/10) of *F. necrophorum*-positive patients. Overall, a larger proportion of positive patients were treated with antibiotics than patients that were negative for group A and C/G streptococci and *F. necrophorum* (29.3% versus 14.0%, respectively, $P = 0.002$) (Table 3).

TABLE 3 Patients for whom antibiotics were prescribed, by diagnosis

Diagnosis	No. of patients (%) for whom antibiotics were prescribed
All (n = 300)	51 (17)
F. necrophorum or group A and C/G streptococci positive (n = 92)	27 (29.3)
F. necrophorum positive (n = 10)	1 (10)
Negative[a] (n = 207)	29 (14.0)

[a]Negative for *F. necrophorum* and group A and C/G streptococci.

DISCUSSION

In response to the resurgence of *F. necrophorum* infections, including Lemierre's syndrome, institutions in Europe have initiated screening for *F. necrophorum* in adolescents and young adults presenting with bacterial pharyngitis as a preventative measure (6, 7, 22). Despite a rather low prevalence rate of 3.3% in our study compared with those from past studies (6, 11, 12, 22), we identified a disparity among the percentages of *F. necrophorum* in certain age groups. Only 1.9% of patients <14 years old were positive for *F. necrophorum*. In contrast, albeit a small cohort, we identified a prevalence rate of 13.5% in patients 14 to 20 years old. This correlates with findings from previous studies that reported prevalences ranging from 10% to 48% in adolescents and young adults (7, 9, 11, 12, 17). This study also confirms that *F. necrophorum* may be an important pathogen in young children, as indicated by the rare reports of Lemierre's syndrome in preadolescent patients (18, 19). Interestingly, Lemierre's syndrome in adolescents and young adults appears to be preceded by pharyngitis, whereas cases of Lemierre's syndrome in preadolescents often present with otitis media. None of the five positive patients <10 years old in this study reported ear pain. All five patients reported fevers and absence of coughs, and 4/5 patients also presented with sore throats. The fifth patient had a very low PCR titer, and it is possible that the patient had a mild infection, was near clinical recovery upon presentation to the ED, or was a false positive.

Interestingly, the recovery of *F. necrophorum* by culture was comparable to that by PCR and is concordant with results from another study demonstrating that PCR is mostly beneficial for detecting low concentrations of *F. necrophorum* (23). A systematic review of studies using molecular detection and culture-based methods to detect *F. necrophorum* in patients with acute tonsillitis showed rates of recovery of *F. necrophorum* that were similar at 22.2% versus 20.3% (P = 0.462), respectively (26). Because clinical pharyngeal samples are expected to contain high numbers of commensal bacteria, antibiotic-selective media may have an advantage if employed in routine screening. In our study, the EYKVJ selective medium showed an improved inhibition of contaminating normal oral flora, without inhibiting the growth of *F. necrophorum*, and eased the screening for *F. necrophorum* colony morphology after 48 h of growth. Hemolysis on the FSA was difficult to discern, and hence was not helpful for differentiating *F. necrophorum* from other organisms. On the basis of our findings, the EYKVJ medium has the potential to be a screening method for *F. necrophorum*. Prior to implementation, laboratories must account for the benefits and limitations of PCR versus selective media for isolating *F. necrophorum* at their institutions.

The high prevalence findings from a Denmark study prompted the routine screening for *F. necrophorum* by culture, resulting in a recovery of *F. necrophorum* from >15% of patients aged 18 to 32 years with tonsillitis (12). Approximately 1 in 400 cases of *F. necrophorum* pharyngitis will progress to Lemierre's syndrome (7), assuming a prevalence of 10% for *F. necrophorum* pharyngitis and an incidence of 14.4 cases per 1 million adolescents (aged 15 to 24 years) per year for Lemierre's syndrome (1). These numbers suggest that Lemierre's syndrome may exceed other pharyngitis complications, such as rheumatic fever. It is postulated that the treatment of *F. necrophorum* pharyngitis would decrease the likelihood of progressing to complications, such as Lemierre's syndrome (7, 14). Thus, a greater emphasis on accurate laboratory diagnosis may be warranted to

assist in the appropriate use of antimicrobial agents. In this study, all of the patients diagnosed with GAS at the time of the visit using a RADT were prescribed antimicrobial therapy, as well as the 36 patients that were RADT negative in the ED, 27 of which were positive for either GAS or *F. necrophorum* by PCR. Long-term studies are needed to determine the impact of the early detection of *F. necrophorum* on preventing the progression to Lemierre's syndrome or other complications.

There were some limitations identified in this study. Since the RADT was performed in the ED, we cannot guarantee that all positive pharyngeal samples were transported to the clinical microbiology laboratory. Thus, the prevalence of GAS infections in our patient population, as well as the rate of potential GAS and *F. necrophorum* coinfections, may be underestimated. Another limitation is that we did not investigate other bacterial and viral causes of pharyngitis apart from the tests performed per physicians' orders. Only two patients had a respiratory viral panel performed and both were positive for influenza virus A. The majority of group A and C/G streptococci-and *F. necrophorum*-negative patients (78.0%) presented with similar signs and symptoms, suggestive of alternate etiologies not investigated in this study. A large portion of the "negative" patients in this study with signs and symptoms of acute pharyngitis are likely to have had a viral infection, since 102 (34%) of the patients presented with coughs. We previously reported that 28.4% of patients were GAS positive by molecular testing despite the presence of coughs (27), highlighting the importance of only screening appropriate patients.

In summary, we demonstrated that *F. necrophorum* is circulating in our patient population, all of whom presented with true signs and symptoms of bacterial pharyngitis. A 13.5% prevalence rate among patients aged 14 to 20 years was similar to those reported previously, and screening may be warranted in this age group. Although the prevalence was only 1.9% in those <14 years old, additional study is necessary to determine the significance of the recovery of *F. necrophorum* from pharyngeal samples in preventing the progression to severe complications, including Lemierre's syndrome.

MATERIALS AND METHODS

Study population. This prospective study included all pharyngeal samples submitted to the microbiology laboratory at Children's Hospital of Los Angeles (CHLA) in Los Angeles, California. CHLA is a 360-bed free-standing, academic, tertiary care pediatric institution with a large ED that serves a large, diverse pediatric community, as well as a large population of children with special health care needs. Three hundred pharyngeal samples were continuously enrolled between October and December 2015 and February and March 2016. Only one sample per patient was enrolled in the study. Any patients who returned within the study period and had an additional pharyngeal sample collected for GAS testing were excluded from the study. This study was reviewed and approved by the CHLA institutional review board.

All patients with pharyngeal samples collected using ESwab (Copan Diagnostics, Murrieta, CA) as part of standard-of-care testing for GAS pharyngitis were enrolled in the study once samples were received in the clinical microbiology laboratory. During the study period, the standard-of-care testing offered for suspect streptococcal pharyngitis at CHLA consisted of an initial screening for GAS in the ED by point-of-care RADT (Osom Ultra Strep A; Sekisui Diagnostics, San Diego, CA), followed by an automatic reflex to culture on blood agar for GAS and group C/G streptococci. Agar plates were incubated for up to 48 h at 35°C in 5% CO_2, and beta-hemolytic colonies were identified using MALDI-TOF MS (Bruker Corporation, Billerica, MA) or Lancefield grouping (PathoDX Strep group kit; Thermo Fisher Scientific, Lenexa, KS).

Pharyngeal sample in ESwabs were stored at 4°C after the processing of standard-of-care testing, and *F. necrophorum* cultures were set up within 24 h of collection. Clinical specimens at our institution are normally stored at 4°C for 7 days, after which, samples are discarded. After 7 days, any residual specimens were stored at −70°C for PCR testing. Additional PCR testing for GAS and group C/G streptococci (Lyra Direct Strep kit; Quidel Corporation, San Diego, CA) was performed for the study per the manufacturer's recommendation (28).

***F. necrophorum* culture.** In this study, we utilized media specifically prepared for the isolation of *F. necrophorum* (Anaerobe Systems, Morgan Hill, CA). Both subspecies of *F. necrophorum* are recovered in culture but cannot be differentiated. ESwab liquid medium was inoculated onto four selective and/or differential agar plates: (i) EYA, (ii) EYKV, (iii) EYKVJ, and (iv) FSA with josamycin, neomycin, and vancomycin (Anaerobe Systems). The egg yolk-based agars were used to detect lipase activity and contained antibiotics to suppress the majority of normal oral flora. FSA with horse blood and josamycin, neomycin, and vancomycin was used to detect hemolysis by *F. necrophorum*. Samples were processed within 24 h of collection to ensure the viability of any *F. necrophorum* present. The agar plates were incubated at 35°C in an anaerobic chamber (Microbiology International, Frederick, MD) for at least 48 h.

Growth on agar plates was observed at 24, 48, 72, and 96 h, and suspect colonies were identified using MALDI-TOF MS. The following criteria were used to identify *F. necrophorum*: (i) lipase activity on egg yolk-based plates or (ii) hemolysis on FSA. In addition, since EYKVJ is a highly selective medium, all colony types that grew were identified by MALDI-TOF MS.

***F. necrophorum* PCR.** ESwab samples (300 μl) were added to 2 ml NucliSENS lysis buffer, were allowed to incubate for a minimum of 20 min, and were extracted and eluted in 30 μl elution buffer on the NucliSENS easyMAG automated instrument (bioMérieux, Inc., Durham, NC). The *F. necrophorum* real-time PCR assay was developed using primers and probes for the RNA polymerase β-subunit (RPO) gene (*rpoB*) previously published (12, 22) and adapted for the Applied Biosystems (ABI) 7500 real-time PCR system (Thermo Fisher Scientific, Lenexa, KS). This is an *F. necrophorum* species-specific real-time PCR assay. *F. necrophorum* subsp. *funduliforme* ATCC 51357 served as positive control and was included in each PCR run. This strain was also used to determine the limit of detection and linear range of the *F. necrophorum* PCR assay. The human RNase P (RNP) gene was used as the internal extraction and amplification control. The primers and probe sequences for RNP were as follows: RNASEP-F, 5′-AGA TTT GGA CCT GCG AGC G-3′; RNASEP-R, 5′-GAG CGG CTG TCT CCA CAA GT-3′; RNASEP-PR, 5′-/Cy5/TTC TGA CCT GAA GGC TCT GCG CG/3BHQ_2/-3′. The RPO target and RNP internal control were run as multiplex reactions at final concentrations of 300 nM each for RPO forward and reverse primers, 100 nM RPO probe, 80 nM each RNP forward and reverse primers, and 100 nM RNP probe. For the real-time PCR, 5 μl of nucleic acid was combined with 20 μl of TaqMan universal PCR master mix (Thermo Fisher Scientific, Lenexa, KS). Thermal cycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 50 cycles of 95°C for 30 s and 60°C for 60 s. This cycling condition was based on results from a previous study using these primers and probe (12). Amplification data were analyzed using the ABI 7500 v2.05 software (Thermo Fisher Scientific, Lenexa, KS). The linear range of the *F. necrophorum* PCR was determined to be between a C_T of 26 and 46 using *F. necrophorum* subsp. *funduliforme* ATCC 51357. A C_T of $<$43 was considered detected and a C_T of $\geq$ 43 was considered not detected. All negative results were acceptable when the internal control had a C_T of $<$40.

The specificity of the *F. necrophorum* RPO primers and probe were tested against and were negative for the following isolates: *Bacteroides fragilis* group, *Gemella haemolysans*, *Fusobacterium periodonticum*, *Fusobacterium nucleatum*, *Fusobacterium varium*, *Prevotella histicola*, *Prevotella intermedia*, *Prevotella nigrescens*, and *Veillonella dispar*. These organisms were isolated from clinical specimens and identified by MALDI-TOF MS.

Medical chart review. Patients' medical records were reviewed, and patient demographic data were collected, including, age, sex, and comorbidities. The other data collected were presenting signs and symptoms (i.e., fever, cough, lymphadenopathy, and exudate), antibiotics prescribed, and final diagnosis (ICD-10 codes).

Data analysis. Comparisons of proportions and determinations of *P* values were performed using chi-square tests (MedCalc statistical software). A *P* values of $<$ 0.05 was considered statistically significant.

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