ORIGINAL ARTICLE 10.1002/nmi2.39

Fusobacterium necrophorum, an emerging pathogen of otogenic and paranasal infections?

D. Creemers-Schild[1], F. Gronthoud[2], L. Spanjaard[2], L. G. Visser[1], C. N. M. Brouwer[3] and E. J. Kuijper[4]

1) *Department of Infectious Diseases, Leiden University Medical Center, Leiden,* 2) *Department of Medical Microbiology, Academic Medical Center, Amsterdam,* 3) *Department of Pediatrics, Leiden University Medical Center, Leiden and* 4) *Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands*

Abstract

Fusobacterium necrophorum is a rare causative agent of otitis and sinusitis. Most commonly known is the classic Lemièrre's syndrome of postanginal sepsis with suppurative thrombophlebitis of the jugular vein. We report five patients diagnosed recently with a complicated infection with *F. necrophorum* originating from otitis or sinusitis. Two patients recovered completely, one patient died due to complications of the infection, one patient retained a slight hemiparesis and one patient had permanent hearing loss. Diagnosis and management are discussed. A possible factor in the emergence of *F. necrophorum* is proposed.

Keywords: Emerging pathogen, *Fusobacterium necrophorum*, Lemièrre's syndrome, otitis, sinusitis
Original Submission: 9 July 2013; **Revised Submission:** 1 January 2014; **Accepted:** 14 January 2014
Article published online: 25 March 2014
New Microbe New Infect 2014; **2:** 52–57

Corresponding author: D. Creemers-Schild, Department of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands.
E-mail: dinaschild@hotmail.com

Introduction

Fusobacterium necrophorum is an anaerobic, non-spore forming pleomorphic Gram-negative rod which is considered a commensal of the animal and human upper respiratory, gastro-intestinal and female genital tract. It can cause a variety of human infections, but is most commonly known as the main cause of postanginal sepsis with suppurative thrombophlebitis of the jugular vein (Lemièrre's syndrome). Less well known is the otogenic variant with mastoiditis and intracranial complications such as meningitis, abscesses and sinus thrombosis [1]. We report five patients with a recent diagnosis of complicated infection with *F. necrophorum* originating from otitis or sinusitis with significant morbidity and mortality.

Case 1

A 9-year-old healthy girl, presented to the emergency department with fever, headache and vomiting. Two weeks before presentation the general practitioner had diagnosed an acute otitis media which was treated with co-amoxiclav for 7 days. The fever persisted intermittently with otalgia. One day before admission she developed otorrhoea and vomiting. On admission she was acutely ill with a temperature of 39.6°C, purulent discharge of the right ear, postauricular tenderness and nuchal rigidity. Cerebral spinal fluid revealed neutrophilic pleocytosis (2399/μL). Empiric antibiotic treatment was started with ceftazidime. A computed tomography scan of the brain showed total obliteration of the right mastoid, thrombosis of the sigmoid sinus and epidural empyema of 8 mm in the posterior surface of the temporal bone. Gram staining of the middle ear fluid showed pleomorphic Gram-negative rods, suggestive for the presence of *Haemophilus influenzae*. Gram staining of the cerebrospinal fluid did not reveal any bacteria. Shortly after admission an urgent mastoidectomy was performed. Post-operatively the patient did not regain consciousness (Glasgow Coma Scale E1M1Vtube) and there were no pupillary light reflexes. The

cultures after 1 day were negative. Antibiotic therapy was switched to meropenem. Repeated computed tomography of the brain revealed massive cerebral oedema with brainstem herniation. There was a progressive loss of all brainstem reflexes despite maximal therapy and treatment was withdrawn. The patient died 24 h after admission. Autopsy of the brain showed cerebral oedema with herniation, thrombosis of the sigmoid sinus and the transverse sinus and there was purulent discharge mainly in the posterior cranial fossa. The cultures taken during life (middle ear fluid and mastoid) and from the autopsy revealed *F. necrophorum*, identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Microflex, Bruker Daltonik, Bremen, Germany).

Case 2

A previously healthy, 9-year-old girl presented to the emergency department with the suspicion of meningitis. Nine days before admission she started with topical treatment (dexamethasone–framycetin–gramicidin eardrops, Sofradex®; Sanofi-aventis Netherlands B.V., Gouda, the Netherlands) for otitis and otorrhoea. Five days before admission she developed high fever, headache and vomiting and, consecutively, neck pain and stiffness 2 days before admission. She appeared acutely ill, but was fully conscious. Cerebrospinal fluid examination revealed a marked neutrophilic pleocytosis (1 280 000/μL). Empiric antibiotic therapy was started with ceftazidime. When pleomorphic Gram-negative rods were seen in the Gram staining of the cerebrospinal fluid, the antibiotic therapy was switched to meropenem. Computed tomography of the brain showed mastoiditis with thrombosis of the sigmoid sinus and a small empyema. An urgent mastoidectomy was performed. Post-operatively the patient was admitted to the intensive care department. *Fusobacterium necrophorum* was cultured after 24 h from the cerebrospinal fluid, and identified by MALDI-TOF MS. Antibiotic therapy was changed to penicillin. Magnetic resonance imaging (MRI) showed an additional right-sided pontine infarct. She recovered slowly and after 4 days she was transferred to the paediatric ward. The penicillin was continued for 18 days and then switched to clindamycin for another 3 weeks. The patient experienced no diarrhoeal problems during the use of clindamycin. Tinzaparin was started for the sinus thrombosis and continued for 3 months. After 20 days of hospitalization she was discharged, at that moment she experienced urinary and faecal incontinence and a hemiparesis of the left leg. Six months later the urinary and faecal incontinence had recovered, there was still a very mild hemiparesis of the left leg.

Case 3

A 2-year-old boy presented at the Ear, Nose and Throat Clinic with fever, otorrhoea and mastoiditis. Six weeks prior to admission he underwent adenoidectomy and received tympanostomy tubes in another hospital. Post-operatively he was re-admitted with fever and otitis for which he received several antibiotic treatments including amoxicillin, co-amoxiclav and ceftazidime. One week after discharge his symptoms returned. On admission his temperature was 39°C. Since 2 days he had had otorrhoea and mastoiditis was suspected. A computed tomography scan of the mastoid showed a Bezold's abscess with partial thrombosis of the sigmoid sinus and the right vena jugularis. A cortical mastoidectomy was performed with drainage of the abscess. On admission co-amoxiclav and ceftazidime were given. Gram stains of fluid from the right ear and the right mastoid showed slender, small Gram-negative rods. Anaerobic cultures revealed *F. necrophorum* (shown by using MALDI-TOF MS). No aerobic growth was observed. *F. necrophorum* was also cultured from the Bezold's abscess. Blood culture remained negative. Antibiotic treatment was changed to penicillin. The patient showed good clinical improvement. After 2 weeks he was discharged with clindamycin for 1 month. The patient experienced no diarrhoeal problems during the use of clindamycin.

Case 4

A 42-year-old man with a suspicion of mastoiditis and meningitis was transferred from another hospital to the intensive care unit of our hospital. His symptoms, right ear pain and a sore throat, started a few days before his first admission. One day before admission the patient showed an altered mental status, lateralization and lowered level of consciousness. On presentation he was febrile with tachycardia, a lowered level of consciousness, left muscle weakness and right ear discharge, possibly liquorrhoea. A computed tomography scan of the brain showed otitis and mastoiditis with total obliteration of the right mastoid, subdural empyema, two intraparenchymal abscesses in the right frontal and temporal lobe and a midline shift to the left. No sinus thrombosis was present. Right middle ear cultures were taken and amoxicillin, ceftriaxone and metronidazole were administered. Cultures revealed *F. necrophorum* (identified by Vitek 2; bioMérieux, Marcy l'Etoile, France). Amoxicillin was discontinued. Blood cultures remained negative. A hemicraniectomy was performed to drain the subdural empyema. Subsequently, a loss of sensitivity of his right ear and temporal skin was observed

along with a conductive hearing loss. Fourteen days after the operation an MRI scan of the brain showed a fronto-temporal abscess on the right side, increase in subdural effusion on the left side and midline shift to the right. He underwent surgery and antibiotic therapy was changed to meropenem and vancomycin. The patient improved. After 10 days the vancomycin was stopped and he was transferred back to the other hospital in good clinical condition with meropenem. After 8 days the patient was discharged. Antibiotic therapy was continued for 3 months according to the decision of the clinician. He experienced permanent hearing loss of the right ear.

Case 5

A 14-year-old boy was admitted to the neurosurgical ward with ethmoiditis and meningeal irritation. Over the course of several days he had developed a headache, fever 38.5°C, a swollen left eye, vomiting, malaise and dizziness. He had no history of sinusitis. On admission he had a temperature of 39°C, meningeal irritation, a red pharynx, a swollen left eyelid which he was unable to open and a red left conjunctiva. An MRI scan showed an abscess in the paranasal sinus with obliteration of the left orbita and intracerebral frontal involvement with dural enhancement. A blood culture and a nasal swab were taken for culture and empiric antibiotic treatment was started with ceftriaxone and metronidazole. The next day the anaerobic blood culture bottle turned positive with slender pleomorphic Gram-negative rods which were also seen in the Gram stain of the nasal swab. *Fusobacterium necrophorum* (MALDI-TOF MS) was cultured from the blood and nasal swab. After 5 days the patient became somnolent and a subsequent MRI scan of the brain showed a progression of the abscess subcutaneously, left intraorbital and intracranial along with progression of subdural empyema and a suspected thrombosis of the superior sagittal sinus and right sigmoid sinus. A craniotomy was performed. The Gram stain of pus from the temporal pocket showed Gram-negative rods, but cultures remained negative. Ceftriaxone was changed to meropenem. Post-operatively, the patient showed clinical improvement. Four days later he showed increasing headache and a left hemiparesis. Evacuation of right frontal subdural abscess was performed. Meropenem was changed to penicillin. Six days later an intraorbital abscess was evacuated. His left hemiparesis disappeared completely. A low protein S level was found for which further analysis was initiated. Three weeks later the patient was discharged in good condition with penicillin for 1 month (Table 1).

Discussion

We report five patients with a diagnosis of complicated infection with *F. necrophorum* originating from otitis or sinusitis within a time period of 9 months. Only two patients recovered completely, one patient died due to complications of the infection, one patient retained a slight hemiparesis and one patient had permanent hearing loss.

The first description of human systemic infection with *F. necrophorum* was made by Veillon and Zuber in 1898 of a young child with chronic purulent otitis media with septic arthritis of the knee, cerebral abscess and signs of overwhelming systemic infection. Courmont and Cade made the first description in 1900 of human postanginal septicaemic infection with *F. necrophorum*. The disease was more clearly characterized by Lemièrre in 1936, but it was not until 1983 that, for the first time, the term Lemièrre's syndrome was used [1].

The most common presentation of Lemièrre's syndrome is postanginal septicaemia with septic thrombophlebitis of the internal jugular vein and distant septic metastases secondary to an acute oropharyngeal infection. *Fusobacterium necrophorum* is an unusual cause of mastoiditis, sinusitis and meningitis with a limited number of published reports [2–10]. *Fusobacterium necrophorum* can cause invasive disease with severe complications in previously healthy children and adults. Binding and activation of plasminogen, platelet aggregation, and production of haemagglutinin by *F. necrophorum* are all likely to be important in the pathogenesis of abnormal coagulation and inflammation seen in patients with Lemièrre's syndrome [1, 11]. This corresponds with the high rate of thrombotic complications observed in four of five patients in our analysis. In addition, some strains appear to be more virulent due to binding of factor H, a complement controlling glycoprotein [12].

Its uncommon presentation and the difficulties encountered in isolating *F. necrophorum*, with the delay of adequate treatment, contributes to the high morbidity and mortality. In Case 1 appropriate antibiotics for *F. necrophorum* were delayed for 12 h due to the initial suspicion of infection with encapsulated *H. influenzae*. In cases of a severe course of an acute otitis media or sinusitis, especially with thromboembolic complications, abscess formation and pleomorphic Gram-negative rods in the Gram stain, the empiric antibiotic treatment must include agents to treat *F. necrophorum*. Various antibiotics are currently being used, as illustrated in our case series. No uniform guidelines exist, though most authors recommend the combination of penicillin and metronidazole or carbapenems [1]. There are conflicting reports in the literature on the susceptibility of *Fusobacterium* isolates to penicillin. Some older studies report on beta-lactamase-producing strains of *Fusobac-*

TABLE 1. **Clinical characteristics and outcomes of five patients with *Fusobacterium necrophorum* disease**

Patient	Sex	Age (years)	Presenting symptoms	Isolation of *F. necrophorum*[a]	Method of identification	Susceptibility (*E*-test) MIC (mg/L)	Complications	ICU (days)	Empirical treatment (duration in days)	Targeted treatment (duration in days)	Length of hospital stay (days)	Outcome at day 30 (mortality and neurological sequelae)
1	F	9	Otorrhoea, fever, vomiting, headache, neck pain	Middle ear fluid, pus subarachnoid (autopsy)	MALDI-TOF MS log score 2.17	ND	Mastoiditis, meningitis, thrombosis sinus sigmoideus, abscess occipital	1	Ceftazidime 150 mg/kg (0.5), meropenem 120 mg/kg (0.5)	–	1	Died (day 1)
2	F	9	Otorrhoea, fever, headache, vomiting, opisthotonus	Cerebral spinal fluid, middle ear fluid	MALDI-TOF MS log score 2.11	Penicillin 0.02 Metronidazole 0.125 Clindamycin 0.02 Meropenem 0.01	Mastoiditis, thrombosis sinus sigmoideus, small empyema, infarction pons	3	Meropenem 120 mg/kg (5)	Penicillin 300 000 units/kg (18) with switch to clindamycin 40 mg/kg (21)	20	Urinary and faecal incontinence, hemiparesis left leg
3	M	3	Otorrhoea, fever, swelling mastoid	Middle ear fluid, mastoid	MALDI-TOF MS log score 1.86	Penicillin <0.016 Metronidazole 0.032 Clindamycin <0.016	Mastoiditis, Bezold's abscess, thrombosis sinus sigmoideus and vena jugularis	–	Ceftazidime 150 mg/kg IV (1), co-amoxiclav 100/10 mg/kg IV (4)	Penicillin 300 000 units/kg (9) and metronidazole 30 mg/kg (6) with switch to clindamycin 25 mg/kg (32)	17	No sequelae
4	M	42	Otorrhoea, septic, decreased consciousness	Middle ear fluid	Vitek 2	Penicillin 0.016 Metronidazole 0.023 Clindamycin 0.016	Mastoiditis, subdural empyema, midline shift, intracerebral abscess	9	Amoxicillin 12 g (4), ceftriaxone 4 g (16), metronidazole 500 mg thrice daily (14)	Meropenem 6 g (117)	36	Hearing loss
5	M	15	Headache, fever, swollen eye, vomiting	Blood, nose, brain abscess	MALDI-TOF MS log score 2.09	Penicillin <0.016 Metronidazole <0.016 Clindamycin <0.016	Paranasal abscess with intraorbital and intracerebral involvement, sinus thrombosis	1	Ceftriaxone 2 g (4), metronidazole 500 mg thrice daily (4)	Meropenem 6 g (7) with switch to penicillin 24 million units (63), the first 29 days with metronidazole 500 mg qid	41	Temporary hemiparesis

MIC, minimum inhibitory concentration; ICU, intensive care unit; ND, not determined.
[a]In Cases 2 and 4 also a few colonies of skin flora were present in the middle ear fluid, considered as contaminants. In Case 5 the nasal swab also showed *Staphylococcus aureus* which was considered as nasal carriage.

terium isolates [13, 14]. In a recent study, all *F. necrophorum* isolates were susceptible to penicillin (MIC <0.5 mg/L) [15]. In the Netherlands, resistance of *Fusobacterium* isolates to penicillin is very rare and was not found in two previously published cases of *F. necrophorum* infections in the Netherlands [3, 10], nor in our case series.

Although *F. necrophorum* is considered to be a commensal of the human upper respiratory tract, its role as a pathogen of throat infection is also considered. Using real-time PCR, Jensen [16] detected *F. necrophorum* in 48% of 61 throat swabs from patients with non-streptococcal tonsillitis and in 21% of 92 throat swabs from healthy controls. Ludlam *et al.* [17] analysed throat swabs from 411 university students and 103 patients with a sore throat. A swab copy count of 50 000 or more was significantly more frequent in the group of patients with a sore throat (35%) compared with the asymptomatic subjects (4.6%). Using conventional anaerobic culture techniques, two studies in patients with sore throat report the presence of *F. necrophorum* in 4.9% and 9.7%, respectively [18, 19].

Within a short time period (9 months), we encountered five patients with a complicated infection with *F. necrophorum* originating from otitis or sinusitis. Interestingly, two recent studies from Israel also report on the emergence of *F. necrophorum* infections associated with complicated mastoiditis. Yarden-Bilavsky *et al.* [20] describe seven children with acute *F. necrophorum* mastoiditis diagnosed during a 3.5 year period of whom five presented in the last 6 months of the study. In a second study, 17 of 27 *F. necrophorum* infections in children were diagnosed during the last 4 years of a 10 year study period. The most common source of infection was otogenic (70%) [21]. Three older surveys from Denmark (1990–1995), Wisconsin (1995–2001) and France (1995–2006) demonstrated a temporary increase in Lemièrre's syndrome [22–24]. Most authors speculate that the more restricted use of antibiotics and the shift in prescribed antibiotics from traditional antibiotics such as penicillin to macrolides and cephalosporines, which lack activity against *F. necrophorum*, as well as improved identification methods by the use of broad-spectrum 16S rRNA PCR have contributed to this increase in incidence. However, the Netherlands is known for having a restricted antibiotic prescription policy for upper respiratory tract infections well before the apparent increase of *F. necrophorum* infections, which has not changed during the last few years. Although microbiological laboratories in the Netherlands introduced MALDI--TOF MS in 2009, resulting in a significant improvement in the identification of anaerobes [25], it is unlikely that this pathogen has been unrecognized in severe syndromes as occurred in the patients we described when using conventional identification techniques.

Another possible explanation could be the pneumococcal vaccination, which was incorporated in the national immunization programme in the Netherlands in 2006. The nasopharynx of children is colonized by multiple microorganisms. Numerous associations between these viral and bacterial pathogens have been found during nasopharyngeal carriage [26]. It is increasingly recognized that disruption of this microbiota with its synergistic and interfering interactions facilitates respiratory tract infections such as otitis media [27, 28]. Replacement of vaccine serotype pneumococci by non-vaccine serotypes after pneumococcal vaccination and its effects on carriage of and infection with other common pathogens such as *H. influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* has been described in several studies [28, 29]. However, meta-genomic analyses have shown a high variability of the nasopharyngeal microbiota, including the presence of *F. necrophorum* and other less prevalent commensals [30]. We hypothesize that an increase in *F. necrophorum* infections may be explained by a similar disrupting impact of pneumococcal vaccination.

In conclusion, we report five patients diagnosed recently with complicated infection with *F. necrophorum* originating from an otitis or sinusitis. Imaging should be considered in all infections with *Fusobacterium* arising from the upper respiratory tract or head region due to the high incidence of thrombotic complications and abscess formation. Appropriate antibiotics should not be delayed. The reason for the recent emergence of *F. necrophorum* infections is not clearly established.

Conflict of Interest

None declared.

References

1. Riordan T. Human infection with *Fusobacterium necrophorum* (necrobacillosis), with a focus on Lemierre's syndrome. *Clin Microbiol Rev* 2007; 20: 622–659.
2. Garimella A, Inaparthy A, Herchline T. Meningitis due to *Fusobacterium necrophorum* in an adult. *BMC Infect Dis* 2004; 4: 24.
3. Veldhoen ES, Wolfs TF, Vught AJ. Two cases of fatal meningitis due to *Fusobacterium necrophorum*. *Pedriatr Neurol* 2007; 36: 261–263.
4. Morrison A, Weir I, Silber T. Otogenic *Fusobacterium meningitis*, sepsis, and mastoiditis in an adolescent. *South Med J* 2004; 97: 416–418.
5. Pace-Balzan A, Keith AO, Curley JWA, Ramsden RT, Lewis H. Otogenic *Fusobacterium necrophorum* meningitis. *J Laryngol Otol* 1991; 105: 119–120.
6. Cron RQ, Webb KH. Necrobacillosis: an unusual cause of purulent otitis media and sepsis. *Pediatr Emerg Care* 1995; 11: 379–380.

7. Giridharan W, De S, Osman EZ, Amma L, Hughes J, McCormick MS. Complicated otitis media caused by *Fusobacterium necrophorum*. *J Laryngol Otol* 2004; 118: 50–53.
8. Lim SC, Lee SS, Yoon TM, Lee JY. Lemierre syndrome caused by acute isolated sphenoid sinusitis and its cranial complications. *Auris Nasus Larynx* 2010; 37: 106–109.
9. Bentham JR, Pollard AJ, Milford CA, Anslow P, Pike MG. Cerebral infarct and meningitis secondary to Lemierre's syndrome. *Pediatr Neurol* 2004; 30: 281–283.
10. Van Munster MP, Brus F, Mul D. Rare but numerous serious complications of acute otitis media in a young child. *BMJ Case Rep* 2013; doi:10.1136/bcr-2012-008149.
11. Holm K, Rasmussen M. Binding and activation of plasminogen at the surface of *Fusobacterium necrophorum*. *Microb Pathog* 2013; 59-60: 29–32.
12. Friberg N, Carlson P, Kentala E *et al.* Factor H binding as complement evasion mechanism for an anaerobic pathogen, *Fusobacterium necrophorum*. *J Immunol* 2008; 181: 8624–8632.
13. Brook I. Infections caused by beta-lactamase-producing *Fusobacterium* spp. in children. *Pediatr Infect Dis J* 1993; 12: 532–533.
14. Tuner K, Nord CE. Antibiotic susceptibility of anaerobic bacteria in Europe. *Clin Infect Dis* 1993; 16: S387–S389.
15. Sousa ELR, Gomes BPFA, Jacinto JC, Zaia AA, Ferraz CCR. Microbiological profile and antimicrobial susceptibility pattern of infected root canals associated with periapical abscesses. *Eur J Clin Microbiol Infect Dis* 2013; 32: 573–580.
16. Jensen A, Hagelskjaer Kristensen L, Prag J. Detection of *Fusobacterium necrophorum* subsp. funduliforme in tonsilitis in young adults by real-time PCR. *Clin Microbiol Infect* 2007; 13: 695–701.
17. Ludlam H, Howard J, Kingston B *et al.* Epidemiology of pharyngeal carriage of *Fusobacterium necrophorum*. *J Med Microbiol* 2009; 58: 1264–1265.
18. Amess JA, O'Niell W, Giollariabhaigh CN, Dytrych JK. A six-month audit of the isolation of *Fusobacterium necrophorum* from patients with sore throat in a district general hospital. *Br J Biomed Sci* 2007; 64: 63–65.
19. Batty A, Wren MW. Prevalence of *Fusobacterium necrophorum* and other upper respiratory tract pathogens isolated from throat swabs. *Br J Biomed Sci* 2005; 62: 66–70.
20. Yarden-Bilavsky H, Raveh E, Livni G, Scheuerman O, Amir J, Bilavsky E. *Fusobacterium necrophorum* mastoiditis in children: emerging pathogen in an old disease. *Int J Pediatr Otorhinolaryngol* 2013; 77: 92–96.
21. Megged O, Assous MV, Miskin H, Peleg U, Schlesinger Y. Neurologic manifestations of Fusobacterium infections in children. *Eur J Pediatr* 2013; 172: 77–83.
22. Hagelskjaer LH, Prag J, Malczynski J, Kristensen JH. Incidence and clinical epidemiology of necrobacillosis, including Lemierre's syndrome, in Denmark 1990–1995. *Eur J Clin Microbiol Infect Dis* 1998; 17: 561–565.
23. Ramirez A, Hild TG, Rudolph CN *et al.* Increased diagnosis of Lemierre syndrome and other *Fusobacterium necrophorum* infections at a Children's Hospital. *Pediatrics* 2003; 112: e380–e385.
24. Le Monnier A, Jamet A, Carbonelle E *et al. Fusobacterium necrophorum* middle ear infections in children and related complications. *Pediatr Infect Dis J* 2008; 27: 613–617.
25. Van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. *J Clin Microbiol* 2010; 48: 900–907.
26. Bergh MR, Biesbroek G, Rossen JWA *et al.* Associations between pathogens in the upper respiratory tract of young children: interplay between viruses and bacteria. *PLoS One* 2012;7: e47711.
27. Brogden KA, Guthmiller JM, Taylor CE. Human polymicrobial infections. *Lancet* 2005; 365: 253–255.
28. Dunne MD, Smith-Vaughan HC, Robins-Browne RM, Mulholland EK, Satzke C. Nasopharyngeal microbial interactions in the era of pneumococcal conjugate vaccination. *Vaccine* 2013; 31: 2333–2342.
29. Mehr S, Wood N. *Streptococcus pneumoniae*: a review of carriage, infection, serotype replacement and vaccination. *Paediatr Respir Rev* 2012; 13: 258–264.
30. Bogaert D, Keijser B, Huse S *et al.* Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS One* 2011; 6: e17035.