Case Report

Actinomyces neuii Isolated From a 20-Month-Old Girl With Cervical Lymphadenitis

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Actinomycetes are Gram-positive bacteria that can be part of the normal human flora of the gastrointestinal, pulmonary, and genital tract. Infections are rare, slowly progressing and most commonly affect the cervicofacial region. *Actinomyces israelii* is the most frequently isolated species but a number of other species may cause infection. We report the first postnatally acquired case of an actinomycosis caused by *A. neuii* in a child. We also provide a systematic review of all published cases of *A. neuii* infections. In children, there is one case report of a premature infant with perinatally acquired *A. neuii* sepsis. In adults 21 cases have currently been reported and *A. neuii* infection was associated with endophthalmitis after eye surgery, foreign material-associated infection and abscess formation in the inguinal, axillary, and mammary area. Our case highlights that a *A. neuii* infection is also a potential differential diagnosis in children with chronic lymphadenitis.

Key words. *Actinomyces neuii*; cervical lymphadenitis; children; MALDI-TOF; Prevotella.

Actinomycosis is a rare infection in children younger than 10 years of age [1]. It mainly affects the cervicofacial region, but many other sites of infection have been described [2]. Actinomycosis is usually caused by *Actinomyces israelii*, a Gram-positive bacterium that colonizes the oral cavity. With the development of molecular techniques in recent years, it was recognized that some *Actinomyces* species were misclassified (eg, as *Actinobaculum* species), and a number of new *Actinomyces* species have been identified [3, 4]. Atypical coryneform bacteria (initially designated as Centers for Disease Control and Prevention [CDC] fermentative coryneform group 1 [5]) were isolated for the first time in the 1980s from patients with endophthalmitis, and the organism was subsequently named *A. neuii* in 1994 [6]. Interestingly current adult literature suggests that *A. neuii* has a different spectrum of disease and most frequently presents with skin and soft tissue infection or abscesse. In children, there is currently only 1 published case report of *A. neuii* sepsis in a premature infant born to a mother with pelvic infection and chorioamnionitis [7]. We report here the first, to our knowledge, case of a postnatally acquired *A. neuii* infection in a child presenting with cervical lymphadenitis.

CASE

A 20-month-old previously healthy girl presented to our emergency department with a 3-week history of submandibular swelling. Ultrasonographic examination performed 1 week before presentation showed a multilobar calcified structure measuring 1.5 cm in diameter. After a rapid increase of the swelling within 24 hours, the child presented to our hospital. The parents reported that since birth, the child was known to have a small cervical sinus tract that intermittently drained foul-smelling fluid. The child had not had any contact with sick individuals, and her family history was unremarkable.

On physical examination, the girl was afebrile and had a submandibular, firm, nonfluctuant, nontender swelling with a sinus tract. She also had an itching macular rash over the cervical area. Results of the remaining examination were normal. In particular, there were no other enlarged lymph nodes. Her dentition was normal, and there were no lesions in her mouth. A full blood count revealed a hemoglobin value of 117 g/L, a white blood cell count of 13.7×10^9/L (62% neutrophils, 31% lymphocytes, 6% monocytes, 1% eosinophils), a platelet count of 491×10^9/L, and a C-reactive protein level of 9 mg/L. Repeated ultrasonography of the

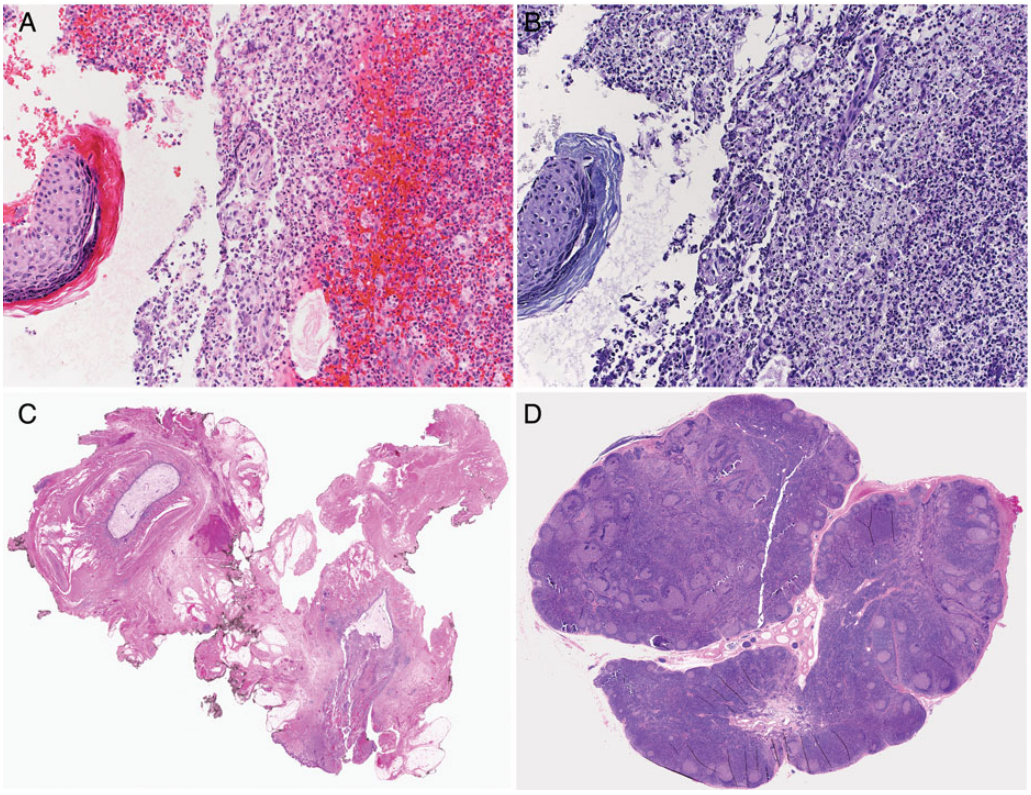

Figure 1. Hematoxylin and eosin (H&E) (A) and periodic acid–Schiff (PAS) (B) staining of histological sections of excised cervical lymph node showing purulent inflammation and small fragments of squamous epithelium (×200 magnification). H&E (C) and PAS (D) staining of histological sections from the second operation showing the remaining sinus tract lined with squamous epithelium cells adjacent to elastic cartilage (C) and follicular hyperplasia of the lymph node (D) (×10 magnification).

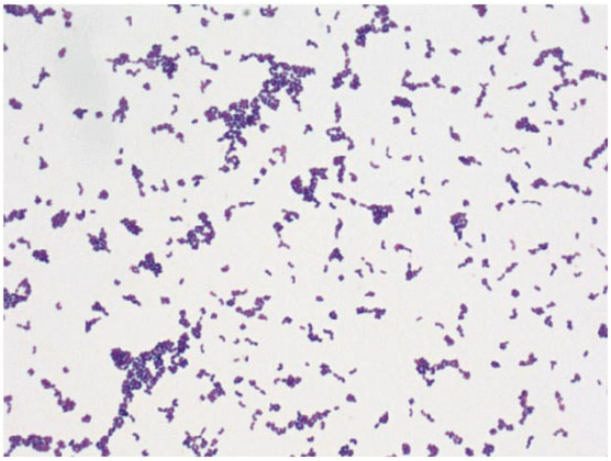

Figure 2. Gram stain of the *A neuii* isolate showing coryneform nonbranching Gram-positive rods (×400 magnification).

neck confirmed the multilobar structure located at the anterior border of the sternocleidomastoid muscle with a diameter of 2 cm and a fistula adjacent to the multilobular structure extending to the skin.

The following day, the mass and fistula were excised. Histopathological examination revealed purulent inflammation and small fragments of squamous epithelium (Figure 1A and B). Sulfur granules were not identified. A Gram stain from several deep cervical swabs showed Gram-positive rods (Figure 2), Gram-positive cocci, and Gram-negative pleomorphic rods. Culture resulted in polymicrobial growth of *Prevotella timonensis*, viridans streptococci, anaerobic Gram-positive cocci, and *A. neuii*. Identification of *A. neuii* was achieved from pure culture on Columbia agar supplemented with 5% sheep blood (BD Diagnostic Systems, Allschwil, Switzerland). The catalase reaction was positive, and the Gram stain revealed coryneform, short rods. In addition, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex LT, Bruker Daltonics) was performed using a short extraction protocol with 1 µL of 70% formic acid added to the smears followed by application of the matrix solution. Analysis of the raw spectral data was performed with MALDI Biotyper software 3.0 (Bruker Daltonics) with reference database version 3.1.2.0 (3995 database entries) and identified *A. neuii* with a score of 2.108. Identification of *P. timonensis* was done using a 16S rRNA gene sequence analysis. The anaerobe cocci and the viridans streptococci identified with culture and Gram staining were not further identified to the species level. Staining for acid-fast bacilli and

Table 1. Details of All Currently Reported Cases of *Actinomyces neuii* Infection Highlighting the Variance of Infection Locations and Treatment

Ref.	Age	Gender	Type of Infection/Underlying Condition	Sample(s) Positive for *A neuii*	Antibiotic susceptibility	Surgical Treatment	Antibiotic Treatment (Dose per d)	Duration of Treatment (d)	Outcome
7	1 d	F	Sepsis/maternal chorioamnionitis	Blood culture, culture of gastric aspirate and residual amniotic fluid in external ear canal	Pen, Cefo, Vanco, Imi, Ery	None	Amp (100 mg/kg), Gent (3 mg/kg) iv	14	Cured
							Pen G (30 mg/kg) oral	28	
Our case	1.6 y	F	Infection of lateral cervical cyst	Culture of intraoperative sample	NS	Drainage with excochleation, secondary selective neck dissection	Amox-Clav (180 mg/kg) iv	4	Cured
							Amox-Clav (80 mg/kg) oral	178	
18	28 y	M	Infection of pilonidal cyst	Culture of purulent fluid	NS	None	Pen V	NS	NS
20	39 y	F	Chronic pericarditis	PCR of pericardial fluid	NS	Pericardial fluid drainage	NS	NS	NS
25	46 y	F	Breast abscess	Culture of fine-needle aspirate	NS	Surgical debridement	Amox (2–3 g) oral	28	Cured
19	48 y	F	Breast abscess	Culture of fine-needle aspirate	NS	None	Amox	21	Cured
18	48 y	F	Breast abscess	Culture of intraoperative sample	NS	Surgical debridement	Pen V	NS	NS
12	58 y	M	Endophthalmitis/ phacoemulsification with posterior chamber intraocular lens implant	Culture of anterior chamber and vitreous body taps	Pen, Amox/Clav, Cefa, Cefu, Ceftr, Vanco, Imi, Oxa, Levo	None	Intravitreous: Vanco (2 mg), Ami (400 µg)	NS	Poor visual acuity (20/40), complicated by central vein occlusion
							Peribulbar: Vanco (25 mg)	NS	
							Ocular: Tobra	NS	
							Cephalexin (2 g)	NS	
17	64 y	F	Mammary prosthesis infection	Culture of swab from mammary prosthesis	Ery, Pen, Tetra, Vanco	Removal of mammary prosthesis	Amox-Clav (4.4 g)	Preop period	Cured
							Amox-Clav (2.4 g)	Postop period	
27	64 y	F	VP-shunt infection	Culture of CSF	Pen, Ceftr, Clinda, Vanco	Removal of VP shunt	Vanco, Cefepime, Amp, Metro	18	Cured
							Pen G (24 Mio IU) iv	42	
							Pen oral	180	
21	66 y	M	Prosthetic valve endocarditis	Blood culture	Pen	None	Pen G (20 Mio IU), Metro (2 g), Ery (4 g)	21	Cured
							Pen G (20 Mio IU) iv	25	
							Amox (2 g) oral	330	
23	67 y	M	Perirenal abscess	Blood culture	NS	Drainage	Amp, followed by Pen and Cipro	37	Cured
22	68 y	M	Endocarditis/aortic paravalvular abscess	Blood culture	Pen, Amp, Ceftr, Vanco, Genta	Open heart surgery	Amp (9 g), Gent (24 mg), Ceftr (2 g) iv	4	NS
							Amp (9 g), Gent (24 mg) iv	5	
							Amp iv	21	
							Ceftr (2 g) iv	63	
							Doxycycline oral	252	
28	68 y	F	Toe ulcer/type 2 diabetes	Cultures of intraoperative samples	Pen G, Cefa, Cefo, Ery, Clinda, Vanco, Teico	Surgical debridement, amputation of toe	Metro (1500 mg), Cipro (200 mg) iv	3	Cured
							Clinda (600 mg) iv	2	
							Clinda (600 mg), Teico (400 mg) iv	15	
							Teico (800 mg) im	10	
29	69 y	F	Bilateral endophthalmitis/ immunosuppression not further specified	Culture of anterior chamber fluid	NS	None	Intravitreous: Vanco, Cefta	1	Limited improvement of visual acuity in right (6/20) and left (6/120) eyes
							Pen G (4 Mio IU) iv, Sulf ocular	21	
14	73 y	M	Chronic endophthalmitis/ phacoemulsification with intraocular lens implantation	Culture of anterior chamber fluid	Ery, Pen, Tetra, Gent, Cefu	Pars plana vitrectomy	Neomycin ocular	21	Satisfactory with visual acuity (6/18)
							Levo (1 g)	NS	
							Azit (500 mg)	NS	
							Chloramphenicol ocular	NS	

13	75 y	M	Chronic endophthalmitis/ cataract surgery	Culture and PCR of aqueous and vitreous fluid	Pen, Cipro, Vanco	None	Intravitreous: Vanco 1 mg/ 0.1 mL and Cefta 2.25 mg/0.1 mL	1	After 6 mo, no symptoms, visual acuity (20/22)
							Oflox, Cefa 600 mg/12 mL ocular	21	
							Cipro (1 g)	14	
24	76 y	M	Chronic osteomyelitis of the calcaneum with fistulation	Culture of bone from curettage	NS	Surgical curettage	Cefa (2 g)	77	Cured
30	78 y	F	Periprosthetic infection/total hip arthroplasty	Culture from joint fluid, intraoperative periprosthetic tissue	Pen, Amp, Clinda, Levo, Vanco, Rif	Surgery (removal of prosthesis, Girdlestone arthroplasty)	Cefa (6 g), Rif (900 mg) iv Pen G (20 Mio IU) iv	7	2 wk after reimplantation, no signs of local infection, no further follow up
							Antibiotic-loaded bone cement (Vanco 2 g, Clinda 1 g, Gent 1 g per 40 g polymethyl methacrylate)	14	
							Amox (3 g) oral	28	
31	79 y	M	Infection of IPP reservoir	Culture of purulent fluid collection around the prosthesis tubing	Amp	Surgery (removal of IPP)	Vanco, Piperacillin/ Tazobactam iv Kan/Cefa, Vanco/Genta, Baci (wound irrigation)	Preop	Cured
							Antibiotic treatment with Vanco iv, cephalexin, Amox-Clav oral, and Amox oral	365	
23	91 y	M	Urosepsis/chronic nephropathy	Blood culture	NS	None	Cefu and mecillinam	9	Cured
32	NS	NS	2 patients with endophthalmitis/ implantation of anterior chamber lenses	Culture of vitreous fluid	Pen, Cefu, Gent	None	None	NS	NS

Abbreviations: iv, intravenous; im, intramuscular; CSF, cerebral spinal fluid; Ami, amikacin; Amox, amoxicillin; Amp, ampicillin; Azit, azithromycin; Baci, bacitracin; Cefa, cefazolin; Cefta, ceftazidime; Ceftr, ceftriaxone; Cefo, cefotaxime; Cefu, cefuroxime; Cipro, ciprofloxacin; Clav, clavulanate; Clinda, clindamycin; Ery, erythromycin; Gent, gentamicin; Imi, imipenem; Kan, kanamycin; Levo, levofloxacin; Metro, metronidazole; Oflox, ofloxacin; Oxa, Oxacillin; Pen, penicillin; Pred, prednisolone; Rif, rifampicin; Sulf, sulfacetamide; Tetra, tetracycline; Teico, teicoplanin; Tobra, tobramycin; Vanco, vancomycin; NS, not stated; VP, ventriculoperitoneal; IPP, inflatable penile prosthesis.

Mycobacterium tuberculosis complex polymerase chain reaction (PCR) remained negative.

Intravenous amoxicillin-clavulanate (180 mg/kg/d) was started. When the swelling and redness subsided, antibiotic treatment was changed to oral amoxicillin-clavulanate (80 mg/kg/d), and the patient was discharged 4 days after surgery. At follow-up 2 weeks later, we noted persistent discharge from the wound that continued during the following 2 months despite local antiseptic and oral antibiotic treatment. A remaining sinus tract was seen, and therefore excision of the remaining fistula and adjacent lymph nodes was performed 3 months after the initial surgery. Pathology examination confirmed a remaining sinus tract lined with squamous epithelium cells adjacent to elastic cartilage (Figure 1C). The resected lymph node was characterized by distinct follicular hyperplasia (Figure 1D). Gram staining did not reveal any bacteria, and culture remained negative. At the next follow-up 2 weeks after the second surgery, the wound had healed and left a small scar (1 cm long). Treatment with amoxicillin-clavulanate was stopped after a total of 6 months, at which time complete resolution of the swelling was documented.

DISCUSSION

A. neuii is a coryneform, nonbranching, aerobically growing, Gram-positive rod that was named in honor of Harold Neu in 1994 [6]. A positive catalase reaction and a positive CAMP test result are key findings in the biochemical identification of this species today. Although the gold-standard method for identification of *A. neuii* is 16S rRNA gene sequencing, recent reports showed that identification with MALDI-TOF MS is excellent even to the species level [8, 9]. Therefore, it has been suggested that for Gram-positive rods, including those of *A. neuii*, a species identification can be accepted without 16S rRNA sequencing analysis if the MALDI-TOF MS cutoff value is higher than 2.0 [8].

Actinomyces spp. are believed to be part of the endogenous flora of mucous membranes in the gastrointestinal, pulmonary, and genital tracts [10]. Recent studies have shown that by the age of 2 years, the oral cavity of every child is colonized with *Actinomyces* spp. [11]. *Actinomyces odontolyticus* and *Actinomyces naeslundii* are the most commonly found species [11]. In contrast, *A. neuii* has not been identified thus far as part of the normal oral flora in the first 2 years of life [11].

After colonization, disruption of the mucosa leading to a microaerophilic environment is thought to promote invasive infection. In adults, a total of 21 cases of *A. neuii* infection have been described in the literature (Table 1). *A. neuii* has been reported most frequently to cause endophthalmitis after eye surgery [12–14], abscess formation, superinfections of ulcers predominantly located in the inguinal, axillary, and mammary areas, and foreign material–associated infections [3, 15–19]. In addition further reports include 3 cases of cardiac infections [20–22], 2 cases of A. neuii bacteremia as a result of a urinary tract infection and a perianal abscess [23] and 1 case of chronic osteomyelitis [23]. Additional details of all previously reported *A. neuii* infections in children and adults are summarized in Table 1.

To our knowledge, only 1 pediatric case of *A. neuii* infection (in a neonate whose infection was caused by maternal bacteremia and subsequent chorioamnionitis) has been reported [7]. Our case represents the first, to our knowledge, postnatally acquired *A. neuii* infection in a child. On the basis of the clinical presentation and the age of the child, infection with atypical mycobacteria was initially suspected, and excision of the enlarged lymph node was performed. The results of culture and PCR remained negative for atypical mycobacteria but showed polymicrobial growth, including growth of *A. neuii*. *A. neuii* is commonly isolated together with other bacterial species, mainly anaerobes. We considered *A. neuii* to be the most important pathogen with potential contribution of the other isolated bacteria. The subspecies of *A. neuii* was not determined. Because the child was afebrile, we did not perform a blood culture; culture results have been shown to be positive in up to 10% of adult patients with *A. neuii* infection [6, 15]. Interestingly, histopathological examination did not reveal any sulfur granules, which are usually a hallmark of actinomycosis. However, the absence of sulfur granules has been reported, particularly in *A. neuii* infections [25]. On the basis of reports on adults, antibiotic treatment with amoxicillin-clavulanate was started. In addition, amoxicillin-clavulanic acid was also considered to be active against the other isolated bacteria. Antimicrobial susceptibility testing for *Actinomyces* spp. is not routinely performed at our microbiology laboratory, because internal data have shown that all *Actinomyces* species are susceptible to amoxicillin-clavulanic acid. Other potential treatment options reported in the literature are ampicillin, penicillin, and cephalosporins [26]. On the basis of experience with infections with other *Actinomyces* spp., we opted for a 6-month antibiotic treatment course with regular follow-ups. Three months after starting treatment, persistent drainage from the lymph node was noted to be a result of a remaining sinus tract rather than treatment failure, because cultures from the second sample remained sterile.

In conclusion, infection with *A. neuii* is a potential differential diagnosis for children with chronic lymphadenitis

and particularly those with presumed atypical mycobacterial infection with negative mycobacterial culture and PCR results from lymph nodes.

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