First Human Case of Meningitis and Sepsis in a Child Caused by *Actinobacillus suis* or *Actinobacillus equuli*

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CASE REPORT

A previously healthy 13-year-old boy who had been suffering from otalgia for 24 h developed fever, headache, photophobia, drowsiness, and neck stiffness and was taken to the emergency department of a local hospital. No cranial trauma was reported. A physical examination found no other meningeval signs, neurological deficits, or papilledema. Otoscopic findings were normal. No petechiae were revealed. Laboratory tests showed neuropsychiatric leukocytosis (white blood cell [WBC] count, 17,700/μL; percentage of neutrophils [N], 85%) and a C-reactive protein (CRP) level of 1.0 mg/dL. Upon worsening drowsiness, non-contrast-enhanced magnetic resonance imaging of the head and neck was performed and the finding was normal. A lumbar puncture yielded opalescent cerebrospinal fluid (CSF) under normal pressure with 650 WBC/μL, mainly polymorphonuclear cells, an elevated total protein level (254 mg/dL), and a normal glucose level (49 mg/dL). The serum glucose level was 113 mg/dL. The patient was empirically given ceftriaxone intravenously (50 mg/kg) and transferred to our tertiary-referral Children’s University Hospital.

Laboratory tests were repeated and showed increased neuropsychiatric leukocytosis (WBC count, 38,560/μL; 95% N) and an elevated C-reactive protein (CRP) level of 14.0 mg/dL. Gram staining of CSF performed at the local hospital revealed a Gram-negative bacillus. Ceftriaxone was then switched to meropenem at 40 mg/kg/dose three times a day, and dexamethasone at 0.1 mg/kg/dose four times a day was added. Blood cultures carried out with the Bactec system with aerobic vials (Becton Dickinson, Milan, Italy) were positive for a Gram-negative bacillus after 24 h. The CSF culture yielded growth of *Neisseria meningitidis* with an identification score of 99.9%.

A physical examination revealed a body temperature of 39°C, a heart rate of 120 beats/min, and a respiratory rate of 25 breaths/min. Laboratory tests were repeated and showed increased neutrophilic leukocytosis (WBC count, 17,700/μL; 95% N) and a C-reactive protein (CRP) level of 1.0 mg/dL. Upon worsening drowsiness, non-contrast-enhanced magnetic resonance imaging of the head and neck was performed and the finding was normal. A lumbar puncture yielded opalescent cerebrospinal fluid (CSF) under normal pressure with 650 WBC/μL, mainly polymorphonuclear cells, an elevated total protein level (254 mg/dL), and a normal glucose level (49 mg/dL). The serum glucose level was 113 mg/dL. The patient was empirically given ceftriaxone intravenously (50 mg/kg) and transferred to our tertiary-referral Children’s University Hospital.

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Real-time PCR tests of DNA extracted from CSF with the EusepScreen kit (Eurospital, Italy) for *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* types B and C, noncapsulated *H. influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus agalactiae*, and *Listeria monocytogenes* were negative.

The 16S rRNA gene sequence, 997 bp long, confirmed the presence of an *Actinobacillus species* (1). To discriminate among *A. ureae*, *A. suis*, and *A. equuli*, a real-time PCR test was performed. The real-time PCR assay amplifies the hypervariable region of the 16S rRNA gene specific for *A. suis* (2) and, as recently reported, for *A. equuli*. The 23S rRNA gene sequences were obtained from the NCBI GenBank database (accession no. EU333989.1 and CP007715.1). The real-time PCR assay was performed with 25-μL reaction volumes containing 2× TaqMan Universal master mix (Applied Biosystems). Primers were used at a concentration of 300 nM; the 6-carboxyfluorescein (FAM)-labeled probe was used at a concentration of 25 nM. Six microliters of DNA was used in each reaction mixture. All reactions were performed in triplicate. DNA was amplified in an ABI 7500 sequence detection system (Applied Biosystems) with the following cycling parameters: 95°C for 10 min, followed by 45 cycles of a two-stage temperature profile of 95°C for 15 s and 60°C for 1 min. The primers and probe used (analyzed with the Primer Express 2.0 program) are shown in Table 1.

Antimicrobial susceptibility was determined by the broth microdilution method, and results were interpreted on the basis of the EUCAST pharmacokinetic/pharmacodynamic (PK/PD; nonspecies-related) breakpoints (http://www.eucast.org, Table v5.0). The data obtained are shown in Table 2.

We obtained a new careful history that revealed that the patient...
had visited a farm 3 days before the onset of symptoms, although close contact with horses or swine was denied.

Two days later, the patient’s condition improved, with fever resolution. Headache, neck stiffness, and drowsiness subsided in 4 days. Dexamethasone was reduced and discontinued in 1 week.

The child was discharged after 12 days of treatment, and a 6-month follow-up examination did not show any signs and symptoms of disease. A complete audiologic evaluation was performed, and the results were normal. T and B cell subsets, immunoglobulin levels, and neutrophil function were evaluated and were completely normal.

We report a case of sepsis and meningitis due to A. suis or A. equuli in a previously healthy 13-year-old boy. To the best of our knowledge, this is the first description of A. suis or A. equuli invasive infection of a child.

Actinobacillus is a Gram-negative coccobacillus, a member of the Pasteurellaceae family. Humans can be rarely colonized or infected by A. hominis or A. ureae (3, 4). A. hominis and A. ureae have been reported as uncommon commensals of the upper respiratory tract and can rarely cause severe infections in humans (3, 4). Twenty-seven cases of infection, including 14 cases of meningitis due to A. ureae, in humans have been reported in the literature (5).

### Table 1 Sequences of the primers and probe used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5’ to 3’ )</th>
<th>Reverse primer</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>23S-rRNA</td>
<td>299CGG TAT TCG AAG TGT CTA TTG TGG TA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>389GAT GGT CCC CCC ATC TTC A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>FAM-&lt;sup&gt;a&lt;/sup&gt; AAC GAC TAG GGC GGG ACA CGA&lt;sup&gt;b&lt;/sup&gt; - TAMRA&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> GenBank accession no. EU333989.1.

<sup>b</sup> TAMRA, 6-carboxytetramethylrhodamine.

### Table 2 Antimicrobial susceptibility of the Actinobacillus isolate described in this report

<table>
<thead>
<tr>
<th>Antibiotic(s)</th>
<th>MIC (µg/ml)</th>
<th>Susceptibility&lt;sup&gt;a&lt;/sup&gt; according to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanic acid (2:1 ratio)</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>≤2/4</td>
<td>S</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.25</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.25</td>
<td>S</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.25</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacit</td>
<td>0.25</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.12</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤4</td>
<td>IE</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤1</td>
<td>IE</td>
</tr>
</tbody>
</table>

<sup>a</sup> S, susceptible; IE, insufficiency of evidence that the species in question is a good target for therapy with the drug.

<sup>b</sup> Interpretation was based on the EUCAST PK/PD breakpoints, version 5.0 (http://www.eucast.org/).

<sup>c</sup> Interpretation was based on the CLSI breakpoints for the HACEK group (Haemophilus spp., Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and Kingella kingae) in reference 15.

A. ureae meningitis occurred mainly in immunocompromised patients or after skull injury (4).

A. suis is an opportunistic pathogen of swine. A. suis is an early colonizer of the upper respiratory airways of swine and can also cause a wide range of invasive infections, including arthritis, pleuropneumonia, septicemia, and meningitis in pigs of all ages (6, 7, 8). A. equuli is a commensal of horses and is a common cause of septicemia in foals (9). A. suis and A. equuli have been reported to cause wound infections in humans after pig or horse bites (10, 11). A case of A. equuli septicemia in a 53-year-old butcher after he sustained a cut has been described (12). However, A. suis and A. equuli have never been reported to cause meningitis and sepsis in children. The most likely source of A. suis or A. equuli is the farm visited by the child 3 days before the onset of symptoms, although physical contact with swine or horses was denied.

The patient presented sepsis and the hallmark symptoms of meningitis, i.e., fever, headache, photophobia, neck stiffness, and severe drowsiness. No alteration of coagulation was observed. No characteristic signs or symptoms that indicate A. suis or A. equuli infection were identified. Empirical treatment with ceftriaxone was started as soon as meningitis was suspected, in accordance with an international guideline (13). However, when Gram staining revealed a Gram-negative bacillus, an Escherichia coli infection was suspected. Therefore, as a high prevalence of extended-spectrum β-lactamase-producing bacteria has been reported in our country (14), a switch to meropenem was made. Nevertheless, A. suis or A. equuli antimicrobial susceptibility showed low MICs of all of the antibiotics tested, including cephalosporins and carbapenems.

The clinical response was optimal, and the patient recovered without any sequelae.

When A. suis or A. equuli was identified, an immunological defect was suspected. A careful immunological evaluation, including T and B cell subsets, immunoglobulin levels and subclasses, and neutrophil function, was performed, and the immunological profile was completely normal. Moreover, the patient had experienced no other significant condition suggestive of immunological defects in the past.

As identification by conventional biochemical profiling with Vitek2 yielded an identification of A. ureae while identification of A. suis or A. equuli could only be suspected on the basis of MALDI-TOF mass spectrometry and confirmed by molecular identification, we suggest that at least some of the previously reported invasive infections by A. ureae could have been caused by A. suis or A. equuli.

In conclusion, we report the first case of A. suis or A. equuli sepsis and meningitis in a child. We did not identify any clinical characteristics that indicate A. suis or A. equuli infection. Misdiagnosis of this pathogen by the Vitek2 system could be possible. MALDI-TOF mass spectrometry and molecular biology tech-
niques may provide the proper diagnosis of this uncommon pathogen.

ACKNOWLEDGMENT

We have no conflicts of interest to report.

REFERENCES


