Septic Arthritis Caused by Noncapsulated *Haemophilus influenzae*

Sandra Le Quellec,a Olivier Gaillot,a Françcois Chotel,a Anne-Marie Freydière,a Frédéric Laurent,a,b François Vandenesch,a,b,g
Anne Doleans-Jordheim,a,b

Hospices Civils de Lyon, Laboratoire de Bactériologie, Centre de Biologie et de Pathologie Est, Lyon, France; Research Group on Bacterial Opportunistic Pathogens and the Environment, UMR 5557, Ecologie Microbienne, CNRS, Université Lyon 1, VetAgroSup, Université de Lyon, Lyon, France; Service de Traumatologie et d’Orthopédie Pédiatrique, Hôpital Femme-Mère-Enfant, Lyon, France; Laboratoire de Bactériologie-Hygiène, Centre de Biologie-Pathologie, Centre Hospitalier Régional Universitaire de Lille, Lille, France; Hospices Civils de Lyon, Laboratoire de Bactériologie de l’Hôpital de la Croix-Rousse, Lyon, France; INSERM, U851, Lyon, France; Centre National de Référence des Staphylocoques, Faculté de Médecine Lyon Est, Université de Lyon, Lyon, France

Since the introduction of type b *Haemophilus influenzae* vaccination, noncapsulated *H. influenzae* has become responsible for most cases of invasive *H. influenzae* diseases. In our two cases of septic arthritis, we isolated strains with β-lactamase-positive amoxicillin-clavulanate resistance (BLPACR). Thus, the increasing prevalence of BLPACR should be taken into account when empirical therapy is chosen for septic arthritis.

**CASE REPORTS**

**Patient 1.** A 1-year-old girl was admitted to the Pediatric Hospital in Lyon, France, in November 2011 with fever and inflammatory signs in her left elbow. She was born prematurely at 31 weeks of amenorrhea and had received 3 injections of diphtheria–tetanus–whole-cell pertussis–*Haemophilus influenzae* type b (DTPw/Hib) vaccine during her first year of life. One week before admission, she presented with a bilateral conjunctivitis associated with a progressive cutaneous rash. The diagnosis of aviral infection was entertained, and she was treated with acetaminophen (international nonproprietary name, paracetamol). A day before admission, she had a swollen, erythematous left elbow with a limited range of motion. Oral amoxicillin treatment was initiated.

Upon admission, the patient was febrile (38.5°C) and asthenic. The left elbow was flexed, swollen, warm, slightly erythematous, and painful upon mobilization. The white blood cell count was 109/liter, with 70% neutrophils (15.42 × 10⁹/liter), 20% lymphocytes, and 10% monocytes, and the C-reactive protein (CRP) concentration was 235.3 mg/liter. An X ray of her left elbow was normal, and an ultrasound scan showed a moderate intra-articular effusion and a superior radial metaphysis subperiostal abscess. Aspirate from the articulation was purulent. Arthrotomy was performed promptly to drain the elbow joint, and empirical antibiotic treatment with intravenous cefamandole (150 mg/kg of body weight/day) was initiated.

*Haemophilus influenzae* was identified from the elbow aspirate and from blood cultures by using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) technology (Vitek mass spectrometry [MS]; bioMérieux, France) on strains collected on chocolate agar plates (bioMérieux, France). The *H. influenzae* antiserum type b was negative, and the nitrocefin test was positive. According to the antibiogram realized with an ATB Haemo EU (08) panel (bioMérieux, France), the strain was resistant to amoxicillin but sensitive to amoxicillin-clavulanate, cefotaxime, moxifloxacin, tetracycline, rifampin, co-trimoxazole, and chloramphenicol. Upon receipt of the antibiotic, the antibiotic treatment was immediately modified to intravenous ceftriaxone (50 mg/kg/day) for 14 days. The patient’s temperature settled within a week, and the CRP concentration slowly decreased. The patient was discharged with an oral antibiotic treatment with amoxicillin-clavulanate (80 mg/kg/day) for 5 more weeks.

The National Reference Center of *Haemophilus influenzae* (Lille, France) identified a noncapsulated biotype III *Haemophilus influenzae* strain. The noncapsulated characteristics were confirmed by PCR (negative for the *bexA* gene). Antimicrobial susceptibility testing was carried out using the agar diffusion method. The isolated strain had a penicillinase and a mutation of the penicillin-binding protein 3 (PPB3) gene, which conferred resistance to ampicillin and amoxicillin-clavulanate (MIC = 2 μg/ml). The nonenzymatic resistance to β-lactam due to a mutation of the PPB3 gene was based on the increase in MIC and confirmed by PCR amplification of the *fisl* gene, encoding PPB3. According to the new antibiogram, the antibiotic treatment was modified to oral co-trimoxazole (30 mg kg⁻¹ day⁻¹ sulfamethoxazole and 6 mg kg⁻¹ day⁻¹ trimethoprim) for 2 weeks. At the end of the treatment, i.e., 2 months after admission, the patient’s elbow had improved significantly, with a range of motion back to normal. The leukocyte count had decreased to 11.5 × 10⁹/liter, and the CRP concentration was lower than 0.3 mg/liter. A control ultrasound scan reported a decrease of the intra-articular effusion.

**Patient 2.** A 66-year-old woman with fever and right-hip pain sought medical attention in the emergency department of the Edouard Herriot Hospital (Lyon, France) in November 2011. The patient had a long medical history, with rheumatoid polyarthritis diagnosed at the age of 17 years, and she had been treated with corticosteroids. Her past medical history was also remarkable for corticoid-induced diabetes, dyslipidemia, hypertension, and bilateral total hip and knee replacements. The rheumatoid polyarthritis was associated with rheumatoid vasculitis, which resulted in restrictive respiratory syndrome and ulcers of the lower extremities. The patient had a long medical history, with rheumatoid polyarthritis diagnosed at the age of 17 years, and she had been treated with corticosteroids. Her past medical history was also remarkable for corticoid-induced diabetes, dyslipidemia, hypertension, and bilateral total hip and knee replacements. The rheumatoid polyarthritis was associated with rheumatoid vasculitis, which resulted in restrictive respiratory syndrome and ulcers of the lower extremities.
ities. The ulcers were known to be infected by *Staphylococcus aureus* and had been treated with local wound care. Over the course of her treatment, multiple antibiotic-induced iatrogenic events occurred, including cytolyis, Quincke’s edema, febrile neutrope

Upon admission, the patient presented with fever, right-hip pain, and symptoms of depression. The pelvic X ray was unremarkable. The leukocyte count was 11.6 × 10^9/liter (no differential count of leukocytes was available), and the CRP concentration was 257 mg/liter. The aspirate from the hip was purulent, containing Gram-negative coccobacilli. The causative bacterium and the antibiotic were identified as previously described and showed an *H. influenzae* strain resistant to ampicillin because of a penicillinase (nitrocefin test positive) and sensitive to amoxicillin-clavulanate, cefotaxime, moxifloxacin, tetracycline, rifampin, co-trimoxazole, and chloramphenicol. Blood cultures were sterile. An empirical antibiotic treatment taking into account the patient’s allergy history was initiated and included intravenous imipenem (500 mg 4 times/day) associated with injections of gentamicin (15 mg/kg/day). A right-hip arthrotomy was performed 3 days later. The joint was drained and the right-hip prosthesis replaced at the same time. Blood, bones, and prosthesis material tissue cultures occurred, including cytolysis, Quincke’s edema, febrile neutropenia, and tendinitis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Comorbidity(ies)</th>
<th><em>H. influenzae</em> serotype</th>
<th>Resistance phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>Male</td>
<td>Rheumatoid polyarthritis, long-term corticotherapy</td>
<td>Noncapsulated</td>
<td>BLPACR</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>0.4</td>
<td>Male</td>
<td>Rheumatoid polyarthritis</td>
<td>Type a</td>
<td>β-Lactamase producer</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>46</td>
<td>Female</td>
<td>Rheumatoid polyarthritis</td>
<td>Noncapsulated</td>
<td>Wild type</td>
<td>8</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>Male</td>
<td>Chronic alcoholism, micronodular cirrhosis</td>
<td>Noncapsulated</td>
<td>Wild type</td>
<td>9</td>
</tr>
</tbody>
</table>

The incidence of *H. influenzae* septic arthritis in children has decreased significantly since the introduction of vaccine-based immunization. In a retrospective study in the United Kingdom of 16 cases of *H. influenzae* septic arthritis in children, 14 cases occurred prior to immunization and 2 occurred after immunization (5). Another study reported 8 new cases of *H. influenzae* type b septic arthritis after the introduction of *H. influenzae* type b vaccine, whereas *H. influenzae* type b was responsible for 41% of septic arthritis prior to the immunization programs (10). Only 4 cases of septic arthritis due to non-type b *H. influenzae* have been reported, 2 in children with *H. influenzae* type b vaccination (on schedule for the children’s ages) and in adults whose *H. influenzae* strains were sensitive to antibiotics (Table 1). In the present report, we offer two new cases of septic arthritis, one of which occurred in an immunized child, and both *H. influenzae* strains presented the same two resistance mechanisms to antibiotics.

Empirical treatment for septic arthritis is directed toward the most common causes, i.e., *Staphylococcus aureus*, non-*aureus* streptococci, and *Kingella kingae* among children. Such regimens are frequently ineffective against penicillinase-producing *H. influenzae*, as shown in the two patients reported in this article. Beta-lactamase production was determined with a nitrocefin disk by the chromogenic cephalosporin method. The modification of penicillin-binding protein type 3 (PBP3) is an emergent resistance mechanism presented by *H. influenzae*. PBP3 is one of the eight PBPs in *H. influenzae*, and it is encoded by the *fisI* gene. Several mutations of the *fisI* gene are responsible for amino acid substitutions within the highly conserved motifs in the transpeptidase domain (S379-S-N, K513-T-G) that alter the transpeptidase activity of PBP3 (11–13). These mutations are therefore responsible for amoxicillin-clavulanate resistance (11–13). *H. influenzae* strains with both β-lactamase production and alteration in PBP3 are reported as β-lactamase positive and amoxicillin-clavulanic acid resistant (BLPACR) (11). While the prevalence of BLPACR strains remains low, it is probably underestimated because of the lack of a consensus-defining breakpoint and consistent technical performances (11). The incidence of BLPACR strains is highly varied among countries (Japan, 1.3% to 11%; France, 14%; United States, 0.15%) (11, 13). Dabernat and Delmas described the BLPACR prevalence among all isolates of noncapsulated *H. influenzae* in children of 5 years of age or less, and the prevalence was equal to 6.4% within the 2001-2008 period and to 2.4% among invasive isolates (14). Finally, as reported in 2011, among 141 *H. influenzae* strains with mutations in the transpeptidase domain of the *fisI* gene, 47 (58.8%) were BLPACR (15). The phenotypic description of our strains as BLPACR was not found in the ATB Haemo (08) (bioMérieux, France) method, and our current *H. influenzae* antibiogram method should therefore...
be reevaluated in order to detect the modification of the PBP3 resistance mechanism.

The cases described in this report showed that a noncapsulated H. influenzae strain was capable of emerging as a pathogen causing septic arthritis in a child vaccinated with H. influenzae type b conjugate vaccine and in an immunosuppressed adult. Taking into account the resistance mechanisms occurring in the noncapsulated H. influenzae strain, empirical antibiotic therapy for septic arthritis should include coverage of the resistant strain described herein to avoid further articular and systemic deterioration.

ACKNOWLEDGMENTS
We acknowledge Pascal Rhéaume and Lars Petter Jordheim for assistance with the preparation of the manuscript.

REFERENCES