Septic arthritis caused by non-capsulated *Haemophilus influenzae*:

about two cases

Sandra Le Quellec (1), Olivier Gaillot (4), Franck Chotel (3), Anne-Marie Freydière (1), Frédéric Laurent (5,6), François Vandenesch (1,6,7), Anne Doléans-Jordheim (1,2)*

(1) Hospices civils de Lyon, Laboratoire de bactériologie, Centre de Biologie et de Pathologie Est, Lyon, France

(2) Research group on « Bacterial Opportunistic Pathogens and Environment », UMR 5557 Ecologie Microbienne, CNRS, Université Lyon 1, ENVL, Université de Lyon, F-69008, Lyon, France.

(3) Service de traumatologie et d'orthopédie pédiatrique, Hôpital femme-mère-enfant, Lyon, France

(4) Laboratoire de bactériologie-hygiène, Centre de biologie-pathologie, Centre hospitalier régional universitaire de Lille, France

(5) Hospices Civils de Lyon, Laboratoire de Bactériologie de l'Hôpital de la Croix-Rousse, Lyon, France

(6) INSERM, U851, Lyon 69008, France

(7) Centre National de référence des Staphylocoques, Faculté de Médecine Lyon Est, Université de Lyon, Lyon 69008, France

* corresponding author: Anne Doléans-Jordheim; anne.doleans-jordheim@univ-lyon1.fr

Groupe de Recherche "Bactéries pathogènes opportunistes et environnement", UMR 5557 Ecologie Microbienne

Laboratoire de Mycologie-Microbiologie (Pavillon Nétien 3ème étage)

Faculté de Pharmacie-ISPB

Université Claude Bernard Lyon 1

8, avenue Rockefeller, 69373 Lyon Cedex 08

Tel: +33 (0)4 78 77 75 15; Fax: +33 (0)4 78 77 72 12
Since introduction of type-b *Haemophilus influenzae* vaccination, non-capsulated *H.* influenzae is 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, increasing prevalence of BLPACR should be taken into account while choosing empiric therapy for septic arthritis.

**CASE REPORT**

**Patient 1**: A one-year-old girl was admitted at the Pediatric Hospital (Lyon, France) in November 2011 with fever and inflammatory signs to her left elbow. She was born prematurely at 31 weeks of amenorrhea and had received 3 injections of DTPw/Hib vaccine during her first year of life. One week before admission, she presented a bilateral conjunctivitis associated with a progressive cutaneous rash. The diagnosis of a viral infection was entertained and she was treated with paracetamol. A day before admission, she had a swollen, erythematous left elbow with 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 and slightly erythematous and painful upon mobilization. The white blood cells count was $22.00 \times 10^9$/liter with 70 % neutrophils ($15.42 \times 10^9$/liter), 20 % lymphocytes and 10 % monocytes, and the C-reactive protein (CRP) was 235.3 mg/liter. X-rays of her left elbow was normal, and ultrasound scan showed a moderate intra-articular effusion and a superior radial metaphysis subperiostal abscess. Aspirate from the articulation was purulent. Arthrotomy was performed readily to drain the elbow joint and empirical antibiotic treatment with intravenous cefamandole (150 mg/kg/day) was initiated.

*Haemophilus influenzae* was identified from the elbow aspirate and from blood cultures by using MALDI-TOF technology (VITEK®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) (bioMérieux, France), the strain was resistant to ampicillin but
sensitive to amoxicillin-clavulanate, cefotaxime, moxifloxacin, tetracyclin, rifampicin,
cotrimoxazole and chloramphenicol. Upon reception of the antibiogram, 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 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
biotype III non-capsulated Haemophilus influenzae. The non-capsulated characteristics were
confirmed by PCR (negative for the bexA gene). Antimicrobial susceptibility testing was
carried using agar diffusion method. The isolated strain had a penicillinase and a mutation of
the Penicillin Binding Protein 3 (PBP3) which conferred a resistance to ampicillin and
amoxicillin-clavulanate (minimal inhibitory concentration (MIC) = 2 µg/mL). The non-
enzymatic resistance to β-lactam due to a mutation of PBP3 was based on the increase in MIC
and confirmed by PCR amplification of the ftsI gene encoding PBP3. According to the new
antibiogram, the antibiotic treatment was modified to oral cotrimoxazole (30 mg kg⁻¹ day⁻¹
sulfamethoxazole and 6 mg kg⁻¹ day⁻¹ trimethoprim) for 2 weeks. At the end of the treatment,
\textit{i.e.} 2 months after admission, the patient’s elbow had improved significantly with a range of
motion back to normal. The leucocytes count had decreased to 11.5 x 10⁹/liter and the CRP
concentration was lower than 0.3 mg/liter. A control ultrasound scan reported decrease of
intra-articular effusion.

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

Upon admission, the patient presented fever, right hip pain and symptoms of depression. The pelvic x-ray was unremarkable. The leucocytes count was 11.6 x 10^9/liter (no leucocytes differential count available) and the CRP concentration was 257 mg/liter. The aspirate from the hip was purulent, containing Gram-negative cocco-bacilli. Identification of the causative bacteria and the antibiogram were realized as previously described showing a *H. influenzae* strain resistant to ampicilin because of a penicillinase (nitrocefin test positive), and sensitive to amoxicillin-clavulanate, cefotaxime, moxifloxacin, tetracyclin, rifampicin, cotrimoxazole and chloramphenicol. Blood cultures were sterile. An empiric antibiotic treatment taking into account the patient’s allergy history was initiated and included intravenous imipenem (500 mg x 4/day) associated with injections of gentamicin (15 mg/kg/day). A right hip arthrotomy was performed three days later. The joint was drained and the right hip prosthesis replaced at the same time. Blood, bones and prosthesis material tissue cultures were all negative, but *H. influenzae* was isolated from the hip fluid. Antibiotic treatment was thereby completed by associating intravenous ofloxacin (200 mg x 2/day) to the intravenous imipenem injections. Clinical and biological inflammatory markers improved within two weeks. The patient was discharged with her regular medication associated with an antibiotic treatment comprising subcutaneous ertapenem (1 g x 2/day) and oral ofloxacin (200 mg x 2/day) for 3 months. A mortified tooth was considered to be the source of the *H. influenzae* infection and was extracted.
The *H. influenzae* strain was identified by the National Reference Center of *H. influenzae* as a biotype II non-capsulated *H. influenzae*. It was sensitive to ceftriaxone (MIC = 0.015 µg/mL), moxifloxacin, tetracyclin and cotrimoxazole, and resistant to ampicillin because of a penicillinase and resistant to amoxicillin-clavulanate (MIC = 2 µg/mL) because of a mutation of the PBP3 similar to the strain from patient 1.

Non-type b and more specifically non-capsulated *H. influenzae* are uncommon causes of invasive diseases in children and adults. However, since the introduction of *H. influenzae* type b vaccine, the relative incidence of such infections has increased (1, 2, 3, 4). Non-capsulated *H. influenzae* has mostly been reported in association with septicemia (1, 2, 3, 5), meningitis (1, 3, 4) and pneumonia (1, 2). However, rare cases of septic arthritis have also been also described (6, 7, 8, 9).

The incidence of *H. influenzae* septic arthritis in children has decreased significantly since the introduction of vaccine-based immunization. In a retrospective study realized in United Kingdom on 16 cases of *H. influenzae* septic arthritis in children, 14 cases occurred prior to immunization and 2 occurred after immunization (5). Another study by reported no 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 % cases 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 was on schedule for the age and 2 in adults whose *H. influenzae* strains were sensitive to antibiotics (Table 1). In the present report we offered 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 towards the most common causes, *i.e.* *Staphylococcus aureus* and 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 by the chromogenic cephalosporin method, using a nitrocefin disk. The modification of penicillin-binding-protein type 3 (PBP3) is an emergent resistance mechanism presented by *H. influenzae*. PBP3 is one of the eight PBP in *H. influenzae*, encoded by the *ftsI* gene (11). Several mutations of the *ftsI* gene are responsible for amino-acid substitutions within the highly conserved motifs in the transpeptidase domain (S379-S-N, K-513-T-G) that alter the transpeptidase activity of the PBP3 (11, 13, 14). These mutations are therefore responsible for the amoxicillin-clavulanate resistance (11, 13, 14). *H. influenzae* strains with both β-lactamase production and alteration in PBP3 are reported as β-lactamase-positive amoxicillin/clavulanic acid resistant (BLPACR) (11). While the prevalence of BLPACR remains low, it is probably underestimated because of the lack of a consensus-defining breakpoint and technical performances (11). Incidence of BLPACR strains is highly variable among countries (Japan 1.3 % to 11 %, France 14 %, USA 0.15 %) (11, 14). Dabernat et al (2012) described BLPACR prevalence among all isolates of non-capsulated *H. influenzae* in children of 5 years of age or less equal to 6.4 % within the 2001-2008 period, and to 2.4 % among invasive isolates (15). Finally, as reported in 2011, among 141 *H. influenzae* strains with mutations in the transpeptidase domain of the *ftsI* gene, 47 were BLPACR (58.8 %) (12).

The phenotypic description as BLPACR of our strains was not found using 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 PBP3 resistance mechanism.

Cases described in this report showed the ability of a non-capsulated *H. influenzae* strain to emerge 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 non-capsulated *H. influenzae*, empirical antibiotic therapy for...
septic arthritis should include coverage of the resistant strain described herein to avoid further articular and systemic deterioration.

Acknowledgements:
The authors acknowledge Drs Pascal Rhéaume and Lars Petter Jordheim for assistance on the preparation of the manuscript.

References:


Table 1. Characteristics of septic arthritis due to non-type b *H. influenzae* cases reported among literature.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient A</th>
<th>Patient B</th>
<th>Patient C</th>
<th>Patient D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1</td>
<td>66</td>
<td>3</td>
<td>0.4</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>Sex</td>
<td>woman</td>
<td>man</td>
<td>woman</td>
<td>man</td>
<td>woman</td>
<td>man</td>
</tr>
<tr>
<td>Comorbidity</td>
<td>Born prematurely, underweight</td>
<td>Rheumatoid polyarthritis, long-term corticotherapy</td>
<td>-</td>
<td>-</td>
<td>Rheumatoid polyarthritis</td>
<td>Chronic alcoholism, micronodular cirrhosis</td>
</tr>
<tr>
<td><em>H. influenzae</em> serotype</td>
<td>Non-capsulated</td>
<td>Non-capsulated</td>
<td>Type a</td>
<td>Type f</td>
<td>Non-capsulated</td>
<td>Non-capsulated</td>
</tr>
<tr>
<td>Resistance phenotype</td>
<td>BLPACR</td>
<td>BLPACR</td>
<td>β-lactamase producer</td>
<td>Wild</td>
<td>Wild</td>
<td>Wild</td>
</tr>
<tr>
<td>References</td>
<td>Our study</td>
<td>(6)</td>
<td>(7)</td>
<td>(8)</td>
<td>(9)</td>
<td></td>
</tr>
</tbody>
</table>