APPLICATION OF RRS GENE SEQUENCING TO ELUCIDATE THE CLINICAL SIGNIFICANCE OF EGGERTHELA LENTA INFECTION.

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Eggerthella lenta, a bacterium of the intestinal human gut flora has rarely been reported to cause significant infections. We report two cases of bacteremia and the first case of liver abscess due to Eggerthella lenta identified with the rrs gene analysis.
CASE REPORT

Case 1.

An 82-year-old man was admitted with fecal peritonitis related to intestinal perforation. His past medical history was unremarkable except a senile dementia. He complained from an acute abdominal pain. At examination, abdominal contracture and high grade fever were noted, but blood cultures remained sterile. A bowel colostomy was undertaken and the patient was started on broad spectrum antibiotic therapy (tazocillin 12g/day and ciprofloxacin 500mg/bid). After 10 days of such therapy, the fever relapsed. Urinary bacterial culture was sterile but anaerobic Gram-positive bacilli were isolated from one blood culture and identified by rrS sequencing as Eggerthella lenta. The isolate was susceptible to amoxicillin and clavulanic acid, imipenem, metronidazole and vancomycin. A CT scan did not show any deep abscess and a transoesophageal echocardiography was normal. The patient responded favourably to a three-week course of imipenem (1.5g/day). He still remains well at follow up.

Case 2.

A 33-year-old man was hospitalized with acute appendicitis. No underlying conditions were noted. After surgical removal of the appendix, the histopathologic analyse showed acute inflammation involving appendicular mucosa and submucosa but no suppurative inflammation. The surgical treatment was rapidly complicated by a febrile parietal abscess at the caecum, at the surgical insertion site. Two sets of blood cultures were collected from which two anaerobic bacteria were identified. Fusobacterium mortiferum was identified with conventional methods by using API 20NE system (bioMérieux, France), but the other Gram-positive bacillus was identified as E. lenta by rrS gene sequencing. They were both susceptible to amoxicillin and clavulanic acid, imipenem and vancomycin. The patient received amoxicillin and clavulanic acid (3g/day) with a quick and favourable outcome.
Case 3.

A 42-year-old woman was admitted with fever and abdominal pain. Her medical past history included an ovarian carcinoma with peritoneal metastatic involvement for which she received antimitotic chemotherapy. A gastro-intestinal derivation was placed for intestinal obstruction a few months ago. At admission, an abdominal CT scan confirmed the presence of a liver abscess that was treated by surgical drainage. Collected pus from the abscess grew two anaerobic bacilli, which were identified by rrs gene sequencing as *Clostridium paraputrificum* and *E. lenta*. They were only susceptible to metronidazole and vancomycin. Antibiotic therapy with metronidazole (1.5g/day) was started. The patient responded favourably.

The bacterial strains in these cases were isolated in blood cultures from patient 1 and 2 and from a liver abscess for patient 3. After 2-4 days, Gram-positive bacteria were isolated from anaerobic blood cultures with the BACTEC 9240 automated blood system (Becton Dickinson and Co., Sparks, Md). Then, blood and pus were cultured onto Columbia sheep blood agar (bioMérieux, Marcy l’étoile, France) and incubated in anaerobic atmosphere during 5 days. Small translucent colonies resulted. An identification using API 20A strips (bioMérieux, France) was performed allowing the identification of *E. lenta* between 90% and 96% of identity. In view to accurately identify these micro-organisms, the 16s r RNA gene comparison were performed (Service of Bacteriology, Hospital Timone, Marseille, France), and the 3 bacteria were identified as *E. lenta* with 99% of homology upon 1,499 nt fragment (GENBANK accession AF 292375) for all the 3 strains (1).

The 3 clinical cases that we have reported thus far are reported in Table 1(Patient n°1, 2 and 3), as well as the other 5 cases cited in Lau et al.(2) The median age was 57 (range: 33-82).

Two of our patients had underlying severe diseases notably one have an ovarian carcinoma. In the three cases we reported, all patients had intestinal underlying diseases and only one had a monomicrobial infection. Antibiotic susceptibility was different between the 3 strains. Two
strains were susceptible to Amoxicillin/clavulanic acid, imipenem, metronidazole and vancomycin but one was susceptible to metronidazole and vancomycin only.

DISCUSSION

The genus *Eggerthella* includes anaerobic, none sporulating, gram positive bacilli. They were named in honor of Arnold Eggerth, who made the first description in 1935 (3). *Eggerthella* gen. nov. was proposed to substitute for *Eubacterium lentum* in 1999 on the basis of the rrs sequence divergence from *Collinsella aerofaciens* and *Coriobacterium glomerans* and the presence of unique phenotypic characters (4,5). *Eggerthella lenta* comb. nov. is one species of this new group.

*E. lenta* (formaly *Eubacterium lentum*) belongs to the intestinal microflora of humans. Few cases due to *E. lenta* have been reported in human. Examples of pathological cases due to *E. lenta* are the following: post- gynaecological surgery, chorioamniotitis after cervical cerclage required for gravid patients and infection of the female genital tract due to intra uterine devices (6). Specific and rare cases have been reported implying the same infectious agent such as: frontal sinusitis with intracranial complications occurred in a young men with sickle cell disease (7), a cutaneous abscess in a IVDU (8) and occurrence of bacteraemia in two single cases, one after a sub gingival irrigation (9) and another in association with a concomitant cytomegalovirus pneumonia (10).

*E. lenta* has also been isolated from appendix tissue samples obtained at surgery from children with suspected appendicitis (11), and isolated in polymicrobial infections with others anaerobic bacteria, notably in colonized aortic aneurysms wall (12). Pelvic inflammatory diseases, infected bed sore might be complicated by *E. lenta* bacteraemia (2).

Throughout the available literature, *Eggerthella sp.* bacteremia was reported in 10 cases,
including 5 due to *E. lenta* only. These 5 cases and in addition 3 more encountered in our unit (infectious and tropical diseases department, Marseille, France) are included in Table 1. *E. lenta* bacteremia has been associated with high risk of morbidity and mortality such as septic shock and disseminated intravascular coagulation (2,13). Among patients with documented bacteraemia, the major risk factors were advanced age and underlying diseases such as neoplasia, immunosuppression and involvement of the gastro-intestinal or genital tract (2,5). In our three cases, patients had a past history of digestive disease.

To our knowledge, *E.lenta* has never been isolated in liver abscess, probably because the samples are currently polymicrobial and *E. lenta* is difficult to isolate with conventional methods.

Molecular techniques are very useful for the specific diagnosis of polymicrobial anaerobic non-sporeulating Gram-positive bacilli bacteraemia. Two novels species, *E. hongkongensis* and *E. sinensis*, have been identified by rrs gene sequencing (13). For the first time, we have been able to identify *E. lenta* in a hepatic abscess using molecular techniques. These 3 cases that we report confirm that *E. lenta* is likely to be a more common pathogen than previously expected. Moreover, it is frequently associated with polymicrobial bacteremia and a remarkable and consistent finding is that *E. lenta* is susceptible to different antibiotics explaining the diversity of antibiotic treatment given to these patients.

Based on our findings, we assume that the application of rrs gene sequencing would thus enable to wider clinical spectrum of *E. lenta* infection.
REFERENCES


Table 1: Characteristics of the patients with *E. lenta* bacteremia.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
<th>4†</th>
<th>5†</th>
<th>6†</th>
<th>7†</th>
<th>8†</th>
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<tbody>
<tr>
<td>Age/sex</td>
<td>M/82</td>
<td>M/33</td>
<td>F/69</td>
<td>M/74</td>
<td>F/75</td>
<td>F/84</td>
<td>F/87</td>
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<td>Diagnosis</td>
<td>Intestinal obstruction</td>
<td>Acute appendicitis</td>
<td>Liver abscess</td>
<td>Primary bacteremia</td>
<td>Primary Bacteremia</td>
<td>Pelvic inflammatory disease</td>
<td>Infected sacral sore</td>
<td>Infected buttock sore</td>
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<tr>
<td>Complications</td>
<td>Intestinal perforation</td>
<td>Parietal abscess</td>
<td>None</td>
<td>None</td>
<td>Septic shock</td>
<td>Disseminated intravascular coagulation</td>
<td>None</td>
<td>Cerebrovascular accident</td>
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<td>Senile demence</td>
<td>None</td>
<td>Ovary carcinoma</td>
<td>Intestinal resection</td>
<td>Immunodepression</td>
<td>Carcinoma of lung</td>
<td>Alcoholic cirrhosis</td>
<td>Gallstones, Gastrointestinal bleeding</td>
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<td>Number of positive blood cultures</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>Mono/polymicrobial sample/bacteremia</td>
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<td>Poly</td>
<td>Poly</td>
<td>Poly</td>
<td>Mono</td>
<td>Poly</td>
<td>Mono</td>
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<td>Concomitant isolates</td>
<td><em>Fusobacterium mortiferum</em></td>
<td><em>Clostridium paraputrificum</em></td>
<td><em>Prevotella intermedia</em></td>
<td><em>Moranella morgani</em></td>
<td><em>Bacteroides splanchnicus, Arcanobacterium haemolyticum</em></td>
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<td>Antibiotic susceptibility</td>
<td>Amoxicillin/ clavulanic acid</td>
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<td>Metronidazole</td>
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<td>Metronidazole</td>
<td>Ticarcillin/Clavulanate</td>
<td>Cefuroxime</td>
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<td></td>
<td>Imipenem, Metronidazole Vancomycin</td>
<td>Imipenem, Metronidazole Vancomycin</td>
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* Patients of our unit.
† (5).