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CASE REPORT

First Case of *Streptococcus lutetiensis* Bacteremia Involving a Clindamycin-Resistant Isolate Carrying the *lnuB* Gene

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Here, we describe the first case of a *Streptococcus lutetiensis* isolate harboring the *lnuB* gene.

CASE REPORT

A 70-year-old male patient had a history of fever, epigastric, and right flank pain of 1 month of evolution. One week prior to admission, residual choledochal microlithiasis without bile duct dilatation was found on echoendoscopy. The patient had had a cholecystectomy 10 years before.

On the night before the emergency room consultation, the patient had postprandial epigastralgia, fever, and chills; therefore, his hospitalization was decided. During hospitalization, endoscopic retrograde cholangiopancreatography (ERCP) was performed, showing microgallstones. They were removed by the use of a Dormia basket and an extraction balloon catheter. The admission diagnosis was cholangitis due to residual lithiasis.

Admission laboratory findings were as follows: white blood cell count, 4,900/mm^3 (with 86% neutrophils); hematocrit, 37%; kaolin partial thromboplastin time (KPTT), 34 s; and prothrombin time, 70%. Liver function test results were as follows: alanine aminotransferase (ALT), 716 U/liter (normal, <35 U/liter); aspartate aminotransferase (AST), 218 U/liter (normal, <35 U/liter); total bilirubin, 0.7 mg/dl (normal, <1.0 mg/dl); direct bilirubin, 0.3 (normal, <0.3 mg/dl); alkaline phosphatase, 353 U/liter (normal, <279 U/liter); and total cholesterol, 103 mg/dl (normal, 150 to 200 mg/dl).

In two blood culture bottles taken on admission, after 24 h of incubation, growth of *Streptococcus infantarius* with *Escherichia coli* was obtained.

Phenotypic identification was carried out by conventional biochemical tests (1). The organism was identified as *Streptococcus infantarius*; however, it was not possible to determine the subspecies by this methodology. In addition, we used the GP card of a Vitek 2 system (bioMérieux, Marcy-l'Etoile, France). The bionumber obtained was 141011164717711, giving an identification of *S. infantarius* subsp. *coli*/*Streptococcus bovis* with an excellent confidence level. Identification was also carried out by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik), showing a spectral score of 2.223 for *Streptococcus lutetiensis* (2).

PCR amplification of the 16S rRNA gene was performed in order to reach definitive identification. A PCR product of the 16S rRNA gene, using the primers described by Weisburg et al. (3), was obtained with the *Taq* DNA polymerase based on the manufacturer's specifications (Promega). Sequencing of the 1.4-kb PCR product was performed on both DNA strands at the sequencing facility of Macrogen, Inc., Seoul, South Korea. The sequences were analyzed using BLAST V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/) and showed 99% identity with the sequences corresponding either to the 16S RNA ribosomal gene of *S. infantarius* subsp. *infantarius* (GenBank accession number EU420174) or to the 16S RNA ribosomal gene of *S. lutetiensis* (GenBank accession number NR_037096). In order to discriminate subspecies, we amplified the *sodA* gene (coding for the manganese-dependent superoxide dismutase) following the methodology described by Poyart et al. (4, 5). A PCR product of 404 bp was obtained using the primers described by those authors (4, 5). Sequence analysis revealed 100% identity with the *sodA* sequence of *Streptococcus lutetiensis* (GenBank accession number AY035713). These results confirmed the species identification. Several different methods were used to arrive at definitive identification because routine phenotypic methods (conventional biochemical tests and automated methods) and 16S rRNA gene sequencing cannot differentiate *S. lutetiensis* from *S. infantarius*.

Disk diffusion was performed following CLSI guidelines (6). Furthermore, susceptibility to 7 antimicrobial agents was determined by the Etest technique (bioMérieux) on Mueller-Hinton agar with 5% sheep blood following the manufacturer's specifications. The MIC breakpoints used in this study were those established by the Clinical and Laboratory Standards Institute (CLSI) in 2012 (6) for the *Streptococcus* subsp. *viridans* group. The MICs (in μg/ml) for *S. lutetiensis* isolate were as follows: for penicillin, 0.032; for ceftriaxone, 0.023; for vancomycin, 0.38; for ciprofloxacin, 0.75; for erythromycin, 0.064; for clindamycin, 2.0; and for lincomycin, 128.

The phenotypic characterization was complemented by a modified triple-disk induction test as previously described (7). In the test, lincomycin and clindamycin disks were placed 15 mm apart at the sides of an erythromycin disk. No inhibition zones were observed around clindamycin and lincomycin disks; no inducible pattern was detected.

To determine the lincosamide resistance mechanism (L-phenotype: erythromycin susceptible but clindamycin resistant), de-

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tection of the *lnuB* gene, encoding the lincosamide nucleotidyltransferase enzyme, was performed. The presence of the *lnuB* gene was detected using primers that have been previously described (8) and was confirmed by sequencing. The nucleotide sequence was deposited in the EMBL/GenBank/DDBJ databases under accession number KC688833. The isolate was also tested for *ermB*, *ermTR*, *lnuB*, and *mefA*/*mefE* with negative results.

Given the clinical picture of cholangitis (fever and epigastric and right flank pain), and with the preliminary report of *Streptococcus infantarius* with *Escherichia coli* isolation in blood cultures, treatment with ampicillin-sulbactam at 3.0 g/6 h administered intravenously (i.v.) plus ciprofloxacin at 400 mg/12 h administered i.v. was started.

After the antibiotic susceptibility report for both microorganisms was received, ciprofloxacin was discontinued.

After 3 days of treatment with this antimicrobial agent, the patient was afebrile and recovered well. He was discharged on ertapenem at 1 g/day administered i.v. for 15 days.

In the nineties, and after several different proposals, the members of the *Streptococcus bovis*/*S. equinus* group were reclassified based on their phenotypic and genotypic differences from *Streptococcus gallolyticus* subsp. *gallolyticus* (9), formerly *S. bovis* biotype I; *Streptococcus lutetiensis*, previously known as *Streptococcus infantarius* subsp. *coli* (4), which in turn corresponds to *S. bovis* biotype II/1; and finally, *Streptococcus gallolyticus* subsp. *pasteurianus* (formerly *S. bovis* biotype II/2) (4). Species from this group are frequently encountered in blood cultures of patients with bacteremia, sepsis, and endocarditis. The clinical significance of *S. bovis* group growing in blood culture is based on the association of S. *gallolyticus* subsp. *gallolyticus* with gastrointestinal disorders, including colon cancer and chronic liver disease, and of *S. gallolyticus* subsp. *pasteurianus* with meningitis and with hepatobiliary infections (although not with colorectal neoplasias) (1, 10–12).

Other species that are also part of this group but less related to human infections are *Streptococcus equinus*, *Streptococcus gallolyticus* subsp. *macedonicus*, *Streptococcus infantarius* subsp. *infantarius*, and *Streptococcus alactolyticus* (13).

In regard to *S. bovis* group antibiotic susceptibility, resistance to erythromycin and to clindamycin in this group has previously been described by Rodríguez-Avial et al. (14). In their study, of a total of 18 isolates, 78% were resistant to erythromycin and 72% were resistant to clindamycin. Among their isolates, the $cMLS_B$ (constitutive MLS_B) phenotype (where "MLS_B" represents "macrolide-lincosamide-streptogramin B") was predominant, and in all of them, the *ermB* gene was detected; the $iMLS_B$ (inducible MLS_B) phenotype was detected in only one erythromycin-resistant isolate. Additionally, differences in the rates of resistance to erythromycin and clindamycin were observed among the different subspecies of *Streptococcus gallolyticus*, *S. infantarius* subsp. *infantarius*, and *Streptococcus lutetiensis* in a study by Romero et al. (15). The highest percentage of resistance was obtained for *S. lutetiensis* (60% resistant to erythromycin and to clindamycin; $MIC_{50} > 2$ μg/ml) (15). These results differ from those reported by Beck et al. (1). In their work, 94% of the isolates of *S. lutetiensis* tested were susceptible to erythromycin (MIC_{90}, 0.12 μg/ml) whereas clindamycin susceptibility was not reported (1). The highest percentages of erythromycin resistance were observed for *S. gallolyticus* subsp. *gallolyticus* isolates ($MIC_{90} > 32$ μg/ml) and for *S. gallolyticus* subsp. *pasteurianus* isolates ($MIC_{90} > 32$ μg/ml); however, no erythromycin resistance mechanism was recorded by those authors (1). In our work, the *S. lutetiensis* isolate was PCR positive only for the *lnuB* gene, representing the first description of this gene within this species. Among streptococci, the *lnuB* gene was also described in *Streptococcus agalactiae* (16, 17) and *Streptococcus dysgalactiae* subsp. *equisimilis* and in *Streptococcus uberis* (18, 19).

The difficulty in differentiating *Streptococcus infantarius* subspecies using conventional biochemical tests has been reported by other authors. Beck et al. (1) found that some features of *S. infantarius* subsp. *coli* (now *S. lutetiensis*) (among 17 isolates studied) were different from the data given by Schlegel et al. (13, 20), especially regarding hydrolysis of esculin and acidity from glycogen, trehalose, and starch. These different characteristics, together with other results from the largest number of S. *infantarius* subsp. *coli* strains to be published, allowed Beck et al. to create an amended species description for *S. infantarius* subsp. *coli* (1). Also, the limitations of the 16S RNA sequencing performed to identify members of *Streptococcus bovis* group have been indicated by Poyart et al. (4). Those authors pointed out that the 16S rRNA gene sequences of strains from *S. infantarius* sp. *coli* were almost identical to those of the type strains of *S. bovis* (99% ± 9%) and *S. infantarius* (99% ± 9%). To differentiate such strains, those authors proposed the use of an alternative single-copy target sequence which exhibits greater sequence divergence than that of 16S rRNA gene: the *sodA* gene of the Gram-positive cocci, which encodes the manganese-dependent superoxide dismutase (Mn-SOD), allows differentiating closely related species belonging to the *Streptococcus* and *Enterococcus* genera (4, 5).

In conclusion, we describe the first case of an *S. lutetiensis* isolate harboring the *lnuB* gene, highlighting that monitoring antibiotic resistance in members of the *S. bovis* group is necessary not only to detect new resistance mechanisms but also in cases in which clindamycin could be a therapeutic option.

Nucleotide sequence accession numbers. The obtained sequences for the *Streptococcus lutetiensis sodA* and *lnuB* genes have been deposited at GenBank under accession numbers KC714048 and KC688833, respectively.

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