Streptococcus lutetiensis Bacteremia. First Clindamycin Resistant Isolate Carrying *lnu*B Gene.

Almuzara M[1], Bonofiglio L[2], Cittadini R[3], Vera Ocampo C[3], Montilla A[2], del Castillo M[3], Ramirez MS[4], Mollerach M[2], Vay C[1]*.

[1]Laboratorio de Bacteriología. Departamento de Bioquímica Clínica. Hospital de Clínicas José de San Martín. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Ciudad Autónoma de Buenos Aires, Argentina.

[2] Cátedra de Microbiología. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires. Argentina.

[3] Sanatorio Mater Dei, Ciudad Autónoma de Buenos Aires, Argentina.

[4] Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina.

*Corresponding author. Mailing address: Avenida Córdoba 2351. First floor. Ciudad Autónoma de Buenos Aires. Zip code: 1120. Argentina. Phone: 54 11 59508663; Fax: 54 11 59508691

E-mail: cavay@fibertel.com.ar

Abstract

Herein, we describe the first case of *S. lutetiensis* isolate harboring the *Inu*B gene

Case report

A 70-year-old male patient had a history of fever, epigastric and right flank pain of one month of evolution. One week prior to admission, residual choledochal microlithiasis without bile duct dilatation was found on echoendoscopy. The patient 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 micro –gallstones. They were removed by Dormia basket and extraction balloon catheter. Admission diagnosis was cholangitis due to residual lithiasis.

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

In two blood sample cultures taken on admission, after 24 hours 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 the

VITEK 2 system (bioMerieux, Marcy-l_Etoile, France). The bionumber obtained was 141011164717711, giving an identification of *S. infantarius* subsp *coli*; now *Streptococcus lutetiensis* (4)/*Streptococcus bovis* with an excellent confidence level. Identification was also carried out by matrix-assisted laser desorption ionization–time-of-flight (MALDITOF) mass spectrometry (MS) (Bruker Daltonik) showing a spectral score of 2.223 for *Streptococcus lutetiensis* (2).

PCR amplification of the 16S rRNA was performed in order to reach definitive identification. 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 Macrogen, Inc., Seoul, Korea sequencing facility. The sequences were analyzed using the Blast V2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/), showing a 99% identity with the sequences corresponding either to the 16S RNA ribosomal gene *S. infantarius* subsp. *infantarius* (GenBank accession number EU420174) or to the 16S RNA ribosomal gene *S. lutetiensis* (GenBank accession number NR 037096). In order to discriminate subspecies, we amplified the *sod*A 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 these authors (4, 5). Sequence analysis revealed 100% identity with the *sod*A sequence of *Streptococcus lutetiensis* (GenBank accession No. AY035713). These results confirmed the species identification.

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 2012 (6) for *Streptococcus* spp. *viridans* group. The MICs for *S. lutetiensis* isolate were as follows (µg/mL): penicillin 0.032; ceftriaxone 0.023; vancomycin 0.38; ciprofloxacin 0.75; erythromycin 0.064; clindamycin 2.0 and 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 at the sides of an erythromycin disk 15 mm apart. 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), detection of *lnu*B gene, encoding the lincosamide nucleotidyl-transferase enzyme was performed. The presence of *lnu*B gene was detected using the previously described (8) primers and confirmed by sequencing. The nucleotide sequence was deposited in the EMBL/GenBank/DDBJ databases under accession number KC688833.

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 3.0 g/6 hours/IV plus ciprofloxacin 400 mg/12 hours/IV was started.

After receiving the antibiotic sensitivity report of both microorganisms, ciprofloxacin was discontinued.

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

In the nineties, and after several different proposals, the group *Streptococcus bovis / equinus* was reclassified based on their phenotypic and genotypic differences in: *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 *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 *S. gallolyticus* subsp. *pasteurianus* with meningitis (1, 10-12). Other species that are also part of this group but less related to men are *Streptococcus equinus*, *Streptococcus gallolyticus* subsp. *macedonicus*, *Streptococcus infantarius* subsp. *infantarius*, and *Streptococcus alactolyticus* (13).

As regards *S. bovis* group antibiotic susceptibility, a high rate of resistance to erythromycin and to clindamycin in this group has previously been described by Rodríguez-Avial et al. (14). In their study, on a total of 18 isolates, 78% were resistant to erythromycin and 72% were resistant to clindamycin. Among their isolates, the $cMLS_B$ phenotype was the most predominant and in all of them, the *erm*(B) gene was detected; the $iMLS_B$ phenotype was only detected in one erythromycin-resistant isolate. Additionally, differences in the rates of resistance to erythromycin and clindamycin were observed among the different subspecies

141 of *Streptococcus gallolyticus*, *S. infantarius* subsp. *infantarius* and *Streptococcus lutetiensis*
142 in the Romero et al. study (15).The highest percentage of resistance was obtained for *S.*
143 *lutetiensis* (60 % resistant to erythromycin and to clindamycin, $MIC_{50} > 2$ µg/ml) (15).
144 These results differ from those reported by Beck et al. (1). In their work, 94% of the
145 isolates of *S. lutetiensis* tested were susceptible to erythromycin (MIC_{90} 0.12 µg/ml) while
146 clindamycin susceptibility was not reported (1). The highest percentages of erythromycin
147 resistance were observed on *S. gallolyticus* subsp. *gallolyticus* (MIC90 > 32 µg/ml) isolates
148 and on *S. gallolyticus* subsp. *pasteurianus* isolates (MIC90 > 32 µg/ml); however
149 erythromycin resistance mechanism was not recorded by these authors (1). In our work, *S.*
150 *lutetiensis* isolate was PCR positive only for *lnu*B gene, representing the first description of
151 this gene within this species. Among streptococci, *lnu*B gene was also described in
152 *Streptococcus agalactiae* (16, 17); *Streptococcus dysgalactiae* ssp. *equisimilis* and in
153 *Streptococcus uberis* (18, 19).
154 The difficulty to differentiate *Streptococcus infantarius* subspecies using conventional
155 biochemical tests has been reported by other authors. Beck et al. (1) found that some
156 features of *S. infantarius* subsp. *coli* (now *S. lutetiensis*) (on 17 isolates studied) were
157 different from the data given by Schlegel, L. et al. (13, 20) especially regarding hydrolysis
158 of esculin and acidity from glycogen, trehalose and starch. These different characteristics,
159 together with the largest number of S. *infantarius* subsp. *coli* strains published, allowed
160 Beck et al. to create an amended species description for *S. infantarius* subsp. *coli* (1). Also,
161 the limitations of the 16S RNA sequencing to identify members of *Streptococcus bovis*
162 group has been indicated by Poyart et al. (4). These authors pointed out that the 16S rDNA
163 sequences of strains from *S. infantarius* sp *coli* were almost identical to those of the type of

S. bovis (99±9%) and *S. infantarius* (99±9%) strains. To differentiate such strains, these authors proposed the use of an alternative single-copy target sequence which exhibits greater sequence divergence than that of 16S rDNA: The *sod*A 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* (4,5) genera.

In conclusion, we describe the first case of *S. lutetiensis* isolate harbouring the *Inu*B gene highlighting that antibiotic resistance in *S. bovis* group monitoring is necessary not only to detect new resistance mechanisms but also for a better therapeutic management when clindamycin is indicated.

Nucleotide sequence accession number. The obtained sequences for the *Streptococcus lutetiensis sod*A and *lnu*B genes have been deposited at GenBank under accession numbers KC714048 and KC688833, respectively.

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