Lactococcus garvieae endocarditis in a native valve identified by MALDI-TOF MS and PCR-based 16s rRNA in Spain: A case report

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Abstract

Lactococcus garvieae is a Gram-positive, catalase negative coccus arranged in pairs or short chains, well-known as a fish pathogen. We report a case of Infective Endocarditis (IE) by *L. garvieae* in a native valve from a 68-year-old male with unknown history of contact with raw fish and an extensive history of heart disease. This case highlights the reliability of MALDI-TOF MS compared to conventional methods in the identification of rare microorganisms like this.

keywords: Endocarditis, Lactococcus garvieae, MALDI-TOF, native valve, PCR

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Introduction

Lactococcus garvieae is a Gram-positive, catalase-negative coccus arranged in pairs or short chains that was formerly included in the lactic group of Streptococcus. However, this coccus was eventually excluded from this group in 1985 basing on 16S rRNA sequencing data. Since *L. garvieae* is a well-known fish pathogen, human infections are usually related to contact with raw fish. Infective Endocarditis (IE) by *L.garvieae* is extremely unusual and may involve both native and prosthetic valves. Due to its morphology and biochemical similarities with the *Enterococcus* genus, *L.garvieae* IE is probably underdiagnosed.

Case

We present the case of a 68-year-old male with hypertension, dyslipidemia, Hodking Lymphoma in remission and a long history of heart disease: implantation of a DDD-mode pacemaker due to a complete atrioventricular block, aortic valve replacement with a mechanical prosthetic Sorin 18mm-valve as a result of severe degenerative aortic stenosis, and significant peri-prosthetic regurgitation requiring percutaneous repair with an Amplatzer-type device.

In February 2014, the patient presented to the Emergency Department with fever of unknown origin of ten days duration. Laboratory data revealed elevation of acute phase reactants (PCR 24.9mg/dl, PCT 0.72ng/ml), as well as neutrophilic leukocytosis, so one set of blood culture was taken. The patient was discharged home with a prescription for empirical antimicrobial treatment with cefditoren 400mg/12h.

Blood culture grew Gram-positive, catalase-negative cocci arranged in short chains with gamma-haemolysis on blood agar after 24h incubation and blue colonies on CHROMAGAR (BD®). Susceptibility testing directly performed from blood culture revealed its sensitivity to vancomycin, ampicillin, cefotaxime, and oxacillin, so we discarded the *Enterococcus* genus. MALDI-TOF MS mass spectrometry (Bruker Daltonics®) identified it as *Lactococcus garvieae* with a good score (2.126).

Two weeks later, the patient returned to the Emergency Department in bad clinical condition compatible with acute heart failure (IV NYHA functional class). On suspicion of IE, the patient was admitted in Cardiology Department, where an emergency transthoracic echocardiography (TTE) was performed, revealing a calcified mitral valve with vegetations and possible rupture of the posterior leaflet, with moderate-high failure. Thus, empirical antibiotic therapy was started with daptomycin 850mg/48h, ampicillin 2g/6h and ceftriaxone 2g/12h and new blood cultures were taken.

Next, we performed the susceptibility testing according the CLSI guidelines employing the Epsilon-Test method to evaluate the following antibiotics (Table 1): penicillin-I (MIC: 0.75mg/dl) cefotaxime-S (MIC: 0.38mg/dl), erythromycin-S (MIC: 0.25mg/dl), vancomycin-S (MIC: 1mg/dl), daptomycin-S (MIC: 1mg/dl), levofloxacin-S (MIC: 1.5 mg/dl) and clindamycin-R (MIC: 1mg/dl). Due to the lack of experimental data for the interpretation

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TABLE 1. Susceptibility pattern of *Lactococcus garvieae* isolated in our patient carried out by E-test method

Antibiotic	MIC (μg/ml)	Interpretation
Penicillin	0.75	I
Cefotaxime	0.38	S
Erythromycin	0.25	S
Vancomycin	1	S
Daptomycin	1	S
Levofloxacin	1.5	S
Clindamycin	1	R

of MICs in the *Lactococcus* genus, we used *Streptococcus spp*. breakpoints as a reference [1]. The susceptibility pattern confirmed that the microorganism was *Lactococcus garvieae* [2]. Following confirmation of the bacterial species, gentamicin was added to improve the treatment of IE. However, gentamicin was discontinued as a result of acute renal failure after three days of treatment. All blood cultures collected on Cardiology Department were negative.

Later, a transesophageal echocardiography (TEE) was performed, showing the presence of mitral abscesses in addition to what was observed in TTE. The patient was referred for replacement surgery of the native mitral valve with a prosthetic Sorin 27mm-valve. The course was complicated involving persistent shock and multiorganic failure resulting in patient's death the same day of surgery.

The mitral valve removed in the surgical intervention was sent to the Microbiology Department of our institution for analysis. Culture of the valve was negative with no microorganisms observed in Gram stain. To confirm the diagnosis of IE caused by *L. garvieae*, a fragment of heart valve tissue was sent to Clinical Microbiology Department of University Hospital Gregorio Marañón, in Madrid, for analysis only by 16S rRNA gene PCR and sequencing. These tests confirmed the presence of *Lactococcus garvieae* in the native mitral valve.

Discussion

Lactococcus spp. is mainly associated to infective endocarditis, but it may be involved in lumbar osteomyelitis [3], hepatic abscess [4] and hip prosthetic infections [5]. Generally, *L. garvieae* IE has been associated with contact with raw fish [6–9], but in our case, the origin was unknown. Digestive pathologies such as colonic diverticulosis [9], duodenal ulcer [10] and colonic polyps [11] could be risk factors for *L. garvieae* IE.

In most reported cases of IE caused by this pathogen, the treatment of choice was gentamicin in combination with a beta-lactam or vancomycin for at least four weeks, with good clinical outcomes [9]. A case of death has been described in a patient with native valve endocarditis who was treated with ceftriaxone alone. In our case, the incorporation of gentamicin to the antimicrobial treatment had to be interrupted due to acute renal failure and the patient ultimate death.

Lactococcus spp. is commonly misidentified as *Enterococcus faecalis,* [2,11] because both have similar morphological characteristics. Furthermore, they are catalase-negative, PYR-positive, grow on 6.5% NaCl and can hydrolyze esculin. Differentiating *Lactococcus spp*. from *Enterococcus faecalis* can be difficult even through carbohydrate fermentation. Both are mannitol, maltose and fructose positive, but arabinose and raffinose negative [12,13]. The main reproducible difference is the fermentation of sorbitol, which is negative in the former and positive in the latter. It is also possible to use two other carbohydrates -xylose and rhamnose-, but five to seven days are required to see any difference: *Lactococcus spp*. is xylose negative and rhamnose positive, whereas *Enterococcus faecalis* is xylose positive and rhamnose weakly positive. It is also known that *Lactococcus* grows slower than *Enterococcus* at 45°C, although this difference is not observed in some cases [6]. Furthermore, it is possible to discriminate between *L. garvieae* and *L. lactis* because the former is resistant to clindamycin and has intermediate susceptibility to penicillin, whereas the latter is susceptible to both antibiotics [7,9].

The use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) at our institution let us a simple and rapid identification of the microorganism. The later confirmation through sequencing of the heart valve supported the reliable identification from MALDI-TOF.

The extraction of only one set of blood culture hampered the evaluation of this pathogen, because of the possibility of being a contamination as usually happens when species from the *Streptococcus* genus are isolated. The subsequent blood cultures taken to confirm the isolation of *L. garvieae* were negative, probably due to the antimicrobial treatment previously received.

There are few reported cases of *L. garvieae* IE in the world (currently only two cases have been described in Spain) [14], but it appears to be an emerging pathogen that matches with the development of whole-cell protein patterns and nucleic acid amplification technologies, such as mass-spectrometry MALDI-TOF MS and PCR and sequencing of 16s rRNA gene, respectively.

In conclusion, the best way to identify *L. garvieae* is through MALDI-TOF MS and 16s rRNA gene PCR, in addition to susceptibility profile, which allowed us to differentiate it from *Enterococcus* spp. Also, we would like to emphasize the importance of the extraction of two sets of blood cultures to perform a reliable assessment of the microorganism isolated, and the importance to discard IE when L. garvieae is isolated

from blood cultures, even when it grows only in one set of blood cultures, if the patient's clinic is compatible.

Conflict of interest

None declared.

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