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A Case of Endocarditis Caused by *Lactococcus garvieae* and Suggested Methods for Identification

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Lactococcus garvieae **is a Gram-positive coccus that has morphological and biochemical similarities to enterococci. *L. garvieae* strains rare human pathogens, with only a few cases reported in the literature, mainly as a cause of infective endocarditis. *L. garvieae* is well known as a fish pathogen, and in some of the reported cases, the patients had a history of contact with raw fish. Some of the reported endocarditis patients had valvular damage as a predisposing condition. We report a case of *L. garvieae* endocarditis in a patient with no history of contact with raw fish and with history of valvular repair in an unaffected heart valve.**

CASE REPORT

We present the case of a 64-year-old male with an extensive cardiac history, including mitral valve repair and coronary artery bypass grafting (CABG) 3 years prior to presentation and an intracardiac defibrillator (ICD) placed 2 years prior to presentation. His past medical history was also significant for hypertension, diabetes mellitus type II, and chronic obstructive pulmonary disease. The patient was referred to our institution from an outside hospital with the diagnosis of aortic valve endocarditis complicated by hypotension.

The patient had presented to the outside facility with a history of progressive fatigue, weight loss, and anorexia over several weeks. The symptoms worsened gradually, and he ultimately developed a significant decline in physical abilities and lost the ability to ambulate. After admission, an echocardiogram showed 4+ aortic insufficiency with vegetations on the aortic valve. One of two blood cultures drawn grew Gram-positive cocci in chains. The patient was started on vancomycin and transferred to our institution for further management.

The patient recalled having had a dental procedure 6 months prior. He had received 3 weeks of prophylactic antibiotics at that time. No other relevant history was found.

At our institution, the surgical management included a median sternotomy with open heart and aortic valve replacement with a Carpentier-Edwards valve, as well as ascending aorta repair with bovine pericardial patch and ICD lead and pacemaker removal. The heart valve obtained during surgery was positive in culture for the same organism that was later detected in the same tissue by universal PCR followed by sequencing. However, multiple blood cultures drawn at our institution were negative. The positive blood culture at the outside laboratory reported a gamma-hemolytic streptococcus; the Microscan Walk Away system (Dade Behring, Inc., West Sacramento, CA), was used for identification without success in this case. Susceptibility testing, also performed with the Microscan system, reported full resistance to clindamycin and intermediate resistance to penicillin and ampicillin using *Streptococcus* sp. breakpoints.

At our laboratory, small to medium-sized, white transparent colonies with a smooth and shiny surface and alpha-hemolysis were seen on the blood agar plate after 48 h of incubation of the valvular tissue. At 24 h, growth was incipient. A Gram stain of the colonies showed Gram-positive cocci in pairs and chains with a slight elongated appearance. With this Gram morphology and a positive pyrrolidonylarylamidase (PYR) test, the presumptive identification was *Enterococcus* spp.; however, our automated identification system, Vitek 2 (bioMérieux, Marcy l'Etoile, France), identified the organism as *Lactococcus garvieae*. To confirm the identification, the isolate was also sent to our research laboratory to be tested on the Bruker (Bruker Daltonics) matrix-assisted laser desorption ionization–time of flight mass spectrometer (MALDI-TOF MS). The result was obtained the same day with the identification of *Lactococcus garvieae* with a score of 2.201, which represents a good identification. In addition to culture, universal PCR followed by sequencing was performed on the valvular tissue at a major referral laboratory, which confirmed our identification. No susceptibility testing was performed due to the lack of standard methods and interpretative breakpoints for *L. garvieae*.

The hospital course was uncomplicated, with good response to empirical antibiotic therapy. The patient was discharged home in good condition with intravenous antibiotics. On a follow-up visit, he was doing very well clinically after completion of a 6-week course of vancomycin. No fevers, chills, or diarrhea were reported. His appetite and physical performance had returned to normal.

The presumptive identification originally made in this case illustrates the difficulties that arise during identification of *Lactococcus* spp. Phenotypically lactococci tend to be pyrrolidonylarylamidase (PYR) positive and grow in 6.5% salt and bile esculin media (BEM) (1). Organisms with this profile can easily be misidentified as *Enterococcus* spp. Published literature on *Lactococcus* spp. (2, 3, 11) suggest the possibility of using the ability of enterococci to grow at 45°C as a tool to separate them from lactococci,

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TABLE 1 Carbohydrate fermentation with Andrade's broth over 7 days of testing

Isolate	Carbohydrate fermentation result with 1%[a]:											
	Xylose	Mannitol	Sorbitol	Sucrose	Maltose	Fructose	Salicin	Arabinose	Rhamnose	Lactose	Tetrahelose	Raffinose
E. faecalis	+[b]	+	+	+	+	+	+	−	Weak[c]	+	+	−
Case report isolate	−	+	−	−	+	+	+	−	+	−	+	−
Isolate 2 (blood)	−	+	−	+	+	+	+	−	+	−	+	−
Isolate 3 (urine)	−	+	−	+	+	+	+	−	+	−	+	−

[a] +, positive; −, negative. Unless otherwise stated, positive acidification of the medium was observed after 24 h of incubation at 37°C in O_2.
[b] Turned positive at day 5.
[c] Weak response from day 1 to day 7.

since the latter will not grow at 45°C in ≤48 h. Additionally, *Lactococcus* spp. should produce acid from mannitol but not from sorbitol like the most commonly encountered enterococci do.

Three and 5 months after this isolate of *L. garvieae* was received, two other *L. garvieae* isolates were identified at our laboratory. The first isolate was obtained from a blood culture on a patient with bacteremia, and the second was from the urine of a patient with a urinary tract infection. On both occasions, the microorganisms were identified to the species level by Vitek 2 with a good percentage of probability (>99%). On both occasions, the identifications were confirmed using Vitek MALDI-TOF MS. Phenotypical testing in both occasions was not fruitful, due to the multiple variable reactions that *Lactococcus* spp. have and the lack of data for some of the testing. Guided by review of the literature (2–4) on identification of lactococci and using standard laboratory practices, we tested these three isolates together along with our *Enterococcus faecalis* control, ATCC 29212. No differences were found in Gram morphology after incubation in thioglycolate broth; the *E. faecalis* control as well as our three isolates showed Gram-positive cocci in pairs and chains with a slight elongated appearance. All isolates grew as alpha-hemolytic white shiny colonies on blood agar and tested PYR positive. All isolates were able to grow with incubation at a 45°C as well as at room temperature. As previously observed by Texeira et al. (4), the *Lactococcus* colonies grew slightly slower and smaller than the *E. faecalis* colonies at 45°C; however, all tested isolates had positive growth 24 h after incubation. All isolates grew on 6.5% NaCl as well as on BEM, and all were able to hydrolyze esculin. When incubated on Andrade's carbohydrate broth and indicator (Remel, Lenexa, KS) with various sugars, differences were seen in the fermentation of sorbitol, which is used by the *E. faecalis* control but was not utilized by any of our *Lactococcus* isolates. Fermentation of lactose by our *E. faecalis* control isolate was present at 24 h, whereas no fermentation was observed with the three *Lactococcus* isolates, contrary to previous reports (4). Xylose fermentation was observed at day 4 with the *E. faecalis* control, whereas no fermentation was seen with any of our *Lactococcus* isolates. A weak reaction for the fermentation of rhamnose was present with the *E. faecalis* control that remained unchanged for 7 days; this was not observed with any of the *Lactococcus* isolates.

All of the *Lactococcus* isolates had very similar reactions with all tested biochemicals, except for sucrose, which our case report isolate did not ferment, whereas the other two isolates readily did so (Table 1).

Routine testing of *Lactoccocus* spp. remains a challenge in the laboratory. Unfortunately, our limited experience with three isolates received in our laboratory could not suggest improvements in the current routine identification practices of these organisms. Of the reported phenotypical differences between *Enterococcus* spp. and *L. garviae*, the fermentation of sorbitol remains a reproducible difference; we could not reproduce differences in growth temperature or fermentation of arabinose and maltose as previously described in the literature (2, 3). The weak fermentation of rhamnose not seen in any of the three isolates and observed in our *E. faecalis* control isolate might also represent a reproducible difference if a larger number of isolates are tested comparatively. We found previously not described differences in the fermentation of lactose and xylose that also merit more studies (Table 1).

In most routine clinical microbiology laboratories, many of these tests would not be performed outside a "kit" system or automated identification system, which, as happened with the initial blood culture of our case, will result in a lack of identification of presumptive *Enterococcus* isolates. Encountering such circumstances in other clinical laboratories should raise the suspicion of *Lactococcus* spp., especially in blood or cardiac tissue. Misidentification of *Lactococcus* spp. as E*nterococcus* spp. may also occur. Genus-level identification of *Lactococcus* by any automated method or commercial kit should be confirmed with a different method, although we are not aware of having such experiences in our laboratory. When performing an identification workup on a presumptive *Enterococcus* isolate, any weak or slow reactions with PYR, salt tolerance, growth on BEM, or esculin hydrolysis should raise the suspicion for *Lactococcus* spp. as previously suggested in the literature (5). Use of molecular tests or whole-cell protein analysis may afford more reliable identification methods for these organisms; however, these methods are not available in all laboratories. Our case exemplifies one of the great advantages of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), a newer technology that achieves bacterial identification using mass spectrometry. Mass spectrum results are graphed as peaks based on the mass/charge ratio of the ionized bacterial proteins; comparison of the mass spectrum profile to a reference database provides the final identification. After 48 h of laboratory workup and nondefinitive, variable results from our routine methods, we tested the isolate in our research MALDI-TOF instrument. In about 30 min, we were able to obtain an identification that had an excellent reliability score. The identification as *L garvieae* was later confirmed through universal bacterial sequencing of the heart valve as well as 16S RNA sequencing performed on the isolate. Although we could not use the MALDI-TOF identification results, since we had not yet implemented this procedure in our routine laboratory, the confirmation by sequencing did support the answer derived from MALDI-TOF MS.

No contact with raw fish was reported by the patient or his

immediate family. The majority of the reported cases of *L garvieae* infection had such association (6–10). No other peculiarities were present in the patient's history that could point in the direction of a possible exposure to *L. garvieae*. His long cardiac history with multiple invasive procedures is certainly a predisposing factor for endocarditis; however, to us the relationship between the causative agent and the patient in this case is not clear. It is possible that lactococci exist in environments other than those previously described; being able to identify them as pathogens will help us understand their relationship with human disease.

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