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Endocarditis caused by Thalassospira sp.

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Introduction

Infective endocarditis (IE) refer to the infections of the inner surface of the heart. They are mostly caused by bacteria, typically staphylococci, streptococci, enterococci and HACEK-related organisms [1]. The microbiological diagnostic of IE relies on the culture of blood samples and infected tissues (when surgery is performed). In 10 % cases, no etiology for IE is found, and culture-independent, molecular methods such as broad-range PCR and more recently, metagenomic sequencing can be used [2].

Case report

A 53-year-old man living in Bora-Bora (French Polynesia) and working as a fisherman (apnea fishing, i.e. spending hours per day in sea water) presented at the Cardiology department of Papeete hospital (French Polynesia) in October 2020 with dyspnea of

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ABSTRACT

We report a case of an infective endocarditis caused by a *Thalassospira* sp. in a 53-year-old man with preexisting valvular lesions and living in French Polynesia as a fisherman. The strain was identified with DNA-sequecing methods while it was not by mass spectrometry.

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progressive worsening over 6 months. He has a medical history of hypertension and rheumatic fever from childhood Trans-thoracic and trans-esophageal echocardiography found a mixed calcific aortic valve disease combining severe stenosis and moderate regurgitation, dilatation of the ascending aorta at 49 mm and a circulating retro-aortic false aneurysm of 50 by 15 mm. Left ventricular ejection fraction was 40 %. The patient did not report any fever.

On admission, C-reactive protein was 25 mg/L with $9000/\mu$ L leukocytes. The patient was transferred to the Bichat-Claude Bernard Hospital (Paris, France) to undergo surgery. There, transoesophageal echocardiography and CT scan confirmed the posterior peri-aortic false aneurysm. A positron emission tomography (PET) scan showed no evidence of cardiac valve or vascular hypermetabolism, and did not reveal any embolic lesion. He developed cardiogenic shock and was operated on October 26th. Repeated blood cultures (total of three pairs before surgery) remained sterile. The surgery consisted of aortic valve replacement by bioprosthesis, reconstruction of the aortic ring by patch, placement of a supracoronary tube and tricuspid ring annuloplasty. The Gram stain examination of the aortic valve revealed a high number of Gram-negative, spiral and

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Case report







Fig. 1. Gram staining of the aortic valve showing the curved (arrow 1) and spiral (arrow 2) shapes of *Thalassospira* sp. (panel A) and aspects of the colonies after 48 -h incubation on COH agar medium (panel B).

curved-shaped bacilli (Fig. 1, panel A). Intravenous amoxicillinclavulanic acid 2 g - 200 mgx6/24 h, cefotaxime 12 g/24 h and gentamicin 3 mg/kg daily were started.

In 48 h, the culture of the valve sample on Columbia agar supplemented with 5% horse blood (COH, bioMérieux, Marcyl'Etoile, France) under aerobic conditions yielded mucoid, translucent colonies (Fig. 1, panel B). A Gram stain on the colonies confirmed Gram-negative bacilli with curved shape. Identification with Matrix Assisted Laser Desorption Ionization - Time of Flight mass spectrometry (MALDI-TOF, Brüker Daltonics, Bremen, Germany) failed both with the in vitro diagnostic (IVD) and research use only (RUO) databases despite proper spectra were obtained. The strain did not grow under anaerobic conditions nor on Drigalski agar medium under aerobic conditions. We inoculated an aerobic and an anaerobic Bactec vials (Becton-Dickinson, Rungis, France) with 10² or 10⁶ colony-forming units of the strains and incubated the vials in a BactecFX. None turned to be positive after 8 days of incubation. Antimicrobial susceptibility testing was performed by the disc diffusion method (I2A, Montpellier, France) and E-tests strips (bioMérieux) on Mueller-Hinton medium (Table 1). The strain was apparently susceptible to penicillins and did not produce any detectable beta-lactamase It was also apparently susceptible to extended-spectrum cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines and cotrimoxazole. It was apparently resistant to cefazolin,

Table 1

Antimicrobia	l susceptibility	testing of the	Thalassospira	sp.
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Antimicrobial family	Antimicrobial	Minimal inhibitory concentration (µg/mL)
Beta-lactam	Amoxicillin	<0.016
	Amoxicillin-clavulanate	<0.016
	Ticarcillin	<0.032
	Piperacillin	3
	Temocillin	512
	Cefazolin	32
	Cefoxitin	32
	Cefotaxime	0.094
	Ceftazidime	0.25
	Ertapenem	0.008
	Imipenem	0.25
Aminoglycoside	Gentamicin	0.19
	Amikacin	1
	Tobramycin	0.19
Fluoroquinolone	Levofloxacin	0.008
	Ciprofloxacin	0.002
Tetracycline	Tetracycline	0.023
	Minocycline	0.016
	Tigecycline	0.016
Other	Cotrimoxazole	0.064
	Fosfomycin	>1024

cefoxitin, temocillin and fosfomycin. The antimicrobial regimen was adapted with amoxicillin 12 g/24 h and gentamicin 3 mg/kg daily for 2 weeks followed by amoxicillin 12 g/24 h and oral levofloxacin 750 mg daily for 4 weeks, with a favorable outcome until now (the patient was discharged home in December 2020).

At the time the strain had grown but kept being unidentified, we extracted the DNA of the valve sample using the Molzym Ultra-deep microbiome kit (Molzym, Bremen, Germany) which depletes in eukaryotic DNA. The DNA of the strain was also extracted using the EZ1 instrument (QIAgen, Courtaboeuf, France). The DNA was sequenced overnight on a MK1C device using a single Flongle flow-cell (Oxford Nanopore Technologies) for the valve and the strain. The reads were identified using Minimap2 [3], the RefSeq database and Kraken [4]. The turn-around time for the Nanopore sequencing on Flongle and the Kraken results was 24 h. Whole genome sequencing of the strain and the valve identified a Thalassospira sp. The Sanger sequencing of 1316 bp of the 16SrRNA encoding gene from the strain has also been done and confirmed the presence of Thalassospira povalilytica (100 % nucleic acid identity). The genome and valve reads have been made available under the access number PRJNA678853.

Discussion

Thalassospira is a Gram-negative, oxidase-positive, mobile, curved or spiral-shaped bacterium which lives in marine waters. From the Tara Oceans consortium metagenomic data, Thalassospira spp. make up to 0.1 % of all marine bacteria [5]. While Thalassospira has been recently spotted for potential beneficial properties (e.g. long-chain polyunsaturated fatty acids production for fish feed or oil degradation), it has only been reported once as a potential human pathogen (a case of otitis evolving as a mastoiditis and meningitis involving Thalassospira profundimaris [6]). Like our present report, the patient had been in close contact with marine water. Indeed, our patient worked as a fisherman using apnea fishing and thus spent hours in sea waters. The patient reported experiencing chronic skin lesions due to the time spent in saline water, especially at the legs. Our current assumption is that symptomatic or asymptomatic cutaneous lesions may have been an opportunistic entry point for marine bacteria to reach the bloodstream and to fix onto pre-existing lesions on the cardiac valves. An alternative route of acquisition could have been the ingestion of contaminated sea food. Skin of soft tissues infections caused by marine or fresh waters bacteria and possible complications such as bloodstream infections and endocarditis have repeatedly been reported, and have usually involved bacteria such as Aeromonas spp., Shewanella spp. or Vibrio vulnificus [7]. The preexisting valvular lesions may have favored the fixation of Thalassospira onto the valve, as reported in an Aeromonas

salmonicida endocarditis where the patient had rheumatic heart disease and bathed in well water [8] and in *Shewanella* endocarditis where the patient had bioprosthetic mitral and aortic valves and fished in saline waters [9].

Nonetheless unlike those reports and that of Marchese et al., our patient did not present any fever or significant biological inflammatory syndrome that could have led to the suspicion of endocarditis at presentation. Moreover, the strain was unable to grow on Bactec vials and the diagnostic of infective endocarditis was only possible by culturing valve specimens. In conclusion, we report here a case of aortic endocarditis caused by *Thalassospira* sp., an unusual bacterium only reported once in human infection. The bacterium has likely been acquired through bathing in saline water and has attached to pre-existing valvular lesions. While conventional methods were challenged by the absence of the strain in MALDI-TOF databases, we showed that rapid, low-cost (<100 \in) metagenomic and genomic sequencing using Nanopore Flongle flow-cells could help.

Declaration of Competing Interest

The authors declare no conflict of interest.

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None.

Ethical approval

The patient authorized the publication of the case by a signed agreement on December 1.

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