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Molecular diagnosis of native mitral valve endocarditis due to *Corynebacterium striatum*

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Corynebacterium striatum is a relatively rare causal agent of infective endocarditis (IE). Its association with IE is complicated by its relatively slow growth, its role as a possible contaminant in such cases, and the phenotypic difficulty in laboratory identification.

The present study reports a case of culture-positive endocarditis due to *C. striatum* in a 77-year-old woman who showed no risk factors for endocarditis. To date, there have been 12 reports of endocarditis due to *C. striatum*, which are discussed and summarised.

Currently, use of molecular microbiological methods has been limited, but such techniques have been adopted in several clinical microbiology laboratories. Molecular methods of identification using the polymerase chain reaction (PCR) and sequencing of 16S ribosomal DNA (rDNA) from the causal agent isolated by blood culture may be very useful in the identification of causal agents in culture-positive endocarditis, which prove difficult to identify using a conventional approach.

The 77-year-old patient presented to hospital with a chronic three-month history of weight loss, fatigue and arthralgia. A history was taken in accordance with the

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questionnaire criteria for endocarditis, as described previously by Paturel *et al*.[1] The patient had no risk factors for IE nor any underlying disease. She was febrile, anaemic and demonstrated septic embolisation. On auscultation, she had a new cardiac murmur, which was shown to originate from leakage from her native mitral valve. In addition, the presence of a vegetation on this valve was demonstrated.

On presentation, she had an elevated C-reactive protein (CRP) of 244.7 mg/L. This rose to 255.5 mg/L and then decreased to 46.4 mg/L nine days after commencement of intravenous antibiotics. Three sets of blood cultures were taken, all of which grew a Gram-positive rod provisionally identified by conventional techniques as a *Corynebacterium* sp. Antibiotic treatment was continued for a further seven weeks and the patient was discharged without any signs or symptoms of IE.

Phenotypic identification of the Gram-positive organism, isolated from three sets of blood cultures (isolate identifier: CAHE68720) was performed using the API *Corynebacterium* system (bioMérieux, Las Halles, France). It gave the profile 3140345 and an identification of *Brevibacterium* sp. (47%). A repeat test gave the API profile 3100305, yielding *Corynebacterium* group G (55.6%). The organism was sensitive to vancomycin, teicoplanin, gentamicin and rifampicin by standard *in vitro* antibiotic disc diffusion susceptibility assay.

Given the relatively poor phenotypic identification obtained, the isolate was forwarded for molecular identification through PCR amplification and direct sequencing of a large but partial region of the 16S rRNA gene, corresponding to the base position of approximately 811–1374 of *Escherichia coli* ATCC 25922 16S rRNA (GenBank accession number: X80724). All DNA isolation procedures were carried out in accordance with the DNA contamination management guidelines of Millar *et al*.[2] and in a Class II biological safety cabinet (MicroFlow, England). This was situated in a room separated from that used to set up nucleic acid amplification reaction mixes, and also from the 'post-PCR' room, in order to minimise contamination and the possibility of false-positive results.

Bacterial DNA was extracted from the isolate using the Roche high-purity PCR template preparation kit (Roche, England) in accordance with the manufacturer's instructions. Extracted DNA was transferred to a clean tube and stored at –80°C prior to PCR. For each batch of extractions, a negative extraction control containing all reagents other than the isolate was performed. Reaction mixes (50 µL) were set up as follows: 10 mmol/L Tris–HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L $MgCl_2$ 200µmol/L (each) dATP, dCTP, dGTP and dTTP, 1.25 units *Thermus aquaticus* (*Taq*) DNA polymerase (Amplitaq, Perkin Elmer), 0.2 µmol/L appropriate 'broad range' primers (PSL[3] [forward] 5′ -AGG ATT AGA TAC CCT GGT AGT CCA-3′ and P13P[4] [reverse] 5′ - AGG CCC GGG AAC GTA TTC AC -3′) and 4 µL DNA template.

Prior to PCR cycling, sealed tubes containing DNA template and all PCR reagents were introduced to the thermal cycler at 96°C to avoid non-specific annealing during the initial ramp stage. The reaction mixtures were subjected to the following thermal cycling parameters in a Perkin Elmer Cetus 2400 thermocycler: 96°C for 3 min followed by 40 cycles at 96°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min. During each run, molecular-grade water was included randomly as several negative controls and DNA templates from *Staphylococcus aureus* were included as a positive control, as appropriate.

Following amplification, samples (15 µL) were removed from each reaction mixture and examined by electrophoresis (80V, 45 min) in gels composed of 2% (w/v) agarose (Gibco, UK) in TAE buffer (40 mmol/L Tris, 20 mmol/L acetic acid, 1 mmol/L EDTA [pH 8.3]), and then stained with ethidium bromide (5 µg/100 mL).

Gels were visualised under ultraviolet (UV) illumination using a gel image analysis system (UVP Products, England) and all images were archived as digital graphic (.bmp) files. Amplicons chosen for automated sequencing were purified using a QIAquick PCR purification kit (Qiagen, UK) and eluted in Tris–HCl (10 mmol/L [pH 8.5]) prior to sequencing, in order to remove dNTPs, polymerases, salts and primers. The amplicon was sequenced on the ALF II Express automated sequencer using the primer PSL which was labelled with Cy-5 fluorescent dye and used in conjunction with the Sequenase fluorescence-labelled primer cycle sequencing kit (Thermo, Amersham, UK).

The resulting sequence obtained (570 bp) was compared with those stored in the GenBank data system using FASTA alignment software (www.ebi.ac.uk), and deposited in GenBank (accession number DQ018338). On BLAST analysis in combination with previously reported criteria used for interpretation of partial 16S rRNA gene sequences,[5] the sequence gave a 100% identification for *C. striatum* (GenBank accession number AY008302) followed by *C. xerosis* X81906 (99% identity) and an uncultured *Rhodococcus* sp. (98% identity).

At present, there are approximately 73 recognised species in the genus *Corynebacterium*, including *C. striatum*. This species was first described by Chester in 1901 as *Bacterium striatum*, and then by Eberson in 1918.[6] Several *Corynebacterium* spp. have been described as causal agents of IE.[7] Over the past 15 years, however, there have only been 10 cases of *C. striatum* endocarditis. In a review of the 12 cases in the literature, seven patients were male. The median age was 62.6 years for females (age range: 46–72 years) and 59 years for males (age range: 24–76 years), as shown in Table 1.

Native valves have been most frequently infected (three mitral, three aortic, one pulmonary and one tricuspid); however, there have been three cases of prosthetic valve endocarditis (two aortic, one mitral). In total to date, two deaths have been attributed to *C. striatum* endocarditis, with aortic valve involvement (Table 1[8–17]).

The current case highlights the important role of molecular methods in the correct identification of the causal agent, namely *C. striatum*. Identification of the genus *Corynebacterium* and differentiation of species in the genus is usually based on differential biochemical tests.[18] However, in most diagnostic laboratories, the API system is employed for the rapid and convenient speciation of isolates in this genus.

Previously, Funke *et al*.,[19] although concluding that the API Coryne system is a useful tool for identifying the diverse group of coryneform bacteria encountered in the routine clinical laboratory, noted that additional tests were required when using version 2.0 of the kit instead of version 1.0, in order to identify strains completely. In the case reported here, the API Coryne system was not able to identify the isolate correctly, even to the genus level, yielding the close

Table 1. A review of the literature describing cases of *Corynebacterium striatum* endocarditis previously reported.

Patient (age, sex)	Predisposing condition for IE	Echocardiographic findings	Valve	Antibiotic therapy	Surgery	Survival	Ref
72, F	RF in childhood. Mitral valvotomy and prosthetic mitral valve replacement 30 y later. Culture-negative IE, 18 m previously	TOE: inconclusive due to what was believed to be calcified vegetations from previous episode of culture-negative IE	NK	Vancomycin/rifampicin	None	Yes	(8)
61, F	RF in childhood	Partially calcified vegetation on mitral valve	Mitral	Vancomycin/gentamicin	None	Yes	(8)
50, M*	Previous mycotic aneurysm for which he underwent surgical treatment.	TOE: 15 mm vegetation on aortic valve visible before and after antibiotic therapy	Aortic	Antibiotic therapy unsuccessful. i. Penicillin/gentamicin ii. Vancomycin/netilmicin iii Vancomycin/gentamicin/ doxycycline	Aortic valve replaced	Yes	(9)
62, F	Diastolic murmur after surgery for a bioprosthetic aortic valve replacement a few years previously	TOE: No evidence of vegetation or abscess formation	Bioprosthetic aortic valve	Vancomycin	None	Yes	(10)
72, F	Aortic valve replacement with a metallic prosthesis	TTE indicated a 15 mm vegetation on the mitral valve; however, this was not confirmed at post mortem	Periprosthetic aortic valve	i.Vancomycin/gentamicin, ii benzyl penicillin	None	No	(11)
68, M	Mitral valve regurgitation (2 y)	TTE: moderate left ventricular dysfunction, mitral valve regurgitation. TOE: mitral regurgitation and vegetation on atrial aspect of anterior mitral valve leaflet	Mitral	i.Vancomycin, ii. Penicillin	None	Yes	(12)
73, M	Pacemaker 6 y previously. Batteries replaced and electrode cut, although distal part left in place. Draining sinus tract	TTE: normal TOE: vegetation on old electrode wire which remained after previous removal TOE after surgical removal of wire. Vegetation visible on tricuspid valve	Tricuspid	i. Vancomycin ii. Oral co-trimoxazole/ rifampin iii. Vancomycin due to penicillin resistance	Removal of old electrode wire via jugular vein	Yes	(13)
24, M	Ventriculoatrial shunt	TTE/TOE: 10 mm vegetation on pulmonary valve close to distal extremity of ventriculoatrial shunt catheter; TTE after 7 w therapy: vegetation unchanged; TOE 20 m later: no vegetations	Pulmonary	i. Amoxicillin/netilmicin ii. Amoxicillin iii Amoxicillin, netilmicin/teicoplanin iv. Oral amoxicillin	Bed sore treated (source of infection) Endovascular process attempted to remove catheter in pulmonary artery (failed)	Yes	(14)
54, M	None	TTE: moderate aortic insufficiency following detection of new murmur. TTE/TOE: ruptured aortic tricuspid valve cusp with severe aortic insufficiency	Aortic	i. Ampicillin/vancomycin ii. Ampicillin + gentamicin iii. Vancomycin alone (due to rash)	Aortic valve replacement	Yes	(7)
76, M†	No history of heart disease	Echo: aortic/tricuspid insufficiency	Aortic	Parenteral ampicillin/ gentamicin	Died prior to planned emergency surgery to replace aortic valve	No	(15)
46, F	Unknown	Unknown	Unknown	Initial treatment failure with linezolid. Switched to daptomycin/ rifampicin	None	Yes	(16)
68, M	Aortic and mitral prosthetic valves	TTE: 10 mm vegetation on prosthetic mitral valve	Prosthetic mitral valve	Vancomycin due to antibiotic pan-resistance	None	Yes	(17)

RF: rheumatic fever; IE: infective endocarditis; TTE: transthoracic echocardiography; TOE: transoesphageal echocardiography; NK: not known.

*Polymicrobial, multiresistant infective endocarditis due to *Staphylococcus epidermidis* and *Corynebacterium striatum*.

†Causative organism most closely resembled *Corynebacterium striatum*.

phylogenetic neighbour *Brevibacterium* sp. (47%) on first testing and eventually *Corynebacterium* group G (55.6%) on subsequent testing.

Where conventional blood culture yields a viable culture, it is important to be able to identify it reliably to aid patient management, in particular antimicrobial susceptibility and the prescribing of an appropriate regimen of anti-infective chemotherapy, and for epidemiological reporting of aetiological agents of IE. Molecular methods may be introduced as part of a polyphasic approach to aid the identification of a culture-positive case, but are not required in the primary diagnostic setting.

Conventional phenotypic characterisation of cultures can be problematic (e.g., speciation of enterococci and coagulase-negative staphylococci) and molecular identification primarily by sequence analysis allows these organisms to be speciated in such cases. Additionally, occasional employment of these molecular methods may help to quality assure the correct phenotypic identification of cultures from patients with IE.

Although primary diagnostic laboratories may be able to detect and isolate a significant organism from the blood cultures of patients with IE, these laboratories may have problems with the correct and reliable identification of such isolates. This may be due to the fact that the cultures are unusual in that they are not observed frequently in the clinical laboratory, either in the context of IE or other infections (e.g., *Weissella confusa*[20]) or where the organism is new (e.g., *Streptococcus nov*. sp.[21]).

Where a new organism is identified by 16S rRNA sequence-based methods, further work is required to fully characterise and describe the new taxon, whether a new species or indeed a new genus. Generally, this necessitates the forwarding of such isolates to a specialist taxonomy laboratory, as even those primary diagnostic laboratories with specialist molecular ability are often not suited for an in-depth analysis of the taxonomic positioning of such isolates. Limitations of routine identification systems (e.g., API, Vitek, etc.) in primary diagnostic laboratories create problems in identifying difficult-to-identify organisms reliably, and employment of 16S rRNA gene sequencing has proved reliable in such cases.[22]

In conclusion, this report demonstrates the value of molecular methods in the correct identification of phenotypically difficult-to-identify causal agents of IE. Furthermore, it represents the first instance in which PCR and direct sequencing has been employed to aid the identification of *C. striatum* endocarditis. Diagnostic clinical laboratories should be familiar with the existence of such techniques and have a mechanism in place to permit the referral of such organisms to a specialist/reference laboratory with experience in these molecular techniques. □

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