Case report

A 40-year-old male had undergone aortic (19 mm Sorin Bicarbon) and mitral (25 mm Sorin Bicarbon) valve replacement surgery for rheumatic valvular disease 9 years previously. He was slow to recover from his initial surgery, suffering from fever and lethargy post operation. Blood cultures were negative but echocardiography showed a mitral valve vegetation. The patient was treated for culture-negative endocarditis. The objective of this report is to emphasize the use of a broad-range PCR technique for bacterial 16S rRNA genes in identifying the causative pathogen, thus enabling targeted antimicrobial treatment.

Nine years later, the patient presented with abdominal pain, dark urine, melaena, jaundice, epistaxis and intermittent pyrexia. Examination revealed a murmur of mitral regurgitation but no peripheral stigmata of endocarditis. Blood tests confirmed haemolytic anaemia. Trans-oesophageal echocardiography revealed partial mitral prosthesis dehiscence with severe paraprosthetic regurgitation, but no vegetations were seen. The aortic prostheses was functioning normally, but there was significant tricuspid regurgitation. The patient underwent urgent mitral valve re-replacement and tricuspid valve repair. During surgery, vegetations were noted to be present on the mitral prosthesis. Microscopy and Gram staining of the explanted valve tissue showed no microorganisms, and valve bacterial culture was initially negative.

DNA was extracted from the resected valve using the tissue protocol of the QIAamp DNA mini extraction kit (Qiagen). Amplification of the 16S rRNA gene was performed using the primers FD1, 5′-AGAGTTTGATCCTG-3′ (Weisburg et al., 1991), and UR, 5′-CTACGGATTTACGCTAC-3′ (Hendolin et al., 2000), with Brilliant SYBR QPCR MasterMix (Stratagene). The PCR was performed using the MX3000P QPCR platform (Stratagene) using the following programme: activation at 95 °C for 10 min followed by 40 cycles of 95 °C for 30 s, 62 °C for 1 min and 72 °C for 1 min 30 s and a final extension step at 72 °C for 10 min. The amplicons were purified using a MinElute PCR purification kit (Qiagen) and the nucleic acid sequence was determined at Lark Technologies, Essex, UK. PCR amplification and nucleotide sequencing of the bacterial 16S rRNA gene identified the organism as M. hominis from the excised tissue. The nucleotide sequences were compared with those available through the NCBI database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The nucleotide sequence showed 99.8 % (669/670 bp) 16S rRNA gene sequence similarity to that of M. hominis.

An active infection was subsequently confirmed when M. hominis was cultured from valve tissue and detected by a species-specific PCR. Characteristic fried-egg-shaped colonies were observed when a section of the valve was plated directly onto Mycoplasma solid media, or after culturing in Mycoplasma liquid broth (Mycoplasma Experience), and incubated at 37 °C in a 5 % CO₂ atmosphere. The identity of the organism was confirmed as M. hominis by species-specific PCR (van Kuppeveld et al., 1992) using DNA extracted from isolated colonies, liquid broth cultures and the resected valve itself. The initial empirical antibiotic regimen of intravenous vancomycin, ciprofloxacin and rifampicin was changed to oral doxycycline, 200 mg once daily, and clindamycin, 450 mg four times per day, which were administered for 8 weeks to treat late prosthetic valve endocarditis.

The patient recovered well from surgery and remained well 12 months post operation with normal prosthetic valve function.
Discussion

*M. hominis* is part of the human microbial flora of the genito-urinary and respiratory tracts (Ladefoged, 2000). This organism does not grow, or may not be detected, in routine blood culture systems (Houpikian & Raoult, 2005). It can be isolated on blood agar but colonies may not be apparent until after the standard 48 h incubation period. *M. hominis* is usually susceptible to tetracyclines, and resistant to erythromycin and penicillin-like drugs (Kenny, 2010; Harwick & Fekety, 1969). Reports of the development of tetracycline-resistant strains of *M. hominis* (Cummings & McCormack, 1990) and the fact that tetracycline is bacteriostatic led us to empirically co-administer clindamycin.

*Mycoplasma* species are a rare cause of endocarditis, with nine cases previously reported (Houpikian & Raoult, 2005; Popat et al., 1980; Scapini et al., 2008). Only six of these were due to *M. hominis* (Cohen et al., 1989; DiSesa et al., 1990; Blasco et al., 2000; Fenollar et al., 2004; Dominguez et al., 2006; Hidalgo-Tenorio et al., 2006). In each case, the patient had undergone recent valve surgery and then developed *M. hominis* infection acutely, suggesting that the organism has a predilection for causing prosthetic rather than native valve endocarditis. At least one reported death from *M. hominis* endocarditis was related to an inappropriate antibiotic regime and the organism was identified from valve culture posthumously (Blasco et al., 2000). The present case differs from previous reports because of the patient’s much later presentation of the illness, valve surgery having been performed 9 years previously.

In five of the previous cases, *M. hominis* was identified by culturing valve tissue excised during surgery, and in the other cases, the pathogen was identified by using 16S rRNA gene PCR (Fenollar et al., 2004). Broad-range bacterial PCR uses primers to recognize conserved sequences of bacterial chromosomal genes encoding rRNA (Dicuono, 1999). This method is now considered a reliable clinical test for identifying the specific causative species of endocarditis when resected heart valve material is available (Nicolson et al., 1998; Millar et al., 2001; Houpikian & Raoult, 2005).

Conclusion

16S rRNA PCR gave a *de novo* microbiological diagnosis of *M. hominis* endocarditis in this case, further emphasizing the importance of this molecular method in making precise microbiological diagnoses and guiding antimicrobial therapy.

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References


