Case of aortic endocarditis caused by \textit{Lactobacillus casei}

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A case of \textit{Lactobacillus} aortic valve endocarditis in a 53-year-old immunocompetent patient with past history of rheumatic fever is reported. Clinical symptoms began after a dental extraction and the patient’s diet included several yogurts per day. Blood, bone marrow cultures and the replaced aortic valve were positive for \textit{Lactobacillus}. The clinical isolate was identified as \textit{Lactobacillus casei} by 16S rDNA sequencing.

Introduction

\textit{Lactobacillus} species include a diverse assemblage of Gram-positive, catalase-negative, non-spore-forming, aerobic or facultative anaerobic, rod-shaped bacteria (Collins \textit{et al.}, 1991). They inhabit a wide variety of habitats, including the gastrointestinal tracts of animals and phytophase, and are traditionally used in the manufacture of fermented foods and, in functional foods, as probiotics (Mattila-Sandholm \textit{et al.}, 1999). These micro-organisms are rarely infectious and their presence as commensals in the gastrointestinal tract is associated with protection against pathogens, stimulation of the immune system and positive effects on colonic health and host nutrition (Falk \textit{et al.}, 1998; Hooper \textit{et al.}, 2001; De Champs \textit{et al.}, 2003). Although lactobacilli are usually considered contaminants in blood cultures, they have been identified in some clinical reports as causal agents of dental caries, infectious endocarditis, urinary tract infections, corioamnionitis, endometritis, meningitis and intra-abdominal, liver and spleen abscesses (Husni \textit{et al.}, 1997). Commonly, these infections can be correlated with previous illnesses (recent surgery, transplants, valvulopathy, diabetes mellitus, AIDS and cancer) with either immunosuppressive therapy or antibiotic treatment, which could promote the development or the selection of the micro-organism. Here, we describe a case of aortic valve endocarditis caused by \textit{Lactobacillus casei} in a 53-year-old immunocompetent patient with a past history of rheumatic fever.

Case Report

A 53-year-old male was admitted to hospital with a history of persistent fever. The patient had a past medical history of rheumatic fever and reported a dental extraction 3 months before admission. Further history was unremarkable, except for his diet, which included several yogurts per day. Two weeks after dental extraction, the patient noted fever associated with muscle and joint pain. He improved markedly on treatment with oral doxycycline (100 mg twice a day) for 2 weeks. However, symptoms reappeared shortly after treatment, and he was admitted to the Department of Infectious Diseases.

On examination, he had a high fever (38.3 °C), a heart murmur and splenomegaly, without any other abnormalities. Results from blood tests showed anaemia (Hb 11.2 g dl\(^{-1}\)) and a raised erythrocyte sedimentation rate (82 mm h\(^{-1}\)). Three aerobic blood and bone marrow cultures yielded a Gram-positive rod, which was sensitive to chloramphenicol and tetracycline, and resistant to penicillin and vancomycin. The patient was treated again with doxycycline for 2 weeks, without changes in the fever pattern. At this point, a change in the existing heart murmur was noted. Although the previous transthoracic cardiac ultrasound had shown no abnormalities, the continued presence of fever, anaemia and a changing heart murmur did not allow us to exclude the
possibility of an endocarditis. A repeated transoesophageal cardiac ultrasound showed vegetation on the aortic valve. A diagnosis of endocarditis caused by a Gram-positive rod was made.

The patient was started on doxycycline and gentamicin, followed by piperacillin-tazobactam and finally imipenem. However, on this treatment, he showed no clinical improvement but, rather, continued with progressive weight loss, and finally became haemodynamically unstable. The patient underwent valve replacement surgery and was treated with doxycycline. The same Gram-positive rod isolated from the blood and bone marrow cultures was isolated from the culture of the removed valve. Eight months after surgery, the patient was fully recovered.

Microbiological Investigation

Blood cultures were collected into BacT/ALERT FA bottles and the heart valve was cultured in brain heart infusion broth. A Gram-positive non-haemolytic bacillus was isolated from all samples. The isolated bacillus was subcultured onto Columbia blood agar (bioMérieux) at 37 °C in aerobiosis. Biochemical tests performed on the isolate showed that reactions for catalase and oxidase were negative. Susceptibility tests were performed by the BSAC comparative method (Andrews, 2001) on DST agar (Oxoid) supplemented with 5 % blood. The isolate was susceptible to chloramphenicol, tetracycline, piperacillin-tazobactam, imipenem, rifampicin, erythromycin, clindamycin and gentamicin, and resistant to penicillin, ampicillin, cephradine, cefuroxime, cefotaxime, ceftazidime, vancomycin, amikacin, colistin, ciprofloxacin and fusidic acid. Phenotypic tests performed with API Coryne and API 32A systems (bioMérieux) did not result in an acceptable identification, although API 32A suggested the possibility of *Lactobacillus acidophilus*.

The need for a reliable diagnosis confirmation prompted sequencing of the 16S rDNA of the organism. Cells used for DNA extraction were grown for approximately 16 h at 30 °C in 20 ml MRS broth (Merck). Genomic DNA was isolated by the guanidium thiocyanate method (Pitcho et al., 1989). 16S rDNA was amplified with universal primers pA and pH (Edwards et al., 1989). The PCR amplification protocol was 35 cycles of 1 min denaturation at 94 °C, 1 min annealing at 50 °C and 2 min extension at 72 °C, after an initial denaturation step of 94 °C for 4 min, with a final extension step (72 °C for 5 min). The amplified fragment was purified with Concert rapid PCR purification system (Gibco-BRL) and cloned into pGEM-T Easy cloning vector (Promega), according to the manufacturer’s instructions. Recombinant plasmids were obtained by blue-white selection of *Escherichia coli* MRF’ clones. Plasmid DNA was extracted with Concert high purity plasmid miniprep system (Gibco-BRL). Selected clones were sequenced in an automated DNA capillary sequencer CEQ 2000-XL (Beckman Coulter) by a dye-labelled dideoxy termination method (DTCS, dye terminator cycle sequence start kit; Beckman Coulter). Five sequencing reactions were performed using vector primers T7 and SP6 and the internal primers 519R (5′-GWATTACC GCGGCGKCTG-3′), 907R (5′-CCGCAATTCTMTTTRAGTTT-3′) and 926F (5′-AAACTYAAKGAATTTGACGG-3′). The sequence was compared with GenBank entries using BLAST (Altschul et al., 1997), which indicated 99 % similarity to *L. casei*; the strain was therefore identified as *L. casei* HSM357872. The 1562 bp sequence of the 16S rDNA determined in this study was deposited in the GenBank database under accession number AF526388.

Discussion

Although infections are dependent on the aetiological agent, the degree of illness may be related to the clinical history of the patient (Husni et al., 1997; Wallet et al., 2002), namely underlying diseases or immunocompromised states. So, infections caused by micro-organisms usually regarded as non-invasive tend to be underestimated and, in some conditions, become true severe illness. Reports of human infections caused by micro-organisms that are usually not pathogenic have become more frequent in recent years (Ha et al., 1999; Olano et al., 2001; Frebourg et al., 2002; Flaherty et al., 2003). Usually, immunocompromised children and elderly individuals are more susceptible to this kind of infection. Bacteraemia caused by probiotic organisms is rare but underestimated, since they are normally regarded as contaminants and their role as primary invaders is also not always easy to establish. Other drawbacks in the identification of unusual disease agents by conventional methods are (i) that they are fastidious, (ii) that many involve some degree of subjective evaluation and (iii) that the regular procedures used in hospitals are, obviously, not designed for them (Wallet et al., 2002). As the 16S rRNA nucleotide sequence is available in databases for most bacterial species, 16S rDNA sequencing becomes a valuable identification strategy for such micro-organisms.

Since no other micro-organism was isolated from all clinical samples (blood, bone marrow and aortic valve), patient bacteraemia was caused primarily by *Lactobacillus* with no association to polymicrobial infections, contrary to other reports (Antony et al., 1996; Husni et al., 1997). A correlation was also found between the consumption of several yogurts per day and the bacteraemia after dental extraction, caused by bacteria usually related to probiotics. Although no direct link for the development of endocarditis by *L. casei* and yogurt ingestion could be established, the past history of rheumatic fever could be responsible for a locus of minor resistance in the aortic valve, allowing the establishment of the bacteria and the development of the endocarditis. Although *Lactobacillus* sp. bacteraemia is rarely considered life-threatening, the further development of serious and fatal illness can be promoted by several underlying clinical conditions, in which immunosuppressive therapy and use of sub-optimal antimicrobials or antibiotics without activity against lactobacilli are usually associated (Bayer et al., 1978; Husni et al., 1997; Wallet et al., 2002). Since the patient was
immunocompetent and the *Lactobacillus* isolate was susceptible *in vitro* to the antibiotics used in clinical treatment, the hypothesis of sub-optimal dosages cannot be ruled out.

Although the increased consumption of dairy products does not appear to promote an increase in *Lactobacillus* sp. bacteraemia (Salminen *et al.*, 2002), it is noteworthy that a probiotic strain of *Lactobacillus rhamnosus* has been involved in several bacteraemia cases (Salminen *et al.*, 2002) and *Lactobacillus* populations can survive in gastrointestinal tracts of humans after oral administration (De Champs *et al.*, 2003). Regarding the association of *Lactobacillus* endocarditis with ingestion of dairy products and dental extraction procedures, conflicting results have been reported (Presterl *et al.*, 2001; Wallet *et al.*, 2002).

Even though the consumption of probiotics and dental manipulations cannot be considered risk factors in the development of diseases caused by bacteria usually considered non-pathogenic, specific individual clinical histories (namely, underlying diseases and the usage of specific treatments) should be taken into account.

This report of a *L. casei* aortic valve endocarditis in an immunocompetent individual should alert both clinicians and microbiologists to the possibility of unusual pathogens causing serious illnesses. 16S rDNA molecular identification represents a powerful tool in diagnosis confirmation of infrequent pathogens.

**References**


