Enterococcus faecium small colony variant endocarditis in an immunocompetent patient

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Abstract

Small colony variants (SCV) are slow-growing subpopulations of bacteria usually associated with auxotrophism, causing persistent or recurrent infections. *Enterococcus faecalis* SCV have been seldom described, and only one case of *Enterococcus faecium* SCV has been reported, associated with sepsis in a leukaemia patient. Here we report the first case described of bacteraemia and endocarditis by SCV *E. faecium* in an immunocompetent patient.

Keywords: Endocarditis, *Enterococcus*, *Enterococcus faecium*, sepsis, small colony variant
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Introduction

The genus *Enterococcus* has medical relevance because of its increasing incidence and antibiotic resistance. Clinical *E. faecalis* strains possess multiple pathogenicity determinants, while only *esp* and *gelE* determinants are usually identified in *E. faecium* medical isolates [1,2].

Small colony variants (SCV) are slow-growing subpopulations of bacteria that form phenotypically different, small colonies on usual culture media compared to conventional isolates belonging to the same species. This phenotype is often unstable [3]. The biochemical basis of these phenotypes is auxotrophism (e.g. menadione, haemin, thymidine), though SCV where auxotrophism cannot be defined have also been described [3,4]. Because of their capacity to persist in host cells and their metabolic and antibiotic resistance features, they are frequently associated with persistent or recurrent infections [5]. *Staphylococcus aureus* SCV have been the most extensively studied, but SCV have also been described in other staphylococci and in Gram-negative microorganisms [3]. A very small number of *Enterococcus faecalis* SCV have been described associated with animal or human infections [6,7], and only one case of *Enterococcus faecium* SCV [5] has been reported, in an acute myeloid leukaemia patient who developed sepsis. We report a case of bacteraemia and endocarditis by SCV *E. faecium* in an immunocompetent patient.

Case report

A 71-year-old woman with a history of hypertension, ischaemic heart disease and myocardial infarction was treated with two percutaneous transluminal coronary angioplasty stents. She had severe tricuspid and mitral regurgitation, which was treated with mitral bioprosthesis and tricuspid annuloplasty.

Six months after heart surgery, she presented to the emergency department with a blood pressure of 125/54 mm Hg, heart rate of 100 beats per minute, oxygen saturation of 100% and temperature of 38°C. She complained of midthoracic discomfort and tenderness in the area of the surgical scar, and referred to asthenia and anorexia that had existed since her heart surgery. Heart auscultation detected a systolic multifocal murmur. Lung auscultation was normal. C-reactive protein was 4.73 mg/dL.

Blood culture grew two morphotypes of Gram-positive, catalase-negative cocci (Fig. 1). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) identified both morphotypes as *Enterococcus faecium*. A further blood culture 4 days later yielded the same result.

Both isolates were susceptible to penicillin, ampicillin, vancomycin and daptomycin, and had a minimum inhibitory concentration of gentamicin allowing synergism with β-lactams. Susceptibility profiles were identical for both morphotypes.

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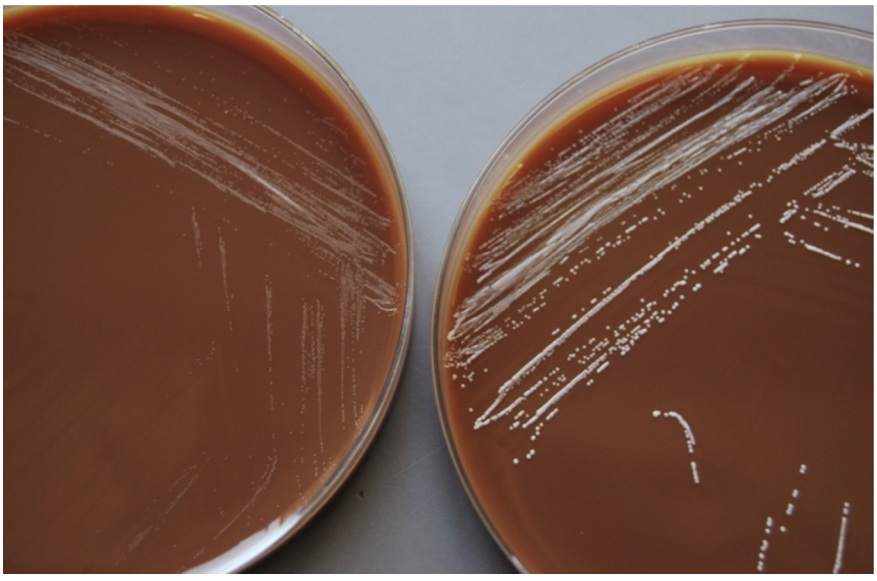

FIG. 1. Normal phenotype and small colony variants of *Enterococcus faecium* isolated from patient with endocarditis.

Transthoracic echocardiography revealed a decreased ejection fraction (about 40%) with normal-functioning mitral bioprosthesis and competent tricuspid annuloplasty. Transesophageal echocardiography revealed a broken chordae tendineae but did not show any other images clearly suggesting endocarditis.

Computed tomography ruled out other infectious foci. The patient was admitted to the cardiology ward with a diagnosis of possible endocarditis according to Duke criteria, with ampicillin (2 g every 4 hours administered intravenously) and gentamicin (1 mg/kg every 8 hours administered intravenously) provided for 6 weeks. Fever decreased soon after initiation of treatment (temperature decreased to 37.5°C), and blood cultures were repeatedly negative. The patient became afebrile and was discharged soon after the end of treatment.

For the microorganisms isolated, both the SCV and the normal phenotype were stable and did not reverse after several subcultures. While the normal phenotype showed α-haemolysis, the SCV did not show any haemolysis. The SCV grew more slowly both in agar plates and broth cultures. The growth curves of both phenotypes confirmed the slower growth of the SCV (10- to 15-fold slower during the first 6 hours). Multilocus sequence typing sequences were identical for both morphotypes. Auxotrophism study with nicotinamide adenine dinucleotide (NAD), haemin, menadione, thiamine, timodine and oleic acid did not yield significant colony size differences.

Discussion

SCV have been described in a wide range of species, but SCV *E. faecalis* was first reported and studied in detail in 2009 [6] and *E. faecium* in 2012 [5]. *E. faecalis* SCV are more heterogeneous in size and shape than normal enterococci. Their cell walls are thicker than those of normal cells and lack the bilayer structure, and auxotrophism tests suggest dependence on different substrates [6]. Growth curves show that SCV grow more slowly [3]. Just like *S. aureus* SCV, *E. faecalis* SCV have a higher ability to invade and persist in mammalian cells. *E. faecalis* SCV have been mainly described in endocarditis [6,7].

The only case previously described of infection associated with SCV *E. faecium* [5] was in a patient with an acute myeloid leukaemia evolved from a myelodysplastic syndrome; the patient developed a urinary tract infection and fever during chemotherapy. *E. faecium* was cultivated from the urine and blood cultures with the same susceptibility profile. *E. faecium* isolated from blood cultures grew with a normal and a SCV phenotype. Meropenem plus vancomycin treatment was started and was further expanded to include liposomal amphotericin B and acyclovir. The patient became afebrile on day 35 and was discharged on day 61 after admission.

As in our case, the SCV was stable, showed almost no haemolysis, and grew more slowly in time–growth curves. Our isolate did not show any auxotrophism for NAD, haemin, menadione, thiamine, timodine or oleic acid. SCV enterococci previously described were auxotrophic for haemin [6] (*E. faecalis*), NAD and thymidine (*E. faecium*) [5]. SCV for which auxotrophism could not be defined had not been described for enterococci although they have been described for other microorganisms [3].

MALDI-TOF correctly identified the isolate. Previous reports suggest that conventional biochemical tests have some

difficulty correctly identifying SCV [5]. Thus, the increasing use of MALDI-TOF in clinical microbiology might increase the detection and study of SCV enterococci.

As frequently happens with SCV, our isolate was associated with endocarditis [3,6,7]. Though SCV are frequently resistant to aminoglycosides, our isolate was susceptible to gentamicin concentrations allowing synergism with β-lactams, and β-lactam plus gentamicin treatment resulted in cure. Though vancomycin resistance is becoming a serious problem in *E. faecium*, usually associated with *van* genes, both our case and the other SCV *E. faecium* case described in humans [5] were vancomycin susceptible.

A suspicion of SCV when several morphotypes appear in culture, especially in persistent or recurrent infections, and their reliable identification with MALDI-TOF may allow better diagnosis and treatment of these patients, as well as better knowledge about the species in which this behavior can be expected and the pathogenicity and susceptibility features of these isolates.

Conflict of Interest

None declared.

References

[1] Eaton TJ, Gasson MJ. Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol 2001;67:1628–35.

[2] Baldisserotto Comerlato C, Costa Carvalho de Resende M, Caierão J, Alves d'Azevedo P. Presence of virulence factors in *Enterococcus faecalis* and *Enterococcus faecium* susceptible and resistant to vancomycin. Mem Inst Oswaldo Cruz 2013;108:590–5.

[3] Proctor RA, von Eiff C, Kahl BC, Becker K, McNamara P, Herrmann M, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat Rev Microbiol 2006;4:295–305.

[4] Kahl B, Herrmann M, Everding AS, Koch HG, Becker K, Harms E, et al. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. J Infect Dis 1998;177:1023–9.

[5] Gröbner S, Beck J, Schaller M, Autenrieth IB, Schulte B. Characterization of an *Enterococcus faecium* small-colony variant isolated from blood culture. Int J Med Microbiol 2012;302:40–4.

[6] Wellinghausen N, Chatterjee I, Berger A, Niederfuehr A, Proctor RA, Kahl BC. Characterization of clinical *Enterococcus faecalis* small-colony variants. J Clin Microbiol 2009;47:2802–11.

[7] Benes J, Dzupova O, Setina M, Feuereisl R, Svec P, Pantucek R. Relapsing endocarditis caused by *Enterococcus faecalis* forming small colony variants. Scand J Infect Dis 2013;45:800–3.