Dwarfs in disguise: multiple spinal abscesses and spondylodiscitis caused by an Enterococcus faecium small-colony variant

Steffen Höring1,*, Katharina Sobotta1, Sylke Schneider2, Bettina Löffler1 and Jürgen Rödel1

Abstract
Small-colony variants are slow-growing subpopulations of bacteria known to be involved in latent or recurrent infections, especially in deep-seated foci. Their atypical growth in small colonies can hamper prompt and correct identification in clinical specimens. Here, we present the first case of multiple spinal abscesses and spondylodiscitis associated with an Enterococcus faecium small-colony-variant in an immunocompetent patient. This case demonstrates the diagnostic challenges when encountering this phenotype in the diagnostic laboratory.

INTRODUCTION
Small-colony variants (SCVs) are slow-growing subpopulations of bacteria known to be involved in latent or recurrent infections. By lowering their virulence and metabolic activity, SCVs escape the host immune defence and are able to persist in deep-seated foci [1]. Their atypical growth in small colonies on agar medium can hamper prompt and correct identification when cultivated from clinical specimen. While SCV infections are well described for various bacteria, such as Staphylococcus aureus or Pseudomonas aeruginosa, only few clinical case reports exist so far for enterococci [1–7]. Here, we present the first case of multiple spinal abscesses and spondylodiscitis associated with an Enterococcus faecium SCV in an immunocompetent patient. This case underlines the importance of SCVs in deep-seated infections and demonstrates the diagnostic challenges experienced when encountering this phenotype in the diagnostic laboratory.

CLINICAL CASE
A 75-year-old male patient presented at our emergency department with acute febrile illness and worsening of general condition. Physical examination yielded febrile body temperature (39.1 °C) and pain on percussion over the lower back. The underlying diseases were a chronic obstructive pulmonary disease due to chronic nicotine abuse (50 pack years), mild dementia and a history of alcohol abuse considered as a predisposing factor for infectious diseases. Furthermore, a compression fracture of the 12th thoracic vertebral body associated with osteoporosis was described in the previous medical history. Basic laboratory testing revealed a mild normocytic anaemia, mild leukocytosis (11.200 cells/l), thrombocytosis (406.000 cells/l) and elevated serum levels of C-reactive protein (143.6 mg l⁻¹). Urinary tract infection was ruled out by urine dipstick test and chest radiography showed no pulmonary focus of infection. Blood cultures were drawn before the initiation of intravenous antibiotic therapy with amoxicillin/clavulanate 875 mg/125 mg BID.

On day two of treatment, four out of four blood culture flasks were flagged positive and Gram-staining showed Gram-positive cocci. Final blood culture results, including pathogen identification as Enterococcus faecium and antibiotic susceptibility testing, were submitted on day four of treatment. In consequence, screening for a source of infection was extended. Transesophageal echocardiography revealed no indications of endocarditis and urine samples tested as sterile. Magnetic resonance tomography (MRT) of the lumbosacral spine showed multiple fluid accumulations representing small abscesses located inter-, para- and praevertebral between the third lumbar and the first sacral vertebral body (Fig. 1). The patient was transferred to our orthopaedic department and surgical source control with intervertebral abscess removal.

Received 09 November 2018; Accepted 07 February 2019; Published 27 March 2019

Author affiliations: 1 Institute of Medical Microbiology, Jena University Hospital, Am Klinikum1, D-07747 Jena, Germany; 2 Clinic for Internal Medicine, Waldkrankenhaus ‘Rudolf Elle’, Klosterlausnitzer Straße 81, D-07607 Eisenberg, Germany.

* Correspondence: Steffen Höring, Steffen.Hoering@med.uni-jena.de

Keywords: small-colony variant; SCV; Enterococcus faecium; spondylodiscitis; spinal abscess.

Abbreviations: AST, antibiotic susceptibility testing; BID, bis in die; MIC, minimum inhibitory concentration; MRT, magnet resonance tomography; SCV, small-colony variant.

000012 © 2019 The Authors
This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Höring et al., Access Microbiology 2019;1

After interlumbar body fusion (L5/S1) and spondylodesis (L3-S1) was performed on day 12. Antibiotic therapy was broadened by applying intravenous linezolid 600 mg BID.

The patient was dismissed in good general condition without signs of infection on post-operative day 15.

**MICROBIOLOGICAL FINDINGS**

Two sets of aerobic/anaerobic blood culture flasks drawn on the day of admission were flagged positive by the automated incubation system (BACTEC 9000; Becton Dickinson, USA) after 30.3 h (minimum) and 35.3 h (maximum) of incubation at 37 °C. All tests performed and results yielded refer to the isolate with the internal identification code BK001612. Microscopic examination of Gram-stained blood culture smears showed Gram-positive cocci in pairs and short chains. Overnight subcultures yielded γ-haemolytic grey colonies that were round and pointy in shape with a diameter of less than 0.1 mm on Columbia agar containing 5% sheep blood and chocolate agar (both Oxoid, Thermo Fisher Scientific, Germany), as shown in Fig. 2. No growth was observed on Drigalski’s lactose agar (Oxoid, Thermo Fisher Scientific, Germany). The catalase test produced a negative result.

Preliminary antibiotic susceptibility testing performed with the BK001612 isolate, applying the agar diffusion method, showed susceptibility to amoxicillin and vancomycin, amongst others.

Due to its colony morphology and antibiotic resistance pattern, the pathogen was misidentified as non-haemolytic streptococci in the first place. However, biochemical identification with the VITEK-2 system (BioMérieux, France) using the VITEK-2 GP ID card resulted in *E. faecium* with excellent test confidence.

The identification results were subsequently confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis and 16s rRNA sequencing. For MALDI-TOF analysis, mass spectra were obtained with the VITEK MS system (BioMérieux, France) and compared against the VITEK MS IVD database. 16s rRNA sequence analysis was performed using the National Center for Biotechnology Information database (NCBI BLAST). The sequence was deposited in NCBI GenBank under the accession number MK138375.

Automated susceptibility testing performed using the VITEK-2 platform was aborted without results when applying the VITEK-2 AST-ST03 and the AST-P587 test card. A final resistogram was yielded by determining the minimal inhibitory concentrations (MICs) with E-test strips (bestbion dx, Germany) and result categorization according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints version 8.0, as shown in Table 1.

Single auxotrophism testing was carried out on Mueller–Hinton agar (Oxoid, Thermo Fisher Scientific, Germany) using standard haemin discs and blank discs loaded with 1.5 µg thymidine and 1.5 µg menadione (all Sigma-Aldrich, USA). No switch of phenotype or enhancement of growth was observed around the discs.

**DISCUSSION**

We present the first case of multiple spinal abscesses and spondylodiscitis caused by an *E. faecium* SCV.

Of interest, the SCV strain described in our case was susceptible to all of the cell wall active agents tested. This is to some extent exceptional, because in the last three decades high-level ampicillin resistance in clinical *E. faecium* isolates rose to over 90% in Germany and ampicillin-susceptible strains are only rarely isolated in our laboratory [8]. Further, for bacteria other than enterococci, SCVs are described to be rather more resistant to antibiotics than their related wild-type phenotype. For example, high-level gentamicin resistance is common in *Staphylococcus aureus* SCV strains [9] and increased resistance levels towards beta-lactam antibiotics have also been described for SCVs [10, 11]. The few case reports existing so far report ampicillin-resistant isolates as well as an ampicillin-susceptible *E. faecium* SCV isolate [3, 7].
Ciprofloxacin resistance as in our case is a common trait in *E. faecium* and is found in the majority of strains isolated in Germany [12]. Subinhibitory dosing of ciprofloxacin has previously been described to induce SCV formation and the expression of adhesion factors facilitating pathogen persistence [13, 14]. Thus, the high ciprofloxacin MIC of the BK001612 *E. faecium* strain might have contributed to expression of the SCV phenotype. However, a prior treatment with ciprofloxacin is not documented in the medical history of this case.

Furthermore, it is relevant to mention the difficulties we encountered in performing automated susceptibility testing (AST) using the VITEK-2 system. The VITEK-2 platform is in widespread use in medical microbiology laboratories and has proven to be reliable for susceptibility testing of enterococci [15]. Nevertheless, in our case AST was aborted repeatedly when using the *E. faecium* SCV BK001612 strain. Most likely this was due to slow growth of SCVs, which was also indicated by the long time-to-positivity of the blood culture flasks, which hinders correct interpretation of growth curves by the VITEK-2 system. Therefore, in cases of suspected SCV infection, manual susceptibility testing using the E-strip test seems to be the preferred approach.

Misidentification of SCVs, as happened in our case, has been described previously. While in previous cases the biochemical profile or single biochemical tests led to wrong conclusions [16–19], in our case the morphology of the bacterial colonies and the antibiotic resistance pattern were misleading characteristics. A similar mistake has been described by Ogihara et al., who misidentified an *E. faecalis* SCV isolate with non-haemolytic streptococci. The authors also emphasized the potential clinical impact of species misidentification through choosing inappropriate antibiotic therapy [5]. Our case gives another good example of SCVs misleading clinical microbiologists and potentially impairing patient care. Therefore, greater awareness among clinical microbiologists of SCVs as a potential phenotype is necessary.

This case provides relevant observations regarding the antibiotic resistance of SCVs and the diagnostic challenges medical microbiologists face when encountering this phenotype. Furthermore, it provides additional proof of the

| Antibiotic         | MIC           | Interpretation |
|--------------------|---------------|----------------|
| Vancomycin         | 1 mg l⁻¹      | S              |
| Linezolid          | 2 mg l⁻¹      | S              |
| Amoxicillin        | 0.25 mg l⁻¹   | S              |
| Ampicillin/sulbactam | 0.094 mg l⁻¹ | S              |
| Ciprofloxacin      | 6 mg l⁻¹      | R              |
| Gentamicin high-level | 4 mg l⁻¹   | S              |

**Table 1.** Antibiotic susceptibility testing results for *E. faecium* SCV BK001612.
clinical relevance of SCVs in deep-seated infections caused by enterococci.

**Conclusion**

In summary, we describe the first case of spondylodiscitis and spinal abscess caused by an Enterococcus faecium SCV. The aim of this report is to raise awareness among clinical microbiologists of the small-colony phenotype in enterococci. In particular, when encountering bacterial colonies resembling γ-haemolytic streptococci with contradictory resistance patterns or ambiguous biochemical test results in deep-seated infections, SCVs of enterococci should be taken into consideration.

**Funding information**

This study was financed by internal funding.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

Our study consists solely of retrospective observations and no intervention to patient care was made. The patient’s informed consent was obtained before submitting this report for publication.

**References**

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

2. Wellinghausen N, Chatterjee I, Berger A, Niederfuehr A, Proctor RA et al. Characterization of clinical Enterococcus faecalis small-colony variants. *J Clin Microbiol* 2009;47:2802–2811.

3. Gröbner S, Beck J, Schaller M, Autenrieth IB, Schulte M. Characterization of an Enterococcus faecium small-colony variant isolated from blood culture. *Int J Med Microbiol* 2012;302:40–44.

4. Benes J, Dzupova Q, Setina M, Feuereisil R, Svec P et al. Relapsing endocarditis caused by Enterococcus faecalis forming small colony variants. *Scand J Infect Dis* 2013;45:800–803.

5. Ogihara S, Saito R, Sawabe E, Hagihara M, Tohda S. First Japanese case of infectious endocarditis due to Enterococcus faecalis small-colony variants. *J Infect Chemother* 2016;22:716–719.

6. Kubota N, Kuzumoto K, Hidaka E, Yoshizawa K, Yamoto K et al. First isolation of oleate-dependent Enterococcus faecalis small-colony variants from the umbilical exudate of a pediatric patient with omphalitis. *J Med Microbiol* 2013;62:1883–1890.

7. Egido SH, Ruiz MS, Inès Revuelta S, Garcia IG, Bellido JLM. Enterococcus faecium small colony variant endocarditis in an immunocompetent patient. *New Microbes New Infect* 2016;6:47–49.

8. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Paul-Ehrlich-Gesellschaft für Chemotherapie e.V. GERMAP 2015–Report on the consumption of antimicrobials and the spread of antimicrobial resistance in human and veterinary medicine in Germany; 2016.

9. Massey RC, Buckling A, Peacock SJ. Phenotypic switching of antibiotic resistance circumvents permanent costs in Staphylococcus aureus. *Curr Biol* 2001;11:1810–1814.

10. Tuschscherr L, Kreis CA, Hoerr V, Flint L, Zachmeister M et al. Staphylococcus aureus develops increased resistance to antibiotics by forming dynamic small colony variants during chronic osteomyelitis. *J Antimicrob Chemother* 2016;71:438–448.

11. Chuard C, Vaudaux PE, Proctor RA, Lew DP. Decreased susceptibility to antibiotic killing of a stable small colony variant of Staphylococcus aureus in fluid phase and on fibronectin-coated surfaces. *J Antimicrob Chemother* 1997;39:603–608.

12. Bundesamt für Verbraucherschutz und Lebensmittelsicherheit P-E-GfCeV. GERMAP 2015–Bericht über den Antibiotikaverbrauch und die Verbreitung von Antibiotikaresistenzen in der Human- und Veterinärmedizin in Deutschland. In: Bundesamt für Verbraucherschutz und Lebensmittelsicherheit P-E-GfCeV (editor). Rheinbach: Antimicrob Agents Chemother 2017:61.

13. Vestergaard M, Paulander W, Inger H. Activation of the SOS response increases the frequency of small colony variants. *BMCRes Notes* 2015;8:749.

14. Sinel C, Cacaci M, Meignen P, Guérin F, Davies BW et al. Subinhibitory concentrations of ciprofloxacin enhance antimicrobial resistance and pathogenicity of Enterococcus faecium. *Antimicrob Agents Chemother* 2017:61.

15. Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, Vitek 2, for identification and antimicrobial susceptibility testing of enterococci. *J Clin Microbiol* 2000;38:2108–2111.

16. Seifert H, von Eiff C, Falkenheuer G. Fatal case due to methicillin-resistant Staphylococcus aureus small colony variants in an AIDS patient. *Emerg Infect Dis* 1999;5:450–453.

17. Seifert H, Wisplinghoff H, Schnabel P, von Eiff C. Small colony variants of Staphylococcus aureus and pacemaker-related infection. *Emerg Infect Dis* 2003;9:1316–1318.

18. Tappe D, Claus H, Kern J, Marzinzig A, Frosch M et al. First case of febrile bacteremia due to Staphylococcus aureus small colony variant of Escherichia coli. *Eur J Clin Microbiol Infect Dis* 2006;25:31–34.

19. Roggenkamp A, Sing A, Hornf M, Brunner U. Autenrieth IB et al. Chronic prosthetic hip infection caused by a small-colony variant of Escherichia coli. *J Clin Microbiol* 1998;36:2530–2534.