Case Report

Isolation of *Helcococcus kunzii* from plantar phlegmon in a vascular patient

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Helcococcus kunzii has previously been considered to belong to the normal skin flora of podiatry patients. Here, *H. kunzii* was isolated in abundance from a pus specimen collected by incision and drainage of plantar phlegmon. This fastidious Gram-positive species was unambiguously identified with the colorimetric VITEK 2 GP card identification system. This suggests that this phenotypic identification system is able to identify promptly *H. kunzii*, which should be considered a potential pathogen.

Case report

A 79-year-old man was hospitalized in December 2006 due to gangrenous ischaemia of the third right toe and a right ankle ulcer. There was a history of hypertension associated with severe intermittent claudication in both legs. Indeed, 3 months before the current admission, in September 2006, the patient underwent a first amputation of the fourth left toe associated with percutaneous transluminal angioplasty (PTA) because of focal stenosis of the left and right superficial femoral and left popliteal arteries. After PTA, the distal blood flow was restored.

On admission in December 2006, the patient had no systemic signs of infection and was metabolically stable. On examination, the right popliteal, dorsalis pedis and posterior tibial pulses were absent, as were the left dorsalis pedis and posterior tibial pulses. The Doppler ultrasound detected no flow in the right posterior and anterior tibial arteries. Plain radiography showed osteitis of the third right toe and amputation was done. One intraoperative bone biopsy was positive by culture for *Enterococcus faecalis*, *Klebsiella oxytoca* and meticillin-susceptible *Staphylococcus aureus*. After completion of 3 weeks of amoxicillin/clavulanate, the patient was discharged home. Four months later, in April 2007, the patient was hospitalized in a re-education centre for local care of a right heel ulcer and non-healing surgical wound of the third right toe amputation. In fact, at the time of admission, the patient exhibited right plantar phlegmon, the temperature was 37.8 °C and blood pressure was 157/57 mmHg. Laboratory findings were a white blood cell count of 24 000 mm⁻³ with 90 % polymorphonuclear leukocytes, a sedimentation rate of 111 min (normal value 3–10 min) and a C-reactive protein level of >200 mg l⁻¹ (normal value <6 mg l⁻¹). The patient was referred to a

vascular surgeon to consider operative exploration. The Doppler ultrasound revealed that the lower limb arteries were occluded bilaterally. The surgeon performed curative surgical debridement and the phlegmon was incised and drained. Wound fluid as well as deep tissue biopsies were collected intraoperatively for microbiological examination.

Microbiological investigation

The examination of a Gram-stained smear of the intraoperative samples showed numerous polymorphonuclear leukocytes and a mixture of Gram-positive cocci and Gram-negative bacilli. The specimens were inoculated onto Columbia agar containing 5 % horse blood, bromocresol purple (BCP) agar and in Brain Heart Infusion (BHI) broth and incubated aerobically for 24 h at 37 °C. A Viande Levure (VL) agar plate was also incubated under anaerobic conditions for 48 h at 37 °C. After 24 h of incubation under aerobic conditions, a heavy growth of pinpoint, slightly grey and α -haemolytic colonies appeared on the Columbia agar associated with a few colonies of coliforms subsequently identified as *Klebsiella oxytoca*, which was also recovered on BCP agar. A Gram stain of the pinpoint colonies showed Gram-positive cocci arranged in pairs and clusters but not in chains. These colonies were catalase- and oxidase-negative. A Gram smear and the subculture of the BHI yielded a similar mixture of Gram-positive cocci and Gram-negative bacilli. In addition to pinpoint colonies and colonies of *K. oxytoca*, strictly anaerobic bacilli, later identified as *Bacteroides fragilis*, grew on the VL agar plate incubated anaerobically. The enzyme profile and biochemical characteristics of the Gram-positive cocci were determined by the colorimetric VITEK 2 GP card identification system (bioMérieux). The isolate gave positive reactions for leucine arylamidase,

alanine arylamidase, L-pyrrolidonylarylamidase, β -galactosidase, D-mannose and β -galactopyranosidase and was identified as *Helcococcus kunzii* with a probability of 99 %. To confirm this identification, partial sequencing of the 16S rDNA was performed. Pair-wise alignments of the 16S rRNA gene showed that the isolate was 99 % identical to *H. kunzii* lodged in GenBank with the accession number DQ082899.1 (Woo *et al.*, 2005). Antimicrobial susceptibility testing as determined by the disc diffusion method using Mueller–Hinton agar supplemented with 5 % sheep blood showed that the isolate of *H. kunzii* was susceptible to penicillin, ampicillin, aminoglycosides and vancomycin.

Discussion

H. kunzii was first described in 1993. This species grows slowly and shares many phenotypic characteristics with other fastidious Gram-positive cocci such as *Aerococcus viridans* (Collins *et al.*, 1993). *H. kunzii* is considered to be part of the normal flora of the skin of the lower extremities (Haas *et al.*, 1997). However, underlying conditions such as diabetes and/or vascular insufficiencies may be linked to colonization with *H. kunzii* and this species might be a component of the polymicrobial flora of lower extremity ulcers (Caliendo *et al.*, 1995; Haas *et al.*, 1997). Thus isolation in mixed superficial culture, particularly with *Staphylococcus aureus*, makes it difficult to demonstrate a clear-cut pathogenic role for this species (Caliendo *et al.*, 1995; Haas *et al.*, 1997) in diabetic or vascular foot ulcers. Here, *H. kunzii* was isolated along with enteric bacteria and anaerobes from a foot infection in a non-diabetic vascular patient, as previously described (Haas *et al.*, 1997). However, the presence of *H. kunzii* by Gram staining and from two separate deep samples, in abundance, suggested that *H. kunzii* may play a pathogenic role in the phlegmon. In addition, some authors reported the isolation of *H. kunzii* in pure culture from life-threatening invasive infections such as primary bacteraemia and empyema thoracis in two intravenous-drug users (Woo *et al.*, 2005). Moreover, *H. kunzii* has also been isolated from an infected sebaceous cyst associated with cellulitis (Peel *et al.*, 1997), from a breast abscess (Chagla *et al.*, 1998) and from a post-surgical foot abscess (Riegel & Lepargneur, 2003) in immunocompetent patients. These reports demonstrated that *H. kunzii* plays a potential pathogenic role. Therefore, routine bacteriological laboratories should be able to

identify promptly this organism. Unfortunately, *H. kunzii* resembles several members of various fastidious cocci and some commercial systems such as API systems can fail to identify this species. Usually, the API 20 STREP profile of *H. kunzii* corresponds to a 'doubtful *Aerococcus viridans*' with a numerical profile of 4100413 (Chagla *et al.*, 1998; Haas *et al.*, 1997; Woo *et al.*, 2005). Similarly, the VITEK 2 system combined with fluorometric cards misidentified *H. kunzii* (Wallet *et al.*, 2005) and the ability of other automated systems such as BD Phoenix to identify correctly *H. kunzii* is unknown. In contrast, the VITEK 2 colorimetric system, which is known to better identify members of the *Streptococcaceae* (Wallet *et al.*, 2005), can provide discriminating identification of *H. kunzii* by analysis of 43 biochemical characters. This last automated system is a good alternative to 16S rRNA sequencing, which may not be routinely available in many laboratories.

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