Case Report

Weissella confusa: a rare cause of vancomycin-resistant Gram-positive bacteraemia

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We describe a case of bacteraemia caused by Weissella confusa in a 48-year-old male who was operated on for adenocarcinoma of the gastro-oesophageal junction and maintained on total parenteral nutrition. Blood cultures were positive for a vancomycin-resistant streptococcus-like organism which was identified as W. confusa by 16S rRNA gene sequencing.

Introduction
Reports of clinical infections due to the lactobacillus-like, vancomycin-resistant, Gram-positive coccobacillus Weissella confusa are rare as it is usually considered a contaminant. Additionally, species level identification is cumbersome and not performed. Due to the unusual Gram-stain morphology of Weissella species, it is usually confused with Lactobacillus- or Leuconostoc-like organisms, which are both vancomycin-resistant. The literature contains 18 case reports implicating W. confusa as the causal agent of abscess, bacteraemia and endocarditis (Lee et al., 2011; Salimnia et al., 2011; Shin et al., 2007; Flaherty et al., 2003). In most of these cases, the patients had a chronic illness with either immunosuppression or long-standing antibiotic therapy, which leads to selection of micro-organisms. W. confusa bacteraemia in a patient on total parenteral nutrition has been reported earlier (Lee et al., 2011; Olano et al., 2001). Here we discuss a case of monomicrobial bacteraemia due to W. confusa in a 48-year-old man who was maintained on total parenteral nutrition following surgery for mucinous adenocarcinoma of the gastro-oesophageal junction.

Case report
A 48-year-old man was referred with a complaint of progressive dysphagia for the previous 3 months. Upper gastrointestinal endoscopy showed a tumour at the gastro-oesophageal junction. Computerized tomography revealed the extension to proximal 4 cm of the stomach with no lymph node involvement. Biopsy confirmed mucinous adenocarcinoma (Sievert type II). The patient was treated by oesophagegastrectomy (Ivor Lewis operation) with a feeding jejunostomy tube left in situ for enteral nutrition. He developed symptoms of sepsis on the fourth post-operative day and blood cultures grew vancomycin-resistant Gram-positive coccobacilli in both aerobic and anaerobic bottles. The isolate was identified later as W. confusa. The patient was successfully treated with intravenous cefoperazone–sulbactam and metronidazole given for 8 days and is on chemotherapy with regular follow-up, without any relapse. The source of bacteraemia in our patient was most likely to be gut flora.

Microbiology
The blood cultures collected on the fourth post-operative day in BacT/Alert bottles flagged positive after 4 days of incubation. Subcultures on sheep blood agar grew small α-haemolytic colonies of catalase-negative, non-motile, Gram-positive cocci. Further biochemical testing revealed that the isolate was negative for cytochrome oxidase and nitrate reduction while aesculin hydrolysis and arginine deamination were positive. Acid was produced from galactose, maltose, sucrose and xylose. The broth Gram-stain morphology showed elongated Gram-positive bacilli. A thermotolerance test done in de Man, Rogosa and Sharpe (MRS) broth was negative at 45 °C. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method on Muller–Hinton agar with sheep blood as recommended by Clinical and Laboratory Standards Institute guidelines (CLSI, 2006). The isolate was sensitive to penicillin, cefuroxime, ceftriaxone, amoxicillin, erythromycin, clindamycin and tetracycline while it...
was resistant to vancomycin. The MIC, determined by Etest (AB Biodisk), for vancomycin was 256 μg ml⁻¹ and that for daptomycin was 0.125 μg ml⁻¹, which was sensitive. Based on the unusual sensitivity pattern, broth Gram-stain morphology and biochemical reactions, the isolate was identified as W. confusa.

The strain identity was further confirmed by the amplification of the 16S rRNA gene. Genomic DNA of the bacterial strains grown in LB medium for 16 h at 30 °C was extracted using the acid guanidinium thiocyanate–phenol/ chloroform extraction method (Pitcher et al., 1989). The 16S rRNA cistrons of this isolate were amplified with the bacterial universal primers 27F (8–27, forward; 5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (1492–1510, reverse; 5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991). The partial sequence of the PCR products was determined with an ABI Prism Cycling Sequencing Kit (BigDye Terminator Cycle). The determined sequences consisted of approximately 323 nt and were compared with the sequences of other Gram-positive, catalase-negative species available in the GenBank database by using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST), which revealed 100% similarity to the sequence of prototype W. confusa strain NH 02 (accession no. AB425970.1). The GenBank accession numbers of the 16S rRNA gene sequences of the isolates from two positive blood cultures in this study are HQ433525 and HQ433526.

**Discussion**

Probiotic organisms are rarely considered pathogens as their ability to cause clinical infection is difficult to establish. Lactobacillus species and Leuconostoc species are increasingly being recognized as a cause of bacteraemia especially in patients with diabetes, cancer, previous exposure to vancomycin, selective gut decontamination and recent gastrointestinal procedures (Lee et al., 2011; Salimnia et al., 2011; Shin et al., 2007). They are seldom identified to the species level due to their fastidious nature and the need to perform additional manual testing, which is laborious and time-consuming (Facklam & Elliott, 1995).

Based on the 16S rRNA gene phylogenetic analysis, Leuconostoc paramesenteroides and related species including Lactobacillus confusus were reclassified in the new genus Weissella with 15 recognized species of which W. confusa and Weissella cibaria have been isolated from human and animal clinical specimens (Collins et al., 1993). Weissella can be differentiated from other homofermentative lactobacilli and lactic acid bacteria by the production of gas from carbohydrates and differentiated from heterofermentative lactobacilli due to the presence of lysine–alanine intrapetide bonds within the cell wall of Weissella species (Collins et al., 1993). Biochemical and physiological properties such as arginine deamination, aesculin hydrolysis, acid production from certain carbohydrates, growth at 42 °C and broth Gram stain morphology can be used to differentiate W. confusa from enterococci, streptococci, Leuconostoc, Lactobacillus and Lactococcus species (Olano et al., 2001). Taxonomically, W. confusa is most closely related to W. cibaria. Both species can produce acid and gas from glucose in MRS broth and NH₃ from arginine. Biochemically, unlike W. cibaria, W. confusa is positive for fermentation of galactose and xylose and negative for fermentation of arabinose and does not grow at 45 °C (Björkroth et al., 2002). Clinical infections by W. confusa such as abscess, peritonitis and endocarditis have been reported previously (Bantar et al., 1991; Green et al., 1990; Olano et al., 2001; Riebel & Washington, 1990; Flaherty et al., 2003). A recent study by Kulwichit et al. (2007) described eight cases of Weissella species infection of which four were bacteraemia: one case each of respiratory tract, urinary tract and bone infection while in one case the source was not mentioned. Clinical details of the patients were not mentioned so their significance remains uncertain. Lee et al. (2011) described 10 cases of W. confusa bacteraemia in which the organism was misidentified as Lactobacillus and Leuconostoc species. However, isolates of Weissella species from blood are considered significant rather than a skin contaminant since they are not part of the normal skin flora (Petti et al., 2005).

W. confusa has a diverse environmental distribution. It has been isolated from a variety of foods such as milk, carrot juice, sugar cane, Malaysian chilli, fermented meat, acid-rich carbohydrate food, garlic mix and banana leaves (Kandler & Weiss, 1984; Paludan-Müller et al., 1999). It has also been isolated from sewage and human faeces and strains are considered normal microflora of the human intestine (Walter et al., 2001). In one particular study, four faecal isolates of W. confusa were recovered from 48 stool samples from children with bacteraemia who were screened for vancomycin-resistant Gram-positive organisms (Green et al., 1990). A close association of total parenteral nutrition and Lactobacillus species bacteraemia has been reported (Husni et al., 1997). To our knowledge, there are only two case reports in the literature of bacteraemia caused by W. confusa in a patient on total parenteral nutrition (Lee et al., 2011; Olano et al., 2001).

Our patient had monomicrobial infection of W. confusa with two positive blood cultures. Both isolates gave identical biochemical reactions and sequencing results. W. confusa sepsis is rarely life-threatening and results from a more severe underlying disease. It is usually susceptible to all classes of antibiotics with the exception of vancomycin, and the antibiotics of choice include penicillin, clindamycin, erythromycin, aminoglycosides and imipenem (Shin et al., 2007). Our isolate was also susceptible to daptomycin, which can be used as an effective alternative for treatment of resistant isolates (Salimnia et al., 2011). This is the second case report of bacteraemia due to W. confusa in a patient on total parenteral nutrition, which may be a risk factor for bacteraemia due to this probiotic micro-organism.
References


