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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 oesophagogastrrectomy (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 Müller–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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the isolates from two positive blood cultures in this study are HQ433525 and HQ433526.

was resistant to vancomycin. The MIC, determined by Etest (AB Biodisk), for vancomycin was $>256 \mu\text{g ml}^{-1}$ and that for daptomycin was $0.125 \mu\text{g ml}^{-1}$, 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'-AGAGTTTGTACCTGGCTCAG-3') and 1492R (1492–1510, reverse; 5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991). The partial sequence of the PCR products was determined with an ABI Prism Cycle 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 intrapeptide 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 NH_3 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 foodstuffs 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

- Bantar, C. E., Relloso, S., Castell, F. R., Smayevsky, J. & Bianchini, H. M. (1991). Abscess caused by vancomycin-resistant *Lactobacillus confusus*. *J Clin Microbiol* **29**, 2063–2064.
- Björkroth, K. J., Schillinger, U., Geisen, R., Weiss, N., Hoste, B., Holzapfel, W. H., Korkeala, H. J. & Vandamme, P. (2002). Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. *Int J Syst Evol Microbiol* **52**, 141–148.
- CLSI (2006). *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*; Approved Guideline. M45-A. Wayne, PA: Clinical and Laboratory Standards Institute.
- Collins, M. D., Samelis, J., Metaxopoulos, J. & Wallbanks, S. (1993). Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J Appl Bacteriol* **75**, 595–603.
- Facklam, R. & Elliott, J. A. (1995). Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. *Clin Microbiol Rev* **8**, 479–495.
- Flaherty, J. D., Levett, P. N., Dewhirst, F. E., Troe, T. E., Warren, J. R. & Johnson, S. (2003). Fatal case of endocarditis due to *Weissella confusa*. *J Clin Microbiol* **41**, 2237–2239.
- Green, M., Wadowsky, R. M. & Barbadora, K. (1990). Recovery of vancomycin-resistant gram-positive cocci from children. *J Clin Microbiol* **28**, 484–488.
- Husni, R. N., Gordon, S. M., Washington, J. A. & Longworth, D. L. (1997). *Lactobacillus* bacteremia and endocarditis: review of 45 cases. *Clin Infect Dis* **25**, 1048–1055.
- Kandler, O. & Weiss, N. (1984). Regular, non-sporing Gram-positive rods. In *Bergey's Manual of Systematic Bacteriology*, 9th edn, vol. 2, pp. 1208–1260. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore, MD: Williams and Wilkins.
- Kulwichit, W., Nilgate, S., Chatsuwat, T., Krajiw, S., Unhasuta, C. & Chongthaleong, A. (2007). Accuracies of *Leuconostoc* phenotypic identification: a comparison of API systems and conventional phenotypic assays. *BMC Infect Dis* **7**, 69.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. London: John Wiley & Sons.
- Lee, M. R., Huang, Y. T., Liao, C. H., Lai, C. C., Lee, P. I. & Hsueh, P. R. (2011). Bacteraemia caused by *Weissella confusa* at a university hospital in Taiwan, 1997–2007. *Clin Microbiol Infect* **17**, 1226–1231.
- Olano, A., Chua, J., Schroeder, S., Minari, A., La Salvia, M. & Hall, G. (2001). *Weissella confusa* (basonym: *Lactobacillus confusus*) bacteremia: a case report. *J Clin Microbiol* **39**, 1604–1607.
- Paludan-Müller, C., Huss, H. H. & Gram, L. (1999). Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. *Int J Food Microbiol* **46**, 219–229.
- Petti, C. A., Polage, C. R. & Schreckenberger, P. (2005). The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. *J Clin Microbiol* **43**, 6123–6125.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Riebel, W. J. & Washington, J. A. (1990). Clinical and microbiologic characteristics of pediococci. *J Clin Microbiol* **28**, 1348–1355.
- Salimnia, H., Alangaden, G. J., Bharadwaj, R., Painter, T. M., Chandrasekar, P. H. & Fairfax, M. R. (2011). *Weissella confusa*: an unexpected cause of vancomycin-resistant Gram-positive bacteremia in immunocompromised hosts. *Transpl Infect Dis* **13**, 294–298.
- Shin, J. H., Kim, D. I., Kim, H. R., Kim, D. S., Kook, J. K. & Lee, J. N. (2007). Severe infective endocarditis of native valves caused by *Weissella confusa* detected incidentally on echocardiography. *J Infect* **54**, e149–e151.
- Walter, J., Hertel, C., Tannock, G. W., Lis, C. M., Munro, K. & Hammes, W. P. (2001). Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* **67**, 2578–2585.