A mixed infection of *Leuconostoc lactis* and vancomycin-resistant *Enterococcus* in a liver transplant recipient

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Bacterial infection in patients who have undergone liver transplantation is a major complication of the procedure. *Leuconostoc* spp. are important pathogenic bacteria in individuals with poor immune function, especially transplant patients. In this report, we describe the case of a 45-year-old Asian male liver transplant recipient who was initially preliminarily diagnosed with infection with *Leuconostoc pseudomesenteroides* by using the microbial tests of the VITEK 2 system and the aesculin hydrolysis test, and with vancomycin-resistant *Enterococcus*. Subsequently, the *Leuconostoc* isolate was identified as *Leuconostoc lactis* by 16S rRNA gene partial sequencing. In this paper, we discuss our identification of *L. lactis* based on physiological characteristics and molecular methodology. Accurate identification of these infections is important for the outcome; use of 16S rRNA gene sequence analysis offers a rapid and precise diagnostic approach. Administration of the drug linezolid may be useful for the treatment of both *Leuconostoc* spp. and vancomycin-resistant *Enterococcus* infections. We suggest that clinical analysts should use molecular methods in addition to biochemical tests in order to identify *Leuconostoc* at the species level more accurately.

**Introduction**

Bacterial infection is a major complication after liver transplantation and the incidence is around 48%. Vancomycin prophylaxis against Gram-positive bacteraemia following transplantation may prevent infection. However, with the increasing use of vancomycin in clinical practice, new vancomycin-resistant pathogenic bacteria are likely to appear. *Leuconostoc* spp. are Gram-positive, catalase-negative bacteria and are intrinsically resistant to vancomycin (Facklam & Elliott, 1995; Bernaldo de Quirós et al., 1991). As a consequence of their irregular coccoid morphology, they used to be listed as members of the *Streptococcaceae*, but are now recognized as *Leuconostocaceae* and are placed within the order *Lactobacillales*. These bacteria are found in the environment, e.g. on vegetables and in dairy and other fermented products, but are known to occasionally cause infection in humans. Subsequent to 1985, several infections that resulted from *Leuconostoc* spp., namely *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*, have been reported (Handwerger et al., 1990; Bernaldo de Quirós et al., 1991; Dhodapkar & Henry, 1996). Most reported cases were bloodstream infections; however, these bacteria are also a cause of meningitis, pneumonia, endocarditis, pleural empyema, osteomyelitis or urinary tract infections. However, to date, no deaths related to *Leuconostoc* spp. infection have been reported (Facklam & Elliott, 1995; Bernaldo de Quirós et al., 1991; Montejo et al., 2000; Espinoza et al., 1997; Giraud et al., 1993; Yamazaki et al., 2009).

Some *Enterococcus* spp. are commensal organisms in the intestine. Furthermore, vancomycin-resistant enterococci (VRE) are recognized as some of the most significant nosocomial pathogens in the world because of their rapid spread, high morbidity and mortality rates, and due to the limited treatment options available. VRE are known to colonize the gastrointestinal tract of patients during their stay in intensive care units (ICUs).

In this paper, we report the case of a liver transplant recipient who had a mixed infection with *Leuconostoc lactis* and vancomycin-resistant *Enterococcus*, and detail the
clinical features of this patient. In addition, we report the diagnosis of *L. lactis* infection based on physiological characteristics and molecular identification. To our knowledge, this case is the first documented report of a mixed infection of *L. lactis* and vancomycin-resistant *Enterococcus* in a liver transplant recipient.

**Case report**

The patient was a 45-year-old Asian male liver transplant recipient who had been diagnosed as hepatitis B virus (HBV)-positive in 2009 and who had hepatocirrhosis with malaise, vague abdominal pain and ascites. In July 2010, the patient underwent an hepatocyte transplantation. However, 3 months after that surgery, the patient reappeared with repeated abdominal discomfort, jaundice and fever. The highest temperature recorded was 38°C and blood chemistry demonstrated elevated bilirubin levels. In November 2010, the patient returned with hepatic coma and a blood ammonia level that rose to 47.0 μmol l⁻¹. Comorbidities included syphilis and pulmonary infections. An allogeneic in situ living donor liver transplantation was performed on 1 February 2011 and, due to the patient’s coagulation defects, transfusion with red blood cells and platelets was carried out during surgery. Before the end of the operation, three drainage tubes were placed in the right liver, the Winchester hole and the section of the right liver. At 1 February 2011 and, due to the patient’s coagulation defects, transfusion with red blood cells and platelets was carried out during surgery. Before the end of the operation, three drainage tubes were placed in the right liver, the Winchester hole and the section of the right liver. After surgery, the patient was sent to the ICU. Immunosuppression therapy was used to control rejection; antibiotic therapy included ceftazidime 2.0 g (t.i.d., ivgtt) and vancomycin, 1000 mg (b.i.d., ivgtt). A central venous catheter was placed and parenteral nutrition was administered for supportive treatment. The patient tolerated this procedure well and recovered gradually; however, 9 days later the patient produced a large amount of ascites, but bacterial cultures were negative. Unfortunately, 18 days after surgery, the patient developed a fever with a temperature that rose to 39°C. The blood count showed an intense leucocytosis (15×10⁹ l⁻¹), in which neutrophils accounted for 93.7% of the total cell number, but a normal liver function test. Three blood cultures taken at different times were positive for bacterial growth, and *L. pseudomesenteroides* was identified using the VITEK 2 system. Microbial test results showed that the bacteria were susceptible in vitro to the drugs azithromycin, erythromycin, tetracycline and clindamycin. The bacteria had an intermediate susceptibility to cefotaxime, and were resistant to cefepime, vancomycin and ceftriaxone. Interestingly, the results of the ascites cell count and the biochemical examination were roughly the same as those found 9 days previously; the only difference was that a vancomycin-resistant enterococcus was isolated from the ascites at the later time, which indicated an intra-abdominal infection. In our hospital, the rectal swabs and chromID VRE agar were used as routine screening to detect VRE immediately after the patient was admitted to the ICU ward. We reviewed the result, which was negative, showing that the patient had not been colonized with VRE when he was admitted to the ICU. As a consequence, drug treatment was immediately changed to linezolid (0.6 g b.i.d., ivgtt). After 10 days of linezolid treatment, the patient’s body temperature returned to normal and culture of the ascites was negative for bacteria. One month subsequently, the patient had slowly recovered and was discharged from hospital.

**Microbiological investigations**

In our hospital, sepsis caused by *L. pseudomesenteroides* had not been previously reported; therefore, in order to characterize the bacterium accurately, we performed a series of further experiments. For identification, the isolate was inoculated onto an aesculin iron agar slant, and the positive result again suggested the presence of *L. pseudomesenteroides*. We also carried out the xylose reaction, and the negative result suggested *L. lactis*. Next, we performed PCR to perform partial sequencing of the 16S rRNA gene up to 169 bp for species identification. Sequences of the primers used (Invitrogen) were: forward: 5′-CACGCAAAGGTGCATTGCACCTTTCAAGT-3′; reverse: 5′-CCATCTCTAGGTGACGCCGAAGCGC-3′. Before amplification, template DNA in the PCR mixture was denatured at 95°C for 5 min. Thermal cycling consisted of 30 cycles: denaturation at 95°C for 30 s, and then annealing and extension at 70°C for 1 min. Thererafter, full extension was completed at 72°C for 7 min (Goto *et al.*, 2004). The PCR products were verified on agarose gels stained with ethidium bromide. Sequence analysis of PCR products was performed by Invitrogen. The results were compared with published sequences in the GenBank database using the BLASTN algorithm through the National Center for Biotechnology Information server, and the isolate was found to have a 99% sequence similarity with *L. lactis* strain J212.

The vancomycin-resistant enterococcal strain was first identified by the VITEK 2 system and we used the Etest with vancomycin to confirm this identification. To identify the VRE at the genetic level, a PCR amplification for the D-Ala-D- Ala ligase gene (*ddl*) (Dutka-Malen *et al.*, 1995) was performed. Furthermore, we screened its virulence factors and resistance genes. The genotype for glycopeptide resistance (Dutka-Malen *et al.*, 1995) was determined by multiplex PCR. The characteristic virulence factors, i.e. enterococcal surface protein (*esp*), hyaluronidase (*hyl*) genes and the adhesin of collagen from *Enterococcus faecium* (*acm*) genes, were detected by PCR, as described by Coque *et al.* (2002), Rice *et al.* (2003) and Nallapareddy *et al.* (2008), respectively. The isolate was vanA phenotype (resistant to both vancomycin and teicoplanin) *E. faecium*, harbouring the vanA gene, *esp* and *acm* genes, and no *hyl* gene.

**Discussion**

Vancomycin is a glycopeptide antibiotic that is used as a therapeutic agent to treat infection caused by Gram-positive cocci. With the increasing use of vancomycin in
clinical practice, some new vancomycin-resistant pathogenic bacteria are likely to appear. Compromised immunity (Montejo et al., 2000; Espinoza et al., 1997; Ferrer et al., 1995), use of vancomycin, parenteral nutrition (Bou et al., 2008) and contaminated surgical implanted devices, such as central venous catheters (Bou et al., 2008), have been considered as risk factors for Leuconostoc spp. infection, although Leuconostoc infections have also been documented in otherwise healthy patients. The origin of the pathogenic bacterium in the patient characterized above was not discovered. As liver transplantation is a long-term intervention, the long exposure time of abdominal organs may provide an opportunity for Leuconostoc spp. in the environment to invade the abdominal cavity and migrate further into the blood. For this patient, infection occurred 2 weeks post-operatively, and therefore the long-term exposure of abdominal organs was not the reason in this case. Infection may have been associated with central venous catheterization or as a consequence of parenteral nutrition. VRE isolated from the ascites may have been associated with intestinal colonization (Bou et al., 2008). As the permeability of the gastric cell wall was changed and damage to the skin membrane barrier occurred, colonization of the abdominal cavity was possible. In general, Leuconostoc spp. and VRE carry the same risk factors for patients. However, we suggest that coinfection with these two types of bacteria makes horizontal gene transfer of vancomycin resistance from L. lactis to E. faecium a possibility.

From our search of the literature, we found that six transplant patients were reported to have a Leuconostoc spp. infection (Montejo et al., 2000; Espinoza et al., 1997; Giraud et al., 1993; Yamazaki et al., 2009). We noticed that most case reports did not describe any molecular methods to identify the Leuconostoc spp. In fact, their identification relied on the following criteria based on Gram-positive coccobacilli culture: (1) negative reactions for catalase, pyrrolidonyl arylamidase (PYR), and leucine aminopeptidase (LAP) production. Additional physiological tests included production of acids from arabinose, lactose, maltose, melibiose, salicin, sucrose,trehalose and xylose. In these studies, L. mesenteroides rather than L. lactis was the commonly identified species causing infection. However, it is widely recognized that in some cases there is no correlation between genotype and phenotype for identification of some micro-organisms. Lee et al. (2011) reported that the VITEK 2 system failed to accurately identify Leuconostoc at the species level and that the accuracy rate was only 15% compared with that of 16S rRNA gene partial sequencing. In their study, most L. mesenteroides and L. pseudomesenteroides isolates identified by the VITEK 2 system were found to be L. lactis by 16S rRNA gene sequence analysis. We suggest, therefore, that the clinical analyst should carry out molecular methods as well as automated tests to identify Leuconostoc spp. isolates even more accurately.

With the increasing use of vancomycin, infections caused by Leuconostoc spp. or VRE may occur more frequently, especially in individuals who have poor immune function. The prompt and accurate identification of Leuconostoc spp. and the potential clinical significance of these bacteria are important for clinical diagnosis.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (81000712) and by the Science and Technology Department of Sichuan Province pilla program (2011 SZ0125).

References


