Bacteraemia Due to Vancomycin-Resistant Leuconostoc lactis in a Patient With Pneumonia and Abdominal Infection

Leuconostoc lactis is a gram-positive facultative anaerobic coccus or coccobacillus, catalase and oxidase negative, which grow in pairs and chains, forming colonies morphologically mistaken for Enterococcus or Streptococcus viridans by routine biochemical testing in the clinical microbiology laboratories. This organism is leucine aminopeptidase-positive and produces gas from glucose. L lactis is intrinsically resistant to vancomycin because its pentapeptide cell wall precursors end in a depsipeptide (alanine-lactate) rather than in the alanine-alanine dipetide, which is the binding site for vancomycin in susceptible gram-positive cocci.1 L lactis is usually cross-resistant to teicoplanin. It is found in vegetables, legumes, fruits, and meat and is used by the food industry in the elaboration of dairy products, wine, and sugars; more rarely, it can be found in human stool and vaginal specimens.2

In the past, L lactis was not thought to be pathogenic to humans, but occasional cases of infections caused by this organism such as ventriculitis,3 ostephtyelitis,4 and bloodstream infection5,6 have been reported in recent years. We have found only 3 cases of bacteremia caused by L. lactis. We describe what we believe is the first reported case of bloodstream infection due to L. lactis finally identified by both matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and 16S rRNA gene partial sequencing.

An 83-year-old Asian woman with cholecystolithiasis presented with a month history of abdominal pain, diarrhea, and nausea. Three days after cholecystectomy, this patient was admitted to intensive care unit (ICU) because of pneumonia and abdominal infection with temperature of 39.8°C. A central venous catheter was placed, and parenteral nutrition was administered for supportive treatment. In the first week, she was treated with intravenous meropenem for pneumonia due to extended-spectrum beta-lactamase-producing Klebsiella pneumoniae and with intravenous vancomycin for 17 days because of abdominal infection caused by Enterococcus faecium. Next, she was treated with intravenous cefoperazone/sulbactam and oral minocycline for 2 weeks due to multidrug-resistant Acinetobacter baumannii complex isolated from ascitic fluid and sputum. By the 27th day after admission to the ICU, linezolid was added to the antibiotic regimen because of L lactis isolated from blood samples. On day 32, the patient died because of acute obstructive cholangitis and shock.

The patient was continuously febrile throughout the admission with elevated white blood cell count (high of 16.0 × 10⁹ per liter, 91.0% neutrophils) consistent with a serious infection and/or sepsis. Ascitic fluid analysis also showed 3,612 white blood cells per milliliter (88.0% neutrophils). Her chest computed tomography scan, performed on ICU day 7, revealed bilateral multifocal nodular and patchy consolidation in both lungs and a small right pleural effusion. On ICU day 27, blood cultures were drawn into BACTEC Plus Aerobic/F and Lytic/10 Anaerobic/F Medium (Becton Dickinson, Franklin Lakes, NJ) and incubated in the BACTEC 9240 automated blood culture system (Becton Dickinson, Cockeysville, MD) according to the manufacturer’s instructions. After 24 hours of incubation, the Aerobic/F Medium blood culture was flagged as positive, and gram-positive cocci in chains were seen. However, Anaerobic/F Medium was continuously negative after 120 hours. The fluid was inoculated on bacteriological agars (Oxoid, Basingstoke, United Kingdom) for 24 hours at 37°C. The bacteria formed small, circular, smooth, convex, gray, and alpha-hemolytic colonies on sheep blood agar and did not grow on MacConkey agar or Mueller-Hinton agar. The organisms were catalase-negative, oxidase-negative, gram-positive, ovoid cocci, often seen in pairs or chains. Identification was performed by VITEK 2-compact system (BioMérieux, Marcy l’Etoile, France) using VITEK 2 GP identification card (BioMérieux, Hazelwood, MO), as instructed by the manufacturer. The isolate gave positive reactions for α-galactosidase, β-galactose, N-acetyl-D-glucosamine, d-maltose, d-mannose, d-raffinose, and saccharose and grew in 6.5% NaCl. It was resistant to polymyxin B, optochin, and novobiocin and was identified as Leuconostoc pseudomesenteroides with a probability of 94% after incubation for 8 hours. The catheter tip culture was negative and the follow-up blood cultures were also negative. Subsequently, the isolate was identified as L lactis by 2 MALDI-TOF MS systems, BioMérieux VITEK-MS mass spectrometer (99.9% probability) and Bruker AutoFlex Speed mass spectrometer (log score 2.089). The identity was confirmed as L lactis by 16S rRNA gene partial sequencing. Universal bacterial primers 27F and 1492R were used for amplification.2 Purified DNA from the PCR was sequenced with BigDye Terminator Cycle sequencing kit (Applied Biosystems, Foster City, CA) and Applied Biosystems ABI PRISM 3730 genetic analyser (Applied Biosystems Division). All sequences were compared with those of similar strains using BLAST and EzTaxon.3 The isolates showed 98% sequence similarity to L lactis (GenBank Accession No. AB904777).

There are no CLSI or EUCAST susceptibility criteria for this unusual organism, so it is hard to evaluate susceptibility determinations. Minimal inhibitory concentration was determined by VITEK 2-compact system using VITEK 2 AST-GL card (BioMérieux, Hazelwood, MO). Minimal inhibitory concentrations were as follows: vancomycin, ≥2 μg/mL; erythromycin, ≥1 μg/mL; etanercept, ≥8 μg/mL; meropenem, ≥4 μg/mL; amoxicillin, 1 μg/mL; levofloxacin, 2 μg/mL; moxifloxacin, 0.5 μg/mL; tetracycline, 4 μg/mL; chloromycetin, ≥2 μg/mL; trimethoprim and sulphamethoxazole, ≤10 μg/mL; telithromycin, ≤0.25 μg/mL. Because the patient was on vancomycin, susceptibility to this antibiotic seemed unlikely. Antimicrobial sensitivities were revealed by disk diffusion method on sheep blood agar (Oxoid) and revealed that the isolate was resistant to vancomycin. Zone diameter showed the following: vancomycin, 6 mm; teicoplanin, 6 mm; trimethoprim and sulphamethoxazole, 6 mm; meropenem, 14 mm; imipenem, 16 mm; rifampicin, 11 mm; ciprofloxacin, 16 mm; levofloxacin, 19 mm; erythromycin, 21 mm; linezolid, 23 mm; chloromycetin, 19 mm; cefepime, 19 mm; ceftriaxone, 18 mm; cefotaxime, 21 mm; ampicillin, 24 mm; amikacin, 20 mm; gentamicin, 22 mm; clindamycin, 25 mm; tetracycline, 18 mm; minocycline, 19 mm; ceftazidime, 24 mm; penicillin, 27 mm; piperacillin, 30 mm; cefoperazone/sulbactam, 31 mm; ticarcillin/clavulanic acid, 31 mm; ampicillin/sulbactam, 26 mm; piperacillin/tazobactam, 31 mm.

In the past, Leuconostoc spp. were listed as members of the Streptococcaceae but are now recognized as Lactobacillaceae and are placed within the order Lactobacillales. The identification of L lactis as a cause of infection can be difficult as it is catalase-negative and gram-positive cocci, and colony
morphology resembles a *Streptococcus*. Suspicion of an alternative pathogen is raised only when sensitivities reveal vancomycin resistance. If sensitivities are omitted, the organism may remain misclassified and potentially dismissed as a blood culture contaminant because of its resemblance to *Streptococcus viridans*, or mistreated as an *Enterococcus*. The failure of automated bacterial identification systems to identify the organism may contribute to an underestimate of its pathogenicity. It has been demonstrated 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. We confirm that molecular methods more accurately identify such organisms and demonstrate that MALDI-TOF MS also provides accurate identification.

Risk factors for infection by *Lactis* include central venous catheters, parenteral nutrition, surgery, liver failure, chronic renal insufficiency treated with hemodialysis, extensive burns, compromised immunity, and previous antibiotic therapy, particularly with vancomycin. The skin and digestive tract are believed to play important roles as routes of entry into the body. Vancomycin therapy and long-term intravenous nutrition, through a central venous catheter, might have played some role in the development of *Lactis* bacteraemia in this patient. Long-term vancomycin administration may have contributed to overgrowth of usual gastrointestinal microbial flora by *Lactis* by selectively suppressing the growth of other vancomycin-susceptible gram-positive organisms.

Proper management of the patient with *Lactis* bacteraemia includes the removal of the infected foci of infection such as the central catheter or by the drainage of abscesses, and administration of appropriate antibiotics. There are no standards for selecting antimicrobial agents to treat *Leuconostoc* spp. The treatment of choice seems to be penicillin or ampicillin, but clindamycin, linezolid, macrolides, aminoglycosides, cephalosporins, and tetracyclines have also been used. Susceptibility to trimethoprim and sulphamethoxazole is variable, with some reported cases of infection in patients who were already receiving this drug. We chose to use linezolid for our patient, but we could not evaluate its effect as the patient died because of acute obstructive cholangitis and shock 4 days later. We also could not be certain whether *Lactis* bacteraemia itself was the cause of death for our patient. Because of the rarity of case reports of *Lactis*, the fatality rate of this pathogen is not known.

With the increasing use of vancomycin in clinical practice, some new vancomycin-resistant pathogenic bacteria are likely to appear. We emphasize the importance of performing tests of sensitivity to vancomycin to properly identify *Lactis*. This may allow the reporting of new cases and help to discover the prevalence and frequency of the infection caused by this pathogen. It is almost certainly more common than generally recognized, and the use of an opportunistic pathogen in food fermentation may be questionable.

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**REFERENCES**