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Case Reports

Bacteremia caused by *Leuconostoc* species : 6-case series

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Leuconostoc species are Gram-positive coccobacilli and are used in dairy products and are intrinsically resistant to vancomycin. *Leuconostoc* infections are rare in humans, usually occurring in immune-compromised patients. We describe 6 patients with *Leuconostoc* bacteremia at Dong-A university hospital between 1990 and 2015. One isolate (*L. lactis*) was identified to species level using 16S rRNA gene sequencing analysis. All patients had underlying diseases and 5 patients underwent procedures that interrupted the normal integumentary defense. Four patients died within 30 days after being identified as carrying *Leuconostoc* species.

Key Words: Bacteremia, *Leuconostoc*, Vancomycin resistance

Leuconostoc species *(L. species)* is Gram-positive, anaerobic, catalase-negative, or oxidase-negative coccus or coccobacillus. It has similar biochemical characteristics to that of *enterococcus or streptococcus viridians* and therefore there is a risk of it being identified with either one.[1] It is a natural resident existing in humans' and animals' mucous layers, large intestines, dairy products, beans, and vegetables. In the past it was thought that it did not infect humans, but recently its occurrence in patients with major diseases and immunocompromised patients has been continuously reported and it has been reported that it has infected humans in the form of bacteremia, encephalomeningitis, peritonitis, or pneumonia.[2] The strains of L. species include *L. lactis, L. mesenteroides, L. paramesenteroides, L. oenos, L. pseudomesenteroides, L. citreum, and L. dextranicum.*[3] Its greatest characteristic is that it has natural tolerance against antibiotics of the glycopeptides series like vancomycin.[1]

L. bacteremia mostly occurs in immunocompromised patients who receive anti-cancer treatment, who have taken steroids for a long time, or who have undergone a liver transplant. In Korea, L. bacteremia has been reported in patients who have

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been administered with vancomycin in order to treat pneumonia,[3] patients who have taken steroids in order to alleviate rheumatoid arthritis,[4] and patients who have had a stent inserted in their bile duct.[5] In Korea, there has been no case reported where L. bacteremia was identified based on molecular diagnostics and therefore the researcher intends to additionally investigate five patients with bacteremia caused by L. species who were hospitalized in our hospital in the past and examine their clinical characteristics and prognosis together with a review of the literature.

CASE

Patient 1

The patient was a 75-year-old male and had diabetes mellitus as an underlying disease. He had undergone arthrodesis due to arthritis in the left ankle in another hospital. Thereafter, because of phlegmone in the tissues surrounding the ankle joint, cefazolin (1g BID) was intravenously administered. Four days later, a fever and abdominal pain started and therefore abdominal computed tomography was performed. Cholecystitis was suspected and therefore he visited the emergency room of our hospital in June, 2015. At the time, the body temperature of the patient was 36.5℃. However, his level of white blood cells slightly increased to 10,290 /mm3 (neutrophil: 96.6%). His level of red blood cells, platelets, AST, ALT, ALP, r-GTP, total billirubin, direct billirubin, and c-reactive protein also increased to 13.4 g/dL, 158,000 /mm3, 46 U/L, 127U/L, 786 U/L, r-GTP 291 U/L, 1.9 mg/dL, 1.2 mg/dL, and 31.06 mg/dL, respectively. From the abdominal computed tomography, there were findings of acute cholangitis resulting from a bile duct stone, acute cholecystitis, liver abscess, and fluid retention in the periphery area of the right liver; therefore, imipenem (500 mg QID) was intravenously administered as an empirical antibiotic after a blood culture test.

L. lactis was identified by a blood culture test through VITEK II system. For precise identification of the bacteria, 16S rRNA gene analysis was conducted with a bacterial specimen (DAU-03) cultured in blood and it showed 98.85% similarity to L. lactis, 98.92% similarity to *L. holzapfelii,* 98.61% similarity to *L. citreum*, and 98.70 similarity to *L. palmae*; therefore, it was found that the bacteria were *L. lactis* (Fig. 1). Then, gallbladder drainage insertion was performed but superinfection (*Enterococcus avium, Enterococcus gallinarum*) continuously occurred in the gallbladder fluid culture and it aggravated the intraperitoneal infection. Therefore, percutaneous drainage was performed. To treat the catheter-related bacteremia (*Candida albicans*), antifungal agent anidulafungin (100mg QD) was administered but on the 28th day after detection of *L. lactis,* he died because sepsis worsened.

Patient 2

The patient was a 56-year-old male. He had

received antic-cancer therapy due to metastatic pancreatic cancer and was hospitalized for pancreatic cancer treatment evaluation in October 2013. On the second day of hospitalization, he was suspected of intraperitoneal infection; he had a fever and his level of white blood cells and C-reactive protein was 11,600 /mm^3 (neutrophil: 88.6%) and 17.16 mg/dL, respectively. He was intravenously administered with ceftizoxime (1g BID) and ampicillin/sulbactam (1.5g TID) as it was suspected that he had an intraperitoneal infection. However, bacteria were not identified in a blood culture test using VITEK II system. From then on, he continued to have a fever and therefore antibiotics were sequentially and intravenously administered—ceftazidime (2g TID), isepamicin (200mg BID), meropenem (1g TID), levofloxacin (750mg QD), and then aztreonam (1g TID). In the blood culture test performed using a central venous catheter on the 28th day of hospitalization, *L. pseudomesenteroides* was detected and he was intravenously administered with ticarcillin/clavulanate (3.2g TID) instead of aztreonam (1g TID) on an empirical basis - despite results showing that he was susceptible to antibiotics. Thereafter, in the blood culture test on the 36th day of hospitalization, bacteria were not detected, but on the 15th day, in other words,

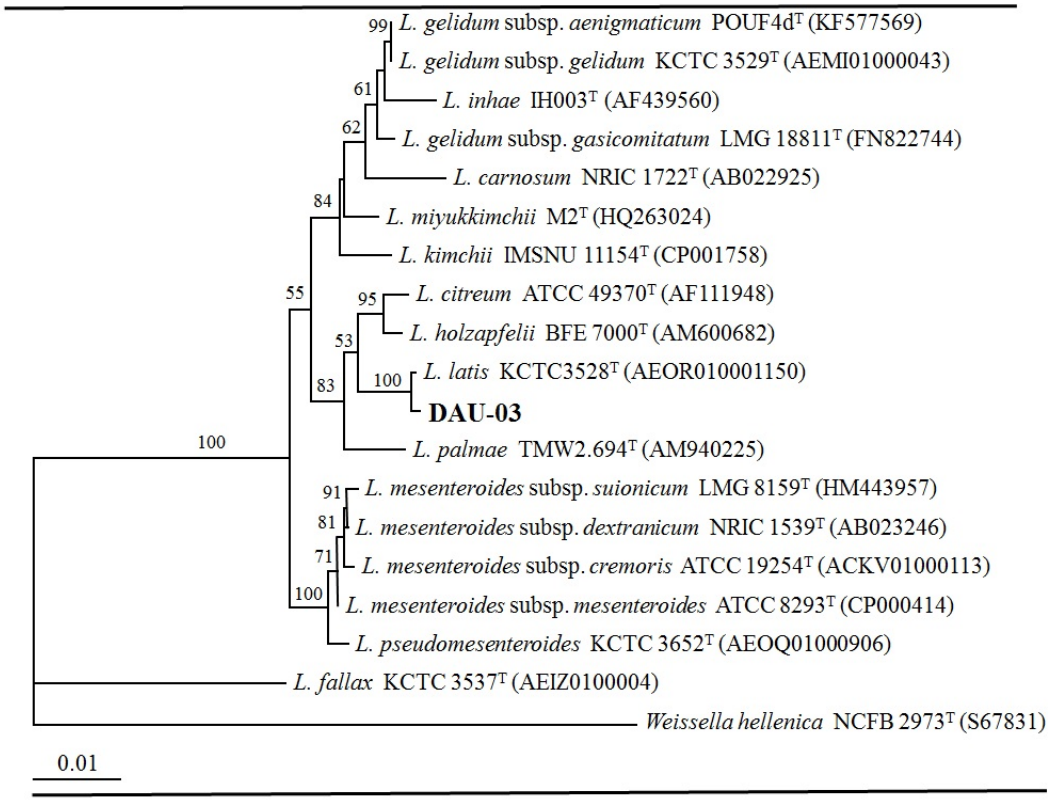

Fig. 1. The phylogenetic relationships of isolate DAU-03 with other related *Leuconostoc* strains based on 16S rRNA gene sequences

on the 40th day of hospitalization, he died due to complications arising from the intraperiotoneal infection.

Patient 3

The patient was an 80-year-old female. She was receiving medication in order to treat diabetes mellitus, hypertension, atrial fibrillation, and hypertrophic cardiomyopathy. She was hospitalized in the Department of Circulatory Internal Medicine in December 2014 with a main complaint of dizziness resulting from atrial fibrillation. At the time of hospitalization, she had no fever but her level of white blood cells and C-reactive protein increased to 11,780 /mm^3 (neutrophil: 78.6%) and 7.21 mg/dL, respectively. Suspected of having acquired an infection, a blood culture test was conducted using VITEK II system and *L. pseudomesenteroides* was identified. From when the bacterial identification was verified, levofloxacin (750mg QD) was empirically and intravenously administered. This was appropriate for antibiotic susceptibility of *L. pseudomesenteroides.* On the 10th day after bacterial identification, her clinical symptoms improved and she was discharged from hospital.

Patient 4

The patient was a 65-year-old male. He had received anticancer therapy and radiological treatment in order to treat cancer found to be present in the external auditory canal and was hospitalized in December 2013 because of phlegmon in the external auditory canal. His body temperature was slightly elevated to 37.6℃ and in his blood test, his level of white blood cells and C-reactive protein had increased a little to 4,250 /mm^3 (neutrophil: 90%) and 5.12 mg/dL, respectively. In his blood culture test using VITEK II system performed at the time of hishospitalization, *L. mesenteroides* was detected and his condition was monitored while the antibiotic cefepime (2g TID) was intravenously administered. In the follow up blood culture test that was conducted, bacteria were not identified and his clinical symptoms improved. He was discharged from hospital on the 24th day of hospitalization.

Patient 5

The patient was a 73-year-old female. She had been diagnosed with aortic regurgitation, aortostenosis, heart failure, and early stage gastric cancer and as a result of which she had received subtotal gastrectomy. She was hospitalized in August 2009 due to occlusion of the junction area resulting from the gastric surgery. After gastrojejunostomy, an antibiotic flomoxef (1g BID) was intravenously administered and her condition was monitored. She had a fever of 37.8℃. In her blood test, her level of white blood cells and C-reactive protein was 10,690 /mm^3 (neutrophil: 88%) and 9.86 mg/dL, respectively. In her blood culture test using VITEK II system performed at the time, *L. citreum* was detected. Nonetheless, regardless of the outcome of susceptibility to antibiotics, ceftriaxone (1g BID) instead of flomoxef (1g BID) was

administered. Thereafter, she had a fever of 38℃ and therefore she received a blood culture test. In the blood in the central venous catheter and peripheral blood, *Candida glabrata* was detected and therefore an antifungal agent anidulafungin (100mg QD) was administered. However, she died because her sepsis worsened.

Patient 6

The patient was a 60-year-old male. He was a patient who completely recovered after surgery to treat common bile duct cancer and progressive gastric cancer. He was hospitalized in November 2013 as was believed to be suffering from bilateral bacterial pneumonia and sepsis. At the time of hospitalization, his body temperature had risen to 38℃ and in his blood test, his level of white blood cells and C-reactive protein had increased to 16,380 /mm3(neutrophil: 92.6%) and 20.66 mg/dL, respectively. *L.* citreum and *Candida lipolytica* were identified together in the peripheral and central venous catheter blood culture tests. From the time of hospitalization, teicoplanin (200mg QD) and meropenem (1g BID) as empirical antibiotics for bilateral pneumonia and sepsis were intravenously administered. Even after identification of L. *citreum*, there was no change in the antibiotic regime. On the 3rd day after identification of *L. citreum*, he died from aggravated sepsis.

DISCUSSION

The authors identified *Leuconostoc lactis (L. lactis)* in the blood culture of a cholecystitis patient and for more precise identification of the bacteria, the authors requested 16S rRNA gene analysis and verified that it was the same bacteria. This led to an investigation on cases where *L.* species was identified in our hospital's culture tests from 1990 to 2015; the bacteria were cultured in seven patients. Excluding one patient whose bacteria were identified in the area of a surgical wound, six patients were suffering from bacteremia. *L. species* is a Gram-positive bacterium and may be a contaminant but it is reasonable to consider the bacteria discussed with regard to the cases in this paper as pathogens rather than contaminants because of indications such as fever, increase in the level of white blood cells, rise in C-reactive proteins, and identification of bacteria in two pairs of blood culture. The strains of *L.* species have been reported to include *L. lactis, L. mesenteroides, L. paramesenteroides, L. oenos, L. pseudomesenteroides, L. citreum,* and *L. dextranicum.*[3] In our hospital, one case of *L. lactis,* two cases of *L. pseudomesenteroides*, one case of *L. mesenteroides*, and two cases of *L. citreum* occurred (Table 1). Four among the six patients were cancer patients and five patients had had a catheter inserted. Within 30 days after the bacteria were identified, four patients had died.

It is known that biochemical characteristics of these bacterial species may produce different outcomes according to the culture condition.[5,6] Lee et al. reported that in an automation test

Table 1. Clinical feature of 6 patients with *L.* species bacteremia

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Organism	*L. lactis*	*L. pseudomes-enteroides*	*L. pseudomes-enteroides*	*L. mesenteroides*	*L. citreum*	*L. citreum*
Age/ Sex	75/M	56/M	80F	65M	73F	60M
Underlying disease						
Malignancies		Pancreas cancer		External auricular cancer	Early gastric cancer	Cholangio-carcinoma, Adavanced gastric cancer
Diabetes	+	+	+	-	-	-
Others	Cholecystitis	Chronic hepatitis B	Congestive heart failure Atrial fibrillation		Congestive heart failure	
Indwelling venous catheter	Central catheter	Venous port		Venous port	Central catheter	Venous port
*WBC(/mm^3)	10,290	11,600	11,780	4,250	10,690	16,380
*CRP(mg/dL)	31.06	17.16	7.21	5.12	9.86	20.66
†Antibiotics	Imipenem	Ticarcillin /clavulanate	Levofloxacin	Cefepime	Ceftriaxone	Teicoplanin, Meropenem
‡All-cause mortality	Died	Died	Survived	Survived	Died	Died

*Reference range: WBC 3,000-10,000(/mm3), CRP 0-0.5(mg/dL)
†Adminitrated after Leuconostoc species identification
‡ All cause mortality in 30 days

through Vitex II system the bacteria were identified as *L. pseudomesenteroides* but all the 15 cases were *L. lactis* in terms of their genotypes as identified through 16S rRNA sequencing analysis.[6] According to Jordan, the sensitivity, specificity, positive predictability, and negative predictability of 16S rRNA polymerase chain reaction (PCR) were 96%, 99.4%, 88.9%, and 99.8%, respectively.[10] According to Sleigh et al., the sensitivity of 16S rRNA PCR was about twice as high as that of existing blood culture techniques.[11] Therefore, if clinical features or reaction to treatment differs according to the strains of *L.* species, a molecular biological diagnosis method may be needed for precise bacterial identification.

In a study by Lee et al., among the 15 patients who succumbed to bacteremia caused by *L.* species, the rate of those who were suffering from cancer was 60% (9/15), the rate of those who had used steroids was 40% (6/15), the rate of those who had a chronic kidney disease was 33% (5/15), and the rate of those who had undergone an organ transplant was 13% (2/15), and the rate of those who had diabetes mellitus was 13% (2/15). The mortality rate within 30 days after bacterial identification was high at 40%.[6] In the cases being reported on in this paper as well, among the six patients, the number of cancer patients was 4, the number of diabetes mellitus patients was 3, and the number of heart failure patients was 2. Four among the six patients died within 30 days after bacterial identification; their mortality rate

was high (Table 1). It is not certain of bacteremia caused by *L.* species was the direct factor causing death in the case of patient 1 and patient 5. Nonetheless, in immunocompromised patients with serious underlying diseases, bacteremia caused by *L.* species may act as a factor that exacerbates their underlying condition. Therefore, if *L.* species is identified in such patients, it should be thought of as a causative bacterium triggering an infection, not a contaminant, and agents suitable for antimicrobial susceptibility should be selected for treatment.

The route by which L. species infiltrates the human body and triggers bacteremia has not been clearly established but bacteremia is thought to be triggered when a normal bacterial flora in a cluster in the skin or the bowel triggers bacteremia when the defense function of normal skin and mucous layers is lost.[7] According to Handwerger et al. nine among 17 patients had a catheter inserted such as a central venous catheter, hemodialysis catheter, peritoneal dialysis catheter, or arteriovenous fistula catheter.[2] In the present study as well, five among the six patients had had either a central venous catheter, a chemoport, or a percutancous transhepatic biliary drainage catheter inserted, which destroyed the skin's defense function, and *L.* species was identified in the blood of the central venous catheter in two patients who had had a chemoport inserted. The chief characteristic of *L.* species is that binding sites where glycopeptides may act do not exist in the cell walls of L. species and so there is a natural resilience to antibiotics of glycopeptides series.[1] It is believed that changes in the normal bacterial flora of patients who have been administered for a long time with glycopeptides like vancomycin leads to the clustering of *L* species; in particular, colonized *L.* species would trigger infection in immunocompromised patients. Due to the increase in the use of vancomycin, the potency and effectiveness of the drug has declined as bacterial resistance to it has increased; as a result, the number of cases in which treatment of immuocompromized patients with antibacterial agents has failed is growing.[7] In a study by Lee et al, the rate of cases where glycopeptides was administered within 30 days before the occurrence of bacteremia caused by *L.* species was 25% (5/20).[6] In a study by Handwerger et al., vancomycin was administered within thirty days before the occurrence of bactermemia in 35% of patients (6/17).[2] On the other hand, in the present study, there was no patient to whom vancomycin was administered within 30 days before the occurrence of bacteremia among the six patients and two patients had been administered with vancomycin eight months and nine months before the occurrence of bacteremia. Therefore, additional research is considered necessary to determine if there is any association between the use of vancomycin and increase in L. species.

It was reported that *L.* species exhibits a natural tolerance against vancomycin but is susceptible to treatment with gentamicin, clindamycin, imipenem, chloramphenicol, erythromycin, and

daptomicin.[4,5] Among them, in particular, the number of strains of *L.* species that are responsive to gentamicin was largest, and other strains exhibited susceptibility to tetracycline, minocycline, and doxycycline as well and they responded well to penicillin treatment.[1,8,9] Kim et al. reported that strains of *L.* species were resistant to trimethoprim-sulfamethoxazole, vancomycin, teicoplanin, but that were responsive to clindamycin, erythromycin, and cephalothin.[3] In the cases presented in this study, strains of *L.* species were resistant to against vancomycin, the third generation cephalosporine, cefotaxime, but responded well to treatment with ampicillin, clindamycin, erythromycin, and tetracycline (Table 2), and these results are similar to results produced in previous research. Nonetheless, resistance to penicillin was increased in all strains except for the one detected in patient 5.

In conclusion, the six patients who had bacteremia that was brought on by *L.* species had a severe underlying disease such as cancer or had a catheter inserted. Four among the six patients died within 30 days after bacterial identification. Thus, in the case where bacteremia caused by *L.* species, which has a natural tolerance to antibiotics, occurs in patients with severe underlying diseases or who have a catheter inserted, *L.* species identified in them should not be regarded as a contaminant and it is necessary to administer appropriate agents that have antimicrobial properties.

Table 2. Antimicrobial susceptibility of isolates of L. isolates

	Patient 1 *L. lactis*	Patient 2 L. pseudomes-enteroides	Patient 3 L. pseudomes-enteroides	Patient 4 *L. mesenteroides*	Patient 5 *L. citreum*	Patient 6 *L. citreum*
Ampicillin	I (4)*	S (< = 0.25)	I (0.5)	I (2)	S (< = 2)	I (4)
Imipenem					S (< = 1)	
Ciprofloxacin					S (1)	
Penicillin G	I (0.5)	I (0.25)	I (0.25)	I (1)	S (1)	I (2)
Vancomycin	R (> = 8)	R (> = 8)	R (> = 8)	R (>=8)	R (> = 32)	R (> = 8)
Clindamycin	I (0.5)	S (< = 0.25)	S (< = 0.25)	S (<=0.25)	S (< = 0.25)	S (< = 0.25)
Erythromycin	S (< = 0.12)	S (< = 0.12)	S (< = 0.12)	S (<=0.12)	S (< = 0.25)	S (< = 0.12)
Tetracyclin	S (2)	S (2)	S (2)	S (2)	S (4)	S (0.5)
Levofloxacin	S (1)	I (4)	S (1)	I (4)	I (4)	S (2)
Teicoplanin					R (> = 32)	
Norfloxacin					R (> = 16)	
Gentamicin					S (†SYN-S)	
Cefazolin					S (2)	
Ceftriaxone	R (> = 8)	R (> = 8)	R (> = 8)	R (> = 8)		R (> = 8)
Cefotaxime	R (4)	R (4)	R (> = 8)	R (> = 8)		R (> = 8)
Linezolid	S (< = 2)	S (< = 2)	S (< = 2)	S (< = 2)		S (< = 2)

*Minimal inhibitory concentration (MIC)
† SYN: Synergistic

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