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Case Report

Lactococcus lactis spp *lactis* infection in infants with chronic diarrhea: two cases report and literature review in children

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Abstract

Lactococcus lactis is a gram-positive, facultative anaerobic coccus that is occasionally isolated from human mucocutaneous surfaces such as the intestines. It is used in the dairy industry for milk acidification and is mostly nonpathogenic in immunocompetent humans, however a number of cases of infection with *L. lactis* have been reported in recent years. In this article, we describe two cases of infection due to *L. lactis* in patients with chronic diarrhea. The first case is a five-month-old boy who was operated on for volvulus on his first day of life and had ileostomy with subsequent diagnosis of chronic diarrhea and bacteremia due to *L. Lactis*. The second case is a six-month-old girl with the diagnosis of chronic diarrhea that developed after a catheter-related bloodstream infection. Both of the infections due to *L. Lactis* spp *lactis* were successfully treated with intravenous vancomycin therapy. Although *Lactococcus* species is mostly known as nonpathogenic, it should be kept in mind as a potential pathogen, especially in patients with gastrointestinal disorders.

Key words: *Lactococcus lactis*; infant, infection; gastrointestinal surgery.

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Introduction

Lactococcus spp. is mostly used in the dairy industry to make cheese and other fermented foods and is considered to be nonpathogenic inhumans. However, in the last two decades there have been reports that they can cause infections, particularly in immunocompromised hosts [1]. Endocarditis and liver abscess were the most common sites of infection caused by *L. Lactis,* however as far as we know, there are few reports of infections in neonates caused by *L. lactis* subsp. *lactis.*

In this report, we summarize two infants who had chronic diarrhea and developed bacteremia and catheter related blood stream infection due to *L. Lactis* spp *lactis*.

Case Report

Case 1

A five-month-old boy had been hospitalized in the pediatric unit because of poor feeding and developmental delay. He was born at a gestational age of 38 weeks weighing 2600 gr. Because of vomiting, abdominal distension and intestinal dilatation detected by prenatal ultrasonographic examination, the patient underwent open surgery with presumed diagnosis of volvulus on first day of his life. Forty cm of nonviable bowel was resected and an ileostomy was performed. He was re-hospitalized when he was five months-old due to poor feeding and developmental delay. At his second hospitalization he weighed 6 kg (below the third percentile), was 62 cm long (at the fifth percentile) and had a head circumference of 40.5 cm (at the fifth percentile). On the tenth day of admission, he had fever and worsening of his clinical condition. The source of fever was not apparent on physical examination. The laboratory results showed a white blood cell (WBC) count of 8700/mm³, hemoglobin level of 8.7 g/dl, platelet count of 154000/mm^3 and C-reactive protein levels of 22 mg/L (0-5 mg/L). Biochemical values including electrolytes, liver and renal function tests were normal. The infant's urine culture did not yield any organism. Moreover, he had no signs of pneumonia. Stool examinations showed no pathogenic microorganisms. Because clinical sepsis was suspected, peripheral venous blood culture was obtained. Blood culture could be obtained from one vein due to difficult

venous access. Empirical antibiotic therapy with vancomycin (60 mg/kg/24h: Q6 h) and cefepime (150 mg/kg/24h: Q8 h) were then commenced. Fourteen hours later, the BacT/Alert device (bioMérieux, Marseille, France) gave a signal indicating the growth of a microorganism in the peripheral vein blood culture. Gram-positive, alpha-hemolytic colonies were seen and they were considered to be members of the viridans group streptococci (VGS). These were first detected by the Vitek 2 and VitekMS systems (bioMérieux, Marseille, France), and were identified as *Lactococcus lactis* spp *lactis* with a reliability of 50% and 99.9%, respectively. The organism was confirmed by 16S rRNA gene sequencing. The bacterial DNA was isolated by heating protocol. The partial nucleotide was amplified by PCR using universal primers 8UA (5'-AGAGTTTGATCCTGGCTCAG-3') and 907B (5'-CCGTCAATTCMTTTAGTTT-3'), and subsequently sequenced with an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Inc.) [2]. BLAST software available at www.ncbi.nlm.nih.gov was used to search for DNA nucleotide sequences against similar nucleotide sequences in the database [3]. The sequence was 99% identical to *L. lactis* spp. *lactis* strain (GenBank accession no.etc. AB008215, HM007591). Minimal inhibitory concentrations (MICs) for vancomycin and penicillin were 0.5 (sensitive) and 0.5 mcg/ml (intermediate), respectively, by e-test in accordance Clinical and Laboratory Standards Institute guideline [4]. Therefore, vancomycin therapy was continued for 10 days and cefepime therapy was stopped. On the third day of the vancomycin therapy, control culture was obtained, and it remained sterile. After completion of 14th day of vancomycin therapy, the patient had fever and clinical worsening again. The source of fever was not found on physical examination. The laboratory results showed a white blood cell (WBC) count of 12700/mm^3, hemoglobin level of 10.9 g/dl, platelet count of 179000/mm^3 and C-reactive protein levels of 12 mg/L (0-5 mg/L). Repeat blood culture taken from peripherial vein yielded *Lactococcus lactis* spp *lactis* again. Vancomycin therapy was restarted for 10 days. No vegetations were seen by echocardiography on the heart valves. No subsequent recurrent infections were observed.

Case 2

A six-month-old girl had been hospitalized in the pediatric unit due to diarrhea and developmental delay. Upon admission she weighed 5.5 kg (below the third percentile), was 61 cm long (below the third percentile) and had a head circumference of 40.5 cm (at the tenth percentile). She was born at a gestational age of 39 weeks weighing 3200gr. Due to diarrhea she was hospitalized in another hospital on the sixteenth day of her life. The source of diarrhea was not found on colonoscopy or laboratory findings. After 100 days in another hospital, she was referred to our tertiary university hospital. She had been fed through nasogastric tube with formula. Central venous line (5.5 Fch Multi-lumen-3, ArrowR) was inserted in the juguler vein because the patient required total parenteral nutrition. On the 45th day of admission, she had fever and worsening clinical condition. The source of fever was not found on physical examination. The laboratory results showed a white blood cell (WBC) count of 13400/mm^3, hemoglobin level of 11,9 g/dl, platelet count of 149000/mm^3 and C-reactive protein levels of 10 mg/L (0-5 mg/L). Biochemical values including electrolytes, liver and renal function tests were normal. Urinalysis was normal, urine culture did not yield any organism and she had no signs of pneumonia. Stool examinations showed no pathogenic microorganisms. Because clinical sepsis was suspected, blood cultures were taken both from peripherial vein and central venous catheter. Nine hours later, the Bact-Alert device (bioMérieux, Marseille, France) gave a signal indicating the growth of a microorganism in the catheter blood culture. Peripheral vein blood cultures remained sterile. The central venous catheter was removed. Gram positive, alfa hemolytic colonies were seen and colonies were considered as viridans group streptococci and identified by Vitek MS system (bioMérieux, Marseille, France) and Vitek MS result was *Lactococcus lactis* spp *lactis* with a reliability of 99.9%. The organism was confirmed by 16S rRNA gene sequencing. The bacterial DNA was isolated by heating protocol. The partial nucleotide was amplified by PCR using universal primers 8UA (5'-AGAGTTTGATCCTGGCTCAG-3') and 907B (5'-CCGTCAATTCMTTTAGTTT-3'), and subsequently sequenced with an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Inc. Foster City, USA) [2]. BLAST software available at www.ncbi.nlm.nih.gov was used to search for DNA nucleotide sequences against similar nucleotide sequences in the database [3]. The sequence was 99% identical to *L. lactis* spp. *lactis* strain (GenBank accession no.etc. AB008215). Empirical antibiotic therapy with vancomycin (60 mg/kg/24h: Q6 h) was started. Antibiogram tests were obtained on second day of antibiotic therapy and the microorganism was found to be susceptible to penicilin, vancomycin and clindamycine. Minimal inhibitory concentrations (MICs) for vancomycin was 0.5

(sensitive) by e-test in accordance Clinical and Laboratory Standards Institute guideline [4]. Therefore, vancomycin therapy was continued for 10 days. Control culture of peripheral blood samples were obtained on the third day of vancomycin therapy and they were remained sterile. No vegetations were seen by echocardiography on the heart valves.

Results and Discussion

Lactococcus lactis is a facultative anaerobic, spherical-shaped gram-positive bacterium that was originally isolated from milk and surfaces of plants. It is widely used in the dairy industry to make cheese and other fermented foods such as milk and yogurt [5]. This microorganism is employed as a vector for vaccine delivery systems [6,7]. It may be a part of the normal flora because it is occasionally isolated from human mucocutaneous surfaces such as intestine [8,9]. There are five species in the genus *Lactococcus; L. lactis, L. garvieae*, *L. piscium*, *L. plantarum*, and *L. raffinolactis L. lactis* has two subspecies, namely *L. lactis* spp *cremoris* and *L. lactis* spp. *lactis* [10]. In recent years there have been case reports of infections with *L. lactis* in adults presenting with endocarditis, liver abscess, septic arthritis, septicemia, cerebellar abscess, deep neck infection, osteomyelitis, canaliculitis, necrotizing pneumonitis, cholangitis and subdural empyema [11-14]. On the other hand, infections in children are limited. A PubMed search identified only five cases of infection due to *Lactococcus* species in children. [Table 1] The first case is a 19-month-old immunocompetent female child with a brain abscess caused by *L. lactis cremoris*. The patient was treated with abscess drainage and antibiotic therapy including vancomycin and meropenem and she recovered completely [15]. The second case is a newborn who developed bacterial meningitis and septicemia due to *Lactococcus lactis* that was successfully treated with vancomycine and cefotaxime without any neurological sequelae [16]. The third case is a 9-month-old girl who was hospitalized in the neonatal intensive care unit since birth. Her early neonatal course was complicated by necrotizing enterocolitis and then she developed short bowel syndrome requiring total parenteral nutrition. She developed catheter-related bacteremia with *Lactococcus lactis* and was treated with vancomycin and cefotaxime without removal of catheter [17]. The fourth case is an 8-year old boy who developed cerebral abscess after sinusitis and was successfully treated with surgical management in addition to vancomycin, ceftriaxone and metronidazole [18]. The fifth case is a one-year-old boy with diagnosis of Down syndrome and Hirschprung who developed catheter-related bloodstream infection and was successfully treated with vancomycin [19].

In this study, we described two infants with infection due to *L. lactis* spp. *Lactis*. The bacteria were identified by Vitek2 and Vitek MS and the biochemical identification was strengthened with the demonstration of 16S ribosomal RNA gene. The second patient who developed catheter-related bloodstream infection had been hospitalized since the sixteenth day of her life, for this reason finding a peripheral vein line was exceedingly hard. Therefore, we could only collect a small blood sample from a peripheral vein line. However the blood sample from the catheter lumen was

Table 1. *Lactococcus lactis* infections in children.

Cases	Identification of *L.lactis*	Site of infection	Source of infection	Treatment	Clinical outcome	References
19-month, female	*L.lactis* spp.*cremoris*	Brain abscess	Unknown	Surgical management and Vancomycin+ meropenem	Recovered	12
Newborn, female	*L.lactis* (no information)	Meningitis, septisemia	Unknown	Vancomycin + cefotaxime	Recovered	13
9-month, female	*L.lactis* spp.*lactis*	Catheter-related bacteremia	Unknown	Vancomycin+ cefotaxime	Recovered	14
8-year, boy	*L.lactis* spp.*cremoris*	Brain abscess	Frontal Sinusitis	Surgical management and Vancomycin+ Ceftriaxone+ metronidazole	Recovered	15
1-year old boy	*L.lactis* spp.*lactis*	Catheter-related blood-stream infection	Unknown	Vancomycin	Recovered	16
5-month, boy	*L.lactis* spp.*lactis*	Bacteremia	Unknown	Vancomycin	Recovered	Case 1
6-month, girl	*L.lactis* spp.*lactis*	Catheter-related blood-stream infection	Unknown	Vancomycin	Recovered	Case 2

easily obtained. We believe this may be the reason the peripheral vein blood cultures remained sterile. In both cases, microorganisms yielded within the first 24 hours and for this reason we didn't assume the results as contamination or colonization.

The source and the mechanism of infection is not clear. Humans may become infected with exposure to unpasteurized dairy products. Bacterial translocation from the gut is the most probable mechanism of bacteremia in patients with short bowel syndrome [20]. Our patients were not fed any dairy products, we think that intestinal resection was the facilitating factor for bacterial translocation from the gut. It is difficult to explain the accurate source of bacteria in our patients as is explained in other cases in literature.

Standard therapeutic regimen of *L. lactis* infection is not well-established. In the previously reported cases, therapeutic regimen mostly based on the result of susceptibility tests. Available limited studies reported that they show a natural resistance to the aminoglycosides and no resistance to vancomycin [21,22]. We used vancomycin as therapeutic agent and the patients recovered completely.

Lactococcus species should be kept in mind as a potential pathogen because it is occasionally isolated from human mucocutaneous surfaces such as the intestines.

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