Case report

An unusually encountered bacterium causing diarrhea, *Kluyvera ascorbata*: A case report

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ABSTRACT

Kluyvera is a recently described member of the family Enterobacteriaceae. It can be found in soil, water, sewage, and the healthcare environment. Though associated with human infection, it is poorly studied. We present a case of a 4-year-old child diagnosed with pre-B cell acute lymphoblastic leukemia on induction chemotherapy who presented in the third week of treatment with diarrhea. When a stool sample was examined for diarrheal etiology, we identified the isolated organism as *K. ascorbata* by generic identification, using manual biochemicals and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Antimicrobial susceptibility of the organism was performed by both disc diffusion and VITEK and was confirmed to be a sensitive strain of *K. ascorbata*. The diarrhea was resolved within a few days after receiving appropriate treatment. Although it is a normal flora of the gastrointestinal tract, it has been significantly associated with opportunistic infections in immunocompromised individuals, including children and the elderly. *K. ascorbata* is a less frequently encountered organism causing diarrhea and is probably underestimated because its biochemical identification patterns are similar to those of other related genera. This organism sometimes remains unidentified, leading to the usage of inappropriate antibiotics and gaining antibiotic-resistant genes, which in turn give rise to multidrug-resistant *K. ascorbata.*

Keywords: Acute lymphoblastic leukemia; *Kluyvera ascorbate*; diarrhea; immunocompromised patients; stool.

INTRODUCTION

Kluyvera is a newly defined genus, taxonomically placed into the family of Enterobacteriaceae (1). In 1956, *Kluyvera* was proposed as a genus by Asai and his coworker (2). In the past, this genus comprised two species- *Kluyvera citrophila* and *Kluyvera noncitrophila*, which were classified based on the differences in utilizing citric acid as a sole carbon and energy source (3). They were named *Kluyvera* due to their ability to produce a large amount of α-ketoglutaric acid on glucose fermentation (3). Their capability to utilize glucose and carry out the Krebs cycle acting as a source of glutamate and glutamine stimulates protein synthesis.DNA-DNA hybridization technique was used to characterize the genus *Kluyvera* (3). Currently, the genus *Kluyvera* consists of *Kluyvera ascorbata*, *Kluyvera cryocrescens*, *Kluyvera georgiana* and *Kluyvera cochleae* (2,4). *Kluyvera* is mainly isolated from sputum, urine, stool, blood cultures, peritoneal fluid, and wound specimen (2). It has been regarded as a saprophytic, opportunistic, or pathogenic organism (1). *Kluyvera* spp. cause infection irrespective of age, i.e., there is no significant difference in bloodstream infection or urinary tract infection between children and adults (5). *Kluyvera* infections in children describe a range of clinical infections, including UTI, diarrhea, peritonitis, neonatal meningitis and sepsis, skin and soft tissue infection, pneumonia, and emphysematous gastritis (4). *Kluyvera* spp. also has been implicated in opportunistic infections of immunocompromised patients, specifically diarrheal disease in adults with neutropenia (4). However, no specific virulence factors have been identified or associated with mounting an infection. *Kluyvera* possesses lipopolysaccharides and surface antigens that confer immunogenicity and virulence like most of the genera in Enterobacteriaceae (1). We report a case of diarrhea caused by this organism in a child diagnosed with acute lymphoblastic leukemia.

CASE REPORT

A 4-year-old child presented with pathological fracture of fibula with on and off low-grade fever and petechial rash over the body. For the above complaints, child underwent a complete blood count, which revealed anemia, thrombocytopenia, and peripheral smear showed 25% blast cells. He was later referred to our hospital, where further confirmatory investigations, including bone marrow aspiration, confirmed pre-B cell acute lymphoblastic leukemia. Child was initiated on induction chemotherapy as per institutional protocol, which constitutes four-drug chemotherapy.

On physical examination, the child's weight was 14.2 kg, heart rate 86 bpm, respiratory rate 22/min. On clinical examination, the child had pallor, hepatosplenomegaly, and phimosis. During the treatment, the patient experienced diarrhea, and stool sample was examined for diarrheal etiology.

METHODOLOGY

Faecal sample was primarily inoculated to selenite faecal broth, Mac Conkey agar, blood agar, and on a selective media for enteric pathogens, Heckton enteric agar (HEA), following the standard operative procedure of our diagnostic lab. No intestinal pathogen of interest was isolated on these plated solid media initially. On the other hand, Selenite F broth subculture on Mac Conkey agar yielded growth of a single organism forming large, irregular, moist, low convex, opaque, and lactose fermenting colonies. Gram staining of the colonies showed gram-negative bacilli. The hanging drop technique revealed the motility of the organism. To know the colony morphology on other media, the colony was subcultured on blood agar and HEA. It produced small, circular, and non-haemolytic colonies on blood agar and yielded yellow colonies on HEA media.

The isolate was oxidase negative, catalase-positive, reduced nitrate, utilized citrate, decarboxylated ornithine, fermented glucose with gas production, and did not produce H_2S (6). It shares most of its biochemical characteristics with *Buttiauxella agrestis* and *Citrobacter spp* (6). *Kluyvera ascorbata* was differentiated from *Citrobacter* by its inability to produce arginine dihydrolase enzyme (6). On the other hand, it was differentiated from *Buttiauxella agrestis* by its ability to produce gas from glucose and ferment sucrose (6).

Based on manual biochemical tests, the organism was identified as *Kluyvera spp* (3). Additionally, the positive ascorbate test and inability to grow at 5°C differentiated the *Kluyvera ascorbata* from *Kluyvera cryocrescens* (3). Ascorbate test was devised by Farmer et al. to test the ability to utilise the ascorbic acid (3). MALDI-TOF confirmed the isolated pathogen as *Kluyvera ascorbata* (3).

Antimicrobial susceptibility test (AST) was performed according to CLSI guidelines by Kirby-Bauer's disc diffusion method. Vitek MIC confirmed AST. The zone of inhibition and MIC values of the organism are stated below.

Antimicrobials	Disc diffusion		Vitek MIC	Interpretation
	Potency	Zone of inhibition (mm)		
Ampicillin	10 µg	19	16	Intermediate
Amoxicillin-clavulanic acid	30 µg	31	≤ 2	Sensitive
Piperacillin/Tazobactam	100/10 µg	26	≤4	Sensitive
Cefuroxime	30 µg	6	32	Resistant
Ceftriaxone	30 µg	32	≤1	Sensitive
Cefepime	30 µg	35	≤1	Sensitive
Imipenem	10 µg	29	≤0.25	Sensitive
Gentamicin	10 µg	28	≤1	Sensitive
Ciprofloxacin	5 µg	38	≤0.25	Sensitive
Trimethoprim/Sulfamethoxazole	25 µg	32	≤20	Sensitive

DISCUSSION

Kluyvera is a small, flagellated, gram-negative bacilli belonging to the family Enterobacteriaceae (1). It is a part of the normal flora of the human intestine but less abundant (1). Therefore, its isolation in the clinical sample is very rare (1). The strains of *Kluyvera spp*. are very uniform in their biochemical reactions, and it is challenging to distinguish from other described species of Enterobacteriaceae, which is the reason it has been reported as unidentified and later defined Enteric Group 8 (3).

It is difficult to associate the pathogenicity of *Kluyvera ascorbata* with the severity of infection or mortality of the patients with *Kluyvera* infection. Since *Kluyvera* causes infection mainly in immunocompromised individuals, if mortality occurs, it may be due to the underlying disease because even multidrug-resistant *Kluyvera* is not virulent enough to cause mortality. *Kluyvera* has been known to cause diarrhea to some extent with the virulence factors it possesses. *Kluyvera* spp can colonise the intestine and can be found in food, but their potential ability to cause diarrhea requires further evaluation (7).

Infections with *Kluyvera* spp. are considered a threat in debilitated individuals if they carry genes for antimicrobial resistance. It is capable of invading multiple organs and has a tendency to form abscesses. The strain of *Kluyvera ascorbata* that we have isolated is resistant to ampicillin and cefuroxime but susceptible to third-generation cephalosporin, ceftriaxone which indicated the non-existence of CTX-M resistance genes in the isolated strain. Since the isolated strain was susceptible, the diarrhea was cured within a few days on treatment with appropriate antibiotics. Based on the literature, *Kluyvera* species are known to be one of the sources of CTX-M type β-lactamases (5). Genes encoding for CTX-M-1 and CTX-M-2 enzymes have been detected in some strains of *K. ascorbata* (5). The strains possessing these genes show high resistance to third-generation cephalosporins. Apart from β-lactamases, *Kluyvera* species have also been reported to produce carbapenamase (5). The production of carbapenemase occurs due to the increased use or exposure to carbapenems (8). Recently, KPC-2 producing *K. ascorbata* that inactivates carbapenems have been known to cause human infections (5).

Though *Kluyvera* is rarely isolated, it is studied widely due to its ability to transfer the gene encoding for ESBLs (9). Though the isolated strain is sensitive to most antibiotics, it possesses the ability to transform into multidrug-resistant strain by taking up the genes encoding for the enzymes hydrolysing many antibiotics. Clinicians should be aware of the increasing antibiotic resistance in *Kluyvera* and should be given additional importance (9). Further research data are required to understand the pathogenicity, clinical manifestations, and antimicrobial treatment of such diarrheal infections.

We isolated and identified an unusual pathogen from the stool. *K. ascorbata* has been associated with opportunistic infections in immunocompromised patients like ours. Though diarrhoea resolved within two days of appropriate treatment with cotrimoxazole, *K. ascorbata* as a causative agent of diarrhoea is probably underestimated in this population as the biochemical identification patterns are similar to those of other related genera. Appropriate antibiotic therapy usually results in quick clinical recovery.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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