


CASE REPORT

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Bacteremia caused by *Enterobacter asburiae* misidentified biochemically as *Cronobacter sakazakii* and accurately identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: a case report

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Abstract

Background: Biochemical analyses of causative bacteria do not always result in clear identification, and new technologies aimed at improving diagnostic accuracy continue to be developed. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is a rapid and accurate technique for bacterial identification. Misidentification of *Cronobacter sakazakii* is related to clinical and industrial problems. Here, we encountered a case of rare bacteremia in which the causative organism *Enterobacter asburiae* was biochemically misidentified as *C. sakazakii* before being correctly identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Case presentation: An 87-year-old Asian man with no diabetes or active disease developed bacteremia and was admitted to our hospital. While the route of infection could not be determined despite various examinations, the clinical course was good following antibiotic therapy. Biochemical analyses identified the causative organism as *C. sakazakii*, but colonies on the blood agar medium showed a grayish coloration, differing from the yellowish coloration of typical *Cronobacter* colonies. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was therefore performed, identifying the bacterium as *E. asburiae* on three independent analyses. This result was confirmed by multilocus sequence analysis using five housekeeping genes.

Conclusions: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry may reduce misidentification of bacteria as *C. sakazakii* and improve the reporting rate of *E. asburiae*. This technique should be considered when biochemical bacterial misidentification is suspected.

Keywords: *Enterobacter asburiae*, *Cronobacter sakazakii*, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Background

Identification of the causative bacteria for infectious diseases using biochemical examinations is sometimes inaccurate, and new technologies aimed at improving diagnostic accuracy continue to be developed. Matrix-assisted laser desorption/ionization time-of-flight mass

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spectrometry (MALDI-TOF MS) allows rapid, accurate identification of the causative organism. This technique analyzes the patterns of proteins that are extracted from bacteria, which can reveal bacteria at the genus, species, and sometimes even subspecies levels [1].

Both *Cronobacter sakazakii* and *Enterobacter asburiae* are Gram-negative, rod-shaped, motile bacteria. *C. sakazakii* is an opportunistic pathogen that can cause lethal infection in newborns and the elderly, so accurate identification is crucial and misidentification by biochemical examination represents a critical problem [2]. *C. sakazakii* was initially identified under the genus *Enterobacter* before being recategorized under the genus *Cronobacter* [3]. Distinguishing between *Cronobacter* and *Enterobacter* is difficult due to the similarities in biochemical phenotypes [2]. Rapid, reliable identification of genus *Cronobacter* and differentiation from genus *Enterobacter* is important for epidemiological research. Here, we report a case of bacteremia in which the causative *E. asburiae* was initially misidentified as *C. sakazakii* by biochemical analyses before eventual correct identification by MALDI-TOF MS.

Case presentation

An 87-year-old Asian man visited his primary-care physician with a 3-day history of fever and severe malaise. He had no history of diabetes mellitus or active disease. He was admitted and treated with intravenous meropenem (MEPM) at 1 g/day and oral levofloxacin at 500 mg/day by his primary-care physician on day 1. The next day, a Gram-negative bacillus was detected from two sets of blood culture bottles. The patient was then transferred to our hospital for further examination and treatment. He was conscious. Body temperature was 37.7 °C, heart rate was 80 beats per minute, respiratory rate was 14 breaths per minute, and blood pressure was 113/70 mmHg. There were no significant findings on physical examination, chest X-ray, plain computed tomography of the head, whole-body contrast-enhanced computed tomography, transthoracic echocardiography, or colonoscopy. Blood testing revealed a white blood cell count of 8080 cells/ μ l, a C-reactive protein (CRP) level of 18.01 mg/dl, and a procalcitonin level of 1.02 ng/ml. Blood and urine cultures were negative. Intravenous MEPM (3 g/day) was administered at our hospital owing to a lack of improvement on the previous treatment (day 2). He was afebrile on day 3. CRP level decreased to 4.05 mg/dl on day 5. A blood culture taken by the previous physician reportedly revealed *C. sakazakii*. Antibiotic therapy was changed to ceftriaxone at 2 g/day based on the results of antimicrobial susceptibility testing on day 6 (Table 1). This treatment was stopped on day 10 after confirming normalization of CRP levels and negative results for blood

Table 1 Antibacterial susceptibility test results of the strain isolated in the present case

Antimicrobial agent	MIC (μ g/ml)	Interpretation according to CLSI 2012 criteria
Ampicillin	> 16	R
Piperacillin	\leq 16	S
Cefazolin	> 16	R
Cefotiam	> 16	R
Cefotaxime	\leq 1	S
Cefepime	\leq 8	S
Imipenem/cilastatin	\leq 1	S
Gentamicin	\leq 4	S
Minocycline	\leq 4	S
Levofloxacin	\leq 2	S

MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; R, resistant; S, susceptible.

culture. After discontinuation of antibiotic therapy and discharge from our hospital, the patient showed good progress without recurrence of fever. Biochemical analyses using the MicroScan WalkAway 96 system (Beckman Coulter, Brea, CA, USA) identified the causative organism as *C. sakazakii* (Table 2). However, the colonies on the blood agar medium unexpectedly showed grayish coloration, differing from the yellowish coloration of typical *Cronobacter* colonies (Fig. 1). MALDI-TOF MS using Microflex LT with the Biotyper v3.1 database (Bruker Daltonics, Bremen, Germany) was therefore conducted. MALDI-TOF MS identified the bacterium as *E. asburiae* on three independent analyses (log score values of 2.19, 2.08, and 2.20, matching with *E. asburiae* type strain DSM 17506). Finally, multilocus sequence analysis using five housekeeping genes (*fusA*, *gyrB*, *leuS*, *rpoB*, and *hsp60*) [4] confirmed our isolate as *E. asburiae* (TWCC 57976). Nucleotide sequence data reported are available under the DNA Data Bank of Japan (DDBJ) accession numbers LC427844 to LC427849.

Discussion and conclusions

In this case, biochemical analyses misidentified *E. asburiae* as *C. sakazakii*. As *C. sakazakii* can contaminate powdered infant formula and may cause fatal infections in newborns, biochemical screening for *Cronobacter* contamination is recommended [5]. Although accurate identification of *C. sakazakii* is extremely important to prevent potentially fatal infections of newborns, a previous study suggested that biochemical test panels are unreliable for identifying *Cronobacter* species [6]. To avoid misidentification, methods other than biochemical analyses need to be considered.

Table 2 Biochemical phenotypes of *E. asburiae* and *C. sakazakii* in reference to the MicroScan WalkAway 96 system and results for the strain isolated in the present case

	<i>E. asburiae</i>	<i>C. sakazakii</i>	Present case
Glucose	99	99	+
Sucrose	99	99	+
Sorbitol	99	5	–
Raffinose	50	90	+
Rhamnose	5	99	+
Arabinose	95	99	+
Inositol	10	75	+
Adonitol	1	1	–
Melibiose	5	90	+
Urease	1	1	–
Hydrogen sulfide	1	1	–
Indole	1	1	–
Lysine decarboxylase	1	1	–
Arginine decarboxylase	25	75	–
Ornithine decarboxylase	95	95	+
Tryptophan deaminase	1	1	–
Esculin hydrolysis	90	95	+
Voges–Proskauer	1	95	+
Citrate	25	99	+
Malonate	1	10	–
β-Galactosidase	99	99	+

Each number represents the probability of biochemical reaction.

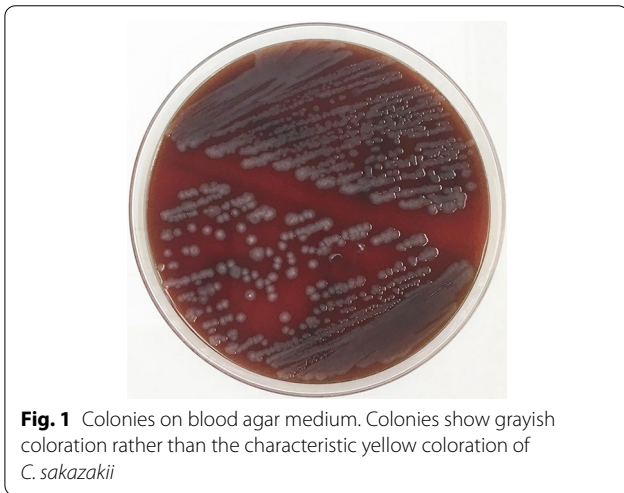


Fig. 1 Colonies on blood agar medium. Colonies show grayish coloration rather than the characteristic yellow coloration of *C. sakazakii*

The biochemical misidentification in this case may have been caused by biochemical heterogeneity of bacteria. In the MicroScan WalkAway system, negativity for sorbitol fermentation and positivity for melibiose, rhamnose, and inositol fermentation and the

Voges–Proskauer reaction matched the typical biochemical phenotype of *C. sakazakii*, but not that of *E. asburiae*. *E. asburiae* belongs to the *Enterobacter cloacae* complex. Six species of bacteria belong to this complex, which share similar biochemical phenotypes [7]. *C. sakazakii* was previously categorized as *Enterobacter sakazakii*, but was later recategorized into genus *Cronobacter* [3]. A previous report described *C. sakazakii* biochemically misidentified as *E. hormaechei*, belonging to the *E. cloacae* complex [8]. They suggested that misidentification of *E. hormaechei* as *C. sakazakii* may cause unnecessary financial losses for manufacturing companies. The activities of enzymes that determine the biochemical phenotype are heterogeneous and can cause misidentification of *Cronobacter* [9]. Biochemical misidentification among related strains has been reported in other bacteria, such as *Pseudomonas aeruginosa* [10] and enterohemorrhagic *Escherichia coli* [11]. Such misidentifications can lead to the prescription of inappropriate antibiotics and serious infection. Additional tests in consideration of the possibility of misidentification by biochemical analyses should be considered.

MALDI-TOF MS is a mass spectrometry technique that involves ionizing a sample using laser light and patterning constituent molecules of the target protein. Bacteria can be identified by comparing the constituent molecular pattern (mass spectrum) of the obtained material with a database [1]. This method is simple and quick compared with DNA-sequence-based methods. The effects of reducing hospitalization days and mortality rates through the proper use of antibiotics based on MALDI-TOF MS results have also been reported [12]. Further, MALDI-TOF MS allows quick identification of bacteria that show poor cultivation rates or long cultivation periods, improving the bacterial identification rate [13].

In our case, *E. asburiae* was initially biochemically misidentified as *C. sakazakii* before correct identification by MALDI-TOF MS. Although the significance of *E. asburiae* bacteremia has not been elucidated, its prevalence may be underestimated due to the misidentification of *E. asburiae* bacteremia. MALDI-TOF MS has the possibility of reducing misidentification and improving reporting rates of *E. asburiae*. This technique may also help elucidate the natural history of the bacteria. When biochemical misidentification of bacteria is suspected, MALDI-TOF MS should be considered for rapid and accurate identification.

Abbreviations

MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MEPM: Meropenem; CRP: C-reactive protein.

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Authors' contributions

NH collected and analyzed the data and drafted the manuscript. SS and TT collected the data and participated in developing the concept of the manuscript. AY, KK, and AN analyzed the data. EM participated in developing the concept of the manuscript and revised the article for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Ethics approval was not required by the ethics committees at Almeida Memorial Hospital and Oita University.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and the accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

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