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 spectrometry (MALDI-TOF MS) is a rapid and accurate technique for bacterial identification, making it a valuable tool in clinical microbiology. However, misidentification of certain bacteria, particularly *Cronobacter* species, remains a significant issue. *Cronobacter sakazakii*, a common pathogen in newborns, can cause serious infections such as meningitis and sepsis, while *Enterobacter asburiae* is less commonly associated with human disease. Biochemical misidentification of *C. sakazakii* can lead to delays in appropriate antibiotic treatment and negative outcomes for patients. Therefore, the development of more accurate and rapid methods for bacterial identification is crucial.

In this case report, a 87-year-old Asian man was admitted to our hospital with bacteremia. Despite various examinations, the route of infection could not be determined. Biochemical analyses initially 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. To confirm the identification, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was performed, identifying the bacterium as *E. asburiae* on three independent analyses. This result was confirmed by multilocus sequence analysis using five housekeeping genes, providing strong evidence for the correct identification of the causative organism.

The use of MALDI-TOF MS in this case helped to avoid misidentification of the bacterium and ensured appropriate antibiotic treatment, which led to a good clinical course for the patient. This case highlights the importance of using advanced diagnostic techniques to improve the accuracy of bacterial identification and clinical outcomes.
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/μl, 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 on day 3. CRP level decreased to 4.05 mg/dl on day 5. Ament on the previous treatment (day 2). He was afebrile administered at our hospital owing to a lack of improvement. 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 administered at our hospital owing to a lack of improvement on the previous treatment (day 2). He was afebrile administered at our hospital owing to a lack of improvement on the previous treatment (day 2). He was afebrile.

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

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

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

**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.
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.

**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

|                      | *E. asburiae* | *C. sakazakii* | Present case |
|----------------------|---------------|----------------|--------------|
| Glucose              | 99            | 99             | +            |
| Sucrose              | 99            | 99             | +            |
| Sorbitol             | 99            | 5              | −            |
| Raffinose            | 50            | 90             | +            |
| Rhamnose             | 5             | 99             | +            |
|阿拉伯糖               | 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*.

**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 ANI 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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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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