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CASE REPORT

Persistent *Acinetobacter bereziniae* Bacteremia in a Pregnant Woman

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SUMMARY

Background: **Persistent *Acinetobacter bereziniae* bacteremia in a pregnant woman has not previously been reported.**
Methods: **A 25-year-old pregnant Kyrgyz woman developed a fever after McDonald operation. Serial blood cultures were performed.**
Results: **Multidrug-resistant *Acinetobacter* was isolated. She was prescribed meropenem. *A. bereziniae* was successfully identified by MALDI-TOF MS with an updated library, and 16S rRNA gene sequencing analysis supported the result.**
Conclusions: **The multidrug-resistant features of *A. bereziniae* and the therapeutic concentration of antibiotic agents during pregnancy had to be considered in the present case.**
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KEY WORDS

Acinetobacter bereziniae, bacteremia, MALDI-TOF MS, pregnancy, multidrug resistance

INTRODUCTION

Acinetobacter bereziniae is a potential nosocomial pathogen [1]. Persistent *A. bereziniae* bacteremia in a pregnant woman has not previously been reported. Clinically, multidrug-resistant features of *A. bereziniae* and the therapeutic concentration of antibiotic agents during pregnancy had to be considered in the present case. This study was approved by the Institutional Review Board (KHUH-2019-05-061) of Kyung Hee University Hospital, Seoul, Korea, which waived the need for written informed consent from the patient.

CASE PRESENTATION

A 25-year-old pregnant Kyrgyz woman (20th gestational week) presenting with lower abdominal pain was admitted to the obstetric department of Kyung Hee Uni-

versity Hospital in South Korea. Four years ago, the patient received a McDonald operation for incompetent internal os of cervix and short cervix. In a same year, she underwent an emergency cesarean section due to preterm labor associated with a bicornuate uterus at the 34th gestational week. After admission, she was diagnosed with preterm labor, then received a second Mc Donald operation on the 3rd hospital day. On the 15th postoperative day, the patient developed a fever of up to 38.6°C. Laboratory examinations showed a white blood cell count of 21.95 x 10^9/L (98% neutrophil) and C-reactive protein level was 185.43 nmol/L. Amniotic fluid analysis revealed a white blood cell count of 1 cell/μL, and both Gram stain and bacterial culture of amniotic fluid were negative. She received meropenem (3 g per day) for 4 days. Two sets of blood cultures were performed at the time of fever using BD Bactec Plus Aerobic/F and BD Bactec Plus Anaerobic/F bottles and a Bactec FX Instrument (Becton Dickinson, Sparks, MD, USA). On day 3 after fever onset, the antibiotic therapy was changed to ampicillin-sulbactam (12 g per day) for 7 days upon isolating *Acinetobacter baumannii/haemolyticus* (98.88% probability) from two sets of culture bottles and determining its susceptibility by a MicroScan Walkaway 96 system with a NC63 panel (Siemens, Deerfield, IL, USA). The intermittent fever was sustained for 3 days after the onset of fever. Then, she was prescribed meropenem (3 g per day) again for the next 2 weeks due to persistent *Acinetobacter* species bacteremia. Then, blood cultures with two sets of bottles were performed on days 4, 7, and 8 after onset of fever, and the numbers of culture-positive bottles were 1, 1, and 2, respectively. All the isolates from the subsequent culture were identified as *Acinetobacter bereziniae* by a Bruker MicroFlex LT (Bruker Daltonik GmbH, Leipzig, Germany) with Biotyper software 2.3 and an MBT 6903 MSP Library (cutoff score range; 2.473 - 2.488). The patient became apyretic and vital signs stabilized on day 7 after fever onset with a normal white blood cell count. The level of CRP came within the reference interval on day 12 after fever onset.

The 16S rRNA gene sequencing was performed [2] retrospectively for all four isolates. Based on the NCBI BLASTn, the four isolates showed the highest match with *A. bereziniae* (accession: NR 117625), exhibiting similarity percentages of 99.8% (1452/1455), 99.9% (1471/1473), 99.9% (1471/1473), and 99.9% (1468/1470) in isolation order. The second highest match was with *Acinetobacter guillouiae* (accession: NR 117626), showing similarity percentages of 99.2% (1444/1455), 99.3% (1463/1473), 99.4% (1463/1472), and 99.3% (1460/1470), respectively. The neighbor-joining tree generated by MEGA-X [3] also showed *A. bereziniae* as the nearest taxa (Figure 1).

DISCUSSION

A. bereziniae is a gram-negative, strictly aerobic, non-motile coccobacillus which was previously known as *Acinetobacter* genospecies 10 [4]. *A. bereziniae* is closely related to *A. guillouiae,* which is known as *Acinetobacter* genomic species 11 [4]. These two species are considered monophyletic taxa based on sequencing analysis, and they share similar properties in phenotypic tests [4]. However, the characteristic that *A. bereziniae* grows at 38°C and not *A. guillouiae* [4] can be helpful in distinguishing between two bacteria in clinical laboratories that generally incubate cultures at 35 ± 2 °C. The interspecies sequence difference between *A. bereziniae* and *A. guillouiae* as determined by 16S rRNA sequencing is less than 0.8% [5]. In our case, a maximum difference of 0.62% was shown, which was not sufficient to distinguish between these species. For better discrimination in sequencing, analyzing the RNA polymerase *β*-subunit (*rpoB*) gene would be helpful [4,6]. It was noted that Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis of *A. bereziniae* and *A. guillouiae* shows specific signals, which demonstrate a clear separation of these species' respective mass spectra [4]. We could identify *A. bereziniae* with the version of MBT 6903 MSP Library of Bruker MicroFlex LT (Bruker Daltonik GmbH), which contains the reference spectrum of both *A. bereziniae* and *A. guillouiae.*

The use of automated biochemical systems such as Vitek 2 (bioMérieux, Marcy l'Etoile, France) or MicroScan (Siemens) for identifying non-*A. baumannii* to the species level were reported to be unsatisfactory [7]. The first isolate of our case which was identified as *Acinetobacter baumannii/haemolyticus* by MicroScan (Siemens) was re-identified as *A. bereziniae* (cutoff score 2.474 by MALDI-TOF MS and 99.2% similarity in the 16S rRNA gene).

Clinical infections caused by *A. bereziniae* are rare, but *A. bereziniae* may cause potentially lethal infections in immunocompromised hosts [8]. To the best of our knowledge, persistent *A. bereziniae* bacteremia in a pregnant woman has not been reported. The source of bacteremia was uncertain. It was unclear whether her persistent bacteremia was related to the characteristics of *A. bereziniae* or suboptimal blood concentrations of antibiotics. While transplacental passage of meropenem was shown to be low in an *ex vivo* model, rapid elimination of beta-lactam drugs, such as ampicillin-sulbactam, which was administered to the case patient after the susceptibility results were released, has been shown in pregnancy [9,10].

In our case, *A. bereziniae* isolated from the patient was susceptible to ampicillin-sulbactam, cefepime, and tobramycin, but was not susceptible to ceftazidime, amikacin, and ciprofloxacin according to the CLSI M100-S28 [11]. The first isolate of *A. bereziniae* was susceptible to meropenem, but the following three isolates were found to be resistant to meropenem. This raises a

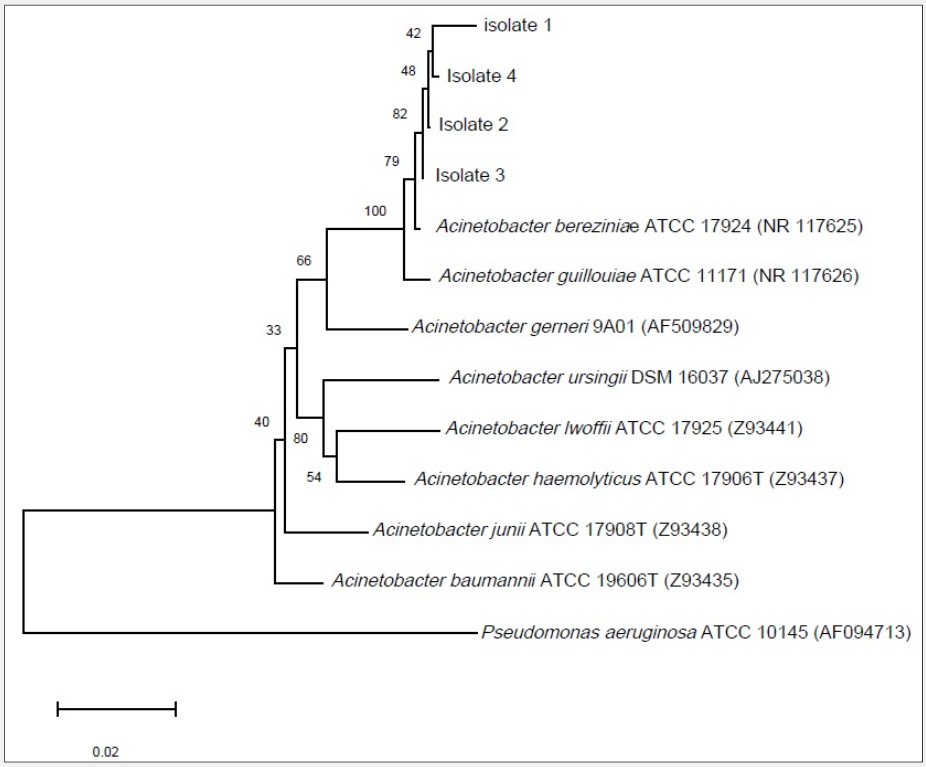

Figure 1. Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences of four isolates and other related species was constructed using MEGA-X software [3], and *Pseudomonas aeruginosa* ATCC 10145 was used as an outgroup. The genetic distance was determined according to Kimura's two-parameter model [14]. Bar indicates 0.02 changes per nucleotide position. GenBank accession numbers are given in parentheses.

concern that non-*baumannii Acinetobacter* species, including *A. bereziniae,* may become reservoirs of clinically relevant resistance genes [12]. It was reported that β-lactamase-mediated resistance of *A. bereziniae* to carbapenem was associated with production of metallo-β-lactamase and class D β-lactamase, and these genes encoding β-lactamase can be examined as an additional method for identifying *A. bereziniae* [13]. The genetic analysis of multidrug resistance for *A. bereziniae* was not conducted, which is a limitation of our study.

CONCLUSION

We presented the case of persistent *A. bereziniae* bacteremia in a pregnant woman. *A. bereziniae* was successfully identified by MALDI-TOF MS with an updated library, and 16S rRNA gene sequencing analysis supported the result. The multidrug-resistant features of *A. bereziniae* and choosing the optimal concentration of antibiotic agents during pregnancy posed a therapeutic challenge.

Declaration of Interest:
All authors declare no conflicts of interest regarding this article.

References:

1. Choi JY, Kim Y, Ko EA, et al. *Acinetobacter* species isolates from a range of environments: species survey and observations of antimicrobial resistance. Diagn Microbiol Infect Dis 2012;74(2): 177-80 (PMID: 22902160).

2. Yang HS, Kim YJ, Lee MS, Lee HJ. *Globicatella sanguinis* Bacteremia in a Non-Immunocompromised Patient Identified by 16S rRNA Gene Sequencing: First Case in Korea. Clin Lab 2016;62 (9):1825-7 (PMID: 28164584).

3. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 2018;35(6):1547-9 (PMID: 29722887).

4. Nemec A, Musilek M, Sedo O, et al. *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. Int J Syst Evol Microbiol 2010;60(Pt 4):896-903 (PMID: 19661501).

5. CLSI. Interpretive Criteria for Identification of Bacteria and fungi by DNA target sequencing, 2nd ed. CLSI document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

6. Gundi VA, Dijkshoorn L, Burignat S, Raoult D, La Scola B. Validation of partial *rpoB* gene sequence analysis for the identification of clinically important and emerging *Acinetobacter* species. Microbiology 2009;155(Pt 7):2333-41 (PMID: 19389786).

7. Lee SY, Shin JH, Kim SH, Shin MG, Suh SP, Ryang DW. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry-based VITEK MS system for the identification of *Acinetobacter* species from blood cultures: comparison with VITEK 2 and MicroScan systems. Ann Lab Med 2015;35 (1):62-8 (PMID: 25553282).

8. Kuo SC, Fung CP, Lee YT, Chen CP, Chen TL. Bacteremia due to *Acinetobacter* genomic species 10. J Clin Microbiol 2010;48 (2):586-90 (PMID: 19955266).

9. Chamberlain A, White S, Bawdon R, Thomas S, Larsen B. Pharmacokinetics of ampicillin and sulbactam in pregnancy. Am J Obstet Gynecol 1993;168(2):667-73 (PMID: 8438948).

10. Hnat M, Bawdon RE. Transfer of meropenem in the ex vivo human placenta perfusion model. Infect Dis Obstet Gynecol 2005; 13(4):223-7 (PMID: 16338783).

11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 28th Infomational supplment. CLSI document M100-S28. Wayne, PA, USA: Clinical and Laboratory Standards Institue 2018.

12. Grosso F, Silva L, Sousa C, Ramos H, Quinteira S, Peixe L. Extending the reservoir of *bla* IMP-5: the emerging pathogen *Acinetobacter bereziniae*. Future Microbiol 2015;10(10):1609-13 (PMID: 26439605).

13. Bonnin RA, Ocampo-Sosa AA, Poirel L, Guet-Revillet H, Nordmann P. Biochemical and genetic characterization of carbapenem-hydrolyzing beta-lactamase OXA-229 from *Acinetobacter bereziniae*. Antimicrob Agents Chemother 2012;56(7):3923-7 (PMID: 22508298).

14. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16(2):111-20 (PMID: 7463489).