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Brief communication

Risk of inaccurate species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and of false carbapenem resistance by automated susceptibility analysis of *Enterobacter* spp.

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Abstract

Enterobacter species were isolated from the sputum of an 84-year-old female patient with fever, chills, cellulitis in the right elbow, and pyogenic arthritis in the left shoulder. The bacteria were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and whole-genome sequencing (WGS). This strain was defined as a carbapenem-resistant *Enterobacterales* (CRE) by the MicroScan NG MIC 44 panel without applying the revised guidelines of the Korea Disease Control and Prevention Agency properly. MALDI-TOF MS is commonly used to determine species, but the accurate identification of *Enterobacter* subspecies remains challenging. An Etest or disk diffusion test is recommended to confirm ertapenem resistance when an isolate exhibits resistance solely to ertapenem among carbapenems.

Keywords: Carbapenems, Drug resistance, *Enterobacter*, Matrix-assisted laser desorption-ionization mass spectrometry

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The *Enterobacter cloacae* complex (ECC) represents a group of closely related species within the *Enterobacter* genus, including *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, and *E. nimipressuralis* [1]. These species are commonly associated with hospital-acquired infections and exhibit significant resistance to multiple antibiotics, particularly carbapenems, which are often used as the last line of defense against multidrug-resistant organisms [2]. The accurate identification of these species and subspecies remains a challenge in clinical microbiology because of their phenotypic similarities.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a widely used tool for species identification in clinical laboratories because of its speed and accuracy [3]. However, distinguishing closely related species within the ECC using MALDI-TOF MS can be difficult, leading to potential misidentification [4]. This is particularly concerning in the context of carbapenem-resistant *Enterobacteriales* (CRE), which requires accurate identification for appropriate treatment decisions. Among carbapenems, ertapenem resistance is especially important for monitoring because it can serve as a marker for broader carbapenem resistance.

In this study, we report the case of an 84-year-old female patient presenting with fever and chills, with cellulitis at the right elbow and pyogenic arthritis at the left shoulder. *Enterobacter* species were isolated from the sputum and identified using MALDI-TOF MS and whole-genome sequencing (WGS). After piperacillin-tazobactam (empiric antimicrobial treatment) was changed to ciprofloxacin (definitive antimicrobial treatment) based on results of the antimicrobial susceptibility test, the clinical symptoms and radiologic findings improved. Herein, we discuss the challenges in accurately identifying ECC species and their implications for clinical management, particularly in the context of carbapenem resistance.

Colonies grown on MacConkey agar were pink, lactose-positive, and mucoid. Gram staining revealed the presence of gram-negative bacilli. The bacteria were identified as *Enterobacter bugandensis* (score = 2.26) or *Enterobacter asburiae* (score = 2.24) using MALDI-TOF MS biotyper (Bruker Biotyper, Bruker Daltonics GmbH). The mass spectrum profile followed the identification standard Method 1.1 (Bruker). *Enterobacter cloacae* (50%) and *E. asburiae* (50%) were equally identified with MALDI-TOF VITEK MS (bioMérieux) in the sample.

To accurately identify the species, the whole genome of this strain was sequenced using a NextSeq 550 instrument (Illumina) as previously described [5]. ECC species were determined using Species Finder 2.0, and Kmer Finder 3.2 from the Center for Genomic Epidemiology website [6]. The Species Finder prediction of bacterial species uses the S16 ribosomal DNA sequence, whereas Kmer Finder uses a fast Kmer algorithm. Sequence type (ST) 53 *E. cloacae* was identified using multilocus sequence typing (MLST) 2.0 (*dnaA*_40/*fusA*_17/*gyrB*_39/*leuS*_15/*pyrG*_46/*rplB*_11/*rpoB*_10) [6].

This strain was defined as a carbapenem-resistant organism, exceeding 1 μg/mL the minimal inhibitory concentration (MIC) of ertapenem, using the MicroScan NG MIC 44 panel (Siemens Healthcare Diagnostics Inc.) in MicroScan WalkAway 96 Plus (Siemens). However, the organism was susceptible to doripenem, imipenem, and meropenem, with an MIC of 1 μg/mL or lower. In this case, ertapenem resistance was confirmed using a disk diffusion test, and this strain showed intermediate ertapenem susceptibility, with an inhibition zone of 19 mm. Although a disk diffusion test could simply be applied to a single carbapenem, the Etest showed higher sensitivity and specificity than the disk diffusion test [7]. Carbapenemase production was tested using NG-Test Carba 5 immunochromatography (NG Biotech), and none of the five carbapenemases (NDM, IMP, VIM, OXA-48, and KPC) were detected. In WGS, only $bla_{\text{ACT-1}}$ was identified as an acquired resistance gene.

WGS and MLST are not part of the routine diagnostics for patients. Differentiating specific species of ECC using phenotypic methods and 16S rDNA sequencing is difficult. Distinguishing subspecies using common MALDI-TOF MS systems in the laboratory is challenging, suggesting that alternative approaches would be helpful [4]. Unfortunately, no method is adequate for routine analysis. Sequence analysis of *oriC, gyrB, rpoB,* and *hsp60* resulted in distinct genetic clusters, but not a specific species. Genomic hybridization analysis, MLST, and WGS are labor-intensive and expensive. In this case, we reported the ECC species based on WGS results and ST for species identification.

The *Enterobacter* genus was first described in 1960. However, changes in taxonomy have occurred over the last 50 years and 22 species have been identified in the genus *Enterobacter* to date. Davin-Regli et al. [8] updated the *Enterobacter* taxonomy. This nomenclature is based on the shared phenotypic and genotypic

characteristics determined by whole-genome DNA-DNA hybridization. Based on this nomenclature, *E. bugandensis, E. timonensis, E. massiliensis, E. chengduensis, E. sichuanensis,* and *E. roggenkampii* are recently described as part of the existing 7-member ECC complex [8]. *E. bugandensis* is occasionally identified in clinical samples, and MALDI-TOF MS is commonly used in clinical microbiology laboratories. Owing to the frequent changes and complexity of the *Enterobacter* genus, species identification has become difficult for clinical microbiology laboratory workers.

Among *Enterobacter* spp., *E. cloacae* and *E. aeruginosa* frequently develop antimicrobial resistance to β-lactam, and clinical isolates collected in the patient during antimicrobial therapy show decreased susceptibility to cephalosporins and carbapenems [9]. However, information on the antimicrobial resistance of other *Enterobacter* species is limited. *E. bugandensis* was reported to be responsible for a 3-month outbreak of septicemia in a neonatal ward in Tanzania, and its multidrug-resistant phenotype has been studied [9]. Recently, multidrug-resistant *E. asburiae* has been increasingly isolated from clinical patients, harboring both *mcr-10* and $bla_{\text{NDM-1}}$ and showing high-level resistance against carbapenem and colistin [10].

CRE should be reported according to the guidelines of the Korea Disease Control and Prevention Agency (KDCPA) if the isolate shows resistance to carbapenems such as doripenem, ertapenem, imipenem, and meropenem [11]. Recently, the ertapenem MIC breakpoint for resistance was changed from 1 to 2 μg/mL according to the breakpoint in Clinical and Laboratory Standards Institute M100 ED33 [12]. The criteria for carbapenems other than ertapenem did not change and the test panels contained adequate antimicrobial concentration ranges. However, the available MicroScan GN MIC panels (Siemens) contain ertapenem at concentrations only up to 1 μg/mL. Therefore, without confirming the 2 μg/mL MIC level, this automated susceptibility analyzer could give false ertapenem resistance results.

In this case, we found that MALDI-TOF MS identification strongly depends on the quality of the database used, and the accurate identification of subspecies of *Enterobacter* spp. remains challenging. Before CRE is reported based on the KDCPA guidelines, ertapenem resistance should be confirmed with an Etest or disk diffusion test if the isolates show resistance only to ertapenem in carbapenems with MicroScan GN MIC panels (Siemens).

Ethics statement

This study was approved by the Institutional Review Board of National Health Insurance Service Ilsan Hospital, Goyang, Korea as required by hospital policy (IRB No. 2024-11-017).

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Data availability

The datasets generated during the current study are available from the corresponding author upon request.

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