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Evaluation of Various Antimicrobial Susceptibility Tests for Novel β-Lactam/β-Lactamase Inhibitors against Carbapenem-Resistant Pathogens

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Background: Infections caused by carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) have severely limited therapeutic options. Recently, Korea introduced novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations, such as ceftazidime-avibactam (CTZ-AVI, Zavicefta; Pfizer, Korea) and ceftolozane-tazobactam (CFZ-TAZ, Zerbaxa; MSD, Korea), increasing the demand for antimicrobial susceptibility testing. This study aims to evaluate various antimicrobial susceptibility tests for CTZ-AVI and CFZ-TAZ against carbapenem-resistant pathogens.

Methods: A total of 132 carbapenem-resistant pathogens (76 CRE and 56 CRPA) were included. Antimicrobial susceptibility to CTZ-AVI and CFZ-TAZ was determined using the Etest (Liofilchem, USA) and compared with results from broth microdilution (Sensititre; Thermo Fisher Scientific, USA) or an automated system (VITEK 2 with Gram-negative extension panel; bioMérieux, France). Carbapenemase genotypes were identified using the Carba NP test (NG Biotech, France) or PCR sequencing.

Results: Comparison of Etest and microdilution methods showed concordance rates of 100% for CTZ-AVI and 92.3% for CFZ-TAZ among 26 CRE isolates. For seven CRPA isolates, results were consistently accurate for both CTZ-AVI and CFZ-TAZ. When comparing Etest with automated methods, concordance rates were 98% for CTZ-AVI and 94% for CFZ-TAZ among 50 CRE isolates, and 100% for CTZ-AVI and 98% for CFZ-TAZ among 49 CRPA isolates. The susceptibility of most CRE isolates to CTZ-AVI and CFZ-TAZ could be predicted based on carbapenemase genotype. In CRPA, the presence of New Delhi metallo-β-lactamase and imipenemase appeared to contribute to resistance to both agents.

Conclusions: All three methods are reliable for susceptibility testing of novel BL/BLI combinations against carbapenem-resistant pathogens.

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Key Words Bacterial sensitivity test, Ceftazidime-avibactam, Ceftolozane-tazobactam, Carbapenem resistance, Enterobacterales, *Pseudomonas aeruginosa*

INTRODUCTION

Carbapenem-resistant Gram-negative bacilli infections are emerging as a major public health challenge worldwide [1]. Infections caused by carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) are associated with treatment failures, increased healthcare costs, prolonged hospital stays, and a significant socioeconomic burden [2]. According to the Korean Antimicrobial Resistance Surveillance System (Kor-GLASS), resistance rates to imipenem in *Klebsiella* pneumoniae and *Escherichia coli* remain low in Korea; however, carbapenem resistance rates exceed 20% among *P. aeruginosa* blood isolates [3].

In the United States and Europe, newly developed antibiotics are prioritized for moderate to severe infections, given their activity and effectiveness against CRE [4]. In contrast, the use of novel β-lactam/β-lactamase inhibitor (BL/BLI) combinations such as cefiderocol, eravacycline, and plazomicin remains limited in Korea, partly due to challenges including drug pricing and insurance coverage. Recently, ceftazidime-avibactam (CTZ-AVI, Zavicefta; Pfizer, Seoul, Korea) and ceftolozane-tazobactam (CFZ-TAZ, Zerbaxa; MSD, Seoul, Korea) have been introduced in Korea, creating an increased demand for antimicrobial susceptibility testing. This study aims to evaluate various antimicrobial susceptibility tests for these novel BL/BLIs against carbapenem-resistant pathogens, providing potential reference data for microbiology laboratories.

MATERIALS AND METHODS

1. Study strains

This study included a total of 132 carbapenem-resistant pathogens (76 CRE and 56 CRPA), isolated from patients at two general hospitals in Gyeonggi Province between July 2023 and October 2024 (905 and 661 beds, respectively). In addition, 49 CRPA isolates collected during the Kor-GLASS study in 2022 were included [5]. For CRE isolates, specimen sources were stool (n=25), blood (n=19), respiratory (n=13), urine (n=14), and other (n=5). For CRPA isolates, specimen sources included blood (n=50), respiratory (n=4), and other (n=2). The Enterobacterales species comprised *Klebsiella pneumoniae* (n=57), *E. coli* (n=11), *Citrobacter koseri* (n=3), *Citrobacter braakii* (n=1), *Klebsiella aerogenes* (n=1), *Klebsiella oxytoca* (*n*=1), *Klebsiella variicola* (*n*=1), and *Serratia marcescens* (n=1). Species identification was performed using the MALDI Biotyper (Bruker Daltonik, Bremen, Germany).

2. Antimicrobial susceptibility tests

Etests were performed using CTZ-AVI 0.016-256 MIC Test Strips (Liofilchem, Waltham, MA, USA) and CFZ-TAZ 0.016-256 MIC Test Strips (Liofilchem), following the manufacturer's instructions for all study strains. Broth microdilution was carried out using Sensititre-Gram Negative GN6F AST Plates (Thermo Fisher Scientific, Waltham, MA, USA) for 26 CRE isolates and 7 CRPA isolates. The VITEK 2 system (bioMérieux, Marcy-l'Étoile, France), equipped with the AST-XN09 Gram-negative extension panel, was used as the automated method for 50 CRE isolates and 49 CRPA isolates, according to the manufacturer's instructions. Interpretive breakpoints were applied following Clinical and Laboratory Standards Institute guidelines [6].

3. Genotypes of carbapenem-resistant genes

For all 76 CRE isolates, carbapenemase genotypes were determined using the Carba NP test (NG Biotech, Guipry-Messac, France), which detects the production of *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase (IMP), and oxacillinase-48 (OXA-48). For 49 CRPA isolates, genotypes were identified using multiplex PCR–based sequencing to detect KPC, Guiana extended-spectrum carbapenemases (GES), NDM, IMP, and VIM [5].

4. Ethics statement

The Institutional Review Board of the National Health Insurance Service Ilsan Hospital approved this study (NHIMC 2024-12-024), and a waiver of informed consent was obtained.

RESULTS

1. Comparison of the Etest and Sensititre methods

When comparing the Etest with the Sensititre method, concordance rates were 100% for CTZ-AVI and 92.3% for CFZ-TAZ across 26 CRE isolates. Two isolates showed discrepancies, demonstrating intermediate susceptibility (I) with Etest but resistance (R) with Sensititre for CFZ-TAZ. Among CRPA isolates (n=7), results were fully consistent for both CTZ-AVI and CFZ-TAZ, with concordance rates of 100%.

2. Comparison of Etest and an automated method

In the comparison between Etest and the automated method, concordance rates were 98% for CTZ-AVI and 94% for CFZ-TAZ among 50 CRE isolates (Table 1). One KPC-producing *K. pneumoniae* isolate was susceptible (S) with Etest but R with the automated method for CTZ-AVI. One KPC-producing *C. braakii* isolate was resistant with Etest but susceptible with the automated method for CFZ-TAZ. Two KPC-producing *E. coli* isolates were I with Etest and S with the automated method for CFZ-TAZ.

For CRPA isolates (n=49), concordance rates were 100% for CTZ-AVI and 98% for CFZ-TAZ (Table 1). One CRPA isolate without a carbapenemase gene was I with Etest and S with the automated method for CFZ-TAZ.

Table 1. Concordance rates between Etest and VITEK 2 methods in CRE and CRPA

Etest vs. VITEK 2	CTZ-AVI	CFZ-TAZ
All CRE (n=50)	98 (49/50)	94 (47/50)
No carbapenemase (n=9)	100 (9/9)	100 (9/9)
KPC (n=26)	96 (25*/26)	89 (23†/26)
OXA (n=8)	100 (8/8)	100 (8/8)
NDM (n=7)	100 (7/7)	100 (7/7)
All CRPA (n=49)	100 (49/49)	98 (48/49)
No carbapenemase (n=11)	100 (11/11)	91 (10/11)
NDM (n=23)	100 (23/23)	100 (23/23)
GES (n=11)	100 (11/11)	100 (11/11)
IMP (n=3)	100 (3/3)	100 (3/3)
NDM+GES (n=1)	100 (1/1)	100 (1/1)

Values are presented as percentages, with numbers in parentheses indicating (n/N).
Abbreviations: CTZ-AVI, ceftazidime-avibactam; CFZ-TAZ, ceftolozane-tazobactam; CRE, carbapenem-resistant Enterobacterales; KPC, *Klebsiella pneumoniae* carbapenemase; OXA, oxacillinase; NDM, New Delhi metallo-β-lactamase; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; GES, Guiana extended-spectrum carbapenemases; IMP, imipenemase.
*One CRE isolate that produced KPC-producing *K. pneumoniae* was susceptible (S) with Etest and resistant (R) with an automated method to CTZ-AVI. †One KPC-producing *Citrobacter braakii* isolate was R with Etest and S with an automated method to CFZ-TAZ. Two KPC-producing *Escherichia coli* isolates were I with Etest and S with an automated method to CFZ-TAZ.

3. Comparison between CTZ-AVI/CFZ-TAZ susceptibility and carbapenemase genotypes

For CTZ-AVI, most CRE isolates were S; however, eight NDM-producing CRE isolates were resistant. For CFZ-TAZ, most CRE isolates were R, although two OXA-producing *K. pneumoniae* and two KPC-producing *E. coli* isolates showed I or S results.

Among CRPA isolates (n=49), overall resistance rates to both CTZ-AVI and CFZ-TAZ were 59%. All NDM-producing isolates (including one co-producing NDM and GES) and all IMP-producing isolates were resistant to both agents (Table 2). In contrast, all CRPA isolates without known carbapenemases were susceptible, and GES-producing isolates showed low resistance rates to both CTZ-AVI and CFZ-TAZ (Table 2).

Table 2. Resistance rates CTZ-AVI and CFZ-TAZ in various carbapenemase genotypes of *Pseudomonas aeruginosa*

Resistant rate	CTZ-AVI*	CFZ-TAZ*
All CRPA (n=49)	59 (29/49)	59 (29/49)
No carbapenemase (n=11)	0 (0/11)	0 (0/11)
NDM (n=23)	100 (23/23)	100 (23/23)
GES (n=11)	18 (2/11)	18 (2/11)
IMP (n=3)	100 (3/3)	100 (3/3)
NDM+GES (n=1)	100 (1/1)	100 (1/1)

Values are presented as percentages, with numbers in parentheses indicating (n/N).
Abbreviations: CTZ-AVI, ceftazidime-avibactam; CFZ-TAZ, ceftolozane-tazobactam; CRPA, carbapenem-resistant *P. aeruginosa*; NDM, New Delhi metallo-β-lactamase; GES, Guiana extended-spectrum carbapenemases; IMP, imipenemase.
*All CRPA isolates showed the same susceptibility results to CTZ-AVI and CFZ-TAZ.

DISCUSSION

For patients with carbapenem-resistant Gram-negative bacterial infections, treatment options are severely limited and often associated with high mortality [1,2]. The introduction of novel BL/BLIs such as CTZ-AVI, meropenem/vaborbactam, imipenem/relebactam, and CFZ-TAZ offers important last-resort options for treating CRE and CRPA infections [4]. Recently, CTZ-AVI and CFZ-TAZ became available in Korea for the management of these infections.

To assess current resistance rates to CTZ-AVI and CFZ-TAZ, all available CRE and CRPA isolates were analyzed without exception, based on samples collected from patients admitted to two general hospitals in Gyeonggi Province between July 2023 and August 2024. The resistance rate to CTZ-AVI among CRE isolates was 2.9% (1/35), with one NDM-1-producing resistant strain identified, while all isolates were resistant to CFZ-TAZ (data not shown). Among CRPA isolates, resistance rates were 60.0% (9/15) for CTZ-AVI and 41.7% (10/24) for CFZ-TAZ (data not shown). Given these high and variable resistance rates, routine susceptibility testing of CRPA isolates against CTZ-AVI and CFZ-TAZ is strongly recommended.

In contrast, the susceptibility of CRE to CTZ-AVI and CFZ-TAZ is more predictable. It is well established that CTZ-AVI has no therapeutic effect against metallo-β-lactamase producers (NDM, VIM, IMP, etc.) [7], while CFZ-TAZ is generally considered ineffective against CRE regardless of carbapenemase genotype [7]. Therefore, susceptibility testing for CTZ-AVI or CFZ-TAZ may not be necessary when the carbapenemase genotype of a CRE isolate is already known. However, in many laboratories where genotyping is impractical due to limited capacity, CTZ-AVI susceptibility testing is highly recommended.

So far, CTZ-AVI remains effective against KPC- or OXA-48–producing CRE [7]. However, numerous KPC variants have recently been reported, contributing to resistance in clinical CRE isolates [7]. In CTZ-AVI–resistant KPC variants, point mutations, insertions, and deletions have been identified at various hot spots [8]. This has heightened the need for antimicrobial susceptibility testing in CRE isolates as well as in CRPA.

Etest and Sensititre are widely used in many laboratories at the request of clinicians, while the automated AST-XN09 Gram-negative extension panel has only recently been introduced. Results from Etests were consistent with those of microdilution methods for both CTZ-AVI and CFZ-TAZ in CRE and CRPA isolates, although the number of CRPA isolates studied was too small to draw firm conclusions. Concordance rates between Etest and the automated method were also high for both CTZ-AVI and CFZ-TAZ in CRE and CRPA isolates. However, in one case, a KPC-producing *K. pneumoniae* isolate tested S by Etest but R by the automated method for CTZ-AVI. Such inconsistencies, although rare, cannot be completely dismissed.

Two OXA-48–producing *K. pneumoniae* isolates and two KPC-2–producing *E. coli* isolates exhibited I or S results for CFZ-TAZ, possibly due to the low activity of OXA-48 carbapenemase [9] and the relatively low resistance levels of KPC-producing *E. coli* [10]. Given the uncertain therapeutic effects of CFZ-TAZ against CRE isolates that test I or S in vitro, CFZ-TAZ susceptibility testing for CRE is not recommended.

Clinical laboratories play an essential role in the prudent use of novel BL/BLIs by providing reliable susceptibility test results. Etest and Sensititre are typically performed manually upon clinician request, and in laboratories without Sensititre systems, results may depend on the individual's interpretation, sometimes overlooking faint colony growth. In contrast, automated tests allow all Enterobacterales and *P. aeruginosa* isolates to be evaluated, regardless of carbapenem resistance.

In this study, results from the three antimicrobial susceptibility testing methods (Etest, microdilution, and automated testing) for CTZ-AVI and CFZ-TAZ were generally consistent, with only a few exceptions. In conclusion, all three methods are useful for susceptibility testing of novel BL/BLIs in carbapenem-resistant pathogens.

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REFERENCES

1. Jean SS, Harnod D, Hsueh PR. Global threat of carbapenem-resistant gram-negative bacteria. Front Cell Infect Microbiol 2022;12:823684.
2. Martin-Loeches I, Diaz E, Valles J. Risks for multidrug-resistant pathogens in the ICU. Curr Opin Crit Care 2014;20:516-24.
3. Kim D, Yoon EJ, Hong JS, Choi MH, Kim HS, Kim YR, et al. Major bloodstream infection-causing bacterial pathogens and their antimicrobial resistance in South Korea, 2017-2019: phase I report from Kor-GLASS. Front Microbiol 2021;12:799084.
4. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America guidance on the treatment of Extended-Spectrum beta-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa). Clin Infect Dis 2021;72:e169-83.
5. Lee H, Yoon EJ, Kim D, Jeong SH, Shin JH, Shin JH, et al. Establishment of the South Korean national antimicrobial resistance surveillance system, Kor-GLASS, in 2016. Euro Surveill 2018;23:1700734.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 35th ed. https://clsi.org/standards/products/microbiology/documents/m100/ (Accessed May 27, 2025).
7. van Duin D, Bonomo RA. Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation beta-lactam/beta-lactamase inhibitor combinations. Clin Infect Dis 2016;63:234-41.
8. Hobson CA, Pierrat G, Tenaillon O, Bonacorsi S, Bercot B, Jaouen E, et al. Klebsiella pneumoniae carbapenemase variants resistant to ceftazidime-avibactam: an evolutionary overview. Antimicrob Agents Chemother 2022;66:e0044722.
9. Boyd SE, Holmes A, Peck R, Livermore DM, Hope W. OXA-48-like beta-lactamases: global epidemiology, treatment options, and development pipeline. Antimicrob Agents Chemother 2022;66:e0021622.
10. Kim YA, Qureshi ZA, Adams-Haduch JM, Park YS, Shutt KA, Doi Y. Features of infections due to Klebsiella pneumoniae carbapenemase-producing Escherichia coli: emergence of sequence type 131. Clin Infect Dis 2012;55:224-31.