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Comparison of Two Quinupristin–dalfopristin Susceptibility Testing Methods and Two Interpretive Criteria for *Enterococcus faecium* Bloodstream Isolates from Korean Hospitals

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Enterococcus faecium, particularly in its multidrug-resistant forms, causes invasive nosocomial infections. Given the limited data comparing the effectiveness of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the CLSI clinical breakpoints (CBPs) for quinupristin–dalfopristin (QD) resistance and the need to evaluate their practical application, we retrospectively investigated the susceptibility patterns of 287 *E. faecium* bloodstream isolates from Korean hospitals to QD using the updated EUCAST and CLSI CBPs and two antimicrobial susceptibility testing methods: disk diffusion (DD) and Sensititre broth microdilution (Sensititre). QD resistance rates were 5.9% (CLSI) and 18.8% (EUCAST) for DD and 22.6% (CLSI) and 28.2% (EUCAST) for Sensititre. The most prevalent QD resistance gene types among QD-resistant isolates were *ermB*+*msrC*+ or *ermB*–*msrC*+. Categorical agreement between DD and Sensititre ranged from 77.7% to 90.7%, depending on the testing method and CBPs applied. The EUCAST zone diameter CBPs more effectively help identify QD-resistant *E. faecium* isolates using the DD method than the CLSI zone diameter CBPs. In comparison, the CLSI minimum inhibitory concentration (MIC) CBPs provide more reliable results for resistance classification in the Sensititre method than EUCAST MIC CBPs. These findings would help improve clinical decision-making for treating multidrug-resistant *E. faecium* infections.

Key Words: Broth microdilution, Clinical breakpoint, CLSI, Disk diffusion, *Enterococcus faecium*, *ermB,* EUCAST, *msrC*, Quinupristin–dalfopristin

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Enterococcus faecium, commonly found in the gastrointestinal tract, causes an increasing number of severe opportunistic infections, including bacteremia, urinary tract infections, and infective endocarditis [1]. Intrinsic resistance to multiple antibiotics, including low-dose penicillin, ampicillin, aminoglycosides, and cephalosporins, significantly limits treatment options [2, 3].

Since the late 1980s, vancomycin-resistant enterococci (VRE) have emerged in hospitals worldwide, further restricting treatment options for enterococcal infections [4, 5]. Given its clinical importance, the WHO listed vancomycin-resistant *E. faecium* as a high-priority pathogen in 2017 and 2024 [6, 7]. Quinupristin–dalfopristin (QD) remains a viable option for treating multidrug-resistant *E. faecium*, along with linezolid, tigecycline, and daptomycin [8–10]. *E. faecium* QD resistance genes such as *ermB* and *msrC* are predominantly found in clinical isolates, whereas *vatD, vatE, vgbA*, and *vgbB* are rarely detected [10, 11]. QD susceptibility testing results are interpreted using clinical breakpoints (CBPs) from the CLSI or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [12, 13]. Despite similarities in susceptibility testing protocols, CLSI and EUCAST employ distinct criteria. We retrospectively investigated the susceptibility patterns of *E. faecium* bloodstream isolates to QD using the CLSI disk diffusion (DD) method and Sensititre AMRENT broth microdilution (BMD) method (Sensititre, Thermo Fisher Scientific, Waltham, MA, USA), comparing results based on CLSI and EUCAST CBPs. Additionally, we examined the distribution of QD resistance genes (*ermB* and *msrC*) based on QD resistance phenotypes. This study was conducted at Chonnam National University Hospital (CNUH), Gwangju, Korea, and approved by the institutional review board (approval No. CNUH-2020-080).

In total, 287 non-duplicated *E. faecium* bloodstream isolates were collected from 11 hospitals in Korea between October 2020 and June 2021. All isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with an ASTA MicroIDSystem (ASTA, Suwon, Korea). QD minimum inhibitory concentrations (MICs) and zone diameters (ZDs) were determined using the Sensititre AMRENT BMD and CLSI DD methods. MIC and ZD results were interpreted according to CBPs in CLSI M100-ED34 and EUCAST breakpoint table version 14.0 [12, 13]. Genomic DNA was extracted as previously described [14] and amplified using primers ermB-1 (5'-CATTTA ACGACGAAACTGGC-3'), ermB-2 (5'-GGAACATCTGTGGTATGGCG-3'), msrC-fw (5'-AAGGAATCCTTCTCTCTCCG-3'), and msrC-rv (5'-GTAAA CAAAATCGTTCCCG-3'). Each 50-μL PCR mixture contained 100 ng genomic DNA, 2.5 U Taq polymerase (Genetbio, Daejeon, Korea), 5 μL of 10× buffer, 10 mM deoxynucleoside triphosphates, and 25 μM of each primer. The thermal cycles were 94°C for 5 mins, 30 cycles of 94°C for 40 secs, 50°C for 1 min, and 72°C for 50 secs, followed by 74°C for 10 mins. The amplification products were purified using a commercial kit (GeneAll Biotechnology, Seoul, Korea).

Antimicrobial susceptibility testing and genetic analysis results are summarized in Table 1. QD resistance rates in DD tests were 5.9% and 18.8% based on CLSI and EUCAST CBPs, respectively. Using the Sensititre method, QD resistance rates were 22.6% and 28.2% when applying CLSI and EUCAST CBPs, respectively. Among all isolates, 277 (96.5%) harbored *ermB+msrC+* or *ermB–msrC+*. The *ermB+msrC+* combination was more common than *ermB–msrC+*, regardless of phenotypic resistance. Among resistant isolates, 100% and 94.4% harbored *ermB+msrC+* or *ermB–msrC+* in DD tests (CLSI and EUCAST CBPs, respectively), whereas 90.8% and 88.9% harbored these genes when using Sensititre (CLSI and EUCAST CBPs, respectively). Among susceptible isolates, the proportions were 96.5% and 97.0% in DD tests

Table 1. Quinupristin–dalfopristin susceptibility categorization based on CLSI and EUCAST CBPs using disk diffusion and Sensititre, along with resistance gene analysis in 287 *E. faecium* bloodstream isolates from Korean hospitals

Method	Interpretive criteria	Categorical interpretation	No. of strains (%)	No. of strains harboring resistance genes (%)	
				ermB+msrC+	*ermB–msrC+*
Disk diffusion	CLSI ZD CBP (mm)	R (≤15)	17 (5.9)	17 (100)	0 (0)
		I (16–18)	17 (5.9)	15 (88.2)	1 (5.9)
		S (≥19)	253 (88.2)	175 (69.2)	69 (27.3)
	EUCAST ZD CBP (mm)	R (<22)	54 (18.8)	33 (61.1)	18 (33.3)
		S (≥22)	233 (81.2)	174 (74.7)	52 (22.3)
Sensititre	CLSI MIC CBP (μg/mL)	R (≥4)	65 (22.6)	33 (50.8)	26 (40.0)
		I (2)	16 (5.6)	10 (62.5)	3 (18.8)
		S (≤1)	206 (71.8)	164 (79.6)	41 (19.9)
	EUCAST MIC CBP (mg/L)	R (>1)	81 (28.2)	43 (53.1)	29 (35.8)
		S (≤1)	206 (71.8)	164 (79.6)	41 (19.9)

Abbreviations: QD, quinupristin–dalfopristin; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CBP, clinical breakpoint; ZD, zone diameter; MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible.

(CLSI and EUCAST CBPs, respectively) and 99.5% in Sensititre tests (CLSI and EUCAST CBPs), indicating no significant difference between resistant and susceptible isolates.

Table 2 presents the comparative results of the two susceptibility testing methods under different interpretive criteria. When using CLSI ZD CBPs, categorical results of the DD method showed 77.7% agreement with those of Sensititre, regardless of whether CLSI or EUCAST MIC CBPs were applied. When using EUCAST ZD CBPs, 90.6% agreement was observed between the DD method and Sensititre. Among 287 isolates, 31 (10.8%) classified as susceptible by CLSI ZD CBPs were found to be resistant using CLSI MIC CBPs in the Sensititre method. This proportion increased to 16.4% (47/287) when Sensititre results were interpreted with EUCAST MIC CBPs. When DD results were interpreted using EUCAST ZD CBPs, and Sensititre results were interpreted using CLSI MIC CBPs, only 3.8% (11/287) of isolates classified as susceptible in DD tests were found resistant in Sensititre.

Table 2. Comparison of susceptibility testing results between disk diffusion and Sensititre using CLSI and EUCAST CBPs

Testing method and CBP	Sensititre				
	CLSI MIC CBP (μg/mL)			EUCAST MIC CBP (mg/L)	
	R (≥4)	I (2)	S (≤1)	R (>1)	S (≤1)
Disk diffusion					
CLSI ZD CBP (mm)					
R (≤15)	17	0	0	17	0
I (16–18)	17	0	0	17	0
S (≥19)	31	16	206	47	206
EUCAST ZD CBP (mm)					
R (<22)	54	0	0	54	0
S (≥22)	11	16	206	27	206

Abbreviations: EUCAST, European Committee on Antimicrobial Susceptibility Testing; CBP, clinical breakpoint; ZD, zone diameter; MIC, minimal inhibitory concentration; R, resistant; I, intermediate; S, susceptible.

Fig. 1 illustrates correlations between DD ZDs and Sensititre MICs, highlighting differences in interpretation based on CLSI versus EUCAST CBPs. The x-axis represents the testing method results (mm or μg/mL), and the y-axis represents the number of isolates. Bar colors indicate susceptibility categories based on MIC from Sensititre (Fig. 1A) or ZD from DD (Fig. 1B). EUCAST ZD CBPs yielded higher agreement with MIC results determined by CLSI and EUCAST MIC CBPs (represented by red-colored bars) than CLSI ZD CBPs (Fig. 1A). In contrast, CLSI MIC CBPs showed higher agreement with ZD results determined by CLSI and EUCAST ZD CBPs (red-colored bars) than that with EUCAST MIC CBPs (Fig. 1B).

Despite the declining clinical use of QD for treating VRE, QD

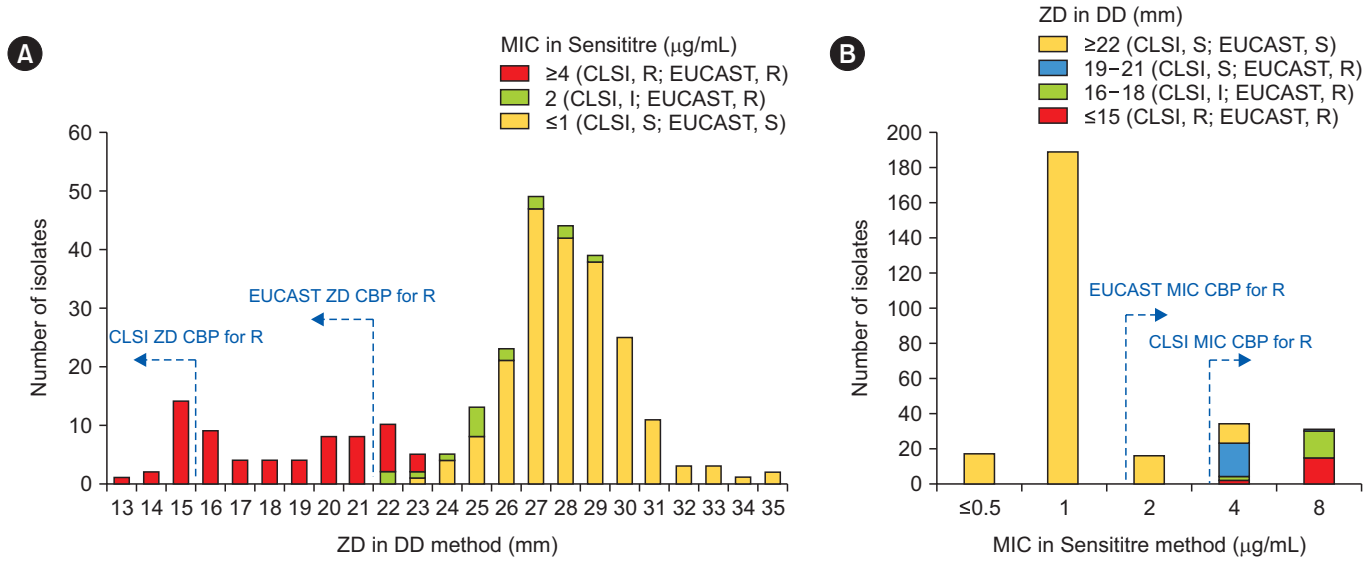

Fig. 1. Relationships between DD ZD and Sensititre MIC results. (A) Distribution of DD ZD and corresponding Sensititre MIC results. Blue arrows indicate the region of the ZD CBPs for CLSI (≤15 mm) and EUCAST (<22 mm) for resistance. (B) Sensititre MIC results corresponding to DD ZD results. Blue arrows indicate the region of the MIC CBPs for CLSI (≥4 μg/mL) and EUCAST (>1 mg/L) for resistance. The bar colors represent categories of susceptibility based on MIC from Sensititre (A) and ZD from the DD method (B).
Abbreviations: DD, disk diffusion; EUCAST, European Committee on Antimicrobial Susceptibility Testing; R, resistant; I, intermediate; S, susceptible; ZD, zone diameter; CBP, clinical breakpoint; MIC, minimum inhibitory concentration.

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remains a valuable alternative, particularly in combination therapy for multidrug-resistant *E. faecium* [8, 15]. Oh, *et al*. reported a 10.0% QD resistance rate among 249 *E. faecium* isolates from Korean hospitals in 2005 [16]. However, limited studies have analyzed QD resistance rates and CBP-based differences in Korea. We addressed both aspects by evaluating QD resistance in *E. faecium* bloodstream isolates from Korean hospitals using DD and Sensititre and comparing CLSI and EUCAST CBPs. QD resistance rates were 5.9% (CLSI) and 18.8% (EUCAST) in DD and 22.6% (CLSI) and 28.2% (EUCAST) in Sensititre. The discrepancy between CLSI- and EUCAST-based results narrowed when using Sensititre. Similar to our findings, in a study on 865 *E. faecium* isolates from Greek hospitals, 28.9% were classified as intermediate resistant to QD (MICs = 1.5–4 mg/L) using the CLSI method [9]. In contrast, Wang, *et al*. [10] reported that 1.0% (9/911) of *E. faecium* clinical isolates from China were resistant to QD, with MIC values ranging from 4 to 64 mg/L, based on susceptibility testing using the Vitek 2 system (bioMérieux, Marcy-l'Étoile, France) and CLSI DD methods and interpretation according to CLSI CBPs. These differences may be attributed to various factors, including regional variation, clinical sample types, and differences in time periods, the methods used for susceptibility testing, and the CBPs applied. *E. faecium* infections are particularly challenging to treat because *E. faecium* has a higher rate of multidrug resistance than *Enterococcus faecalis*, significantly narrowing available treatment options, especially when linezolid or daptomycin fail or are unavailable [17–19]. The relatively low QD resistance rates observed in this study highlight the ongoing clinical importance of QD as a valuable treatment option for resistant *E. faecium* infections.

Our findings revealed that most *E. faecium* bloodstream isolates were *ermB*+*msrC*+ or *ermB*–*msrC*+, regardless of QD resistance, testing method, or CBPs. While 88.9%–100% of QD-resistant and 96.5%–99.5% of susceptible isolates carried these genes, Wang, *et al*. reported that 88.9% (8/9) of QD-resistant isolates were positive for both *ermB* and *msrC* [10]. However, no data were available for susceptible isolates in that study. Moreover, while *ermB* and *msrC* mediate resistance to the B component (quinupristin) and *vatD* and *vatE* confer resistance to the A component (dalfopristin), resistance to the A component alone is sufficient for resistance to streptogramin A and B combinations [20]. This suggests that, in *E. faecium* bloodstream isolates in Korea, the mechanisms of QD resistance may more strongly rely on *vatD*, *vatE*, or other unidentified mechanisms than on *ermB* and *msrC*. Further studies are required to investigate the potential mechanisms; the lack of exploration of this aspect represents a limitation of this study.

The distributions of ZDs and MICs of *E. faecium* isolates revealed that, when applying the EUCAST ZD CBPs in the DD method, most isolates classified as resistant were also identified as resistant according to both CLSI and EUCAST MIC CBPs (represented using a red-colored bar in Fig. 1A). However, when applying CLSI ZD CBPs, most isolates represented by the red-colored bar were classified as non-resistant (Fig. 1A). In the Sensititre method, some isolates classified as susceptible by the DD method (yellow-colored bar in Fig. 1B) were identified as resistant under both CLSI and EUCAST MIC CBPs. The number of isolates represented using a yellow-colored bar were classified as resistant was lower when applying CLSI MIC CBPs than when applying EUCAST MIC CBPs (Fig. 1B). These findings suggest that the EUCAST ZD CBPs are more effective in identifying resistant isolates using the DD method. In contrast, CLSI MIC CBPs provide more reliable results for resistance classification in the Sensititre method.

In conclusion, our findings support QD as an alternative for treating *E. faecium* bloodstream infections, given its relatively low resistance rate, and offer insights into selecting the most suitable CBPs based on the susceptibility testing method used when evaluating QD resistance prior to treatment. These findings highlight the importance of continuous evaluation and standardization of CBPs to enhance the accuracy of QD susceptibility testing for *E. faecium*.

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AUTHOR CONTRIBUTIONS

Kwon YJ and Lim HJ analyzed the data, drafted the manuscript, and visualized the results. Kim SH designed and supervised the study and reviewed the manuscript. Byun SA, Lee GY, and Kim G-G contributed to data curation. Jeong SH, Shin JH, Kim YA, Uh Y, and Shin JH collected clinical isolates and participated in the manuscript review.

CONFLICTS OF INTEREST

None declared.

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