ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2005, p. 5176–5178 Vol. 49, No. 12
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.12.5176–5178.2005

High Rate of Resistance to Quinupristin-Dalfopristin in *Enterococcus faecium* Clinical Isolates from Korea

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Received 14 July 2005/Returned for modification 19 August 2005/Accepted 20 September 2005

We tested the in vitro susceptibilities of 603 enterococcal isolates from eight tertiary-care hospitals in Korea. The quinupristin-dalfopristin resistance rate in *Enterococcus faecium* was very high (25 isolates, 10.0%). It was suggested that both clonal spread and the sporadic emergence of quinupristin-dalfopristin-resistant isolates may explain the high prevalence of quinupristin-dalfopristin resistance in Korea.

Enterococci have become a more important cause of nosocomial infections with the emergence of multidrug-resistant strains in recent years (17). For instance, infections caused by vancomycin-resistant enterococci have resulted in increased morbidity and mortality due to limited treatment options (15). According to recent nationwide surveillance studies in Korea, the rate of vancomycin-resistant *Enterococcus faecium* (VREF) isolates has increased from 4% in 1997 to 16% in 2002 (11, 12). Quinupristin-dalfopristin (QD) could be useful in clinical practice as one of a few therapeutic options. Although several surveillance studies have reported increases in resistance to QD, the resistance rate still remains low in most parts (8, 13, 18). This recent multicenter surveillance study reports a high prevalence of QD resistance among *E. faecium* isolates from Korea.

Enterococcal isolates. As part of a multicenter surveillance study during 2 months (August and September) in 2004, a total of 603 nonduplicate enterococcal isolates (330 *Enterococcus faecalis* isolates, 249 *E. faecium* isolates, and 24 other isolates) were collected from eight tertiary-care hospitals in various regions of Korea. In vitro susceptibility testing was performed by a broth microdilution test according to CLSI guidelines (2). Eleven antimicrobial agents were tested: vancomycin, teicoplanin, ampicillin, tetracycline, erythromycin, ciprofloxacin, chloramphenicol, rifampin, QD, streptomycin, and gentamicin. For streptomycin (1,000 mg/liter) and gentamicin (500 mg/liter), high-level resistance was tested. Susceptibility interpretive criteria used were those established in CLSI standard M100-S15 (2). *E. faecalis* strain ATCC 29212 and *Staphylococcus aureus* strain ATCC 29213 were used as control strains. The chi-square test and Fisher's exact test were used to determine the significance of resistance differences where appropriate.

Molecular characterization. Multilocus sequence typing was performed as described previously (4, 9). To determine the number of variations in the *esp* A and C repeats, two different primer combinations were used, esp_{fs}7F-esp_{fm}5R and esp_{fm}5F-esp_{fs}3R, respectively (10). A genotypic clone was defined by coupling sequence type in multilocus sequence typing and the number of *esp* A and C repeats (9). Two virulence genes of *E. faecium*, enterococcal surface protein (*esp*) and hyaluronidase (*hyl*) genes, were detected by the duplex PCR method as described previously (23).

The results of the antimicrobial susceptibility test are summarized in Table 1. Sixty-three (25.3%) of 249 *E. faecium*

TABLE 1. Antibiotic resistance of *E. faecium* and *E. faecalis* isolates

Antimicrobial agent[a]	*E. faecium* (n = 249)		*E. faecalis* (n = 330)	
	MIC_{90} (mg/liter)	Resistance (%)	MIC_{90} (mg/liter)	Resistance (%)
Vancomycin	>64	63 (25.3)	2	6 (1.8)
Teicoplanin	64	53 (21.3)	0.5	5 (1.5)
Ampicillin	>64	233 (93.6)	8	16 (4.8)
Tetracycline	4	21 (8.4)	>64	262 (79.4)
Erythromycin	>32	227 (91.2)	>32	210 (63.6)
Ciprofloxacin	>64	235 (94.4)	64	92 (27.8)
Chloramphenicol	16	8 (3.2)	32	88 (26.7)
Rifampin	>16	240 (96.4)	16	122 (37.0)
Quinupristin-dalfopristin	4	25 (10.0)	16	269 (81.5)
Streptomycin-HLR	NA[b]	162 (65.1)	NA	106 (31.2)
Gentamicin-HLR	NA	228 (91.6)	NA	178 (53.9)

[a] HLR, high-level resistance.
[b] NA, not available.

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TABLE 2. Comparison of antimicrobial resistance between VREF and VSEF isolates

Antimicrobial agent[c]	No. of resistant isolates (%)		*P* value
	VREF (n = 63)	VSEF (n = 186)	
Teicoplanin	53 (84.1)	0	<0.01[a]
Ampicillin	63 (100)	170 (91.4)	0.01[a]
Tetracycline	10 (15.9)	11 (5.9)	0.01[a]
Erythromycin	60 (95.2)	167 (89.8)	0.19[a]
Ciprofloxacin	63 (100)	172 (92.5)	0.02[b]
Chloramphenicol	5 (7.9)	3 (1.6)	0.03[b]
Rifampin	62 (98.4)	178 (95.7)	0.45[b]
Quinupristin-dalfopristin	2 (3.2)	23 (12.4)	0.01[a]
Streptomycin-HLR	45 (71.4)	117 (62.9)	0.22[a]
Gentamicin-HLR	60 (95.2)	168 (90.3)	0.22[a]

[a] Chi-square test.
[b] Fisher's exact test.
[c] HLR, high-level resistance.

isolates were resistant to vancomycin, while only 6 (1.8%) of 330 *E. faecalis* isolates were resistant to vancomycin. Resistance rates to vancomycin in *E. faecium* markedly varied by hospital, ranging from 0% to 54.7%. Isolates of VREF showed significantly higher resistance rates than vancomycin-susceptible *E. faecium* strains (VSEF) to teicoplanin, ampicillin, tetracycline, ciprofloxacin, and chloramphenicol (Table 2).

In this study, the most prominent piece of data was a high rate of resistance to QD in *E. faecium* isolates in Korea (10.0%). This rate was significantly higher than those in North America, South America, and Europe, which ranged from 0% to 3.8% (7, 13, 19, 20, 22). Previous data from Korea with 56 *E. faecium* isolates also showed that only one isolate was resistant to QD (6). Based on previous reports, Taiwan showed very high rates of resistance to QD in *E. faecium* isolates, ranging from 9% to 51% (5, 14). Recent data from the SENTRY project in the Asian-Pacific region confirmed the high QD resistance rate in *E. faecium* from Taiwan (19.0%) and also showed increasing resistance to QD (29.4%) in Korea, with 17 isolates of *E. faecium* (J. M. Bell and J. D. Turnidge, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1361, 2004). Our study confirmed the increasing tendency of QD resistance in *E. faecium* isolates in Korea. Such high QD resistance rates in *E. faecium* in Taiwan and Korea were not observed in other Asian-Pacific countries (Bell and Turnidge, 44th ICAAC, abstr. C2-1361).

Our data showed a much higher QD resistance rate (12.9%) in VSEF than in VREF (3.2%), which was consistent with previous data (1). This implies that the QD resistance in *E. faecium* is not associated with the recent use of QD in the hospital for the treatment of vancomycin-resistant enterococci. Actually, the emergence of QD resistance even before its com-

TABLE 3. Genotypic characteristics and antimicrobial resistance in 25 QD-resistant *E. faecium* isolates from Korea

Hospital[a]	Isolate	ST (allelic profile)[b]	*esp* repeat (A-C)	*hyl*	Antimicrobial resistance[c]
SMC	01-27	78 (15-1-1-1-1-1-1)	5-6	+	Van, Tei, Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-34	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-37	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-67	203 (15-1-1-1-1-20-1)	8-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-93	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str
	01-106	NEW (1-12-1-1-1-1-1)	—[d]	−	Amp, Pen, Tet, Ery, Cip, Rif, QD, Str
	01-107	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-118	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-121	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str
	01-122	78 (15-1-1-1-1-1-1)	6-5	−	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-142	NEW (1-12-1-1-1-1-1)	—	−	Amp, Pen, Tet, Ery, Cip, Rif, QD, Str
	01-148	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-158	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-167	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-176	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	01-196	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
SVH	02-03	78 (15-1-1-1-1-1-1)	5-6	−	Tet, Chl, Gen
	02-25	203 (15-1-1-1-1-20-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	02-34	203 (15-1-1-1-1-20-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
Kyungpook	06-14	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
Chonnam	08-20	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	08-81	203 (15-1-1-1-1-20-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
Chungbuk	10-14	192 (15-1-1-1-1-7-1)	5-6	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	10-24	78 (15-1-1-1-1-1-1)	6-5	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen
	10-32	78 (15-1-1-1-1-1-1)	—	+	Amp, Pen, Ery, Cip, Rif, QD, Str, Gen

[a] SMC, Samsung Medical Center, SVH, Seoul Veterans Hospital; Kyungpook, Kyungpook National University Hospital; Chonnam, Chonnam National University Hospital; Chungbuk, Chungbuk National University Hospital.
[b] ST, sequence type (*atpA-ddl-gdh-purK-gyd-pstS-adk*).
[c] Van, vancomycin; Tei, teicoplanin; Amp, ampicillin; Pen, penicillin; Tet, tetracycline; Ery, erythromycin; Cip, ciprofloxacin; Chl, chloramphenicol; Rif, rifampin; Str, streptomycin; Gen, gentamicin.
[d] Absence of *esp* gene.

mercial use in the United States suggests that QD resistance might be linked with other reasons. Luh et al. (14) inferred that the high QD resistance rate in *E. faecium* in Taiwan was due to the use of virginiamycin in animal husbandry for many years. In Korea, virginiamycin has also been frequently used as a growth promoter in food animals, which could partly explain the high prevalence of QD resistance in *E. faecium*. However, the use of virginiamycin may not be the sole reason for the high rate of resistance to QD in Korea and Taiwan because Europe and the United States, where virginiamycin has also been used in animal husbandry, showed a low rate of QD resistance (16, 21). In addition, transmission of antibiotic-resistant *E. faecium* isolates from animals to humans is not common (3).

The QD resistance rate in *E. faecium* isolates was the highest at the Samsung Medical Center (16 of 64 isolates). In this hospital, two clones, ST192-A5-C6 in seven isolates and STnew-A0-C0 in two isolates, were identified (Table 3). This may suggest the clonal spread of the resistant strain within that hospital. In addition, two isolates in the Seoul Veterans Hospital also belonged to the same clone. However, there was no evidence that QD-resistant *E. faecium* isolates from other Korean hospitals have been clonally disseminated.

Of 249 *E. faecium* isolates, *esp* and *hyl* genes were detected in 184 (73.9%) and 169 (67.9%) isolates, respectively. The *esp* gene was more frequently found in VREF (58/63 isolates, 92.1%) than in VSEF (67.7%) isolates. The *hyl* gene was present in 37 (58.7%) and 232 (71.0%) VREF and VSEF isolates, respectively. A dual presence of *esp* and *hyl* genes was observed among 31 VREF (49.2%) and 108 VSEF (58.1%) isolates.

In summary, the present study documented a high rate of QD resistance in *E. faecium* from Korea due to both clonal spread and sporadic emergence. Given the clinical importance of multidrug-resistant enterococci, continuous surveillance of QD resistance in *E. faecium* is strongly warranted if QD is to be used to treat *E. faecium*.

This study was partly supported by the Korean Food & Drug Administration (KFDA) and the Asian-Pacific Research Foundation for Infectious Diseases (ARFID).

The eight tertiary-care hospitals participating in this study were the Samsung Medical Center (SMC, Seoul), Seoul Veterans Hospital (Seoul), Kangbuk Samsung Hospital (Seoul), Dong-A University Hospital (Busan), Kyungpook National University Hospital (Daegu), Chungnam National University Hospital (Daejeon), Chonnam National University Hospital (Gwangju), and Chungbuk National University Hospital (Chungju).

REFERENCES

1. **Ballow, C. H., R. N. Jones, and D. J. Biedenbach.** 2002. A multicenter evaluation of linezolid antimicrobial activity in North America. Diagn. Microbiol. Infect. Dis. **43:**75–83.
2. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing, 15th informational supplement. Document M100-S15. CLSI, Wayne, Pa.
3. **Coque, T. M., R. J. L. Willems, J. Fortún, J. Top, S. Diz, E. Loza, R. Cantón, and F. Baquero.** 2005. Population structure of *Enterococcus faecium* causing bacteremia in a Spanish university hospital: setting the scene for a future increase in vancomycin resistance? Antimicrob. Agents Chemother. **49:**2693–2700.
4. **Homan, W. L., D. Tribe, S. Poznanski, M. Li, G. Hogg, E. Spalburg, J. D. A. van Embden, and R. J. L. Willems.** 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. J. Clin. Microbiol. **40:**1963–1971.
5. **Hsueh, P. R., W. H. Chen, L. J. Teng, and K. T. Luh.** 2005. Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and in vitro activities of newer antimicrobial agents. Int. J. Antimicrob. Agents **26:**43–49.
6. **Hwang, S. H., M. N. Kim, C. H. Pai, D. H. Huh, and W. S. Shin.** 2000. In vitro activities of quinupristin/dalfopristin and eight other antimicrobial agents against 360 clinical isolates from Korea. Yensei Med. J. **41:**563–569.
7. **Jevitt, L. A., A. J. Smith, P. P. Williams, P. M. Raney, J. E. McGowan, Jr., and F. C. Tenover.** 2003. In vitro activities of daptomycin, linezolid, and quinupristin-dalfopristin against a challenge panel of staphylococci and enterococci, including vancomycin-intermediate *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. Microb. Drug Resist. **9:**389–393.
8. **Jones, R. N., C. H. Ballow, D. J. Biedenbach, J. A. Deinhart, and J. J. Schentag.** 1998. Antimicrobial activity of quinupristin-dalfopristin (RP 59500, Synercid) tested against over 28,000 recent clinical isolates from 200 medical centers in the United States and Canada. Diagn. Microbiol. Infect. Dis. **31:**437–451.
9. **Ko, K. S., J. Y. Baek, J.-Y. Lee, W. S. Oh, K. R. Peck, N. Y. Lee, W. G. Lee, K. Lee, and J.-H. Song.** 2005. Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolates from Korea. J. Clin. Microbiol. **43:** 2303–2306.
10. **Leavis, H., J. Top, N. Shankar, K. Borgen, M. Bonten, J. van Embden, and R. J. L. Willems.** 2004. A novel putative enterococcal pathogenicity island linked to the *esp* virulence gene of *Enterococcus faecium* and associated with epidemicity. J. Bacteriol. **186:**672–682.
11. **Lee, K., S. J. Jang, H. J. Lee, N. Ryoo, M. Kim, S. G. Hong, and Y. Chong.** 2004. Increasing prevalence of vancomycin-resistant *Enterococcus faecium*, expanded-spectrum cephalosporin-resistant *Klebsiella pneumoniae*, and imipenem-resistant *Pseudomonas aeruginosa* in Korea: KONSAR study in 2001. J. Kor. Med. Sci. **19:**8–14.
12. **Lee, K., Y. A. Kim, Y. J. Park, H. S. Lee, M. Y. Kim, E. C. Kim, D. Yong, and Y. Chong.** 2004. Increasing prevalence of vancomycin-resistant enterococci and cefoxitin-, imipenem- and fluoroquinolone-resistant gram-negative bacilli: a KONSAR study in 2002. Yensei Med. J. **45:**598–608.
13. **Low, D. E., N. Keller, A. Barth, and R. N. Jones.** 2001. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY antimicrobial surveillance program, 1997–1999. **32**(Suppl. 2)**:**S133–S145.
14. **Luh, K. T., P. R. H. Hsueh, L. J. Teng, H. J. Pan, Y. C. Chen, J. J. Lu, J. J. Wu, and S. W. Ho.** 2000. Quinupristin-dalfopristin resistance among grampostive bacteria in Taiwan. Antimicrob. Agents Chemother. **44:**3374–3380.
15. **Malathum, K., and B. E. Murray.** 1999. Vancomycin-resistant enterococci: recent advances in genetics, epidemiology and therapeutic options. Drug Resist. Updates **2:**224–243.
16. **McDonald, L. C., S. Rossiter, C. Mackinson, Y. Y. Wang, S. Johnson, M. Sullivan, R. Sokolow, E. Debess, L. Gilbert, J. A. Benson, B. Hill, and F. J. Angulo.** 2001. Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. N. Engl. J. Med. **345:**1155–1160.
17. **Murray, B. E.** 1990. The life and times of the *Enterococcus*. Clin. Microbiol. Rev. **3:**46–65.
18. **Mutnick, A. H., D. J. Biedenbach, and R. N. Jones.** 2003. Geographic variations and trends in antimicrobial resistance among *Enterococcus faecalis* and *Enterococcus faecium* in the SENTRY Antimicrobial Surveillance Program (1997–2000). Diagn. Microbiol. Infect. Dis. **46:**63–68.
19. **Sader, H. S., R. N. Jones, C. H. Ballow, D. J. Biedenbach, R. F. Cered, and the GSMART Latin America Study Group.** 2001. Antimicrobial susceptibility of quinupristin/dalfopristin tested against gram-positive cocci from Latin America: results from the global SMART (GSMART) surveillance study. Braz. J. Infect. Dis. **5:**21–31.
20. **Simonsen, G. S., K. Bergh, L. Bevanger, A. Digranes, P. Gaustad, K. K. Melby, and E. A. Hoiby.** 2004. Susceptibility to quinupristin-dalfopristin and linezolid in 839 clinical isolates of Gram-positive cocci from Norway. Scand. J. Infect. Dis. **36:**254–258.
21. **Soltani, M., D. Beighton, J. Philpott-Howard, and N. Woodford.** 2000. Mechanisms of resistance to quinupristin-dalfopristin among isolates of *Enterococcus faecium* from animals, raw meat, and hospital patients in Western Europe. Antimicrob. Agents Chemother. **44:**433–436.
22. **Torres-Vierak, C., and L.-M. Dembry.** 2004. Approaches to vancomycin-resistant enterococci. Curr. Opin. Infect. Dis. **17:**541–547.
23. **Vankerchhoven, V., T. V. Autgaerden, C. Vael, C. Lammens, S. Chapelle, R. Rossi, D. Jabes, and H. Goossens.** 2004. Development of a multiplex PCR for the detection of *asa1*, *gelE*, *cylA*, *esp*, and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. J. Clin. Microbiol. **42:**4473–4479.