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Quinupristin-Dalfopristin Resistance among Gram-Positive Bacteria in Taiwan

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To understand quinupristin-dalfopristin resistance among clinical isolates of gram-positive bacteria in Taiwan, where this agent is not yet available for clinical use, we evaluated 1,287 nonduplicate isolates recovered from January 1996 to December 1999 for in vitro susceptibility to quinupristin-dalfopristin and other newer antimicrobial agents. All methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates were susceptible to quinupristin-dalfopristin. High rates of nonsusceptibility to quinupristin-dalfopristin (MICs, ≥2 μg/ml) were demonstrated for the following organisms: methicillin-resistant *S. aureus* (MRSA) (31%), coagulase-negative staphylococci (CoNS) (16%), *Streptococcus pneumoniae* (8%), viridans group streptococci (51%), vancomycin-susceptible enterococci (85%), vancomycin-resistant *Enterococcus faecalis* (100%), vancomycin-resistant *Enterococcus faecium* (66%), *Leuconostoc* spp. (100%), *Lactobacillus* spp. (50%), and *Pediococcus* spp. (87%). All isolates of MSSA, MRSA, *S. pneumoniae*, and viridans group streptococci were susceptible to vancomycin and teicoplanin. The rates of nonsusceptibility to vancomycin and teicoplanin were 5 and 7%, respectively, for CoNS, ranging from 12 and 18% for *S. simulans* to 0 and 0% for *S. cohnii* and *S. auricularis*. Moxifloxacin and trovafloxacin had good activities against these isolates except for ciprofloxacin-resistant vancomycin-resistant enterococci and methicillin-resistant staphylococci. In Taiwan, virginiamycin has been used in animal husbandry for more than 20 years, which may contribute to the high rates of quinupristin-dalfopristin resistance.

Antimicrobial resistance among gram-positive bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative staphylococci (CoNS), penicillin-resistant *Streptococcus pneumoniae* and viridans group streptococci, and ampicillin- or vancomycin-resistant enterococci (VRE), has complicated the treatment of infections due to these organisms (12–16, 18, 20, 23, 28, 30). In the last 2 decades these multidrug-resistant pathogens have been emerging rapidly worldwide, and vancomycin has become the first-line agent for the management of these infections (18, 20, 23). Acquired resistance to vancomycin among gram-positive bacteria, such as enterococci and staphylococci, has been known in recent years (7, 18, 20, 23); P. A. Evans, C. W. Norden, S. Rhoads, J. Deobaldia, and J. L. Silber, Letter, Antimicrob. Agents Chemother. **41:**1406, 1997). Isolates belonging to lactic acid bacteria such as *Leuconostoc, Pediococcus*, and *Lactobacillus* spp., which are commonly found as natural microflora in the mucous membranes of humans and animals and in dairy products, are increasingly recognized as opportunistic pathogens involved in invasive infections in humans (2, 6, 8–11, 17, 19, 26). These genera of bacteria are well documented to be intrinsically resistant to vancomycin but susceptible to other antimicrobial agents.

Quinupristin-dalfopristin is a semisynthetic mixture of streptogramin A and B compounds. It has recently been licensed for clinical use in the United States and Europe for the treatment of infections caused by multidrug-resistant gram-positive pathogens, including vancomycin-resistant *Enterococcus faecium* (27). Virginiamycin, another mixture of streptogramin A and B compounds, has long been used as a growth promoter in animal feed in many European countries (27, 29). Previous studies showed that extensive use of virginiamycin in animal husbandry might contribute to the emergence of quinupristin-dalfopristin resistance among human isolates of gram-positive bacteria (27, 29).

The purpose of this study was to determine the in vitro activities of glycopeptides, linezolid, moxifloxacin, trovafloxacin, quinupristin-dalfopristin (the last four agents are not available in Taiwan), and other antimicrobial agents against 1,287 recent clinical isolates of gram-positive bacteria in Taiwan.

MATERIALS AND METHODS

Bacterial isolates. A total of 1,287 isolates of gram-positive bacteria were recovered from various clinical specimens of patients treated mainly at National Taiwan University Hospital (NTUH) from January 1996 to December 1999 (Table 1). These isolates included 80 blood isolates of MRSA, 68 blood isolates of methicillin-susceptible *S. aureus* (MSSA), 405 of CoNS, 267 of *S. pneumoniae*, 140 of viridans group streptococci, 64 of vancomycin-susceptible enterococci (VSE), 150 of VRE (vancomycin MICs of ≥32 μg/ml), 35 of *Leuconostoc* spp., 8 of *Pediococcus* spp., and 69 of *Lactobacillus* spp. The *S. pneumoniae* isolates were obtained from five major teaching hospitals in Taiwan as previously reported (15). Of the 150 VRE isolates, 92 were recovered from patients treated at NTUH and the other 58 were from patients seen at Tri-Service General Hospital and National Cheng-Kung University Hospital, which are located in the northern and southern parts of Taiwan, respectively. Isolates other than *S. pneumoniae* or VRE were all recovered from patients seen at NTUH.

These isolates were identified to the species or genus level by means of conventional methods as previously described, as well as by using the following commercial identification systems: the API 20 Strep system and API 32 Strep system (for identification of streptococci), the API 150 CH system (for the three lactic acid bacteria), and the Vitek GPI system and API Staph system (for staphylococci) (bioMerieux Vitek, Inc., Hazelwood, Mo.) (7, 14, 17, 25). The isolates were stored at −70° in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) supplemented with 15% glycerol before being tested.

Antimicrobial agents. The following antimicrobial agents were provided by the manufacturers for use in this study: penicillin, gentamicin, and rifampin (Sigma Chemical Co., St. Louis, Mo.); vancomycin (Eli Lilly & Co., Indianapolis, Ind.); teicoplanin and cefotaxime (Marion Merrell Dow, Cincinnati, Ohio); trovafloxa-

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TABLE 1. Sources of clinical isolates of gram-positive bacteria recovered from hospitals in Taiwan (January 1996 to December 1999)

Source	No. (%) of isolates of:									
	MRSA	MSSA	CoNS	*S. pneumoniae*	Viridans group streptococci	VRE	VSE	*Leuconostoc* spp.	*Pediococcus* spp.	*Lactobacillus* spp.
Blood	80 (100)	68 (100)	301 (74)	19 (7)	78 (56)	20 (13)	12 (16)	15 (43)	1 (13)	14 (20)
Respiratory tract	0	0	0	230 (86)	0	0	0	0	0	0
Cerebrospinal fluid	0	0	6 (1)	4 (2)	12 (9)	0	0	5 (14)	0	0
Bile	0	0	0	0	10 (7)	12 (8)	17 (26)	1 (3)	0	1 (1)
Wound	0	0	87 (21)	8 (3)	20 (14)	45 (30)	35 (58)	1 (3)	0	0
Rectal swab or stool	0	0	0	0	0	73 (49)	0	8 (23)	7 (87)	43 (62)
Other	0	0	12 (3)	6 (2)	20 (14)	0	0	5 (14)	0	11 (16)
Total	80 (100)	68 (100)	406 (100)	267 (100)	140 (100)	150 (100)	64 (100)	35 (100)	8 (100)	69 (100)

cin (Pfizer Inc., New York, N.Y.); ciprofloxacin and moxifloxacin (Bayer Co., West Haven, Conn.); quinupristin-dalfopristin (Rhone-Poulenc Rorer, Collegeville, Pa.); and linezolid (Pharmacia & Upjohn, Kalamazoo, Mich.). For *S. pneumoniae* isolates, only ciprofloxacin, moxifloxacin, quinupristindalfopristin, and linezolid were tested in this study.

Susceptibility testing. MICs of these agents for the 1,287 isolates of gram-positive bacteria were determined by means of the agar dilution method according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (21). The isolates were grown overnight on Trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) at 37°C. Bacterial inocula were prepared by suspending the freshly grown bacteria in sterile normal saline and adjusted to a 0.5 McFarland standard. For susceptibility testing of staphylococci for oxacillin, Mueller-Hinton agar (BBL Microbiology Systems) supplemented with 2% NaCl was used. For *S. pneumoniae* and viridans group streptococci, Mueller-Hinton agar supplemented with 5% sheep blood (BBL Microbiology Systems) was used. For susceptibility testing of staphylococci for other antimicrobial agents and of enterococci and the three lactic acid bacteria, unsupplemented Mueller-Hinton agar (BBL Microbiology Systems) was used. Using a Steers replicator, an organism density of 10^4 CFU/spot was inoculated onto the appropriate plate with various concentrations of antimicrobial agents. The following organisms were included as control strains: *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. faecium* ATCC 19434, *S. pneumoniae* ATCC 49619, and *Leuconostoc lactis* ATCC 19256.

Staphylococci, *S. pneumoniae*, viridans group streptococci, and enterococci were categorized into susceptible, intermediate, and resistant strains based on the guidelines of the NCCLS (22). Two vancomycin-resistant phenotypes (VanA and VanB) of enterococci were categorized as follows: for VanA types, vancomycin MICs were ≥ 64 μg/ml and teicoplanin MICs were ≥ 16 μg/ml, and for VanB types, vancomycin MICs were 16 to 512 μg/ml and teicoplanin MICs were ≤8 μg/ml (7). For isolates of *Leuconostoc*, *Pediococcus*, and *Lactobacillus* spp., there were no NCCLS MIC breakpoint criteria for susceptibility or resistance, and MIC breakpoints of trovafloxacin and moxifloxacin for gram-positive bacteria are also lacking (22). In the present report, the MIC breakpoints for streptococci other than *S. pneumoniae* were used to interpret susceptibilities and resistance for the three lactic acid bacteria (22). MIC interpretive criteria for moxifloxacin (susceptible, ≤2 μg/ml; intermediate, 4 μg/ml; resistant, ≥8 μg/ml), trovafloxacin (susceptible, ≤2 μg/ml; intermediate, 4 μg/ml; resistant, ≥8 μg/ml), and linezolid (susceptible, ≤4 μg/ml for staphylococci, ≤2 μg/ml for streptococci and the three lactic acid bacteria, and ≤2 μg/ml for enterococci; intermediate, 4 μg/ml; resistant, ≥8 μg/ml) were used in accordance with the previous reports (1, 5, 10, 18, 25)

RESULTS

The MlCs (particularly those of quinupristin-dalfopristin) for *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619 were all within the NCCLS control ranges (22) The MIC ranges, MICs at which 50% of the isolates were inhibited (MIC_{50}s), MICs at which 90% of the isolates were inhibited (MIC_{90}s), and percentages of 1,287 clinical isolates that were susceptible and resistant to various antimicrobial agents are summarized in Table 2.

All MSSA isolates were susceptible to quinupristin-dalfopristin. High rates of nonsusceptibility to quinupristin-dalfopristin (MIC, ≥2 μg/ml) were demonstrated for the following organisms: MRSA (31%), CoNS (16%), *S. pneumoniae* (8%), viridans group streptococci (51%), VSE (85%), vancomycin-resistant *E. faecalis* (100%), vancomycin-resistant *E. faecium* (66%), *Leuconostoc* spp. (100%), *Lactobacillus* spp. (50%), and *Pediococcus* spp. (87%). The top three *Staphylococcus* spp. (of more than 10 isolates tested) exhibiting nonsusceptibility to quinupristin-dalfopristin were *S. cohnii* (48%), *S. capitis* (39%), and *S. saprophyticus* (36%) (Table 3). Among viridans group streptococci, the majority of the following species were nonsusceptible to quinupristin-dalfopristin: *S. anginosus* (100%), *S. mutans* (75%), *S. oralis* (59%), *S. intermedius* (58%), and *S. sanguinis* (58%) (Table 4).

All isolates of MSSA, MRSA, *S. pneumoniae*, and viridans group streptococci were susceptible to vancomycin and teicoplanin. The rates of nonsusceptibility to vancomycin and teicoplanin were 5 and 7%, respectively, for CoNS, ranging from 12 and 18% for *S. simulans* isolates to 0 and 0% for *S. cohnii* and *S. auricularis* (Table 3).

Among viridans group streptococcus isolates, the majority of *S. mitis*, *S. sanguinis*, *S. oralis*, and *S. salivarius* isolates were nonsusceptible to penicillin (Table 4). Thirty-six isolates of viridans group streptococci tested were nonsusceptible to cefepime; however, only 12% of these isolates were nonsusceptible to cefotaxime. Moreover, more than three-fourths of *Streptococcus constellatus* isolates, which were all susceptible to cefotaxime, had intermediate susceptibility to cefepime.

The majority of MRSA (97%) and vancomycin-resistant *E. faecium* (90%) isolates were nonsusceptible to ciprofloxacin. However, 80% of MSSA, 73% of CoNS, and 58% of vancomycin-resistant *E. faecalis* isolates were susceptible to ciprofloxacin. The potency of trovafloxacin and moxifloxacin was 2- to 16-fold superior to that of ciprofloxacin against all bacteria tested.

The majority of MSSA (77%), MRSA (83%), and CoNS (76%) isolates were susceptible to rifampin. However, 91% of vancomycin-resistant *E. faecium* isolates were nonsusceptible to rifampin. Among the three lactic acid bacteria tested, rifampin exhibited better activity against *Pediococcus* spp. than *Leuconostoc* spp. (MIC_{90}, 16 μg/ml) and *Lactobacillus* spp. (MIC_{90}, 16 μg/ml).

Among all the agents tested, linezolid demonstrated the most potent activity against nearly all (99.0%) of the isolates tested. All MRSA and vancomycin-resistant *E. faecalis* and *E. faecium* isolates of either the VanA or VanB phenotypes were inhibited by linezolid at a concentration of 1 to 2 μg/ml (except one isolate for which the linezolid MIC was 4 μg/ml). Ten isolates (0.78%) with remarkably decreased susceptibilities to linezolid (MICs, >32 μg/ml) included eight isolates of *Staphylococcus haemolyticus* and one each of *Staphylococcus epidermidis* and *S. simulans*. Two of the eight *S. haemolyticus* isolates and the *S. epidermidis* and *S. simulans* isolates were also highly resistant to oxacillin (MICs, >128 μg/ml), vancomycin (MICs, >128 μg/ml), and teicoplanin (MICs, >128 μg/ml).

TABLE 2. In vitro susceptibilities of clinical isolates of gram-positive bacteria recovered from patients seen from January 1996 to December 1999 in Taiwan

Bacterium (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)			% of isolates showing resistance phenotype[b]		
		Range	50%	90%	S	I	R
MSSA (68)	Quinupristin-dalfopristin	0.25–1	1	1	100	0	0
	Oxacillin	0.25–2	0.5	2	100	0	0
	Vancomycin	0.5–4	1	1	100	0	0
	Teicoplanin	0.25–4	1	2	100	0	0
	Gentamicin	0.25–>512	0.25	64	80	1	19
	Ciprofloxacin	0.06–4	1	4	80	8	12
	Trovafloxacin	0.03–2	0.06	0.06	100	0	0
	Moxifloxacin	0.03–1	0.03	0.06	100	0	0
	Rifampin	0.03–32	0.03	16	77	10	13
	Linezolid	0.12–4	2	2	100	0	0
MRSA (80)	Quinupristin-dalfopristin	0.25–4	1	2	69	30	1
	Oxacillin	16–>128	>128	>128	0	0	100
	Vancomycin	1–4	2	4	100	0	0
	Teicoplanin	0.25–8	2	2	100	0	0
	Gentamicin	0.25–>512	>512	>512	9	0	91
	Ciprofloxacin	0.25–64	16	64	3	0	97
	Trovafloxacin	0.06–16	4	8	47	39	4
	Moxifloxacin	0.06–8	2	4	89	5	6
	Rifampin	0.03–>128	0.03	>128	83	4	13
	Linezolid	1–2	2	2	100	0	0
CoNS (406)	Quinupristin-dalfopristin	0.12–>32	0.5	2	84	8	8
	Oxacillin	<0.03–>128	4	>128	16	0	84
	Vancomycin	0.125–>128	1	2	95	2	3
	Teicoplanin	<0.03–>128	2	8	93	4	3
	Gentamicin	<0.03–>128	32	>128	37	5	58
	Ciprofloxacin	0.03–>128	0.5	16	73	4	23
	Trovafloxacin	<0.03–32	0.06	2	93	2	5
	Moxifloxacin	0.03–8	0.12	2	92	6	2
	Rifampin	<0.03–>128	0.03	>128	76	5	19
	Linezolid	0.5–>32	2	2	98	—	—
S. pneumoniae							
All (267)	Quinupristin-dalfopristin	0.06–4	0.25	1	92	6	2
	Linezolid	0.5–2	1	1	100	0	0
	Ciprofloxacin	0.25–64	2	2	96	3	1
	Trovafloxacin	0.03–>256	0.12	0.25	99	0	1
	Moxifloxacin	0.03–>256	0.12	0.25	99	0	1
Penicillin susceptible (64)	Quinupristin-dalfopristin	0.25–4	0.25	1	91	0	3
	Linezolid	0.5–2	0.5	1	100	0	0
Penicillin intermediate (136)	Quinupristin-dalfopristin	0.25–4	0.5	1	96	3	1
	Linezolid	1–2	1	1	100	0	0
Penicillin resistant (67)	Quinupristin-dalfopristin	0.06–4	0.5	1	90	7	3
	Linezolid	0.5–2	1	1	100	0	0
Viridans group streptococci (140)	Quinupristin-dalfopristin	0.25–8	2	4	49	37	14
	Penicillin	0.03–8	0.12	1	66	29	5
	Vancomycin	0.12–1	0.5	1	100	0	0
	Teicoplanin	0.03–0.5	0.12	0.25	—	—	—
	Cefotaxime	0.03–16	0.25	16	88	4	8
	Cefepime	0.03–16	0.5	2	64	22	14
	Gentamicin	1–>128	8	32	—	—	—
	Ciprofloxacin	0.06–8	1	2	—	—	—
	Trovafloxacin	0.03–2	0.12	0.25	100	0	0
	Moxifloxacin	0.03–0.5	0.25	0.5	100	0	0
	Rifampin	0.03–>128	0.06	0.25	—	—	—
	Linezolid	0.03–2	2	2	100	0	0
VSE (64)	Quinupristin-dalfopristin	0.5–32	8	16	15	9	76
	Penicillin	0.06–>128	4	>128	77	0	23
	Vancomycin	0.5–4	1	2	100	0	0
	Teicoplanin	0.5–4	1	4	100	0	0
	Gentamicin	0.5–>512	>512	>512	26	0	74
	Ciprofloxacin	0.06–>128	1	64	60	12	28
	Trovafloxacin	0.12–32	0.5	4	76	16	8
	Moxifloxacin	0.12–16	0.25	4	78	14	8
	Rifampin	0.03–8	1	8	56	22	12
	Linezolid	1–4	2	2	99	1	0

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TABLE 2—*Continued*

Bacterium (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)			% of isolates showing resistance phenotype[b]		
		Range	50%	90%	S	I	R
Vancomycin-resistant *E. faecalis* (50)	Quinupristin-dalfopristin	4–>128	16	128	0	0	100
	Penicillin	1–>128	4	4	94	2	4
	Vancomycin	128–>128	>128	>128	0	0	100
	Teicoplanin	4–>128	32	>128	10	24	66
	Gentamicin	1–>512	>512	>512	49	0	51
	Ciprofloxacin	0.06–64	1	32	58	6	36
	Trovafloxacin	0.06–16	0.5	16	76	8	16
	Moxifloxacin	0.25–16	0.5	16	80	2	18
	Rifampin	0.25–16	1	2	56	34	10
	Linezolid	1–2	2	2	100	0	0
Vancomycin-resistant *E. faecium* (100)	Quinupristin-dalfopristin	0.5–128	4	16	34	15	51
	Penicillin	4–>128	>128	>128	12	0	88
	Vancomycin	32–>128	>128	>128	0	0	100
	Teicoplanin	0.5–>128	32	64	39	5	56
	Gentamicin	4–>512	>512	>512	16	0	84
	Ciprofloxacin	0.06–>128	128	>128	10	7	83
	Trovafloxacin	0.12–32	8	16	19	18	63
	Moxifloxacin	0.25–32	16	32	21	1	78
	Rifampin	0.03–>128	8	16	9	8	83
	Linezolid	1–4	2	2	99	1	0
Leuconostoc spp. (35)[a]	Quinupristin-dalfopristin	2–>128	2	16	0	54	46
	Penicillin	0.5–1	0.5	1	0	100	0
	Vancomycin	>128	>128	>128	0	0	100
	Teicoplanin	>128	>128	>128	0	0	100
	Cefotaxime	0.5–64	8	32	3	14	83
	Gentamicin	0.03–1	0.25	0.5	—	—	—
	Ciprofloxacin	1–>128	1	2	—	—	—
	Trovafloxacin	0.06–0.5	0.25	0.5	100	0	0
	Moxifloxacin	0.12–2	0.25	1	100	0	0
	Rifampin	0.25–16	2	16	—	—	—
	Linezolid	1–4	2	2	97	—	—
Lactobacillus spp. (69)[a]	Quinupristin-dalfopristin	0.25–>128	2	8	50	26	24
	Penicillin	0.06–4	0.5	2	3	91	6
	Vancomycin	0.06–>128	>128	>128	10	—	—
	Teicoplanin	0.03–>128	>128	>128	—	—	—
	Cefotaxime	0.06–128	2	32	34	16	50
	Gentamicin	0.03–8	1	4	—	—	—
	Ciprofloxacin	0.25–16	1	8	—	—	—
	Trovafloxacin	0.03–2	0.25	0.5	100	0	0
	Moxifloxacin	0.12–8	0.25	1	96	1	3
	Rifampin	0.03–>128	0.5	16	—	—	—
	Linezolid	0.06–2	1	2	100	0	0
Pediococcus spp. (8)[a]	Quinupristin-dalfopristin	0.25–128	—	—	13	37	50
	Penicillin	0.12–2	—	—	13	0	87
	Vancomycin	0.03–>128	—	—	13	—	—
	Teicoplanin	0.03–>128	—	—	—	—	—
	Cefotaxime	0.25–32	—	—	37	0	63
	Gentamicin	0.25–2	—	—	—	—	—
	Ciprofloxacin	0.25–16	—	—	—	—	—
	Trovafloxacin	0.03–1	—	—	100	0	0
	Moxifloxacin	0.25–2	—	—	100	0	0
	Rifampin	0.25–1	—	—	—	—	—
	Linezolid	0.5–2	—	—	100	0	0

[a] The MIC breakpoints for streptococci other than *S. pneumoniae* were used to interpret susceptibilities and resistance for *Leuconostoc*, *Lactobacillus*, and *Pediococcus* spp.
[b] S, susceptible; I, intermediate; R, resistant.

Of the vancomycin-resistant *E. faecium* isolates, 61% exhibited the VanA phenotype and 39% showed the VanB phenotype. Of the vancomycin-resistant *E. faecalis* isolates, 90% exhibited the VanA phenotype and the other 10% exhibited the VanB phenotype. More than 90% of the vancomycin-resistant *E. faecalis* isolates but only 10% of the vancomycin-resistant *E. faecium* isolates were susceptible to penicillin. More than 80% of the vancomycin-resistant *E. faecium*, compared to 50% of the vancomycin-resistant *E. faecalis* isolates, showed high-level resistance to gentamicin (MICs, >500 μg/ml).

Thirty-eight of the 39 ciprofloxacin-susceptible (MICs, ≤1 μg/ml) VRE isolates were also susceptible to moxifloxacin and

TABLE 3. In vitro susceptibilities of 10 species of CoNS

Species (no. of isolates)	% Intermediate/% resistant isolates					Linezolid (% nonsusceptible isolates [no. of nonsusceptible isolates])
	Oxacillin	Vancomycin	Teicoplanin	Rifampin	Quinupristin-dalfopristin	
S. epidermidis (101)	0/90	1/3	2/3	9/34	1/5	1 (1)
S. haemolyticus (84)	0/82	2/2	2/6	0/27	1/2	10 (8)
S. hominis (47)	0/68	2/0	2/2	10/9	4/0	0 (0)
S. simulans (34)	0/71	0/12	6/12	9/12	3/12	3 (1)
S. cohnii (33)	0/82	0/0	0/0	3/9	21/27	0 (0)
S. auricularis (26)	0/73	0/0	0/0	0/21	4/0	0 (0)
S. capitis (23)	0/91	4/0	0/0	0/4	22/17	0 (0)
S. warneri (21)	0/52	5/5	5/10	0/10	19/10	0 (0)
S. saprophyticus (22)	0/100	0/5	0/5	0/5	22/14	0 (0)
S. sciuri (14)	0/100	7/0	7/7	28/36	13/0	0 (0)

trovafloxacin. Moxifloxacin and trovafloxacin both had poor activities against ciprofloxacin-nonsusceptible VRE isolates. The MIC_{50}s and MIC_{90}s of moxifloxacin and trovafloxacin for ciprofloxacin-nonsusceptible vancomycin-resistant *E. faecium* isolates were 16 and 32 μg/ml and 8 and 16 μg/ml, respectively. However, the MIC_{50}s and MIC_{90}s of moxifloxacin and trovafloxacin for ciprofloxacin-nonsusceptible vancomycin-resistant *E. faecalis* were 2 and 16 μg/ml and 4 and 16 μg/ml, respectively.

DISCUSSION

In recent years, there has been a dramatic increase in the number of infections due to gram-positive bacteria (18, 20, 23). This is compounded by the rapid emergence of resistance to commonly used antimicrobial agents for these organisms, especially in staphylococci (MRSA and vancomycin-resistant *S. aureus*), enterococci (VRE), pneumococci (penicillin- and extended-spectrum cephalosporin-resistant strains), and viridans group streptococci (penicillin- and extended-spectrum cephalosporin-resistant strains) (12–16, 18, 20, 23, 28, 30). The increase in infections caused by these resistant organisms over the past decade poses problems beyond the lack of available antimicrobial therapy (25). One concern is that interspecies and intraspecies spread of these resistant genes is plausible with continued selective pressure (18, 25). Therefore, there is an urgent need for antimicrobial agents with activity against these multidrug-resistant gram-positive bacteria.

In this study of the in vitro susceptibilities of antimicrobial agents against recent clinical isolates of gram-positive bacteria in Taiwan, four important points were clearly demonstrated. First, contrary to previous studies, quinupristin-dalfopristin resistance among CoNS, viridans group streptococci, VRE (including vancomycin-resistant *E. faecium*), and the three lactic acid bacteria isolated in Taiwan is considerable (3, 5, 15, 27). Second, resistance to glycopeptides among Taiwan CoNS isolates was first documented in this report, and this resistance was distributed in many species of CoNS. Third, compared with previous studies (28, 30), the resistance of viridans group streptococci to penicillin and extended-spectrum cephalosporins continues to increase. Finally, linezolid was the most potent agent against all isolates tested, including glycopeptide- and quinupristin-dalfopristin-resistant isolates.

The in vitro susceptibilities of gram-positive bacteria to quinupristin-dalfopristin have been widely studied (3, 5, 13, 14, 18, 27). Previous reports showed that rates of nonsusceptibility and MIC_{90}s of this drug for recent clinical isolates (1996 to 1997) recovered from the United States and Canada were 0.3% and 0.5 μg/ml for *S. aureus*, 0.3% and 0.5 μg/ml for MSSA, 1% and 1.0 μg/ml for MRSA, 2.3% and 0.75 μg/ml for *S. pneumoniae*, 3% and 0.75 μg/ml for streptococci other than *S. pneumoniae*, 13% for all *E. faecium* isolates, 0.2% and 1 μg/ml for vancomycin-resistant *E. faecium*, and 87% for enterococci other than vancomycin-resistant *E. faecium* (18). Compared with previous findings, our rates of nonsusceptibility of these multidrug-resistant gram-positive bacteria to quinupristin-dalfopristin were remarkably high.

In the European Union, virginiamycin, another streptogramin A and B combination, has been used as a growth promoter in animal feed for many years (27, 29). It selects for virginiamycin-resistant *E. faecium* isolates which are also cross resistant to quinupristin-dalfopristin (27, 29). The Vat(D) and Vat(E) acetyltransferases, which confer resistance to both quinupristin-dalfopristin and virginiamycin, appeared in enterococci from different European countries (27). Although the *vat*(E) gene was documented to be present on plasmids in

TABLE 4. In vitro susceptibilities of nine species of viridans group streptococci to β-lactams, trovafloxacin, and quinupristin-dalfopristin

Species (no. of isolates)	% Intermediate/% resistant isolates					
	Penicillin	Cefotaxime	Cefepime	Trovafloxacin	Quinupristin-dalfopristin[a]	Linezolid
S. intermedius (31)	6/3	0/3	23/3	3/0	48/10	0/0
S. mitis (24)	38/13	13/13	4/38	0/0	25/17	0/0
S. sanguinis (28)	57/0	4/10	7/14	0/0	29/29	0/0
S. constellatus (18)	6/0	0/0	78/0	0/0	39/0	0/0
S. oralis (17)	47/6	6/12	35/18	0/0	53/6	0/0
S. salivarius (8)	50/13	13/0	13/0	0/0	13/0	0/0
S. acidominimus (6)	17/17	0/17	17/33	0/0	17/0	0/0
S. mutans (4)	0/0	0/0	0/0	0/0	50/25	0/0
S. anginosus (4)	0/0	0/0	0/0	0/0	50/50	0/0

[a] MIC breakpoints for susceptibility were adapted from those suggested by the NCCLS for interpreting susceptibility of group A and B streptococci.

E. faecium isolates from farm animals, raw meats, and hospital patients in the United Kingdom, none of the patients had received quinupristin-dalfopristin (27). Previous reports postulated that exchange of resistant strains or resistant genes may occur between *E. faecium* isolates from nonhuman and human sources (27, 29).

In Taiwan, quinupristin-dalfopristin is not available in clinical settings and there are no ongoing clinical trials with this drug. Furthermore, Taiwan does not import meat from any European country where virginiamycin is used. However, virginiamycin has indeed been used in animal husbandry as a growth-promoting agent in Taiwan since 1976, although the amount of consumption of this drug was relatively low (6,250 kg) and ranked sixteenth among all antibiotics used in animal husbandry in 1999. Further studies should be performed to identify the source of this resistance among clinical isolates and to investigate the mechanisms of resistance to quinupristin-dalfopristin among these organisms.

Linezolid is an oxazolidinone agent that inhibits protein synthesis by binding to the 50S ribosome subunit and preventing formation of the initiation complex. In vitro studies have demonstrated that linezolid has significant activity against multidrug-resistant gram-positive cocci (MICs, 0.25 to 8 μg/ml), MRSA (MICs, 0.5 to 8 μg/ml), methicillin-resistant CoNS (MICs, 0.5 to 4 μg/ml), VRE (MICs, 0.5 to 4 μg/ml), and multidrug-resistant *S. pneumoniae* (MICs, 0.25 to 2 μg/ml) (24, 25). Our results were partly in accordance with the above findings. Interestingly, 10 isolates of CoNS, especially *S. haemolyticus*, required extremely high linezolid MICs (>32 μg/ml), which have rarely been reported previously (24, 25). Based on our in vitro results, linezolid is the most potent agent against these multidrug-resistant gram-positive bacteria, though some recent in vivo studies have debated its clinical efficacy, partly due to a lack of in vitro bactericidal activity (4, 24, 25).

When MIC_{90} results were compared, moxifloxacin was demonstrated to be more active than ciprofloxacin against MRSA (16-fold), methicillin-resistant CoNS (32-fold), enterococci (4-fold), viridans group streptococci (16-fold), and *S. pneumoniae* (16-fold) (1). Our study supported these findings. In the present study, we demonstrated that trovafloxacin had better activity (2- to 4-fold) than moxifloxacin against viridans group streptococci, vancomycin-resistant *E. faecium*, and the three lactic acid bacteria.

In the present study, for all tested *Leuconostoc* and *Pediococcus* isolates penicillin MICs were ≤2 μg/ml, and ciprofloxacin MICs for the majority of *Pediococcus* isolates were ≥4 μg/ml. These findings were similar to those reported previously (10, 17, 31). However, Zarazaga and colleagues demonstrated that for 26.2% of *Lactobacillus* spp., penicillin MICs were ≥16 μg/ml, and for 60% of *Lactobacillus* and 72% of *Leuconostoc* isolates, ciprofloxacin MICs were ≥4 μg/ml (31). These findings were discordant with our results.

In conclusion, the results presented here from testing 1,287 clinical isolates of gram-positive bacteria from Taiwan indicate the poor activity of quinupristin-dalfopristin against clinical isolates of gram-positive bacteria. Restricted use of virginiamycin in animal feed is necessary to alleviate the quinupristin-dalfopristin resistance among bacteria from human sources in Taiwan.

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