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In Vitro Activities of Fluoroquinolones against Antibiotic-Resistant Blood Culture Isolates of Viridans Group Streptococci from across Canada

J. C. S. DE AZAVEDO,* L. TRPESKI, S. PONG-PORTER, S. MATSUMURA, THE CANADIAN BACTERIAL SURVEILLANCE NETWORK,† AND D. E. LOW

Department of Microbiology, Mount Sinai and Princess Margaret Hospitals, and Department of Pathobiology and Laboratory Medicine, University of Toronto, Toronto, Ontario, Canada

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Among 418 blood culture isolates of viridans group streptococci obtained between 1995 and 1997, the in vitro rates of nonsusceptibility to penicillin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole were 28, 29, 24, and 14%, respectively. The most prevalent group (125 strains) was *Streptococcus mitis*, followed by *Streptococcus sanguis* (56 strains). For 236 (56%) strains resistant to one or more antibiotics, the ciprofloxacin MIC at which 90% of the isolates were inhibited (MIC_{90}) was 4 μg/ml, whereas the MIC_{90}s of trovafloxacin, grepafloxacin, and gatifloxacin were 0.25 μg/ml.

Viridans group streptococci, although part of the normal human flora, are a common cause of sepsis in neutropenic patients (3) and bacterial endocarditis (17). Recently, there have been several reports describing emerging antimicrobial resistance in this group of streptococci (1, 2, 7, 15). In 1996, 56% of strains in the United States were nonsusceptible to penicillin (7), an antimicrobial that had exhibited good activity against these organisms 20 years ago (4).

This study examined the activities of several antimicrobial agents, comprising those commonly used for treating infections caused by viridans group streptococci and those that could be potentially useful, such as the fluoroquinolones, with enhanced activity against gram-positive organisms (14).

Between May 1995 and March 1997, 418 blood culture isolates of viridans group streptococci were collected through the Canadian Bacterial Surveillance Network, a system of 55 private laboratories and community- and university-affiliated hospitals representing nine provinces across Canada, of which 39 provided 3 or more isolates. Isolates were sent to Mount Sinai Hospital for susceptibility testing and species identification. The latter was carried out with a combination of standard biochemical tests (18), the Vitek GPI system (bioMerieux Vitek Inc., Hazelwood, Mo.), and the API 20 Strep system (bioMerieux sa, Marcy l'Etoile, France) according to the manufacturer's instructions. Susceptibility testing was performed by broth microdilution, in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines (13). Panels were prepared in-house, by using cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 5% lysed horse blood. The in vitro activities of the following agents were tested: penicillin, cefuroxime, ceftriaxone, cefotaxime, erythromycin, clindamycin, vancomycin, tetracycline, trimethoprim-sulfamethoxazole (TM/S [19: 1]), ciprofloxacin (Sigma-Aldrich Canada, Oakville, Ontario), sparfloxacin (Rhone-Poulenc Rorer, Collegeville, Pa.), levofloxacin (Ortho-McNeil Pharmaceuticals, Spring House, Pa.), and gatifloxacin (Bristol-Myers Squibb Company, Princeton, N.J.). *Streptococcus pneumoniae* ATCC 49619 was used as a control. MICs of drugs were read after 20 to 24 h of incubation in ambient air at 35°C.

Relatively high rates of resistance against several antimicrobials were noted (Table 1). Among the 418 isolates, 116 (28%) were nonsusceptible to penicillin, with MICs ranging from 0.12 to 32 μg/ml (MIC at which 90% of the isolates are inhibited [MIC_{90}], 1 μg/ml). Of these, 91 strains (22%) demonstrated intermediate resistance (MIC, 0.25 to 2 μg/ml), and 25 (6%) showed high resistance (MIC, ≥4 μg/ml). Among the strains showing high resistance, the penicillin MICs were 4 μg/ml for 16 strains, 8 μg/ml for 9 strains, and 32 μg/ml for 1 strain. The rate of penicillin nonsusceptibility found in this study is lower than the rates of 56% reported in the United States (7) and 37% in New Zealand (15). In a study in Spain of 410 viridans strains isolated from blood, 34% were found to be nonsusceptible to penicillin (5). Predictably, nonsusceptibilities to both amoxicillin and ceftriaxone were also relatively high at 12%, and the MIC_{90}s of these two agents were the same as that for penicillin at 1 μg/ml.

Interestingly, although 29% of the strains were nonsusceptible to erythromycin, only 4% were nonsusceptible to clindamycin, and these did not show inducible resistance. This suggests that resistance was not due to the presence of an *erm* methylase, which confers resistance to macrolides, lincosamides, and streptogramin B type antimicrobials (22), but is more likely due to the high prevalence of an efflux mechanism, such as is conferred by the *mef* gene, which selectively confers resistance to erythromycin but not to clindamycin (20). High rates of resistance to erythromycin in blood culture isolates have been observed previously in Spain (39%) (1) and the United States (38%) (7); however, susceptibility to clindamycin was not examined. Other antibiotics to which strains showed a high level of nonsusceptibility were tetracycline at 24% and TM/S at 14%.

Emergence of high rates of resistance to various antimicrobials was surprising, considering that 173 (60%) strains were from community-based hospitals (<200 beds) rather than tertiary care hospitals. There was no significant difference (P = 0.8) in levels of penicillin resistance between strains from the

* Corresponding author. Mailing address: Department of Microbiology, 600 University Ave., Rm. 1483, Toronto, Ontario, Canada M5G 1X5. Phone: (416) 586-8549. Fax: (416) 586-8746. E-mail: jdeazavedo@mtsinai.on.ca.

† Members are listed in the Appendix.

TABLE 1. In vitro susceptibility of 418 blood culture viridans group streptococci to selected antimicrobial agents

Antimicrobial agent	No. (cumulative %) of strains for which MIC (μg/ml) was:										% Nonsusceptible	
	≤0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16.0	>16 of ≥32	Intermediate	Resistant
Penicillin	240 (57)	62 (72)	38 (81)[a]	27 (87)[a]	16 (91)[a]	10 (94)[a]	16 (98)	8 (99.7)	0	1 (100)[b]	22	6
Amoxicillin	158 (46)	95 (60)	63 (76)	50 (88)	9 (90)	12 (93)	9 (95)	22 (100)			2	10
Ceftriaxone	126 (30)	130 (61)	74 (79)	36 (87)	25 (93)[a]	14 (97)	9 (99)	4 (100)			6	6
Erythromycin	ND[c]	293 (70)	3 (71)	5 (72)[a]	19 (77)	54 (90)	17 (94)	8 (96)	2 (96)	17 (100)[b]	1	39
Clindamycin	ND	ND	399 (96)	1 (96)[a]	0	0	0	0	0	18 (100)[b]	0.2	4
Tetracycline	ND	ND	ND	ND	291 (70)	16 (73)	13 (77)[a]	16 (80)	16 (84)	66 (100)	3	21
TM/S[d]	ND	ND	208 (50)	69 (66)	51 (78)	32 (86)	25 (92)	23 (98)	10 (100)		20	14

[a] Intermediate resistance.
[b] The MIC was 32 μg/ml.
[c] ND, not determined.
[d] Trimethoprim-sulfamethoxazole at a 19:1 ratio.

two groups of hospitals, and patterns of resistance to the other antibiotics were also similar.

In *S. pneumoniae*, it has been noted that penicillin-resistant strains tend to be resistant to other unrelated antimicrobials, and resistance is generally low in penicillin-susceptible strains (19). However, this did not appear to be the case in this group of viridans streptococci, and rates of resistance to certain antibiotics were relatively high among penicillin-susceptible strains. Of 302 penicillin-susceptible isolates, 60 (20%) showed nonsusceptibility to erythromycin, 68 (23%) were nonsusceptible to tetracycline, and 15 (5%) were nonsusceptible to TM/S. In the case of tetracycline, the resistance rates were almost identical in penicillin-susceptible and nonsusceptible strains at 23 and 25%, respectively, which is different from the pattern found in *S. pneumoniae* strains.

Of the 418 strains tested, 236 showed resistance to one or more of the antimicrobial agents listed in Table 1. These strains were tested against a panel of fluoroquinolones as shown in Table 2. The ciprofloxacin MIC_{50} and MIC_{90} were 4 and 2 μg/ml, respectively, and the ofloxacin MIC_{90} and MIC_{50} were both 2 μg/ml. By comparison, with the exception of levofloxacin (with a MIC_{90} of 1 μg/ml), the MICs of the other fluoroquinolones were 0.5 or less. The MICs of trovafloxacin, grepafloxacin, and gatifloxacin were at least fourfold lower than those of ciprofloxacin, ofloxacin, and levofloxacin. Two strains showed reduced susceptibility to all of the fluoroquinolones tested, with MICs of ciprofloxacin and ofloxacin of 32 μg/ml and MICs of sparfloxacin, gatifloxacin, and grepafloxacin of 4 to 8 μg/ml.

Identification at the group level was carried out with the 236 strains showing resistance to one or more antibacterial agents. Antimicrobial activity against 211 strains belonging to the most prevalent groups is shown in Table 3. Groups which encompassed fewer than 10 strains were not included: *Streptococcus milleri* (7 strains), *Streptococcus bovis* (6 strains), and *Streptococcus mutans* (3 strains). In addition, small numbers of strains (e.g., three or four) which did not fall into easily identifiable groups, such as *Streptococcus acidominus*, were also excluded. The largest number of isolates (59%) belonged to the *Streptococcus mitis* group. Of 125 *S. mitis* isolates, 50 (40%) were nonsusceptible to penicillin and 66 (53%) were nonsusceptible to erythromycin. The two strains which showed reduced susceptibility to all of the fluoroquinolones (Table 2) belonged to the *S. mitis* group. In the *Streptococcus sanguis* group, comprising 56 strains, 55% were nonsusceptible to penicillin.

The results of this study show that 28% of bloodstream isolates of viridans group streptococci are nonsusceptible to penicillin, with as many as 40% of *S. mitis* strains, the most prevalent group, showing nonsusceptibility. The MIC of penicillin for one *S. milleri* strain (*Streptococcus intermedius*) was 32 μg/ml. This is of concern, since *S. pneumoniae* can acquire resistance, as represented by a MIC of penicillin as high as 64 μg/ml, by horizontal transfer from *S. mitis* in vitro (8, 12). The current rate of penicillin resistance (intermediate plus high) in pneumococci in Canada is 14% (6, 19), which is considerably less than that found in this study for viridans group streptococci.

Our data indicate that the newer fluoroquinolones exhibit good in vitro activity against viridans group streptococci, including isolates known to have intermediate or high resistance to penicillin and other β-lactam antibiotics. Currently, there are no interpretative standards with respect to the efficacy of most fluoroquinolones against streptococci. However, trovafloxacin, grepafloxacin, and gatifloxacin showed excellent ac-

TABLE 2. In vitro susceptibility to fluoroquinolones of 236 viridans group streptococci resistant to one or more antibiotics

Antimicrobial agent	No. (cumulative %) of strains for which MIC (μg/ml) was:									
	0.06	0.12	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0
Ciprofloxacin	ND[a]	1 (0.4)	2 (1)	6 (4)	87 (41)	113 (89)	19 (97)	5 (98.7)	1 (99)	2 (100)
Ofloxacin	ND	ND	1 (0.4)	2 (1)	31 (14)	182 (92)	15 (98)[b]	1 (98)	1 (99)	3 (100)[c]
Levofloxacin	3 (1)	0	7 (4)	76 (36)	138 (95)	8 (98)	1 (99)[b]	1 (99)	2 (100)	0
Trovafloxacin	38 (16)	170 (88)	18 (96)	7 (99)	1 (99)	0[b]	2 (100)			
Sparfloxacin	4 (2)	62 (28)	136 (86)	28 (97)	2 (98)	2 (99)	0	2 (100)		
Grepafloxacin	79 (33)	66 (61)	78 (94)	7 (97)	4 (99)[b]	0	0	2 (100)		
Gatifloxacin	57 (24)	125 (77)	48 (97)		2 (98)	2 (99)	2 (100)			

[a] ND, not determined.
[b] Intermediate resistance.
[c] MICs were ≥32 μg/ml.

TABLE 3. Antimicrobial activities of various agents against individual streptococcal species[a]

Antimicrobial (breakpoint)[b]	*S. mitis* (n = 125)			*S. sanguis* (n = 56)			*Streptococcus salivarius* (n = 17)			Nontypeable (n = 13)		
	MIC (μg/ml)[c]		No. (%) nonsusceptible	MIC (μg/ml)		No. (%) nonsusceptible	MIC (μg/ml)		No. (%) nonsusceptible	MIC (μg/ml)		No. (%) nonsusceptible
	50%	90%		50%	90%		50%	90%		50%	90%	
Penicillin (≥0.25)	0.12	4.0	50 (40)	0.25	2.0	31 (55)	0.5	1.0	10 (59)	0.06	0.5	9 (69)
Ceftriaxone (≥1.0)	0.12	2.0	28 (22)	0.12	1.0	7 (13)	0.25	1.0	3 (18)	0.12	1.0	4 (34)
Erythromycin (≥0.5)	1.0	8.0	66 (53)	0.12	4.0	18 (32)	0.12	8.0	7 (41)	0.12	8.0	8 (62)
Tetracycline (≥4)	1.0	>32	49 (39)	2.0	>32	23 (41)	1.0	8.0	6 (35)	1.0	>32	2 (15)
TM/S[d] (≥1.0)	1.0	8.0	58 (46)	1.0	4.0	30 (53)	0.5	1.0	4 (23)	0.25	2.0	4 (31)
Ciprofloxacin	2.0	4.0	NA[e]	1.0	2.0	NA	1.0	4.0	NA	1.0	2.0	NA

[a] In addition to the groups listed here, there were six strains belonging to the *S. bovis* group, seven strains belonging to the *S. milleri* group, and three strains belonging to the *S. mutans* group.
[b] Breakpoint for resistance as defined by the NCCLS.
[c] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[d] Trimethoprim-sulfamethoxazole at a 19:1 ratio.
[e] NA, not applicable, since breakpoints are not defined by the NCCLS.

tivity in vitro, with MIC_{90}s of 0.25 μg/ml, a 4- to 16-fold decrease compared to levofloxacin, ofloxacin, and ciprofloxacin. Trovafloxacin has been shown to decrease bacterial counts of an *S. mitis* strain and an *S. sanguis* strain showing reduced susceptibility to ciprofloxacin in an animal model of streptococcal endocarditis (9). Our work confirms the results of other studies showing that newer fluoroquinolones have better activity against clinical isolates of viridans streptococci (9, 11). However, mutations in the topoisomerase genes *parC* and *gyrA* have been shown to occur both in vitro and in vivo in response to fluoroquinolone exposure and treatment (21). Blood culture isolates of viridans group streptococci with reduced susceptibility to ciprofloxacin (MIC_{90}, 8 to 16 μg/ml) have already been described (9–11, 16), and resistance to newer fluoroquinolones will likely develop. Consequently, ongoing surveillance for the development of fluoroquinolone resistance in viridans streptococci is essential.

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APPENDIX

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