ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2005, p. 820–823 Vol. 49, No. 2
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.2.820–823.2005

In Vitro Activity of Telithromycin against Viridans Group Streptococci and *Streptococcus bovis* Isolated from Blood: Antimicrobial Susceptibility Patterns in Different Groups of Species

Iciar Rodríguez-Avial,* Carmen Rodríguez-Avial, Esther Culebras, and Juan J. Picazo

Servicio de Microbiología, Hospital Clínico San Carlos, Madrid, Spain

Received 12 August 2004/Returned for modification 7 September 2004/Accepted 4 October 2004

The in vitro activities of penicillin, erythromycin, clindamycin, and telithromycin were determined against 155 viridans group streptococci (VGS) and 18 *Streptococcus bovis* blood isolates. Heterogeneity in the susceptibility patterns and macrolide resistance phenotypes and genotypes in the different groups of VGS was detected. We found seven telithromycin-resistant *S. bovis* isolates all harboring the *erm*(B) gene.

Viridans group streptococci (VGS) are the main commensals of the oropharyngeal cavity in humans. They are the leading cause of subacute bacterial endocarditis and have also emerged as important pathogens causing sepsis in neutropenic cancer patients. *Streptococcus bovis* is frequently found as part of the commensal bowel flora. These organisms are also agents of endocarditis. Bacteremia caused by *S. bovis* isolates is associated with malignancies of the gastrointestinal tract.

Recent studies have shown that VGS are becoming increasingly resistant to many antibiotics, and it seems that antimicrobial resistance is not homogeneous among the different members of the group (1, 7, 8, 20). Most studies of VGS report that *Streptococcus mitis*, in addition to being the most frequently isolated group, is also the group that shows the highest rates of penicillin resistance (1, 7, 8, 10, 17). However, reports about the susceptibility of other VGS and *S. bovis* are scarce. The aim of our study was to determine overall rates of VGS susceptibility to penicillin, macrolides, and telithromycin and also to determine whether there are any differences in the susceptibility patterns and erythromycin resistance mechanisms of the different species that make up this group.

A total of 155 VGS and 18 *S. bovis* strains isolated from blood were collected between 1998 and 2003. Of the 173 isolates, 111 were isolated from 1998 to 2002 and were previously characterized in terms of their macrolide resistance (15). All strains were identified by standard methods (16) and by the Rapid ID32 Strep system (bioMérieux, Marcy l'Étoile, France) and then classified according to the review of Coykendall (4).

Susceptibility to penicillin, erythromycin, clindamycin, and telithromycin was determined by the agar dilution method. MICs were interpreted using NCCLS criteria for *Streptococcus* other than *Streptococcus pneumoniae*. For telithromycin, since no NCCLS breakpoints have been established, we used those defined for *S. pneumoniae* (12). Macrolide resistance phenotypes were determined using a double-disk diffusion method (18). The presence of *erm* and *mef* genes was determined by PCR amplification with previously described primers specific for *erm*(A), *erm*(B), *erm*(C), and *mef*(A) (18, 19).

* Corresponding author. Mailing address: Servicio de Microbiología Clínica, Hospital Clínico San Carlos, Plaza de Cristo Rey s/n, 28040 Madrid, Spain. Phone: 34 91-3303486. Fax: 34 91-3303478. E-mail: iciaravial@hotmail.com.

Of the 155 VGS, 72 isolates belonged to the *S. mitis* group; other groups included *Streptococcus anginosus* (49 isolates), *Streptococcus sanguis* (21 isolates), *Streptococcus salivarius* (11 isolates), and *Streptococcus mutans* (2 isolates).

The in vitro activities of the antibiotics tested for the different streptococci, except for the two *S. mutans* group isolates, which were susceptible to all antibiotics tested, are shown in Table 1.

Overall, the rate of nonsusceptibility to penicillin was 29%. Of the 50 penicillin-nonsusceptible strains, 13 (8%) were highly resistant to penicillin and 37 (21%) showed intermediate resistance. *S. mitis* showed the highest rate of penicillin resistance (45%). Moreover, of the 13 highly penicillin-resistant strains, 12 belonged to the *S. mitis* group and one belonged to the *S. salivarius* group. These results agree with other surveys which report that penicillin resistance is more common in *S. mitis* and *S. salivarius* than in *S. anginosus* (1, 7, 8).

The resistance percentages for erythromycin and clindamycin were 45.6 and 27.7%, respectively. High percentages (30 to 50%) of erythromycin resistance have been reported in recent studies of VGS isolated from blood cultures (1, 5–7). Resistance to erythromycin varied by species, and *Streptococcus bovis* group isolates showed the highest rates of resistance to erythromycin and clindamycin. *S. bovis* isolates were found to be more resistant to these antibiotics than other streptococcal species in Denmark were (14). The lowest percentages of erythromycin and clindamycin resistance were found in *S. salivarius* isolates.

Overall the ketolide telithromycin was the most active antimicrobial agent tested, with 96% susceptibility. However, we found seven telithromycin-resistant strains. These belonged to the *S. bovis* group, and the MICs for them ranged from 4 to 128 μg/ml. Telithromycin-resistant VGS have been recently reported in Belgium (11), but, to our knowledge, ours is the first report that describes telithromycin-resistant *S. bovis* strains and the first that shows MICs as high as 128 μg/ml.

Of the 79 erythromycin-resistant strains, 61% displayed constitutive macrolide-lincosamide-streptogramin B ($cMLS_B$) resistance, 35% displayed the M phenotype, and 4% displayed the inducible MLS_B ($iMLS_B$) phenotype (Table 2). The predominance of the $cMLS_B$ phenotype in VGS isolated from blood has been reported from Korea (61.1%), France (77%),

TABLE 1. MIC_{50}, MIC_{90}, ranges, and susceptibility percentages of the antibiotics tested for different groups of species[a]

Antibiotic	MIC	All strains	*Streptococcus* group				
			S. mitis (*n* = 72)	*S. anginosus* (*n* = 49)	*S. sanguis* (*n* = 21)	*S. salivarius* (*n* = 11)	*S. bovis* (*n* = 18)
Penicillin	Range	≤0.03–64	≤0.03–64	≤0.03–2	≤0.03–2	0.06–8	0.03–2
	MIC_{50}	0.06	0.12	0.06	0.12	0.25	0.06
	MIC_{90}	2	4	0.12	0.25	1	0.25
	% S	71.1	55	94	57	64	89
	% I	21.3	28	6	43	27	11
	% R	7.6	17	0	0	9	0
Erythromycin	Range	≤0.03–256	0.06–256	≤0.03–256	0.06–256	0.06–256	0.06–256
	MIC_{50}	0.12	0.12	0.12	1	0.06	256
	MIC_{90}	256	128	256	64	4	256
	% S	52.6	50	63	48	73	22
	% I	1.8	4	0	0	0	0
	% R	45.6	46	37	52	27	78
Clindamycin	Range	≤0.03–256	≤0.03–256	≤0.03–128	≤0.03–64	≤0.03–0.12	≤0.03–256
	MIC_{50}	0.06	0.06	0.06	0.06	0.06	64
	MIC_{90}	256	128	128	32	0.12	256
	% S	72.3	78	69	81	100	28
	% I	0	0	0	0	0	0
	% R	27.7	22	31	19	0	72
Telithromycin	Range	≤0.015–128	≤0.015–2	≤0.015–1	≤0.015–0.25	≤0.015–0.06	≤0.015–128
	MIC_{50}	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	0.12
	MIC_{90}	0.25	0.12	0.12	0.12	0.03	64
	% S	95.5	99	100	100	100	61
	% I	0.5	1	0	0	0	0
	% R	4	0	0	0	0	39

[a] MIC_{50}, MIC at which 50% of the isolates tested are inhibited; MIC_{90}, MIC at which 90% of the isolates tested are inhibited; % S, percent susceptible; % I, percent intermediate; % R, percent resistant. All MICs are in micrograms per milliliter.

and Holland (73%) (3, 9, 22). However, in the United States, Canada, and Latin America (6, 8), the M phenotype was the most common. In VGS isolated from normal flora, there are also differences between phenotypes in the different countries (2, 11, 17)

In our study, the $cMLS_B$ phenotype was the most predominant in *S. anginosus* and *S. bovis*, while in *S. sanguis* and *S. salivarius* the most frequent was the M phenotype. Jacobs et al. (9) reported that 73% of *S. anginosus* strains studied had the $cMLS_B$ phenotype strains, and for *S. bovis*, Rennenberg et al. (14) reported a high percentage of strains with the $cMLS_B$ phenotype. However, in the study by Teng et al. (21) in Taiwan, $iMLS_B$ was the most common phenotype in 38 erythromycin-resistant *S. bovis* isolates.

Therefore, the proportions of the different phenotypes may vary according to country and source of strains and may even depend on the groups of VGS that are included in the studies.

The *erm*(B) gene was amplified in all strains with the $cMLS_B$ and $iMLS_B$ phenotypes and was negative for all isolates with the M phenotype. *mef*(A) was detected in most isolates with the M phenotype as well as in eight isolates with a $cMLS_B$ phenotype. These strains were *S. mitis* (four), *S. anginosus* (three), and *S. bovis* (one). As in previous studies of VGS (8, 11, 13), we did not find any differences in MICs between the strains harboring the *erm*(B) and *mef*(A) genes and those harboring *erm*(B) alone. Therefore, the MIC makes it impossible to know which genes are present in each strain and whether it is necessary to perform a genotypic analysis. No *erm*(B), *erm*(A), *erm*(C), or *mef*(A) genes were detected in one *S. mitis* isolate with the M phenotype.

Figure 1 shows the distribution of macrolide resistance genes according to telithromycin MICs. The strains harboring the *mef*(A) gene showed a range of ≤0.015 to 0.25 μg/ml, while all strains for which the MIC was ≥0.5 μg/ml harbored the *erm*(B) gene, either alone or with the *mef*(A) gene. All telithro-

TABLE 2. Distribution of erythromycin resistance genes according to erythromycin resistance phenotypes and to the different streptococcus groups

Streptococcus group	No. of ER strains[a]	Phenotype	No. (%)	No. with genotype		
				erm(B)	*mef*(A)	*erm*(B) plus *mef*(A)
S. mitis	33	M	17 (51)	0	16	0
		$iMLS_B$	0	0	0	0
		$cMLS_B$	16 (49)	12	0	4
S. anginosus	18	M	2 (11)	0	2	0
		$iMLS_B$	1 (6)	1	0	0
		$cMLS_B$	15 (83)	12	0	3
S. sanguis	11	M	7 (64)	0	7	0
		$iMLS_B$	0	0	0	0
		$cMLS_B$	4 (36)	4	0	0
S. salivarius	3	M	2 (67)	0	2	0
		$iMLS_B$	1 (33)	1	0	0
		$cMLS_B$	0	0	0	0
S. bovis	14	M	0	0	0	0
		$iMLS_B$	1 (7)	1	0	0
		$cMLS_B$	13 (93)	12	0	1

[a] ER, erythromycin resistant.

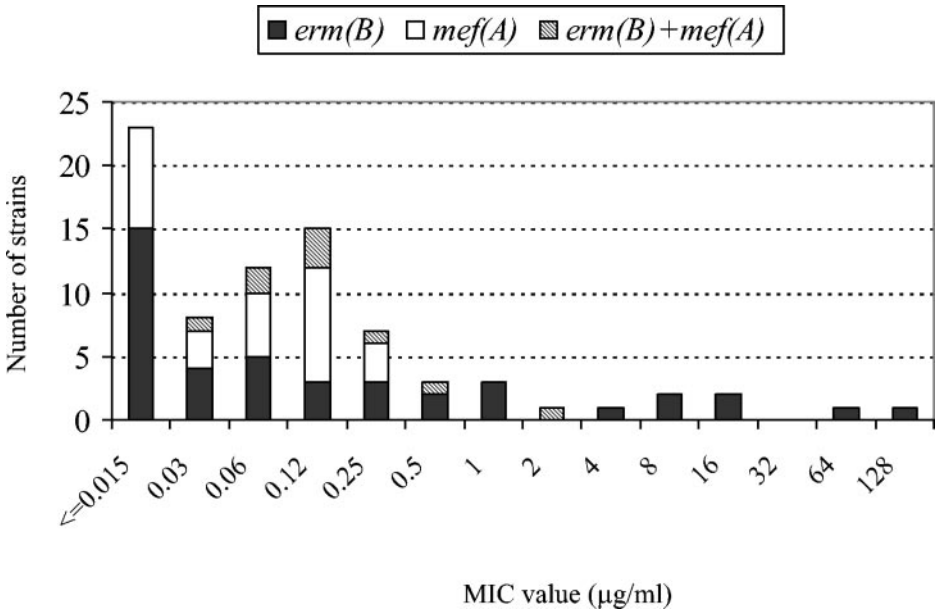

FIG. 1. Distribution of macrolide resistance genes according to the telithromycin MICs.

mycin-resistant *S. bovis* isolates harbored the *erm*(B) gene, although not all *erm*(B)-positive *S. bovis* isolates were resistant to telithromycin.

Walsh et al. (23) found an L22 riboprotein mutation and a 210-bp *erm*(B) attenuator deletion in an *S. pneumoniae* telithromycin-resistant strain which was generated in vitro. Quite recently, there have been reports of *erm*(B)-positive *S. pneumoniae* telithromycin-resistant clinical isolates which also exhibited *erm*(B) attenuator mutations (D. J. Farrell, I. Morrissey, S. Bakker, and D. Felmingham, Abstr. 14th Eur. Cong. Clin. Microbiol. Infect. Dis., abstr. 1465, 2004).

Thus, telithromycin resistance in streptococci might depend either on the species, at the level of *erm*(B) gene expression, as occurs in *S. pneumoniae*, or on an additional mechanism.

The results of the present investigation and the data from the literature suggest that the clinical microbiology laboratory should carry out an accurate identification of VGS at species level, as differences in the patterns of susceptibility to the antimicrobial agents and in the mechanisms responsible for this resistance are observed. The finding of *S. bovis* group isolates showing telithromycin resistance implies the necessity of further studies to determine the mechanisms responsible for this resistance.

This work was supported by grant FIS PI02/0037 from the Fondo de Investigación Sanitaria, Madrid, Spain.

REFERENCES

1. **Alcaide, F., M. A. Benítez, J. Carratalá, F. Gudiol, J. Liñares, and R. Martín.** 2001. In vitro activities of the new ketolide HMR3647 (telithromycin) in comparison with those of eight other antibiotics against viridans group streptococci isolated from blood of neutropenic patients with cancer. Antimicrob. Agents Chemother. **45:**624–626.
2. **Aracil, B., M. Miñambres, J. Oteo, C. Torres, J. L. Gómez-Garcés, and J. I. Alós.** 2001. High prevalence of erythromycin-resistant and clindamycin-susceptible (M phenotype) viridans streptococci from pharyngeal samples: a reservoir of *mef* genes in commensal bacteria. J. Antimicrob. Chemother. **48:**587–595.
3. **Arpin, C., M.-H. Canron, J. Maugein, and C. Quentin.** 1999. Incidence of *mefA* and *mefE* genes in viridans group streptococci. Antimicrob. Agents Chemother. **43:**2335–2336.
4. **Coykendall, A. L.** 1989. Classification and identification of the viridans streptococci. Clin. Microbiol. Rev. **2:**315–328.
5. **de Azavedo, J. C. S., L. Trpeski, S. Pong-Porter, S. Matsumura, the Canadian Bacterial Surveillance Network, and D. E. Low.** 1999. In vitro activities of fluoroquinolones against antibiotic-resistant blood culture isolates of viridans group streptococci from across Canada. Antimicrob. Agents Chemother. **43:**2299–2301.
6. **Diekema, D. J., M. L. Beach, M. A. Pfaller, R. N. Jones, and the SENTRY Participants Group.** 2001. Antimicrobial resistance in viridans group streptococci among patients with and without the diagnosis of cancer in the USA, Canada and Latin America. Clin. Microb. Infect. **7:**152–157.
7. **Doern, G. V., M. J. Ferraro, A. B. Brueggemann, and K. L. Ruoff.** 1996. Emergence of high rates of antimicrobial resistance among viridans group streptococci in the United States. Antimicrob. Agents Chemother. **40:**891–894.
8. **Gershon, A. S., J. C. S. de Azavedo, A. McGeer, K. J. Ostrowska, D. Church, D. J. Hoban, G. K. M. Harding, K. Weiss, L. Abbott, F. Smaill, M. Gourdeau, G. Murray, and D. E. Low.** 2002. Activities of new fluoroquinolones, ketolides, and other antimicrobials against blood culture isolates of viridans group streptococci from across Canada. Antimicrob. Agents Chemother. **46:**1553–1556.
9. **Jacobs, J. A., G. J. van Baar, N. H. H. J. London, J. H. T. Tjhie, L. M. Schouls, and E. E. Stobberingh.** 2001. Prevalence of macrolide resistance genes in clinical isolates of the *Streptococcus anginosus* ("*S. milleri*") group. Antimicrob. Agents Chemother. **45:**2375–2377.
10. **Lyytikäinen, O., M. Rautio, P. Carlson, V.-J. Anttila, R. Vuento, H. Sarkkinen, A. Kostiala, M. L. Vaisanen, A. Kanervo, and P. Ruutu.** 2004. Nosocomial bloodstream infections due to viridans streptococci in haematological and non-haematological patients: species distribution and antimicrobial resistance. J. Antimicrob. Chemother. **53:**631–634.
11. **Malhotra-Kumar, S., C. Lammens, A. Martel, C. Mallentjer, S. Chapelle, J. Verhoeven, M. Wijdooghe, F. Haesebrouck, and H. Goossens.** 2004. Oropharyngeal carriage of macrolide-resistant viridans group streptococci: a prevalence study among healthy adults in Belgium. J. Antimicrob. Chemother. **53:**271–276.
12. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing; 14th informational supplement, M100-S14. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. **Perez-Trallero, E., D. Vicente, M. Montes, J. M. Marimon, and L. Piñeiro.** 2001. High proportion of pharyngeal carriers of commensal streptococci resistant to erythromycin in Spanish adults. J. Antimicrob. Chemother. **48:**225–229.
14. **Rennenberg, J., L. L. Niemann, and E. Gustschik.** 1997. Antimicrobial susceptibility of 278 streptococcal blood isolates to seven antimicrobial agents. J. Antimicrob. Chemother. **39:**135–140.
15. **Rodríguez-Avial, I., C. Rodríguez-Avial, E. Culebras, and J. J. Picazo.** 2003.

Distribution of tetracycline resistance genes *tet*(M), tet(O), *tet*(L) and *tet*(K) in blood isolates of viridans group streptococci harbouring *erm*(B) and *mef*(A) genes. Susceptibility to quinupristin/dalfopristin and linezolid. Int. J. Antimicrob. Agents **21:**536–541.

16. **Ruoff, K., R. A. Whiley, and D. Beighton.** 2003. *Streptococcus*, p. 405–421. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
17. **Seppälä, H., M. Haanperä, M. Al-Juhaish, H. Järvinen, J. Jalava, and P. Houvinen.** 2003. Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. J. Antimicrob. Chemother. **52:**636–644.
18. **Seppälä, H., M. Skurnik, H. Soini, M. C. Roberts, and P. Huovinen.** 1998. A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes*. Antimicrob. Agents Chemother. **42:**257–262.
19. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
20. **Teng, L. J., P. R. Hsueh, Y. C. Cheng, S. W. Ho, and K. T. Luh.** 1998. Antimicrobial susceptibility of viridans group streptococci in Taiwan with an emphasis on the high rates of resistance to penicillin and macrolides in *Streptococcus oralis*. J. Antimicrob. Chemother. **41:**621–627.
21. **Teng, L. J., P. R. Hsueh, S. W. Ho, and K. T. Luh.** 2001. High prevalence of inducible erythromycin resistance among *Streptococcus bovis* isolates in Taiwan. Antimicrob. Agents Chemother. **45:**3362–3365.
22. **Uh, Y., D. H. Shin, I. H. Jang, G. Y. Hwang, M. K. Lee, K. J. Yoon, and H. Y. Kim.** 2004. Antimicrobial susceptibility patterns and macrolide resistance genes of viridans streptococci from blood cultures in Korea. J. Antimicrob. Chemother. **53:**1095–1097.
23. **Walsh, F., J. Willcock, and S. Amyes.** 2003. High-level telithromycin resistance in laboratory-generated mutants of *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **52:**345–353.