Oral Microbiology Immunology 2005: 20: 35–38
Printed in Singapore. All rights reserved

Oral Microbiology and Immunology

Short communication

In vitro activity of telithromycin against *mefA* and *ermB* erythromycin-resistant viridans streptococci isolated from bacteremia of oral origin in Spain

I. Tomás[1], M. Alvarez[2], C. López-Meléndez[2], J. Limeres[1], M. Tomás[3], P. Diz[1]

[1]Department of Special Needs, School of Medicine and Dentistry, Santiago de Compostela University, Santiago de Compostela, [2]Research Laboratory, Department of Clinical Microbiology, Xeral-Cíes Hospital, Vigo, [3]Research Laboratory, Department of Clinical Microbiology, Juan Canalejo Hospital, La Coruóña, Spain.

Tomás I, Alvarez M, López-Meléndez C, Limeres J, Tomás M, Diz P. In vitro *activity of telithromycin against* mefA *and* ermB *erythromycin-resistant viridans streptococci isolated from bacteremia of oral origin in Spain.*
Oral Microbiol Immunol 2005: 20: 35–38. © Blackwell Munksgaard, 2005.

Erythromycin-resistant viridans streptococci are often responsible of bacteremias following dental manipulations. The aim of this study was to evaluate the *in vitro* activity of telithromycin against erythromycin-susceptible and erythromycin-resistant viridans streptococci. Venous blood samples were collected from 84 patients, 30 s after dental extractions. Viridans streptococci were identified by conventional microbiological techniques. A set of 36 viridans streptococci were selected to investigate the *in vitro* activity of telithromycin by the agar dilution method. Macrolide resistance genes were amplified with specific primers for the *mefA* and *ermB* genes and visualized by electrophoresis. For erythromycin-susceptible viridans streptococci, telithromycin minimal inhibitory concentration$_{90}$ (MIC$_{90}$) was <0.008 μg/ml. For erythromycin-resistant viridans streptococci, telithromycin MIC$_{90}$ was 1 μg/ml. The MIC$_{90}$ to telithromycin of the *mefA*-positive and *ermB*-negative viridans streptococci was lower than that of the *mefA*-negative and *ermB*-positive viridans streptococci (0.128 μg/ml *versus* 1 μg/ml). The *in vitro* activity of telithromycin was high, irrespective of the erythromycin susceptibility and the mechanism of erythromycin resistance identified.

Key words: bacteremia; dental extractions; viridans streptococci; telithromycin

Pedro Diz Dios, C./ Panamá 2; 2° dcha, 36203 Vigo, Spain
Tel.: + 34 981 563100, ext. 12344;
fax: +34 981 562226;
e-mail: pdiz@usc.es
Accepted for publication June 18, 2004

The systemic dissemination of oral pathogens to distant body sites may cause bacterial endocarditis, brain abscesses, and other life-threatening infections, with viridans streptococci frequently being implicated (5).

In Spain, erythromycin is considered the antimicrobial of choice in the prophylaxis of focal infections for penicillin-allergic patients with risk factors who undergo certain dental procedures (24, 25). Recently, our group found that after dental extractions there was a high prevalence of bacteremia caused by erythromycin-resistant viridans streptococci (submitted). This is of particular concern since the lack of erythromycin susceptibility is associated with resistance to first-line antimicrobials recommended in the current prophylactic regimes such as long half-life macrolides (azythromycin and clarythromycin) and clindamycin (4, 20).

In this scenario, new antimicrobials such as telithromycin have emerged. Few studies about the *in vitro* activity of telithromycin against viridans streptococci of oral origin have been published (10, 18). The present study evaluated the *in vitro* activity of telithromycin against erythromycin-susceptible and erythromycin-resistant viridans streptococci with a well characterized erythromycin resistance mechanism (*mefA* and *ermB* genes)

isolated from the bloodstream after dental extractions.

Patients and methods

Venous blood samples were collected from 84 patients, 30 s after dental extractions. Viridans streptococci were identified as aerobic cocci that display chains in the Gram stain, were catalase-negative, optochin-resistant, pyrrolidonyl arylamidase-negative, leucine aminopeptidase-positive and did not grow in 6.5% NaCl broth. We used the identification scheme proposed by Ruoff et al. (16, 17), which includes five groups of viridans streptococci:

Streptococcus mitis group, *Streptococcus anginosus* group, *Streptococcus mutans* group, *Streptococcus salivarius* group and *Streptococcus bovis* group.

Eighty-one streptococci were isolated, and their susceptibilities to erythromycin and clindamycin evaluated. A set of 36 of these viridans streptococci were selected to investigate the *in vitro* activity of telithromycin: 11 were erythromycin-susceptible strains, 15 erythromycin-resistant strains and 10 erythromycin- and clindamycin-resistant strains. The following streptococcal groups were identified:

- *Streptococcus mitis* group, n = 25;
- *Streptococcus anginosus* group, n = 7;
- *Streptococcus salivarius* group, n = 2;
- *Streptococcus mutans* group, n = 1;
- *Streptococcus bovis* group, n = 1.

Minimal inhibitory concentrations (MICs) were determined by the agar dilution method (15). An inoculum of 10^4 colony-forming units (CFU)/spot were placed on Mueller-Hinton II agar (Becton Dickinson Microbiology Systems, Cockeysville, MD), pH 7.3, containing 5% sheep blood, with a 19 PIN Hand Inoculator (Mast Laboratories Ltd., Mereyside, UK). The plates, which contained doubling dilutions of telithromycin, were incubated for 18–24 h at 37°C under aerobic conditions. For telithromycin preliminary breakpoints, MICs proposed by the manufacturer (Aventis Pharma, Romainville, France) were applied (susceptible ≤ 0.5 μg/ml; resistant ≥ 4 μg/ml). These breakpoints have been validated by two committees in Europe, MENSURA (Mesa Española de Normalización de la Sensibilidad y Resistancia a los Antimicrobianos) and CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie) (21). *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 were used as controls.

DNA extraction for polymerase chain reaction (PCR) amplification was performed using the InstaGene Matrix (Biorad Laboratories, Hercules, CA) according to the manufacturer's instructions. Macrolide resistance genes were amplified with specific primers for the *mefA* and *ermB* genes (2, 22) and visualized following agarose gel electrophoresis.

Results

The MIC_{50} and MIC_{90} to telithromycin for all viridans streptococci tested were 0.032 μg/ml and 1 μg/ml respectively (MIC range, <0.008–1 μg/ml). Applying the telithromycin preliminary breakpoints, none of the isolates was resistant to telithromycin.

The MIC range, MIC_{50} and MIC_{90} to erythromycin, clindamycin and telithromycin of the erythromycin-susceptible and erythromycin-resistant viridans streptococci with a well characterized erythromycin resistance mechanism (*mefA* and *ermB* genes) is detailed in Table 1. For the erythromycin-susceptible viridans streptococci, the telithromycin MIC_{90} value (<0.008 μg/ml) was lower than the values to erythromycin and clindamycin (0.128 μg/ml for both antimicrobials).

For the erythromycin-resistant viridans streptococci, the telithromycin MIC_{90} was 1 μg/ml (MIC range, <0.008–1 μg/ml). The MIC_{90} to telithromycin of the *mefA*-positive and *ermB*-negative viridans streptococci was lower than that of the *mefA*-negative and *ermB*-positive viridans streptococci (0.128 μg/ml *versus* 1 μg/ml). Only one strain carried both genes, showing a telithromycin MIC of 0.064 μg/ml. The MIC range to telithromycin for *mefA*- and *ermB*-negative viridans streptococci was 0.064–0.256 μg/ml.

Discussion

In agreement with Alcaide et al. (1), telithromycin showed higher *in vitro* activity than erythromycin and clindamycin against erythromycin-susceptible viridans streptococci. Low telithromycin MIC values have been also detected in other erythromycin-susceptible streptococci such as *S. pneumoniae* (26), *Streptococcus pyogenes* (19) and *Streptococcus agalactiae* (11).

Table 1. MIC range, MIC_{50} and MIC_{90} to erythromycin, clindamycin and telithromycin of the erythromycin-susceptible and erythromycin-resistant viridans streptococci

Antimicrobial	Resistance profile to E (no. of VS)	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Erythromycin	E-susceptible VS (11)	0.016–0.128	0.032	0.128
	E-resistant VS (25)	1–256	16	256
	mefA-positive and *ermB*-negative VS (10)	1–256	4	256
	mefA-negative and *ermB*-positive VS (10)	256	256	256
	mefA- and *ermB*-positive VS (1)	256		
	mefA- and *ermB*-negative VS (4)	4–16		
Clindamycin	E-susceptible VS (11)	0.032–0.128	0.032	0.128
	E-resistant VS (25)	0.016–256	0.256	256
	mefA-positive and *ermB*-negative VS (10)	0.016–0.512	0.064	0.512
	mefA-negative and *ermB*-positive VS (10)	16–256	256	256
	mefA- and *ermB*-positive VS (1)	256		
	mefA- and *ermB*-negative VS (4)	0.032–0.256		
Telithromycin	E-susceptible VS (11)	<0.008–0.016	<0.008	<0.008
	E-resistant VS (25)	<0.008–1	0.128	1
	mefA-positive and *ermB*-negative VS (10)	0.016–0.128	0.064	0.128
	mefA-negative and *ermB*-positive VS (10)	<0.008–1	1	1
	mefA- and *ermB*-positive VS (1)	0.064		
	mefA- and *ermB*-negative VS (4)	0.064–0.256		

VS= viridans streptococci, E= erythromycin.

In other viridans streptococci series (6, 18), telithromycin was also active against highly erythromycin- and clindamycin-resistant viridans streptococci. These findings are similar to those detected in erythromycin-resistant *S. pneumoniae* (13) and *S. agalactiae* (3), but differ from those observed in *S. pyogenes* (7, 12). In relation to the erythromycin resistance mechanism, contrary to the findings reported by Seppälä et al. (18), we found that the telithromycin MICs were higher for *ermB*-positive viridans streptococci (MIC_{90}=1 μg/ml) than for strains harboring the *mefA* gene (MIC_{90}=0.128 μg/ml). In previous viridans streptococci and *S. pneumoniae* series, other authors have also found that although all the telithromycin MICs were in the susceptible range, the *in vitro* activity of this ketolide was only slightly affected by the *ermB*-mediated erythromycin resistance mechanism (10, 13). Jalava et al. (7) and Nagai et al. (14) even demonstrated that for some *S. pyogenes* which constitutively express the *ermB* gene, the telithromycin MICs were so high that the strains may be clinically resistant to this ketolide. Structural changes in any of the ribosomal components could help explain the differences found in the effect of the *ermB* genotype on the telithromycin susceptibility among different streptococcal species (18, 27). Furthermore, Liu & Douthwaite (8) have suggested that the disparities in ketolide resistance levels could be correlated with variation in the degree of *ermB*-encoded methylation at nucleotide A2058.

Shortridge et al. (19) found that telithromycin MICs for *S. pneumoniae* harboring both *mefA* and *ermB* genes were low (CMI_{90}= 0.25 μg/ml), suggesting that despite this genetic duplicity, telithromycin activity is similar to that observed against strains with only an efflux resistance mechanism. In the present study, one viridans streptococci carried both genes, showing a telitromycin MIC of 0.064 μg/ml. In our series, the telithromycin MICs for *mefA*- and *ermB*-negative viridans streptococci resistant to erythromycin were low. These strains could harbor other *erm* genes or any other erythromycin resistance mechanism, as has been recently described in *S. pneumoniae* (23) and *S. pyogenes* (9). In this sense, it has been proved that the constitutive expression of other *erm* genes such as *ermA* gene decreases neither the *in vitro* activity to telithromycin (12) nor the presence of mutations in streptococcal rRNA or ribosomal proteins (19).

All the isolates in the present study were susceptible to telithromycin, since they were inhibited at a concentration ≤ 1 μg/ml, irrespective of the erythromycin susceptibility and the mechanism of erythromycin resistance. This high *in vitro* activity, coupled with a favorable pharmacokinetic profile and the lack of inducible properties (27), make this antimicrobial a promising alternative for prophylaxis of streptococcal focal infections associated with certain dental procedures when the administration of beta-lactam agents is inappropriate.

References

1. Alcaide F, Benítez MA, Carratalá J, Gudiol F, Liñares J, Martín R. *In vitro* activities of the new ketolide HMR 3647 (telithromycin) in comparison with those of eight other antibiotics against viridans group streptococci isolated from blood of neutropenic patients with cancer. Antimicrob Agents Chemother 2001: **45**: 624–626.
2. Arpin C, Daube H, Tessier F, Quentin C. Presence of *mefA* and *mefE* genes in *Streptococcus agalactiae*. Antimicrob Agents Chemother 1999: **43**: 944–946.
3. Betriu C, Culebras E, Gómez M, Rodríguez-Avial I, Sánchez A, Agreda MC, et al. Erythromycin and clindamycin resistance and telithromycin susceptibility in *Streptococcus agalactiae*. Antimicrob Agents Chemother 2003: **47**: 1112–1114.
4. Dajani AD, Taubert KA, Wilson W, Bolger AF, Bayer A, Ferrieri P, et al. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. JAMA 1997: **277**: 1794–1801.
5. Gendron R, Grenier D, Maheu-Robert L. The oral cavity as a reservoir of bacterial pathogens for focal infections. Microbes Infect 2000: **2**: 897–906.
6. Gershon AS, de Azavedo J, McGeer A, Ostrowska KI, Church D, Hoban DJ, et al. Activities of new fluoroquinolones, ketolides and other antimicrobials against blood culture isolates of viridans group streptococci from across Canada 2000. Antimicrob Agents Chemother 2002: **46**: 1553–1556.
7. Jalava J, Kataja J, Seppälä H, Huovinen P. *In vitro* activities of the novel ketolide telithromycin (HMR 3647) against erythromycin resistant *Streptococcus* species. Antimicrob Agents Chemother 2001: **45**: 789–793.
8. Liu M, Douthwaite S. Activity of the ketolide telithromycin is refractory to *erm* monomethylation of bacterial rRNA. Antimicrob Agents Chemother 2002: **46**: 1629–1633.
9. Malbruny B, Nagai K, Coquemont M, Bozdogan B, Andrasevic AT, Hupkova H, et al. Resistance to macrolides in clinical isolates of *Streptococcus pyogenes* due to ribosomal mutations. J Antimicrob Chemother 2002: **49**: 935–939.
10. Malhotra-Kumar S, Lammens, C, Martel A, Mallentjer C, Chapelle S, Verhoeven J, et al. Oropharyngeal carriage of macrolide-resistant viridans group streptococci: a prevalence study among healthy adults in Belgium. J Antimicrob Chemother 2004: **53**: 271–276.
11. Mikamo H, Yin XH, Ninomiya M, Tamaya T. *In vitro* and *in vivo* antibacterial activities of telithromycin. Chemotherapy 2003: **49**: 62–65.
12. Morosini MI, Cantón R, Loza E, Del Campo R, Almaraz F, Baquero F. *Streptococcus pyogenes* isolates with characterized macrolide resistance mechanisms in Spain: *in vitro* activities of telithromycin and cethromycin. J Antimicrob Chemother 2003: **52**: 50–55.
13. Morosini MI, Cantón R, Loza E, Negri MC, Galán JC, Almaraz F, et al. *In vitro* activity of telithromycin against spanish *Streptococcus pneumoniae* isolates with characterized macrolide resistance mechanisms. Antimicrob Agents Chemother 2001: **45**: 2427–2431.
14. Nagai K, Appelbaum PC, Davies TA, Kelly LM, Hoellman DB, Andrasevic AT, et al. Susceptibility to telithromycin in 1,011 *Streptococcus pyogenes* isolates from 10 central and eastern european countries. Antimicrob Agents Chemother 2002: **46**: 371–377.
15. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th edn. Approved Standard M7-A5. Wayne, PA: NCCLS, 2000.
16. Ruoff KL. Miscellaneous catalase-negative, Gram-positive cocci: emerging opportunists. J Clin Microbiol 2002: **40**: 1129–1133.
17. Ruoff KL, Whiley RA, Beighton D. Streptococcus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of clinical microbiology, 7th edn. Washington DC: Am Soc Microbiol, 1999: 283–295.
18. Seppälä H, Haanperä M, Al-Juhaish M, Järvinen H, Jalava J, Huovinen, P. Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. J Antimicrob Chemother 2003: **52**: 636–644.
19. Shortridge VD, Zhong P, Cao Z, Beyer JM, Almer LS, Ramer NC, et al. Comparison of *in vitro* activities of ABT-773 and telithromycin against macrolide susceptible and resistant streptococci and staphylococci. Antimicrob Agents Chemother 2002: **46**: 783–786.
20. Simmons NA. British Society for Antimicrobial Chemotherapy Working Party report. Recommendations for endocarditis prophylaxis. J Antimicrob Chemother 1993: **31**: 437–438.
21. Soussy CJ, Carret G, Cavallo JD, Chardon H, Chidiac C, Choutet P, et al. Antibiogram Committee of the French Microbiology Society. Report 2000–01. Pathol Biol (Paris) 2000: **48**: 832–871.
22. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother 1996: **40**: 2562–2566.
23. Tait-Kamradt A, Davies T, Appelbaum PC, Depardieu F, Courvalin P, Petitpas J, et al. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob Agents Chemother 2000: **44**: 3395–3401.

24. Tomás I, Diz P, Seoane J, Limeres J. Pautas de profilaxis antibiótica de endocarditis bacteriana en pacientes sometidos a tratamiento odontológico. Rev Clin Esp 2001: **201**: 21–24.
25. Tomás I, Diz P, Limeres J, Outumuro M, Caamaño F, Fernández J, et al. Pautas de profilaxis antibiótica de endocarditis bacteriana recomendadas por los odontólogos en España. Med Oral 2004: **9**: 56–62.
26. Weiss K, Guilbault C, Cortes L, Restieri C, Low DE and the EQUERE project. Genotypic characterization of macrolide-resistant strains of *Streptococcus pneumoniae* isolated in Quebec, Canada, and *in vitro* activitity of ABT-773 and telithromycin. J Antimicrob Chemother 2002: **50**: 403–406.
27. Zhanel GG, Walters M, Noreddin A, Vercaigne LM, Wierzbowski A, Embil JM, et al. The ketolides. A critical review. Drugs 2002: **62**: 1771–1804.