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Activity of Tigecycline against Coryneform Bacteria of Clinical Interest and *Listeria monocytogenes*▽

Carlos Salas,[1] Jorge Calvo,[1] and Luis Martínez-Martínez[1,2]*

Service of Microbiology, Hospital Universitario Marqués de Valdecilla,[1] and Department of Molecular Biology, School of Medicine,[2] Santander, Spain

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The activities of tigecycline and eight other agents were evaluated against 220 coryneform bacteria and 42 *Listeria monocytogenes* isolates. All strains were inhibited by tigecycline at 0.5 μg/ml, except for 11 *Corynebacterium striatum* strains that were inhibited at 1 μg/ml. Tigecycline shows good in vitro activity against coryneform bacteria and *L. monocytogenes*.

Coryneform bacteria may cause a variety of infectious processes, being involved most frequently in soft-tissue infections, bacteremia, respiratory tract infections, and endocarditis (3, 5).

Several reports are available on the activity of antimicrobial agents against coryneform bacteria (6–9, 12). Different methods have been used for susceptibility testing of coryneform bacteria (6–9, 12). Recently, the Clinical and Laboratory Standards Institute (CLSI) has proposed a reference microdilution method (2) for testing these organisms. A large proportion of *Corynebacterium jeikeium*, *Corynebacterium urealyticum*, and *Corynebacterium amycolatum* strains are multiresistant, and very few agents (of which the largest clinical experience has been obtained with glycopeptides) remain universally active against these organisms. Other species may be susceptible in vitro to commonly used antimicrobial agents, but beyond in vitro data, reliable clinical evidence supporting these in vitro observations is lacking.

Tigecycline is the first glycylcycline (a group of agents related to tetracyclines) introduced in clinical use. It has been approved for parenteral treatment of complicated skin and skin structure infections and complicated intra-abdominal infections (13). It shows excellent in vitro activity against both gram-positive and gram-negative bacteria, including multiresistant isolates, and is also active against many organisms resistant to classical tetracyclines.

The objective of this study was to evaluate the in vitro activity of tigecycline in comparison with other compounds against different species of coryneform bacteria and *Listeria monocytogenes* isolated from clinical samples.

The following organisms isolated from clinical samples (one per patient) were evaluated: *C. amycolatum* ($n = 31$), *C. jeikeium* ($n = 25$), *Corynebacterium minutissimum* ($n = 9$), *Corynebacterium pseudodiphtheriticum* ($n = 22$), *Corynebacterium striatum* ($n = 59$), *C. urealyticum* ($n = 23$), *Corynebacterium xerosis* ($n = 5$), *Arcanobacterium haemolyticum* ($n = 26$), *Rhodococcus equi* ($n = 20$), and *L. monocytogenes* ($n = 42$). Organisms had been isolated in the period of 2003 to 2006 at the Hospital Universitario Marqués de Valdecilla, Santander, Spain ($n = 172$) or were provided from several centers in Spain ($n = 90$) (see the acknowledgments for details). Microorganisms were identified according to the method of Funke et al. (3), using API-CORYNE strips (BioMérieux, Marcy l'Étolie, France) and additional phenotypic tests when necessary. Sequencing of the 16S rRNA gene was performed when phenotypic traits were unable to provide a definitive identification. After identification, organisms were maintained in tryptic soy broth-10% glycerol at −80°C. They were subcultured twice on Columbia agar-5% sheep blood (Becton Dickinson) before susceptibility testing.

The MICs of tigecycline (Wyeth, Pearl River, NY), doxycycline (Sigma, Madrid, Spain), minocycline (Sigma), tetracycline (Sigma), vancomycin (Sigma), cefuroxime (Sigma), clindamycin (Sigma), gentamicin (Sigma), and levofloxacin (Aventis Pharma, Antony, France) were determined by microdilution using Mueller-Hinton broth with 3% laked horse blood according to the guidelines of the M45-A document from CLSI (2). Tigecycline, doxycycline, and clindamycin were tested in the range of 0.015 to 16 μg/ml; all other compounds were tested in the range of 0.03 to 32 μg/ml. A fresh solution of tigecycline prepared from reference powder was used every day that a batch of microdilution plates was used, until completion of the study. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains for susceptibility testing assays. MICs for these two strains were within the accepted ranges indicated by the CLSI.

MIC_{50}s, MIC_{90}s, and MIC ranges of tigecycline and comparators against the tested strains are presented in Table 1. The CLSI has defined clinical breakpoints for several agents against coryneform bacteria. These breakpoints are often adapted from those previously established for staphylococci. However, the CLSI, as well as other agencies or committees, have not yet defined breakpoints for tigecycline for coryneform bacteria or *L. monocytogenes*. As a reference, the breakpoint for susceptibility of staphylococci defined by the FDA is 0.5 μg/ml.

All coryneform bacteria, except 11 out of 59 *C. striatum* isolates, were inhibited by 0.5 μg/ml of tigecycline. These 11 *C. striatum* were inhibited at 1 μg/ml of tigecycline. Although the CLSI recommends using blood-supplemented broth for testing

* Corresponding author. Mailing address: Service of Microbiology, Hospital Universitario Marqués de Valdecilla, Avda. de Valdecilla s/n, 39008 Santander, Spain. Phone: 34 942 202580. Fax: 34 942 202660. E-mail: lmartinez@humv.es.

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TABLE 1. Ranges, MIC_{50}s, and MIC_{90}s of antimicrobial agents for coryneform bacteria and *L. monocytogenes*

Organism (*n*)	Drug	MIC (μg/ml)			Organism (*n*)	Drug	MIC (μg/ml)		
		Range	50%	90%			Range	50%	90%
C. amycolatum (31)	Tigecycline	≤0.015–0.5	0.125	0.25	*C. urealyticum* (23)	Tigecycline	≤0.015–0.25	0.03	0.06
	Doxycycline	0.03–1	0.06	0.5		Doxycycline	≤0.015–16	0.125	4
	Minocycline	≤0.03–0.5	0.06	0.25		Minocycline	≤0.03–>32	0.06	0.5
	Tetracycline	0.06–16	0.125	0.5		Tetracycline	≤0.03–>32	0.25	16
	Clindamycin	0.06–>16	>16	>16		Clindamycin	≤0.015–>16	>16	>16
	Vancomycin	0.125–0.5	0.25	0.5		Vancomycin	≤0.03–0.5	0.25	0.5
	Levofloxacin	0.06–>32	>32	>32		Levofloxacin	≤0.03–>32	>32	>32
	Cefuroxime	0.125–>32	0.5	2		Cefuroxime	4–>32	>32	>32
	Gentamicin	0.06–>32	8	16		Gentamicin	0.06–>32	>32	>32
C. jeikeium (25)	Tigecycline	≤0.015–0.125	≤0.015	0.03	*C. xerosis* (5)	Tigecycline	≤0.015–0.125	0.125	NA
	Doxycycline	≤0.015–4	0.03	1		Doxycycline	0.125–1	0.5	NA
	Minocycline	0.06–0.5	0.06	0.25		Minocycline	0.03–0.25	0.25	NA
	Tetracycline	0.06–8	0.06	2		Tetracycline	0.06–1	0.5	NA
	Clindamycin	>16	>16	>16		Clindamycin	0.125–>16	>16	NA
	Vancomycin	0.06–0.25	0.06	0.25		Vancomycin	0.125–0.5	0.25	NA
	Levofloxacin	0.06–>32	4	>32		Levofloxacin	0.125–32	32	NA
	Cefuroxime	2–>32	>32	>32		Cefuroxime	0.125–2	2	NA
	Gentamicin	0.06–>32	>32	>32		Gentamicin	≤0.03–16	8	NA
C. minutissimum (9)	Tigecycline	≤0.015–0.003	≤0.015	NA[a]	*A.haemolyticum*(26)	Tigecycline	≤0.015–0.06	≤0.015	≤0.015
	Doxycycline	0.06–1	0.25	NA		Doxycycline	≤0.015–16	0.125	8
	Minocycline	≤0.03–1	≤0.03	NA		Minocycline	≤0.03–4	≤0.03	4
	Tetracycline	0.125–2	0.25	NA		Tetracycline	≤0.03–16	0.125	16
	Clindamycin	0.06–>16	>16	NA		Clindamycin	≤0.015–0.03	≤0.015	≤0.015
	Vancomycin	0.125–0.25	0.25	NA		Vancomycin	0.125–0.5	0.25	0.5
	Levofloxacin	≤0.03–>32	0.06	NA		Levofloxacin	0.125–1	0.5	0.5
	Cefuroxime	0.25–16	0.5	NA		Cefuroxime	0.06–>32	0.125	0.25
	Gentamicin	≤0.03–4	0.06	NA		Gentamicin	0.25–1	0.5	0.5
C. pseudodiphtheriticum (22)	Tigecycline	≤0.015–0.06	≤0.015	0.03	*R. equi* (20)	Tigecycline	≤0.015–0.5	0.25	0.5
	Doxycycline	≤0.015–0.25	0.125	0.125		Doxycycline	≤0.015–4	1	1
	Minocycline	≤0.03–0.06	0.06	0.06		Minocycline	0.06–0.5	0.25	0.25
	Tetracycline	0.06–0.5	0.125	0.25		Tetracycline	0.06–16	8	8
	Clindamycin	≤0.015–>16	>16	>16		Clindamycin	0.03–>16	2	8
	Vancomycin	0.06–0.5	0.25	0.25		Vancomycin	0.125–2	0.5	0.5
	Levofloxacin	0.06–32	0.25	4		Levofloxacin	0.125–2	1	2
	Cefuroxime	≤0.03–2	0.06	0.125		Cefuroxime	0.25–16	8	16
	Gentamicin	≤0.03–1	0.06	0.06		Gentamicin	0.125–2	0.5	1
C. striatum (59)	Tigecycline	≤0.015–1	0.5	1	*L. monocytogenes* (42)	Tigecycline	0.03–0.25	0.06	0.125
	Doxycycline	0.06–>16	8	>16		Doxycycline	0.03–2	0.125	0.125
	Minocycline	≤0.03–16	4	8		Minocycline	≤0.03–4	0.06	0.06
	Tetracycline	0.06–>32	16	>32		Tetracycline	0.06–4	0.5	0.5
	Clindamycin	≤0.03–>16	>16	>16		Clindamycin	0.125–4	1	2
	Vancomycin	0.125–2	0.25	0.5		Vancomycin	0.125–2	0.5	1
	Levofloxacin	≤0.03–>32	>32	>32		Levofloxacin	0.125–2	0.5	1
	Cefuroxime	0.125–>32	1	2		Cefuroxime	4–>32	>32	>32
	Gentamicin	0.06–>32	2	8		Gentamicin	≤0.03–2	0.25	0.125

[a] NA, not applicable (less than 10 isolates tested).

coryneform bacteria, *C. striatum* (and other species commonly isolated from human samples, including *C. amycolatum*) is able to grow in nonsupplemented Mueller-Hinton broth (5–7). MICs of tigecycline were tested against 33 out of the 59 *C. striatum* isolates included in this study for which the MICs of tigecycline were ≥0.125 μg/ml: in 25 isolates, MICs of tigecycline were two to four times lower in plain Mueller-Hinton broth than in blood-supplemented Mueller-Hinton broth. In the absence of additional clinical information, it is not possible to determine the relevance of these differences. It could be possible that MICs of tigecycline for other nonfastidious corynebacteria are also lower in nonsupplemented Mueller-Hinton broth.

All *L. monocytogenes* strains were inhibited by tigecycline at 0.5 μg/ml, with an MIC_{90} of this compound of 0.125 μg/ml, the same value presented in a recent report where 100 isolates were tested (1).

Ninety-six to one hundred percent of isolates of *C. amycolatum*, *C. jeikeium*, *C. minutissimum*, *C. pseudodiphtheriticum*, *C. xerosis*, and *L. monocytogenes* were susceptible to tetracycline, but a significant number of strains of *C. striatum* (58%), *R. equi* (55%), *A. haemolyticum* (35%), and to a lesser extent *C. urealyticum* (12%) were not susceptible to this compound. Doxycycline and minocycline were also poorly active against *C. striatum* and some strains of *C. urealyticum*. MIC_{90}s of tigecycline were lower than those of tetracycline, doxycycline, and minocycline for most of the tested species (being equal for the remaining ones), which indicates that, as already noted for other organisms, tigecycline maintains in vitro activity against corynebacteria for which tetracyclines are not active. In a previous study (4) on 21 isolates of *Corynebacterium* spp., the MIC_{50} and MIC_{90} of tigecycline were 0.06 and 0.125 μg/ml, respectively, similar to those of minocycline and lower than the corresponding ones for doxycycline and tetracycline.

Few studies are available about the mechanisms of resistance of corynebacteria to tetracyclines. TetM is common in *C. striatum* isolates (10) of clinical origin, and *tetAB* genes have been identified in the 50-kb R-plasmid pTP10 from the clinical isolate *C. striatum* M82B (initially thought to be *C. xerosis*) (14). TetA(Z) has also been identified as a tetracycline resistance determinant of plasmid pAG1 from the soil bacterium *Corynebacterium glutamicum* 22243 (15), and the related Tet 33

was recognized in plasmid pTET3 from *C. glutamicum* LP-6 (16). Additional studies would be helpful for understanding the ability of tigecycline to escape the mechanisms of resistance to tetracyclines in the bacteria we have evaluated.

The activities of other antimicrobial agents against organisms included in this study are in agreement with previous reports (5–8, 12). All strains of corynebacteria and *L. monocytogenes* we studied were susceptible to vancomycin. All isolates of *A. haemolyticum* were susceptible to clindamycin, but for other species, the percentages of susceptibility to this agent were rather low: 0% (*C. jeikeium*), 5% (*C. striatum*), 10 to 13% (*C. amycolatum*, *C. minutissimum*, and *R. equi*), and 20 to 26% (*C. xerosis*, *C. urealyticum*, and *C. pseudodiphtheriticum*). Levofloxacin maintained good activity against *A. haemolyticum* and *L. monocytogenes*, but poor activity of this quinolone was observed against most species of *Corynebacterium*, as already published, which may be related to the presence of mutations in *gyrA* and the absence of topoisomerase IV (11). Gentamicin was poorly active against multiresistant species such as *C. jeikeium* (only 8% of isolates were susceptible) and *C. urealyticum* (30% of isolates susceptible); 65% of *C. amycolatum* and 14% of *C. striatum* isolates were nonsusceptible to this aminoglycoside. Cefuroxime also poorly inhibited *C. jeikeium*, *C. urealyticum*, and *L. monocytogenes*.

In conclusion, tigecycline showed an excellent in vitro activity against coryneform bacteria, including several species of *Corynebacterium*, *A. haemolyticum*, and *R. equi.* This suggests that tigecycline may represent a reasonable therapeutic alternative for infections caused by these organisms. It is also active against many gram-negative bacteria (eventually present in mixed infections) not covered by anti-gram-positive drugs such as glycopeptides, which are often referred to in the literature as drugs of choice for these organisms. In addition, it may be considered as an alternative against multiresistant corynebacteria, for which the current options are rather limited. Tigecycline is also active in vitro against *L. monocytogenes*. Its clinical usefulness against this organism warrants further studies.

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