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Activities of ABT-773 against *Listeria monocytogenes* and Coryneform Bacteria of Clinical Interest

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The in vitro activities of ABT-773 were evaluated against 15 *Listeria monocytogenes* strains and 196 coryneform bacteria isolated from clinical samples. One hundred percent of the *L. monocytogenes* strains were inhibited by ≤0.015 μg of ABT-773/ml. MICs of ABT-773 (μg/ml) at which 50% of the isolates tested were inhibited (MIC_{50}s) and MIC_{90}s for other organisms were 0.125 and 0.5 (*Corynebacterium amycolatum*), 1 and >32 (*Corynebacterium jeikeium*), 0.03 and >32 (*Corynebacterium minutissimum*), >32 and >32 (*Corynebacterium pseudodiphtheriticum* and *Corynebacterium urealyticum*), 0.125 and >32 (*Corynebacterium striatum*), and 0.03 and 0.5 (*Rhodococcus equi*), respectively.

Two relevant aspects related to coryneform bacteria have become significant during the last decade: the recognition of the medical importance of some species, including *Corynebacterium jeikeium*, *Corynebacterium urealyticum*, *Corynebacterium striatum*, *Corynebacterium amycolatum*, and *Corynebacterium minutissimum*, and the changes in the taxonomy of these organisms leading to the recognition of a large number of new species and the redefinition of already known organisms (1, 2, 4, 6, 8). It is critical that studies of the activities of antimicrobial agents be based on testing microorganisms identified according to the new taxonomic criteria, in order to really obtain clinically significant information and to allow the comparison of data obtained from different laboratories (3, 5, 13, 19, 24). Unfortunately, there is yet no standardized methodology for susceptibility testing of coryneform bacteria. The NCCLS has not defined breakpoints for clinical categories of antimicrobial agents against coryneform bacteria, and in the case of *Listeria* spp. only the category of susceptibility to ampicillin and penicillin has been suggested (16). There is scant information on the activities of antimicrobial agents against coryneform bacteria (4–6, 9). *C. jeikeium*, *C. urealyticum*, and *C. amycolatum* are usually multiresistant organisms, and only glycopeptides remain universally active against these species (4–6, 18, 19, 22, 23). Some reports suggest that other species may be susceptible to commonly used antimicrobial agents, but we lack reliable clinical evidence supporting these in vitro observations. It is necessary to evaluate the activities of new antimicrobial agents against coryneform bacteria of clinical importance (3, 7, 10–12, 19).

It has been shown previously that ketolides show a broader spectrum of activity than do reference macrolides, being active against macrolide-susceptible gram-positive cocci and against gram-positive organisms in which macrolide resistance is caused by active efflux or inducible production of methylase (21). In a previous study we showed that the new ketolide telithromycin was more active than were 14- and 16-membered macrolides, azithromycin, or clindamycin against many coryneform bacteria and had high in vitro activity against *Listeria monocytogenes* (10). The objective of this study was to evaluate the in vitro activities of ABT-773 in comparison with other compounds against *L. monocytogenes* and different species of coryneform bacteria isolated from clinical samples.

Two hundred eleven organisms isolated from clinical samples at the Department of Clinical Microbiology, University Hospital Virgen Macarena, Seville, Spain, were evaluated, including the following species (number of strains): *L. monocytogenes* (15), *C. amycolatum* (40), *C. jeikeium* (40), *C. minutissimum* (14), *Corynebacterium pseudodiphtheriticum* (12), *C. striatum* (40), *C. urealyticum* (40), and *Rhodococcus equi* (10). Microorganisms were identified according to the method of Funke et al. (4), by using API-CORYNE strips and additional phenotypic tests when necessary. After identification, organisms were maintained in tryptic soy broth–10% glycerol at −80°C. The following reference strains were also tested: *C. jeikeium* ATCC 43734, *C. striatum* ATCC 6940, and *C. urealyticum* ATCC 43042. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains for susceptibility testing assays. The following compounds were studied: ABT-773 (Abbott), cefuroxime (Sigma), clindamycin (Sigma), co-trimoxazole (Gayoso, Madrid, Spain), erythromycin (Sigma), and vancomycin (Sigma). Solutions of antimicrobial agents were prepared on the same day as testing, according to the manufacturer's instructions. The MICs of the above-indicated antimicrobial agents were determined, as previously described, by in-house microdilution according to NCCLS guidelines (15, 16), with the exception that, when *C. jeikeium* and *C. urealyticum* (lipophilic organisms) were tested, the broth was supplemented with 0.5% Tween 80 (Difco, Detroit, Mich.). Plates were inoculated with a suspension (approximately 5×10^5 CFU/ml) in Mueller-Hinton broth (plus 0.5% Tween 80 in the case of *C. jeikeium* and *C. urealyticum*) prepared from bacteria grown on Columbia agar with 5% sheep blood for 24 to 48 h. Plates were incubated, after inoc-

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ulation, at 35°C for 20 to 24 h, or (in the case of *C. jeikeium* and *C. urealyticum*) for up to 48 h, to allow bacterial growth in control (antibiotic-free) wells of the microtiter plate.

The activities of the agents herein tested have been considered in terms of MIC_{50}s (MICs at which 50% of the isolates tested are inhibited), MIC_{90}s, and MIC ranges (Table 1). Additionally, the numbers and percentages of strains inhibited at each concentration of ABT-773 and the related drugs erythromycin and clindamycin have also been determined (Table 2). All strains of *L. monocytogenes* were inhibited by ≤0.015 μg of ABT-773/ml. MIC_{90}s of ABT-773 were ≥32- and ≥128-fold lower than those of erythromycin and clindamycin, respectively. Interestingly, this value is also much lower than that recently obtained in our laboratory for telithromycin, for which the MIC_{50} and MIC_{90} were 0.125 and 0.25 μg/ml, respectively. Our results are similar to those obtained in a previous study (17), in which all 24 strains of *L. monocytogenes* were inhibited at 0.03 μg/ml. This good in vitro activity of ABT-773 against *L. monocytogenes* contrasts with its relatively high effective dose (100.1 mg/kg of body weight/day) in an animal model of sepsis caused by *L. monocytogenes* (14). Vancomycin and co-trimoxazole also showed good in vitro activities against *L. monocytogenes*. ABT-773 inhibited higher percentages of strains of all *Corynebacterium* species and of *R. equi* evaluated than did erythromycin. At a concentration of 0.5 μg/ml (the breakpoint of susceptibility for erythromycin against *Staphylococcus* spp.) the percentages of inhibition by ABT-773 and erythromycin were 92.5 and 15.0% for *C. amycolatum*, 90.0 and 60.0% for *R. equi*, 62.5 and 20.0% for *C. striatum*, 50.0 and 37.5% for *C. minutissimum*, 47.5 and 2.5% for *C. jeikeium*, 33.3 and 25.0% for *C. pseudodiphtheriticum*, and 7.5 and 5.0% for *C. urealyticum*, respectively. Clindamycin was even less active than erythromycin against the tested strains. We have previously evaluated the activities of telithromycin against coryneform bacteria, with the same methodology used in this study (10). ABT-773 showed an activity similar to that of telithromycin against coryneform bacteria, except in the case of *C. striatum*, for which the MIC_{50} and MIC_{90} of telithromycin (0.03 and 0.06 μg/ml, respectively) were lower than those of ABT-773 (0.125 and >32 μg/ml, respectively). The actual reasons for the difference in the activities of the two ketolides against *C. striatum* are presently unknown. It could well be that ABT-773 is intrinsically less active than telithromycin is against this organism, but since the isolates evaluated in this study were more recent than those in the study with telithromycin, the observed lower susceptibility of *C. striatum* to ABT-773 could be due to a recent increase in the level of resistance of *C. striatum* to ketolides, a situation already described for fluoroquinolones (11).

The differences in susceptibilities to ABT-773 and to erythromycin in the tested strains indicate that macrolide-resistant coryneform bacteria are still inhibited by ketolides. The mechanisms underlying this observation remain undefined, since the mechanisms of resistance to both macrolides and ketolides in coryneform bacteria are poorly known. The *ermC* gene has been reported to be present in most *C. striatum* strains resistant to erythromycin (20). The existence of bimodal populations among *C. minutissimum*, *C. pseudodiphtheriticum*, *C. striatum*, and *C. urealyticum* suggests that these species may

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

Bacterium (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Listeria monocytogenes (15)[a]			
ABT-773	≤0.015	≤0.015	≤0.015
Erythromycin	0.125–0.5	0.25	0.5
Clindamycin	0.5–2	2	2
Co-trimoxazole	0.015–0.03	0.015	0.03
Vancomycin	0.5–1	1	1
C. amycolatum (40)			
ABT-773	≤0.015–2	0.125	0.5
Erythromycin	≤0.06–>128	16	128
Clindamycin	0.25–>64	>64	>64
Cefuroxime	≤0.03–>128	0.25	0.5
Co-trimoxazole	0.25–>16	>16	>16
Vancomycin	0.25–1	0.5	0.5
C. jeikeium (40)			
ABT-773	≤0.015–>32	1	>32
Erythromycin	≤0.06–>128	>128	>128
Clindamycin	0.25–>64	>64	>64
Cefuroxime	0.06–>64	>64	>64
Co-trimoxazole	0.125–>16	>16	>16
Vancomycin	0.5–1	0.5	1
C. minutissimum (14)			
ABT-773	≤0.015–>32	0.03	>32
Erythromycin	≤0.06–>128	32	>128
Clindamycin	0.06–>64	>64	>64
Cefuroxime	0.06–>64	0.5	32
Co-trimoxazole	0.06–>16	2	>16
Vancomycin	0.125–1	0.25	0.5
C. pseudodiphtheriticum (12)			
ABT-773	≤0.015–>32	>32	>32
Erythromycin	≤0.06–>128	>128	>128
Clindamycin	≤0.03–>64	>64	>64
Cefuroxime	≤0.03–1	0.125	0.5
Co-trimoxazole	0.5–>16	4	>16
Vancomycin	0.25–0.5	0.25	0.25
C. striatum (40)			
ABT-773	≤0.015–>32	0.125	>32
Erythromycin	≤0.06–>128	8	>128
Clindamycin	1–>64	>64	>64
Cefuroxime	0.5–>64	2	4
Co-trimoxazole	0.5–16	4	8
Vancomycin	0.125–0.5	0.25	0.25
C. urealyticum (40)			
ABT-773	≤0.015–>32	>32	>32
Erythromycin	≤0.06–>128	>128	>128
Clindamycin	0.125–>64	>64	>64
Cefuroxime	>64	>64	>64
Co-trimoxazole	>16	>16	>16
Vancomycin	0.125–1	0.5	0.5
Rhodococcus equi (10)			
ABT-773	≤0.015–4	0.03	0.5
Erythromycin	≤0.06–>128	0.5	>128
Clindamycin	0.125–>64	4	>64
Cefuroxime	0.5–>64	8	>64
Co-trimoxazole	0.25–>16	16	>16
Vancomycin	0.5	0.5	0.5

[a] Following the NCCLS suggestion (16), MICs of cefuroxime against *L. monocytogenes* are not detailed.

TABLE 2. Percentages of strains of *L. monocytogenes* and coryneform bacteria inhibited at the indicated concentrations of ABT-773, erythromycin, and clindamycin

Bacterium (no. of isolates)	Antimicrobial agent	% of strains inhibited at concn (μg/ml):													
		≤0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
C. amycolatum (40)	ABT-773	30.0	35.0	45.0	75.0	85.0	92.5	95.0	100						
	Erythromycin			15.0						30.0	35.5	55.0	80.0	87.5	100
	Clindamycin					10	15.0							17.5	100
C. jeikeium (40)	ABT-773	7.5	12.5	30	42.5	45.0	47.5	60.0	62.5		65.0			100	
	Erythromycin			2.5					5.0	7.5			15.0	20.0	100
	Clindamycin					2.5									100
C. minutissimum (14)	ABT-773	42.9						57.2					64.3	100	
	Erythromycin			28.6		35.7		42.9					50.0	57.2	100
	Clindamycin			7.2	14.3			28.6	42.9						100
C. pseudodiphtheriticum (12)	ABT-773	33.3											41.7	100	
	Erythromycin			16.7		25.0									100
	Clindamycin		8.3		25.0										100
C. striatum (40)	ABT-773	27.5	30	47.5	57.5	60.0	62.5							100	
	Erythromycin			20					25.0	47.5	55.0	60.0		62.5	100
	Clindamycin						2.5	12.5	20.0						100
C. urealyticum (40)	ABT-773	5.0					7.5	10.0						100	
	Erythromycin			50											100
	Clindamycin				5.0										100
L. monocytogenes (15)	ABT-773	100													
	Erythromycin				6.7	73.3	100								
	Clindamycin						6.7	40.0	100						
R. equi (10)	ABT-773	40.0	60.0	70.0		80.0	90.0			100					
	Erythromycin			20.0		30.0	60.0		70.0						100
	Clindamycin				20.0		30.0	40.0		70.0					100

express a similar determinant of resistance, but further studies are obviously needed in this area.

All coryneform bacteria evaluated were inhibited by 1 μg of vancomycin/ml, in agreement with previous studies (10, 19, 23). On the other hand, co-trimoxazole was poorly active against coryneform bacteria, and for this agent all MIC_{50}s against *C. amycolatum*, *C. jeikeium*, and *C. urealyticum* were >16 μg/ml. MIC_{50}s of co-trimoxazole were higher than 2/38 μg/ml (the breakpoint for staphylococci that may be considered as a reference indicator) for *C. pseudodiphtheriticum*, *C. striatum*, and *R. equi*. Cefuroxime was also poorly active in vitro against most coryneform bacteria. Cefuroxime showed good in vitro activities against *C. amycolatum* (MIC_{90}, 0.5 μg/ml), *C. pseudodiphtheriticum* (MIC_{90}, 0.5 μg/ml), and to a lesser extent *C. striatum* (MIC_{90}, 4 μg/ml). Although the same percentages of *C. amycolatum* and *C. striatum* strains were inhibited by 8 μg of cefuroxime/ml (100%), this compound was more active against the former species, because the percentages of inhibition at 0.5 μg/ml were 92.5% for *C. amycolatum* and 5% for *C. striatum*.

In conclusion, ABT-773 shows very good in vitro activity against *L. monocytogenes* and also inhibits a significant number of coryneform bacteria of clinical relevance. ABT-773 shows good in vitro activities against *C. amycolatum* and *R. equi* and moderate activities against *C. minutissimum* and *C. striatum* and the multiresistant species *C. jeikeium*. ABT-773 is poorly active against *C. pseudodiphtheriticum* and *C. urealyticum*.

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