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Activities of Gemifloxacin and Five Other Antimicrobial Agents against *Listeria monocytogenes* and Coryneform Bacteria Isolated from Clinical Samples

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The in vitro activities of gemifloxacin, ciprofloxacin, ampicillin, doxycycline, gentamicin, and vancomycin were evaluated against 15 *Listeria monocytogenes* strains and 205 coryneform bacteria isolated from clinical samples. The percentages of strains inhibited by gemifloxacin at 0.5 μg/ml were 100% (*L. monocytogenes*), 93.3% (*Brevibacterium* spp.), 90% (*Corynebacterium minutissimum*), 42.5% (*Corynebacterium amycolatum*), 20% (*Corynebacterium striatum*), 12.5% (*Corynebacterium jeikeium*), and 10% (*Corynebacterium urealyticum*). One hundred percent of the *L. monocytogenes* strains were inhibited by 0.25 μg of gemifloxacin per ml, whereas 0% of the strains were inhibited by 0.25 μg of ciprofloxacin per ml. Vancomycin at 2 μg/ml inhibited all strains. Doxycycline and gentamicin at 4 μg/ml inhibited 94 and 49% of the strains, respectively, while ampicillin at 0.5, 2, and 8 μg/ml inhibited 24, 61, and 66% of the strains, respectively. It is concluded that gemifloxacin shows good in vitro activity against *L. monocytogenes* and coryneform bacteria except *C. jeikeium* and *C. urealyticum*.

Interest in coryneform bacteria has increased in recent years, especially after the recognition of the medical importance of some species, including *Corynebacterium jeikeium*, *Corynebacterium urealyticum*, *Corynebacterium striatum*, *Corynebacterium amycolatum, Corynebacterium minutissimum,* and other less frequently isolated organisms (4, 6). There have been profound changes in the taxonomy of this group of bacteria, with the definition of a large number of new species and the redefinition of organisms already known (4, 6). A good example of this situation has been the identification of *Corynebacterium amycolatum* as a very common human pathogen or colonizer and the finding that this taxon includes practically all strains previously identified as *Corynebacterium xerosis* and a significant number of organisms previously identified as *C. minutissimum*, coryneform CDC groups I2 and F2, and, to a lesser extent, *C. striatum* (1, 2).

Data about the activities of antimicrobial agents against coryneform bacteria have been obtained in the past, but data are available from only a limited number of studies (2, 3, 5, 7, 9–11, 15–17, 19). Several studies have compared different methodologies for susceptibility testing of coryneform bacteria (8, 20), but specific breakpoints for clinical categorization of susceptibility testing results are lacking (13, 14). A large proportion of *C. jeikeium*, *C. urealyticum*, and *C. amycolatum* strains are multidrug resistant, and only glycopeptides remain universally active against these species. Some reports suggest that other species may be susceptible to commonly used antimicrobial agents, but we lack reliable clinical evidence to support these in vitro observations. Therefore, it is convenient to evaluate the activities of new antimicrobial agents against coryneform bacteria of clinical importance. It is critical that studies on the activities of antimicrobial agents against coryneform bacteria be based on microorganisms identified according to the taxonomic information obtained over the years in order to generate information of clinical significance and to allow comparison of data obtained in different laboratories.

In previous studies we have observed that new quinolones are more active than older compounds against coryneform bacteria (10, 11). The objective of the study described here was to determine the in vitro activity of the new fluoroquinolone gemifloxacin in comparison with those of other compounds against different species of coryneform bacteria isolated from clinical samples.

The following coryneform species (number of isolates) obtained from clinical samples at our laboratory were evaluated: *Listeria monocytogenes* ($n = 15$), *Brevibacterium* spp. ($n = 15$), *C. amycolatum* ($n = 40$), *C. jeikeium* ($n = 40$), *C. minutissimum* ($n = 20$), *Corynebacterium pseudodiphteriticum* ($n = 10$), *C. striatum* ($n = 40$), and *C. urealyticum* ($n = 40$). All strains except the *C. striatum* strains were isolated from January 1991 to January 1999; *C. striatum* strains were obtained from May 1998 to January 1999. Microorganisms were identified as described by Funke et al. (4) with API CORYNE strips and by additional phenotypic tests when necessary (4, 6). After identification, the organisms were maintained in tryptic soy broth–10% glycerol at −80°C. The following reference strains were also included: *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 tests with gemifloxacin (SmithKline Beecham Pharmaceuticals, Harlow, United Kingdom), ciprofloxacin (Bayer, Leverkusen, Germany), ampicillin (Sigma, Madrid, Spain), gentamicin (Sigma), doxycycline (Sigma), and vancomycin (Sigma). The concentrations of antimicrobial agents ranged from 0.008 to 16 μg/ml (gemifloxacin, ciprofloxacin, vancomycin) or 0.06 to 128 μg/ml (all other agents). MICs were determined by a microdilution assay, as described previously (7, 9–11). Briefly, cation-adjusted Muel-

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ler-Hinton broth (supplemented with 0.5% Tween 80 when *C. jeikeium* and *C. urealyticum* were tested) was used. A suspension with a turbidity equivalent to that of a 0.5 McFarland standard was prepared in saline by using bacteria grown on Columbia agar base with 5% sheep blood agar at 35°C for 18 to 24 h. This suspension was further diluted in the same medium used for the microdilution assay to obtain a final inoculum of ca. 5×10^5 CFU/ml. After inoculation the microdilution plates were incubated at 35°C in air for 24 h (*C. jeikeium* and *C. urealyticum*) or for 18 to 20 h (all other species).

NCCLS has not provided official breakpoints for clinical categorization of susceptibility testing results for coryneform bacteria (13, 14). Wise and Andrews (21) have proposed a breakpoint for susceptibility to gemifloxacin of ≤0.5 μg/ml. The percentages of strains inhibited by ciprofloxacin and gemifloxacin at 0.5 μg/ml, 1 μg/ml (breakpoint for susceptibility to ciprofloxacin for staphylococci), or 2 μg/ml (breakpoint for intermediate susceptibility to ciprofloxacin for staphylococci) were calculated. The ranges of MICs and the MICs that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the strains tested are shown in Table 1.

All 220 strains tested were inhibited by 2 μg of vancomycin per ml, in agreement with previous reports (6, 16, 19). All strains *of L. monocytogenes*, *Brevibacterium* spp., *C. amycolatum*, *C. pseudodiphteriticum*, and *C. jeikeium,* 80% of *C. striatum* strains, 90% *of C. urealyticum* strains, and 95% of *C. minutissimum* strains were inhibited by 4 μg of doxycycline per ml (data not shown). Although tetracycline was not tested in the present study, other reports have indicated that doxycycline is more active than tetracycline against coryneform bacteria (6, 19).

All strains of *L. monocytogenes* were inhibited by 0.25 μg of gemifloxacin per ml and 2 μg of ciprofloxacin per ml; the MICs of gemifloxacin were four to eight times lower than those of ciprofloxacin in the present study and those of levofloxacin in a previous study that used the same methodology (10). All these results indicate the potential usefulness of gemifloxacin against infections caused by *L. monocytogenes*.

The activities of gemifloxacin, ciprofloxacin, ampicillin, and gentamicin against coryneform bacteria were related to the species. Most *C. jeikeium* and *C. urealyticum* strains were multidrug-resistant organisms that were not inhibited by ampicillin, gentamicin, or ciprofloxacin. Although the percentages of inhibition of these two species were similar for gemifloxacin (12.5% of *C. jeikeium* strains and 10% of *C. urealyticum* strains) and ciprofloxacin (10% of strains of both species) when both drugs were used at a concentration of 0.5 μg/ml, gemifloxacin was more active than ciprofloxacin against these two corynebacteria, which is reflected in the lower MIC_{50}s (4 versus >16 μg/ml for both species) and higher percentages of inhibition at 2 μg/ml (28 to 38% for gemifloxacin versus 10% for ciprofloxacin against both species). *C. striatum* was more susceptible to gemifloxacin than to ciprofloxacin: 98, 68, and 20% of the strains tested were inhibited by 4, 2, and 0.5 μg of gemifloxacin per ml, respectively, whereas the rates for ciprofloxacin were 15, 12, and 5%, respectively. Gemifloxacin was also more active than ciprofloxacin against *C. amycolatum* (58 and 32% of the strains were inhibited by the two drugs at 2 μg/ml, respectively, and 43 and 25% were inhibited by the two drugs at 0.5 μg/ml, respectively). Gentamicin and cipro-

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

Bacterium (no. of isolates) and antimicrobial agent	MIC (μg/ml)		
	Range	50%	90%
Total strains (220)			
Gemifloxacin	≤0.008–>16	2	16
Ciprofloxacin	≤0.015–>16	>16	>16
Ampicillin	≤0.06–>128	1	>128
Doxycycline	≤0.06–>128	0.125	4
Gentamicin	≤0.06–>128	8	>128
Vancomycin	0.125–2	0.5	0.5
L. monocytogenes (15)			
Gemifloxacin	0.06–0.25	0.125	0.125
Ciprofloxacin	0.5–2	1	2
Ampicillin	0.125–1	0.5	1
Doxycycline	0.125–1	0.25	0.25
Gentamicin	0.125–2	1	1
Vancomycin	1–2	1	1
Brevibacterium spp. (15)			
Gemifloxacin	≤0.008–2	0.06	0.125
Ciprofloxacin	0.03–>16	1	2
Ampicillin	≤0.06–0.5	≤0.06	0.5
Doxycycline	≤0.06–0.25	≤0.06	0.25
Gentamicin	≤0.06–128	0.25	2
Vancomycin	0.125–0.5	0.25	0.5
C. amycolatum (40)			
Gemifloxacin	≤0.008–>16	1	16
Ciprofloxacin	0.015–>16	16	>16
Ampicillin	≤0.06–4	0.125	1
Doxycycline	≤0.06–4	≤0.06	0.5
Gentamicin	≤0.06–>128	≤0.06	128
Vancomycin	0.25–1	0.25	0.5
C. jeikeium (40)			
Gemifloxacin	≤0.008–16	4	16
Ciprofloxacin	0.125–>16	>16	>16
Ampicillin	0.5–>128	>128	>128
Doxycycline	0.06–4	0.125	4
Gentamicin	≤0.06–>128	>128	>128
Vancomycin	0.125–0.5	0.5	0.5
C. minutissimum (20)			
Gemifloxacin	≤0.008–4	0.015	0.5
Ciprofloxacin	0.03–>16	0.125	16
Ampicillin	≤0.06–1	0.5	1
Doxycycline	≤0.06–16	0.25	0.5
Gentamicin	≤0.06–4	≤0.06	1
Vancomycin	0.25–0.5	0.25	0.5
C. pseudodiphteriticum (10)			
Gemifloxacin	≤0.008–0.125	≤0.008	0.03
Ciprofloxacin	0.015–2	≤0.06	1
Ampicillin	≤0.06–0.5	≤0.06	≤0.06
Doxycycline	≤0.06–0.5	≤0.06	0.25
Gentamicin	≤0.06	0.25	≤0.06
Vancomycin	0.25–0.5	0.25	0.25
C. striatum (40)			
Gemifloxacin	0.015–>16	2	4
Ciprofloxacin	0.06–>16	>16	>16
Ampicillin	0.25–4	1	4
Doxycycline	0.06–16	0.125	16
Gentamicin	≤0.06–>128	128	>128
Vancomycin	0.25–0.5	0.25	0.5
C. urealyticum (40)			
Gemifloxacin	≤0.008–>16	4	>16
Ciprofloxacin	0.03–>16	>16	>16
Ampicillin	≤0.06–>128	>128	>128
Doxycycline	0.125–>128	0.25	4
Gentamicin	≤0.06–>128	>128	>128
Vancomycin	0.25–0.5	0.5	0.5

floxacin exhibited low levels of activity against *C. striatum* and *C. amycolatum*. The results of the present study suggest that the susceptibility of *C. striatum* to the various antimicrobial agents evaluated has decreased over the last few years. In a previous study by our group in which we evaluated 86 strains of *C. striatum* isolated during the period from 1991 to 1994, MIC_{50}s and MIC_{90}s of 1 and 128 μg/ml and 4 and 16 μg/ml for gentamicin and ciprofloxacin, respectively, were obtained, values much lower than those obtained in the present study. The decreased activity of ciprofloxacin against other *Corynebacterium* species (*C. amycolatum*, *C. jeikeium*, *C. minutissimum*, *C. urealyticum*) may also be inferred when data obtained in the present study are compared to data from previous studies conducted by our group (10, 11). For all the strains mentioned above, the MIC_{50}s of ciprofloxacin have increased from 4 to >8 times, whereas the proportions of strains inhibited by ciprofloxacin at 2 μg/ml have decreased from 50 to 32% (*C. amycolatum*), 50 to 10% (*C. jeikeium*), 87 to 75% (*C. minutissimum*), and 44 to 10% (*C. urealyticum*). Other investigators have also previously noticed the increased level of resistance of *C. urealyticum* to fluoroquinolones over time (18). When data from the present study are compared with those from a previous one (10), it may be concluded that gemifloxacin is more active than levofloxacin against *C. minutissimum* and shows activity similar to that of levofloxacin against other *Corynebacterium* species; in terms of absolute MIC_{50}s and MIC_{90}s, gemifloxacin exhibited slightly better activity than levofloxacin against *C. amycolatum* and *C. jeikeium* and was slightly less active against *C. striatum*.

Interestingly, the MIC_{50}s and MIC_{90}s of ampicillin for *C. amycolatum* in the present study were lower than those for *C. striatum* (Table 1). Previous reports have shown that *C. amycolatum* was more often resistant to ampicillin than *C. striatum*, a trait that could be exploited to help in the routine identification of both organisms in the clinical laboratory (12). This might be related to the decreased activity of ampicillin against recent isolates of *C. striatum*.

Gemifloxacin was more active than ciprofloxacin against *C. minutissimum* and *C. pseudodiphteriticum*: 90 and 100% versus 75 and 80% of *C. minutissimum* and *C. pseudodiphteriticum* strains were inhibited by gemifloxacin and ciprofloxacin at 0.5 μg/ml, respectively. Similarly, 94 and 27% of *Brevibacterium* spp. were inhibited by 0.125 μg of gemifloxacin and ciprofloxacin per ml, respectively.

Few reports have investigated the mechanisms of resistance of coryneform bacteria to antimicrobial agents. Detailed studies are required to understand the biochemical and genetic bases of the increased levels of resistance observed in the present study.

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