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MIC and Time-Kill Study of Activities of DU-6859a, Ciprofloxacin, Levofloxacin, Sparfloxacin, Cefotaxime, Imipenem, and Vancomycin against Nine Penicillin-Susceptible and -Resistant Pneumococci

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MIC and time-kill methods were used to test the activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, cefotaxime, imipenem, and vancomycin against nine penicillin-susceptible, -intermediate, and -resistant pneumococci. The MIC of penicillin for penicillin-susceptible strains was 0.016 μg/ml, those for intermediate strains were 0.25 to 1.0 μg/ml, and those for resistant strains were 2.0 to 4.0 μg/ml. Of the four quinolones tested, DU-6859a had the lowest MIC (0.064 μg/ml), followed by sparfloxacin (0.25 to 0.5 μg/ml) and levofloxacin and ciprofloxacin (both 1.0 to 4.0 μg/ml). Vancomycin inhibited all strains at MICs of 0.25 to 0.5 μg/ml. The MICs of imipenem and cefotaxime for penicillin-susceptible, -intermediate, and -resistant strains were 0.004 to 0.008, 0.008 to 0.032, and 0.25 μg/ml and 0.016, 0.125 to 0.5, and 2.0 μg/ml, respectively. DU-6859a was bactericidal at eight times the MICs (0.5 μg/ml) for seven of the nine strains after 4 h and bactericidal for all nine strains after 6 h at eight times the MICs and after 12 h at two times the MICs. By comparison, sparfloxacin, the next most active quinolone, was uniformly bactericidal at two times the MICs only after 24 h, with little activity after 2 h. Levofloxacin and ciprofloxacin were bactericidal against all strains after 12 h at eight times the MICs and against all strains at 24 h at four times the MICs. Imipenem was bactericidal against all strains, at concentrations exceeding the MICs, after 24 h. Cefotaxime was also uniformly bactericidal only after 24 h of incubation at two times the MICs. Vancomycin, despite having uniformly low MICs for all strains irrespective of their penicillin susceptibility, was uniformly bactericidal only at two times the MICs after 24 h.

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in humans and is the leading cause of bacterial pneumonia as well as an important cause of otitis media and meningitis (1). The past two decades, and in particular the past 5 years, have witnessed a dramatic increase worldwide in the incidence of pneumococcal strains which are resistant to penicillin G and other antimicrobial agents. The main foci of penicillin-resistant pneumococci are currently South Africa, Spain, Eastern Europe, and Korea, but wherever these organisms are sought with the correct techniques they are being isolated (1). The problem is exacerbated by the tendency of these organisms to spread from country to country and from continent to continent (11, 12). In the United States, recent surveys have shown an increase in penicillin resistance from <5% before 1989 (with MICs of ≥2.0 μg/ml for <0.02% of isolates) to 6.6% in 1991 to 1992 (with MICs of ≥2.0 μg/ml for 1.3% of isolates) (3, 21). The latter spread is not evenly divided amongst the states: Block and coworkers have recently reported a 28% incidence of resistant pneumococci in middle ear fluids of children with acute otitis media in Kentucky and a 29% incidence of these strains in nasopharyngeal cultures from children with otitis media in Tennessee (2).

There is an urgent need for antimicrobial agents which can be used for oral therapy of infections such as pneumonia, bronchitis, sinusitis, and otitis media caused by penicillin-resistant pneumococci (4–6). Currently available quinolones, such as ciprofloxacin, ofloxacin, and lomefloxacin, are either inactive or marginally active against pneumococci, with MICs above or clustering around the susceptibility breakpoints (15, 16, 18, 20).

DU-6859a is a new fluoroquinolone with an expanded spectrum of activity against aerobic and anaerobic bacteria (7–10, 13, 16, 17). Previous studies performed in our laboratory have documented excellent activity of this compound against pneumococci, with an agar dilution MIC at which 50% of the isolates are inhibited (MIC_{50}) of 0.064 μg/ml and an MIC_{90} of 0.125 μg/ml (16). The aim of the study reported here was to confirm and expand the above findings by testing the activities of DU-6859a, ciprofloxacin, levofloxacin, sparfloxacin, imipenem, cefotaxime, and vancomycin against nine penicillin-susceptible and penicillin-resistant pneumococci by microdilution MIC and time-kill methods.

MATERIALS AND METHODS

Bacteria. Nine isolates of *S. pneumoniae* isolated from clinical specimens were examined. Of these, three were susceptible to penicillin (MIC, <0.1 μg/ml), three were intermediately susceptible to penicillin (MIC, 0.1 to 1.0 μg/ml), and three were resistant to penicillin (MIC, ≥2.0 μg/ml).

Antimicrobial agents. The following antimicrobial agents were supplied as laboratory powders of known potency by the indicated sources: DU-6859a and levofloxacin, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan; ciprofloxacin, Bayer, Inc., West Haven, Conn.; sparfloxacin, Rhône-Poulenc Rorer, Collegeville, Md.; imipenem, Merck, Inc., Rahway, N.J.; cefotaxime, Hoechst-Roussel Pharmaceuticals, Somerville, N.J.; and vancomycin, Eli Lilly & Co., Indianapolis, Ind.

MIC determination. MICs were determined by the microdilution method

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TABLE 1. MICs for individual strains

Strain[a]	MIC (μg/ml)						
	DU-6859a	Ciprofloxacin	Levofloxacin	Sparfloxacin	Imipenem	Cefotaxime	Vancomycin
60 (S)	0.064	2.0	1.0	0.25	0.004	0.016	0.25
149 (S)	0.064	4.0	2.0	0.25	0.008	0.016	0.25
153 (S)	0.064	2.0	1.0	0.25	0.004	0.016	0.5
5 (I)	0.064	4.0	1.0	0.25	0.032	0.125	0.25
95 (I)	0.064	2.0	1.0	0.25	0.032	0.5	0.25
99 (I)	0.064	2.0	2.0	0.25	0.008	0.125	0.5
24 (R)	0.064	1.0	1.0	0.25	0.25	2.0	0.5
37 (R)	0.064	1.0	1.0	0.5	0.25	2.0	0.5
227 (R)	0.064	4.0	4.0	0.5	0.25	2.0	0.25

[a] S, penicillin susceptible; I, penicillin intermediate; R, penicillin resistant.

recommended by the National Committee for Clinical Laboratory Standards (14), using cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 5% lysed defibrinated horse blood. For MIC determinations, suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml of sterile saline. Suspensions were further diluted 1:10 to obtain a final inoculum of 5×10^5 CFU per well. Trays were incubated overnight in ambient air at 37°C. Standard quality control strains (14) were included in each run.

Time-kill assays. For time-kill studies, glass tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (Difco) plus 5% lysed horse blood with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath. Antibiotic concentrations were chosen to comprise 3 doubling dilutions above and 4 dilutions below the agar dilution MIC (15).

Lysed horse blood was prepared by freezing and thawing horse blood (Cleveland Scientific, Bath, Ohio) several times. Equal volumes of lysed blood and sterile, deionized water were then mixed and centrifuged at 12,000 × *g* for 20 min. Appropriate amounts of 50% lysed blood were then added to the cation-adjusted Mueller-Hinton broth to yield a final concentration of 5% lysed horse blood. The bacterial inoculum was prepared by diluting a 16-h broth (medium described above) culture in the same medium (15). Dilutions required to obtain the correct inoculum (5×10^5 to 5×10^6 CFU/ml) were determined by prior viability studies using each strain.

To inoculate each tube of serially diluted antibiotic, 50 μl of diluted inoculum was delivered by pipette beneath the surface of the broth. The tubes were then vortexed and plated for viability counts (0 h). Only tubes containing an initial inoculum within the range of 5×10^5 to 5×10^6 CFU/ml were acceptable.

Viability counts of antibiotic-containing suspensions were performed at 0, 2, 4, 6, 12, and 24 h by plating 10-fold dilutions of 0.1-ml aliquots from each tube in sterile Mueller-Hinton broth onto Trypticase soy agar–5% sheep blood agar plates (BBL Microbiology Systems, Cockeysville, Md.). Recovery plates were incubated for up to 48 h. Colony counts were performed on plates, yielding 30 to 300 colonies. The lower limit of sensitivity of colony counts was 250 CFU/ml.

Time-kill assay results were analyzed by determining the numbers of strains which yielded $\Delta\log_{10}$ CFU/ml of −1, −2, and −3 in comparison with counts at 0 h, for all seven compounds at all five times. Antimicrobial agents were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥ 3 $\log_{10}$ CFU/ml (99.9%) at each of the times and bacteriostatic if the inoculum was reduced by <3 $\log_{10}$ CFU/ml. With the sensitivity threshold and inocula used in these studies, no problems were encountered in delineating 99.9% killing, when present.

RESULTS

The MIC of penicillin for penicillin-susceptible strains was 0.016 μg/ml, those for intermediate strains were 0.25 to 1.0 μg/ml, and those for resistant strains were 2.0 to 4.0 μg/ml. Quinolone and vancomycin MICs were not influenced by penicillin MICs. Of the four quinolones tested, DU-6859a had the lowest MIC (0.064 μg/ml), followed by sparfloxacin (0.25 to 0.5 μg/ml) and levofloxacin and ciprofloxacin (both 1.0 to 4.0 μg/ml). Vancomycin inhibited all strains at MICs of 0.25 to 0.5 μg/ml. Imipenem and cefotaxime MICs against penicillin-susceptible, -intermediate, and -resistant strains were 0.004 to 0.008, 0.008 to 0.032, and 0.25 μg/ml and 0.016, 0.125 to 0.5, and 2.0 μg/ml, respectively (Table 1).

The MICs were all within 1 dilution of bacteriostatic levels. The results of the time-kill studies are presented in Table 2 and Fig. 1. As can be seen, DU-6859a was bactericidal at eight times the MICs (0.5 μg/ml) against seven of the nine strains after 4 h and bactericidal against all nine strains after 6 h at eight times the MICs and after 12 h at two times the MICs. DU-6859a at four times the MICs lowered the colony counts by 90% for all strains after only 2 h of incubation. By comparison, sparfloxacin, the next most active quinolone, was uniformly bactericidal only at two times the MICs after 24 h, with little activity after 2 h. Sparfloxacin lowered the colony counts of all strains by 90% after 6 h at four times the MICs.

Levofloxacin and ciprofloxacin were bactericidal against all strains after 12 h at eight times the MICs and against all strains at 24 h at four times the MICs. Levofloxacin lowered the colony counts of all nine strains by 90% after 4 h at four times the MICs, and ciprofloxacin yielded the same results after 6 h. Imipenem was bactericidal against all strains, at concentrations exceeding the MICs, after 24 h and also lowered the counts of all strains by 90% after 6 h. Cefotaxime was also uniformly bactericidal only after 24-h incubation at two times the MICs, lowering counts by 90% after 6 h at four times the MICs. Vancomycin, despite having uniformly low MICs against all strains irrespective of their penicillin susceptibility, was uniformly bactericidal only at two times the MICs after 24 h, lowering the counts of all strains by 90% after 12 h at two times the MICs.

DISCUSSION

The viable count threshold of a 0.1-ml aliquot placed on a plate is theoretically 10 CFU/ml if one colony grows; however, for statistical accuracy, the lower limit was set at 25 colonies (250 CFU/ml, as in a previous study [15]), and this threshold was therefore used in all experiments. The problem of drug carryover was also addressed. We believe that spreading 0.1 ml of undiluted broth on a plate containing 25 ml of medium would dilute the drug 1:250, further 10-fold dilutions would dilute the drug 1:2,500, 1:25,000, etc. With the concentration of drugs used, only undiluted inocula would have had any potential for drug carryover, and only low counts (<1,000 CFU/ml) would be likely to be affected. We therefore feel that drug carryover was not a confounding factor in the generation of the data.

DU-6859a is a new fluorocyclopropyl quinolone active against a broad spectrum of bacteria, including staphylococci, hemolytic and viridans group streptococci, pneumococci, enterococci, members of the family *Enterobacteriaceae*, gram-negative nonfermentative rods, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and anaerobes (7–10). The current study confirms the findings of a previous study that DU-6859a is very active against pneumococci (agar dilu-

TABLE 2. Cumulative time-kill results for all strains

Drug and concn[a]	No. (%) of strains with the indicated $\Delta\log_{10}$ CFU/ml[b] at:														
	2 h			4 h			6 h			12 h			24 h		
	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
DU-6859a															
8×	9 (100)	6 (66.7)	0	9 (100)	8 (88.9)	7 (77.8)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
4×	9 (100)	1 (11.1)	0	9 (100)	7 (77.8)	4 (44.4)	9 (100)	9 (100)	6 (66.7)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
2×	2 (22.2)	0	0	9 (100)	3 (33.3)	0	9 (100)	8 (88.9)	0	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
1×	1 (11.1)	0	0	5 (55.6)	0	0	6 (66.7)	0	0	8 (88.9)	7 (77.8)	3 (33.3)	7 (77.8)	6 (66.7)	4 (44.4)
0.5×	0	0	0	0	0	0	0	0	0	0	0	0	1 (11.1)	0	0
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	1 (11.1)	0	0
Ciprofloxacin															
8×	5 (55.6)	0	0	8 (88.9)	2 (22.2)	0	9 (100)	7 (77.8)	4 (44.4)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
4×	3 (33.3)	0	0	8 (88.9)	2 (22.2)	0	9 (100)	6 (66.7)	2 (22.2)	9 (100)	9 (100)	8 (88.9)	9 (100)	9 (100)	9 (100)
2×	3 (33.3)	0	0	6 (66.7)	1 (11.1)	0	8 (88.9)	6 (66.7)	0	8 (88.9)	8 (88.9)	6 (66.7)	8 (88.9)	8 (88.9)	8 (88.9)
1×	0	0	0	5 (55.6)	0	0	6 (66.7)	4 (44.4)	0	6 (66.7)	6 (66.7)	3 (33.3)	7 (77.8)	6 (66.7)	6 (66.7)
0.5×	0	0	0	2 (22.2)	0	0	3 (33.3)	1 (11.1)	0	4 (44.4)	1 (11.1)	0	3 (33.3)	1 (11.1)	1 (11.1)
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Levofloxacin															
8×	7 (77.8)	1 (11.1)	0	9 (100)	6 (66.7)	1 (11.1)	9 (100)	9 (100)	4 (44.4)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)
4×	3 (33.3)	0	0	9 (100)	6 (66.7)	0	9 (100)	7 (77.8)	4 (44.4)	9 (100)	9 (100)	8 (88.9)	9 (100)	9 (100)	9 (100)
2×	2 (22.2)	0	0	8 (88.9)	3 (33.3)	0	9 (100)	5 (55.6)	1 (11.1)	9 (100)	9 (100)	7 (77.8)	9 (100)	9 (100)	9 (100)
1×	1 (11.1)	0	0	4 (44.4)	1 (11.1)	0	7 (77.8)	4 (44.4)	1 (11.1)	8 (88.9)	7 (77.8)	5 (55.6)	7 (77.8)	6 (66.7)	2 (22.2)
0.5×	1 (11.1)	0	0	1 (11.1)	0	0	1 (11.1)	0	0	3 (33.3)	1 (11.1)	0	1 (11.1)	0	0
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparfloxacin															
8×	3 (33.3)	0	0	8 (88.9)	6 (66.7)	0	9 (100)	4 (44.4)	0	9 (100)	9 (100)	8 (88.9)	9 (100)	9 (100)	9 (100)
4×	2 (22.2)	0	0	6 (66.7)	1 (11.1)	0	9 (100)	2 (22.2)	0	9 (100)	9 (100)	6 (66.7)	9 (100)	9 (100)	9 (100)
2×	0	0	0	5 (55.6)	1 (11.1)	0	8 (88.9)	1 (11.1)	0	9 (100)	9 (100)	5 (55.6)	9 (100)	9 (100)	9 (100)
1×	0	0	0	3 (33.3)	0	0	4 (44.4)	0	0	7 (77.8)	4 (44.4)	0	3 (33.3)	2 (22.2)	1 (11.1)
0.5×	0	0	0	0	0	0	0	0	0	1 (11.1)	0	0	0	0	0
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Imipenem															
8×	4 (44.4)	0	0	8 (88.9)	1 (11.1)	0	9 (100)	7 (77.8)	0	9 (100)	9 (100)	7 (77.8)	9 (100)	9 (100)	9 (100)
4×	4 (44.4)	0	0	7 (77.8)	1 (11.1)	0	9 (100)	6 (66.7)	0	9 (100)	9 (100)	7 (77.8)	9 (100)	9 (100)	9 (100)
2×	3 (33.3)	0	0	7 (77.8)	0	0	9 (100)	4 (44.4)	0	9 (100)	9 (100)	6 (66.7)	9 (100)	9 (100)	9 (100)
1×	1 (11.1)	0	0	4 (44.4)	0	0	6 (66.7)	2 (22.2)	0	8 (88.9)	7 (77.8)	3 (33.3)	8 (88.9)	8 (88.9)	7 (77.8)
0.5×	0	0	0	0	0	0	0	0	0	0	0	0	2 (22.2)	1 (11.1)	0
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime															
8×	2 (22.2)	0	0	6 (66.7)	0	0	9 (100)	2 (22.2)	1 (11.1)	9 (100)	9 (100)	6 (66.7)	9 (100)	9 (100)	9 (100)
4×	1 (11.1)	0	0	5 (55.6)	0	0	9 (100)	2 (22.2)	0	9 (100)	9 (100)	4 (44.4)	9 (100)	9 (100)	9 (100)
2×	0	0	0	3 (33.3)	0	0	8 (88.9)	2 (22.2)	0	9 (100)	9 (100)	4 (44.4)	9 (100)	9 (100)	9 (100)
1×	0	0	0	1 (11.1)	0	0	7 (77.8)	0	0	7 (77.8)	7 (77.8)	0	6 (66.7)	6 (66.7)	5 (55.6)
0.5×	0	0	0	0	0	0	1 (11.1)	0	0	1 (11.1)	1 (11.1)	0	2 (22.2)	1 (11.1)	1 (11.1)
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vancomycin															
8×	2 (22.2)	0	0	6 (66.7)	0	0	8 (88.9)	7 (77.8)	0	9 (100)	9 (100)	7 (77.8)	9 (100)	9 (100)	9 (100)
4×	1 (11.1)	0	0	6 (66.7)	0	0	8 (88.9)	7 (77.8)	0	9 (100)	9 (100)	6 (66.7)	9 (100)	9 (100)	9 (100)
2×	0	0	0	5 (55.6)	0	0	8 (88.9)	4 (44.4)	0	9 (100)	9 (100)	5 (55.6)	9 (100)	9 (100)	9 (100)
1×	0	0	0	2 (22.2)	0	0	7 (77.8)	1 (11.1)	0	7 (77.8)	7 (77.8)	3 (33.3)	7 (77.8)	7 (77.8)	7 (77.8)
0.5×	0	0	0	0	0	0	0	0	0	0	0	0	1 (11.1)	0	0
0.25×	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Concentrations expressed as multiples of the MIC.
[b] Log_{10} CFU/ml lower than original inoculum (0 h), indicating the following: −1, 90% killing; −2, 99% killing; and −3, 99.9% killing.

tion MIC_{90}, 0.125 μg/ml) (16). In the current study, DU-6859a yielded microbroth MICs of 0.064 μg/ml against all nine strains and was the most active compound as determined by the time-kill method, yielding uniform bactericidal activity after 6 h at a concentration of 0.5 μg/ml and after 12 h at a concentration of 0.125 μg/ml. The bactericidal activity of this compound against pneumococci lies well within levels achievable in serum, with maximum concentrations of drug in serum (in micrograms per milliliter) of 0.29 ± 0.08, 0.51 ± 0.14, 1.0 ± 0.14, and 1.86 ± 0.36 (means ± standard deviations) after single oral doses of DU-6859a of 25, 50, 100, and 200 mg, respectively, given to human volunteers (13).

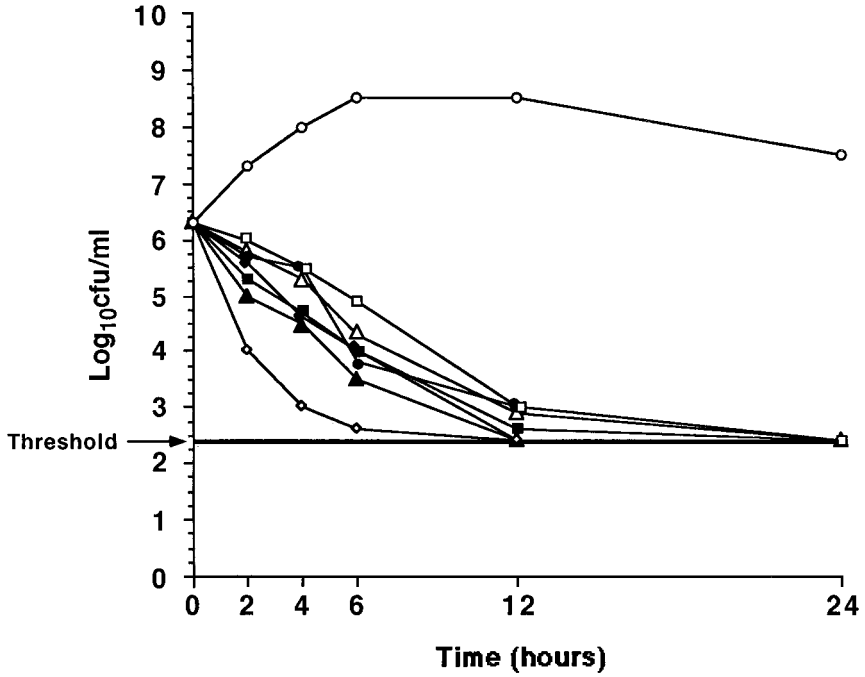

FIG. 1. Time-kill results with strain 37 (penicillin resistant, [MIC, 2.0 μg/ml]) at eight times the MICs of DU-6859a (◇), ciprofloxacin (◆), levofloxacin (▲), sparfloxacin (△), imipenem (■), cefotaxime (□), and vancomycin (●). The growth of a control without drug (○) is also shown.

None of the other quinolones tested showed rapid bactericidal activity as demonstrated by DU-6859a. In addition, the MICs of these compounds were all higher than those of DU-6859a. Sparfloxacin, despite having bactericidal activity later than DU-6859a, had activity which points to possible clinical use, on the basis of MICs, bactericidal concentrations, and levels achievable in serum. Peak sparfloxacin concentrations in human plasma of 0.44, 0.65, and 1.4 μg/ml were observed 2.5 to 4.5 h after single oral doses of 100, 200, and 400 mg, respectively (18). Imipenem had low MICs but showed bactericidal activity against all strains only after 24 h. The clinical significance of this phenomenon is unknown, as few clinical data for this drug in treatment of pneumococcal infections are currently available. Vancomycin MICs were within achievable levels in serum but marginal in the cerebrospinal fluid. This, together with its relatively late bactericidal activity, may help explain unsatisfactory results obtained with vancomycin therapy of pneumococcal meningitis (23). Cefotaxime was most effective against penicillin-susceptible and -intermediate strains (19, 22) but also was bactericidal only after 24 h.

In summary, DU-6859a showed the lowest MICs and most rapid killing of all quinolone and nonquinolone compounds tested. Results point to clinical studies of DU-6859a in infections caused by penicillin-susceptible and -resistant pneumococci.

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REFERENCES

1. **Appelbaum, P. C.** 1992. Antimicrobial resistance in *Streptococcus pneumoniae*—an overview. Clin. Infect. Dis. **15:**77–83.
2. **Block, S., J. Hedrick, P. Wright, R. Finger, R. Leggiadro, M. Appleton, S. Kahn, and R. Hutcheson.** 1994. Drug-resistant *Streptococcus pneumoniae*—Kentucky and Tennessee, 1993. Morbid. Mortal. Weekly Rep. **43:**23–25, 31.
3. **Breiman, R. F., J. C. Butler, F. C. Tenover, J. A. Elliott, and R. R. Facklam.** 1994. Emergence of drug-resistant pneumococcal infections in the United States. JAMA **271:**1831–1835.
4. **Friedland, I. R., and G. S. Istre.** 1992. Management of penicillin-resistant pneumococcal infections. Pediatr. Infect. Dis. J. **11:**433–435.
5. **Friedland, I. R., and G. H. McCracken, Jr.** 1994. Management of infections caused by antibiotic-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. **331:**377–382.
6. **Jacobs, M. R.** 1992. Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. Clin. Infect. Dis. **15:**119–127.
7. **Jolley, A., J. M. Andrews, N. Brenwald, and R. Wise.** 1993. The in-vitro activity of a new highly active quinolone, DU-6859a. J. Antimicrob. Chemother. **32:**757–763.
8. **Korten, V., J. F. Tomayko, and B. E. Murray.** 1994. Comparative in vitro activity of DU-6859a, a new fluoroquinolone agent, against gram-positive cocci. Antimicrob. Agents Chemother. **38:**611–615.
9. **Marshall, S. A., and R. N. Jones.** 1993. In vitro activity of DU-6859a, a new fluorocyclopropyl quinolone. Antimicrob. Agents Chemother. **37:**2747–2753.
10. **Marshall, S. A., R. N. Jones, P. R. Murray, J. A. Washington, S. D. Allen, E. H. Gerlach, and M. E. Erwin.** 1993. In-vitro comparison of DU-6859a, a novel fluoroquinolone, with other quinolones and oral cephalosporins tested against 5,086 recent clinical isolates. J. Antimicrob. Chemother. **32:**877–884.
11. **McDougal, L. K., R. Facklam, M. Reeves, S. Hunter, J. M. Swenson, B. C. Hill, and F. C. Tenover.** 1992. Analysis of multiply antimicrobial-resistant isolates of *Streptococcus pneumoniae* from the United States. Antimicrob. Agents Chemother. **36:**2176–2184.
12. **Munoz, R., J. M. Musser, M. Crain, D. E. Briles, A. Marton, A. J. Parkinson, U. Sorensen, and A. Tomasz.** 1992. Geographic distribution of penicillin-resistant clones of *Streptococcus pneumoniae*: characterization by penicillin-binding protein profile, surface protein A typing, and multilocus enzyme analysis. Clin. Infect. Dis. **15:**112–118.
13. **Nakashima, M., T. Uematsu, K. Kosuge, K. Umemura, H. Hakusui, and M. Tanaka.** 1995. Pharmacokinetics and tolerance of DU-6859a, a new fluoroquinolone, after single and multiple oral doses in healthy volunteers. Antimicrob. Agents Chemother. **39:**170–174.
14. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard. NCCLS publication no. M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1994. Study of comparative antipneumococcal activities of penicillin G, RP 59500, erythromycin, sparfloxacin, ciprofloxacin, and vancomycin by using time-kill methodology. Antimicrob. Agents Chemother. **38:**2065–2072.
16. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1995. Activity of CP

99,219 compared to DU-6859a, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin, tosufloxacin, sparfloxacin and grepafloxacin against penicillin-susceptible and -resistant pneumococci. J. Antimicrob. Chemother. **35:**230–232.

17. **Sato, K., K. Hoshino, M. Tanaka, I. Hayakawa, and Y. Osada.** 1992. Antimicrobial activity of DU-6859a, a new potent fluoroquinolone, against clinical isolates. Antimicrob. Agents Chemother. **36:**1491–1498.
18. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1992. Susceptibilities of penicillin-susceptible and -resistant strains of *Streptococcus pneumoniae* to RP 59500, vancomycin, erythromycin, PD 131628, sparfloxacin, temafloxacin, Win 57273, ofloxacin, and ciprofloxacin. Antimicrob. Agents Chemother. **36:**856–859.
19. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1994. Susceptibilities of 177 penicillin-susceptible and -resistant pneumococci to FK 037, cefpirome, cefepime, ceftriaxone, cefotaxime, ceftazidime, imipenem, biapenem, meropenem, and vancomycin. Antimicrob. Agents Chemother. **38:**898–900.
20. **Spangler, S. K., M. R. Jacobs, G. A. Pankuch, and P. C. Appelbaum.** 1993. Susceptibility of 170 penicillin-susceptible and -resistant pneumococci to six oral cephalosporins, four quinolones, desacetylcefotaxime, Ro 23-9424 and RP 67829. J. Antimicrob. Chemother. **31:**273–280.
21. **Spika, J. S., R. R. Facklam, B. D. Plikaytis, M. J. Oxtoby, and the Pneumococcal Surveillance Working Group.** 1991. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979–1987. J. Infect. Dis. **163:** 1273–1278.
22. **Tweardy, D. J., M. R. Jacobs, and W. T. Speck.** 1983. Susceptibility of penicillin-resistant pneumococci to eighteen antimicrobials; implications for treatment of meningitis. J. Antimicrob. Chemother. **12:**133–139.
23. **Viladrich, P. F., F. Gudiol, J. Liñares, R. Pallarés, I. Sabaté, G. Rufí, and J. Ariza.** 1991. Evaluation of vancomycin for therapy of adult pneumococcal meningitis. Antimicrob. Agents Chemother. **35:**2467–2472.