ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2000, p. 2211–2213 Vol. 44, No. 8
0066-4804/00/$04.00+0
Copyright © 2000, American Society for Microbiology. All Rights Reserved.

Comparative Activities of Ciprofloxacin, Clinafloxacin, Gatifloxacin, Gemifloxacin, Levofloxacin, Moxifloxacin, and Trovafloxacin against Epidemiologically Defined *Acinetobacter baumannii* Strains

BARBARA HEINEMANN,[1] HILMAR WISPLINGHOFF,[1] MICHAEL EDMOND,[2] AND HARALD SEIFERT[1]*

Institute of Medical Microbiology, Immunology and Hygiene, University of Cologne, 50935 Cologne, Germany,[1] and Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia[2]

Received 16 March 2000/Returned for modification 25 April 2000/Accepted 11 May 2000

In vitro activities of seven fluoroquinolones against 140 clinical *Acinetobacter baumannii* isolates representing 138 different strain types were determined. The rank order of activity was clinafloxacin > gatifloxacin > levofloxacin > trovafloxacin > gemifloxacin = moxifloxacin > ciprofloxacin. The 31 outbreak-related *A. baumannii* strains were significantly more resistant than were 109 sporadic strains.

During the past 20 years, *Acinetobacter baumannii* has emerged as a significant nosocomial pathogen (3, 6, 11, 20, 27). These organisms have a particular propensity for nosocomial cross-transmission, and numerous outbreaks of infections have been reported (8, 17, 21, 24, 25). The widespread multiresistance of these organisms is a cause of concern. Aminoglycosides, carbapenems, and fluoroquinolones remain the mainstay of therapy for serious *A. baumannii* infections, although reports of increasing resistance against these agents have appeared (8, 15, 16, 21, 22, 23).

Recently, several new fluoroquinolones with a greater potency against a variety of bacterial species have been developed (4). These agents exert a promising activity against *A. baumannii* (1, 13, 26). The present study was conducted to evaluate the in vitro activity of ciprofloxacin in comparison to those of the novel fluoroquinolone compounds clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin against clinically significant *A. baumannii* isolates that were recovered from blood cultures, tracheal secretions, wound swabs, and urine. *Acinetobacter* species were identified as nonfermentative, gram-negative, nonmotile, oxidase-negative bacilli. Phenotypic identification as *A. baumannii* was performed using the simplified identification scheme of Bouvet and Grimont (5). Possible strain relatedness of the organisms was assessed by molecular typing methods, such as randomly amplified polymorphic DNA (RAPD) analysis, performed with two different primers (ERIC-2 and M13) using Ready-To-Go RAPD Analysis Beads (Pharmacia Biotech, Freiburg, Germany) and/or pulsed-field gel electrophoresis of genomic DNA using the restriction enzyme *Apa*I as described previously (9, 18). The organisms were selected on the basis of exhibiting a unique DNA fingerprint pattern. Usually, only one isolate per patient was included, as was one isolate per given hospital outbreak. A second isolate was considered only if the isolate differed in its ciprofloxacin MIC by three or more twofold dilutions, as was the case for two isolates. In total, 109 sporadic and 31 outbreak-related isolates were selected. Included were 47 isolates that were originally recovered from patients in the Cologne metropolitan area in Germany between 1 July 1990 and 31 December 1998. Twelve of these strains were isolated from 10 well-defined hospital outbreaks caused by 10 different clonal strains. Details of these outbreaks have been described elsewhere (17, 18, 27). Thirty-five strains were sporadic isolates from the same geographic area but were epidemiologically unrelated and represent different strain types (19). Also included were a number of strains related to well-defined hospital outbreaks ($n = 9$), as well as sporadic strains ($n = 13$) from various hospitals in Germany and neighboring European countries, such as Belgium, Denmark, and Great Britain. In addition, 71 *A. baumannii* blood culture isolates recovered from patients throughout the United States between 1 March 1996 and 28 February 1998 were selected. These isolates included 10 outbreak-related strains as well as 61 strains that were epidemiologically unrelated (28). MICs were determined by the agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (12) by using cation-adjusted Mueller-Hinton agar (DIFCO, Augsburg, Germany). Plates were inoculated with a Steers replicator with a final inoculum of approximately 10^4 CFU per spot and incubated for 18 h at 37°C. The MIC was defined as the lowest concentration of drug that prevented visible growth. The following antibiotics were tested at concentrations ranging from 0.03 to 128 mg/liter: ciprofloxacin (Bayer AG, Leverkusen, Germany), clinafloxacin (Parke-Davis Pharmaceuticals, Ann Arbor, Mich.), gatifloxacin (Grünenthal GmbH, Stolberg, Germany), gemifloxacin (SmithKline Beecham, Munich, Germany), levofloxacin (Hoechst AG, Frankfurt, Germany), moxifloxacin (Bayer AG) and trovafloxacin (Pfizer Central Research, Groton, Conn.). The breakpoints used for ciprofloxacin and levofloxacin were those recommended by the NCCLS, while the breakpoints chosen for each of the novel fluoroquinolones were those recommended by the manufacturers (Table 1). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *A. baumannii* ATCC 19606 were used as controls.

The in vitro activities of the different fluoroquinolones against 140 clinical *A. baumannii* isolates are shown in Table 1. While all of the novel fluoroquinolones were 4- to 16-fold more active than ciprofloxacin, the activities of the novel quinolone compounds did not exhibit major differences. The rank order of activity as determined by their MICs at which 90% of the isolates tested are inhibited (MIC_{90}s) was as follows: clinafloxacin > gatifloxacin = levofloxacin > trovafloxacin = gemi-

* Corresponding author. Mailing address: Institute of Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstr. 19-21, 50935 Cologne, Germany. Phone: 0049 221 4783009. Fax: 0049 221 4783067. E-mail: harald.seifert@uni-koeln.de.

Downloaded from http://aac.asm.org/ on February 26, 2021 by guest

TABLE 1. In vitro activities of ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin against 140 *A. baumannii* isolates

Antibiotic	MIC (mg/liter)				% of strains fully susceptible[a]
	Range	50%	90%	Geometric mean	
Ciprofloxacin	≤0.03—>128	0.5	64	19.6	66.4
Clinafloxacin	0.06—32	0.12	4	1.3	79.3
Gatifloxacin	≤0.03—128	0.12	8	3.7	71.4
Gemifloxacin	≤0.03—32	0.12	16	3.2	72.1
Levofloxacin	≤0.03—32	0.25	8	3.2	74.3
Moxifloxacin	≤0.03—64	0.12	16	4.2	73.6
Trovafloxacin	≤0.03—32	0.06	16	3.3	77.9

[a] MIC breakpoints for susceptibility are those defined by NCCLS for ciprofloxacin (≤1 mg/liter) and levofloxacin (≤2 mg/liter) for susceptibility testing of non-*Enterobacteriaceae*. For the other fluoroquinolone agents, no breakpoints have been established. For clinafloxacin (≤1 mg/liter), gatifloxacin (≤1 mg/liter), gemifloxacin (≤0.5 mg/liter), moxifloxacin (≤1 mg/liter), and trovafloxacin (≤1 mg/liter), the breakpoints suggested by the manufacturers were applied.

floxacin = moxifloxacin > ciprofloxacin. The overall respective MIC_{50}s and MIC_{90}s were as follows: ciprofloxacin, 0.5 and 64 mg/liter; clinafloxacin, 0.12 and 4 mg/liter; gatifloxacin 0.12 and 8 mg/liter; gemifloxacin, 0.12 and 16 mg/liter; levofloxacin, 0.25 and 8 mg/liter; moxifloxacin, 0.12 and 16 mg/liter; and trovafloxacin, 0.06 and 16 mg/liter. Although the novel quinolones were considerably more active than ciprofloxacin in terms of MIC_{50}s, MIC_{90}s, and geometric mean MICs, there were only minor differences regarding the percentage of strains susceptible at the respective breakpoints. For example, among the 38 strains resistant to ciprofloxacin (MIC, ≥4 mg/liter), only nine strains were fully susceptible to clinafloxacin (MIC, ≤1 mg/liter).

Most studies reporting data of antimicrobial drug susceptibilities of *A. baumannii* did not consider the high epidemicity of these bacteria at many institutions. Their results were probably biased by the inclusion of isolates that were obtained from different patients but were nevertheless clonally related (10, 13, 16). It is obvious that the analysis of isolates collected consecutively from hospitalized patients even in multicenter studies with the inclusion of highly resistant epidemic strains tends to overestimate the resistance of these microorganisms to antimicrobial agents. We therefore included only isolates that represent different strain types as shown by molecular typing. This may explain why the percentages of strains susceptible to fluoroquinolones, ranging from 66 to 79% in our study, were considerably higher than in other recent studies (2, 13, 15, 16).

If outbreak-related strains were compared to sporadic strains, *A. baumannii* outbreak strains were significantly more resistant to fluoroquinolone agents than sporadic strains. These differences were highly significant for all the quinolones tested ($P \leq 0.0001$, data not shown). Whereas susceptibilities to fluoroquinolones ranged from 76 to 86% among sporadic isolates, only 32 to 55% of outbreak-related *A. baumannii* strains were susceptible to the quinolones tested (Table 2). Villers et al. (25) recently found that previous therapy with a fluoroquinolone was an independent risk factor for infection with epidemic *A. baumannii*. It appeared that the selection pressure caused by the indiscriminate use of fluoroquinolones was responsible for the persistence and epidemic spread of multidrug-resistant *A. baumannii* clones for at least 5 years.

Resistance of *A. baumannii* to the fluoroquinolones has been attributed to changes in the structure of DNA gyrase or topoisomerase IV which are caused by mutations in the *gyrA* or *parC* genes, respectively, which lower the affinity of the drug in the enzyme-DNA complex (7, 14, 23). A second mechanism of resistance has been described with mutations of chromosomally encoded drug influx and efflux systems that determine intracellular drug accumulation (4, 7, 14); these mutations result in either reduced production of outer membrane proteins, which mediate quinolone influx, or stimulation of the cells' efflux system, which leads to active drug expulsion. The basis of the increased activity of the novel quinolones against *A. baumannii* remains to be determined.

In conclusion, the novel quinolones demonstrated superior activity against *A. baumannii* compared to ciprofloxacin. Clinafloxacin was the most active agent against sporadic as well as outbreak-related *A. baumannii* isolates, the latter being significantly more resistant to all agents tested. Our findings demonstrate the need to analyze epidemiologically well-defined *A. baumannii* isolates and to exclude clonally related strains from hospital outbreaks or from nosocomial cross-transmission between patients. In the light of the limited therapeutic options for the treatment of infections caused by *A. baumannii*, clinical studies are required to test the relevance of the increased activities of the novel fluoroquinolones against *A. baumannii* infections.

TABLE 2. In vitro activities of seven fluoroquinolone agents against sporadic and epidemic *A. baumannii* isolates

A. baumannii (no. of strains tested)	Antibiotic	MIC (mg/liter)				% of strains fully susceptible
		Range	50%	90%	Geometric mean	
Sporadic isolates (109)	Ciprofloxacin	≤0.03—>128	0.25	64	13.5	76.1
	Clinafloxacin	0.06—16	0.12	2	0.8	86.2
	Gatifloxacin	≤0.03—64	0.12	8	2.2	80.7
	Gemifloxacin	≤0.03—32	0.12	8	1.9	80.7
	Levofloxacin	≤0.03—32	0.25	8	2.2	83.5
	Moxifloxacin	≤0.03—64	0.12	8	3.2	83.5
	Trovafloxacin	≤0.03—32	0.06	8	2.0	85.3
Outbreak isolates (31)	Ciprofloxacin	0.25—>128	4	128	41.2	32.3
	Clinafloxacin	0.12—32	1	4	2.9	54.8
	Gatifloxacin	≤0.03—128	2	16	9.1	38.7
	Gemifloxacin	0.06—32	2	32	7.5	41.9
	Levofloxacin	0.12—32	4	16	6.6	41.9
	Moxifloxacin	0.06—32	2	32	8.0	38.7
	Trovafloxacin	≤0.03—32	1	32	7.6	51.6

Downloaded from http://aac.asm.org/ on February 26, 2021 by guest

We thank D. Stefanik for excellent technical assistance and P. Gerner-Smidt (Department of Clinical Microbiology, Statens Seruminstitut, Copenhagen, Denmark) for providing some of the *A. baumannii* strains investigated in this study.

REFERENCES

1. **Bauernfeind, A.** 1997. Comparison of the antibacterial activities of the quinolones Bay 12-8039, gatifloxacin (AM 1155), trovafloxacin, clinafloxacin, levofloxacin and ciprofloxacin. J. Antimicrob. Chemother. **40:**639–651.
2. **Bello, H., G. Gonzalez, M. Dominguez, R. Zemelman, A. Garcia, and S. Mella.** 1997. Activity of selected β-lactams, ciprofloxacin, and amikacin against different *Acinetobacter baumannii* biotypes from Chilean hospitals. Diagn. Microbiol. Infect. Dis. **28:**183–186.
3. **Bergognge-Bérézin, E., and K. J. Towner.** 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin. Microbiol. Rev. **9:**148–165.
4. **Blondeau, J. M.** 1999. Expanded activity and utility of the new fluoroquinolones: a review. Clin. Ther. **21:**3–40.
5. **Bouvet, P. J., and P. A. Grimont.** 1987. Identification and biotyping of clinical isolates of Acinetobacter. Ann. Inst. Pasteur Microbiol. **138:**569–578.
6. **Cefai, C., J. Richards, F. K. Gould, and P. McPeake.** 1990. An outbreak of *Acinetobacter* respiratory tract infection resulting from incomplete disinfection of ventilatory equipment. J. Hosp. Infect. **15:**177–182.
7. **Drlica, K., and X. Zhao.** 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol. Mol. Biol. Rev. **61:**377–392.
8. **Go, S. E., C. Urban, J. Burns, B. Kreiswirth, W. Eisner, N. Mariano, K. Mosinka-Snipas, and J. J. Rahal.** 1994. Clinical and molecular epidemiology of Acinetobacter infections sensitive only to polymyxin B and sulbactam. Lancet **344:**1329–1332.
9. **Grundmann, H. J., K. J. Towner, L. Dijkshoorn, P. Gerner-Smidt, M. Maher, H. Seifert, and M. Vaneechoutte.** 1997. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. J. Clin. Microbiol. **35:**3071–3077.
10. **Marques, M. B., E. S. Brookings, S. A. Moser, P. B. Sonke, and K. B. Waites.** 1997. Comparative in vitro antimicrobial susceptibilities of nosocomial isolates of *Acinetobacter baumannii* and synergistic activities of nine antimicrobial combinations. Antimicrob. Agents Chemother. **41:**881–885.
11. **McDonald, L. C., S. N. Banerjee, W. R. Jarvis, and the National Nosocomial Infections Surveillance System.** 1999. Seasonal variation of Acinetobacter infections: 1987–1996. Clin. Infect. Dis. **29:**1133–1137.
12. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. **Pascual, A., I. Lopez-Hernandez, L. Martinez-Martinez, and E. J. Perea.** 1997. In-vitro susceptibilities of multiresistant strains of *Acinetobacter baumannii* to eight quinolones. J. Antimicrob. Chemother. **40:**140–142.
14. **Piddock, L. J. V.** 1995. Mechanism of resistance to fluoroquinolones: state-of-the-art 1992–1994. Drugs **49:**29–35.
15. **Ruiz, J., M. L. Nunez, J. Perez, E. Simarro, L. Martinez-Campos, and J. Gomez.** 1999. Evolution of resistance among clinical isolates of Acinetobacter over a 6-year period. Eur. J. Clin. Microbiol. Infect. Dis. **18:**292–295.
16. **Seifert, H., R. Baginski, A. Schulze, and G. Pulverer.** 1993. Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob. Agents Chemother. **37:**750–753.
17. **Seifert, H., B. Boullion, A. Schulze, and G. Pulverer.** 1994. Plasmid DNA profiles of Acinetobacter baumannii: clinical application in a complex endemic setting. Infect. Control Hosp. Epidemiol. **15:**520–528.
18. **Seifert, H., A. Schulze, R. Baginski, and G. Pulverer.** 1994. Comparison of four different methods for epidemiologic typing of *Acinetobacter baumannii*. J. Clin. Microbiol. **32:**1816–1819.
19. **Seifert, H., and P. Gerner-Smidt.** 1995. Comparison of ribotyping and pulsed-field gel electrophoresis for molecular typing of *Acinetobacter* isolates. J. Clin. Microbiol. **33:**1402–1407.
20. **Siegman-Igra, Y., S. Bar-Yosef, A. Gorea, and J. Avram.** 1993. Nosocomial *Acinetobacter* meningitis secondary to invasive procedures: report of 25 cases and review. Clin. Infect. Dis. **17:**843–849.
21. **Tankovic, J., P. Legrand, G. De Gatines, V. Chemineau, C. Brun-Buisson, and J. Duval.** 1994. Characterization of a hospital outbreak of imipenem-resistant *Acinetobacter baumannii* by phenotypic and genotypic methods. J. Clin. Microbiol. **32:**2677–2681.
22. **Vila, J., A. Marcos, F. Marco, S. Abdalla, Y. Vergara, R. Reig, R. Gomez-Lus, and T. Jimenez de Anta.** 1993. In vitro antimicrobial production of β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase by and susceptibility of clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **37:**138–141.
23. **Vila, J., J. Ruiz, P. Goni, M. A. Marcos, and M. T. Jimenez de Anta.** 1995. Mutation in the *gyrA* gene of quinolone-resistant clinical isolates of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **39:**1201–1203.
24. **Vila, J., J. Ruiz, M. Navia, B. Becerril, I. Garcia, S. Perea, I. Lopez-Hernandez, I. Alamo, F. Ballester, A. M. Planes, J. Martinez-Beltran, and M. T. Jimenez de Anta.** 1999. Spread of amikacin resistance in *Acinetobacter baumannii* strains isolated in Spain due to an epidemic strain. J. Clin. Microbiol. **37:**758–761.
25. **Villers, D., E. Espaze, M. Coste-Burel, F. Giauffret, E. Ninin, F. Nicolas, and H. Richet.** 1998. Nosocomial Acinetobacter baumannii infections: microbiological and clinical epidemiology. Ann. Intern. Med. **129:**182–189.
26. **Visalli, M. A., S. Bajaksouzian, M. R. Jacobs, and P. C. Appelbaum.** 1997. Comparative activity of trovafloxacin, alone and in combination with other agents, against gram-negative nonfermentative rods. Antimicrob. Agents Chemother. **41:**1475–1481.
27. **Wisplinghoff, H., W. Perbix, and H. Seifert.** 1999. Risk factors for nosocomial bloodstream infections due to *Acinetobacter baumannii*—a case-control study in adult burn patients. Clin. Infect. Dis. **28:**59–66.
28. **Wisplinghoff, H., M. B. Edmond, M. A. Pfaller, R. N. Jones, R. P. Wenzel, and H. Seifert.** 2000. Nosocomial blood stream infections due to *Acinetobacter* spp. in US hospitals: clinical features, molecular epidemiology, and antimicrobial susceptibility. Clin. Infect. Dis., in press.

Downloaded from http://aac.asm.org/ on February 26, 2021 by guest