ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2002, p. 3068–3070 Vol. 46, No. 9
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.9.3068–3070.2002

In Vitro Activities of Garenoxacin (BMS-284756) against 170 Clinical Isolates of Nine *Pasteurella* Species

Ellie J. C. Goldstein,* Diane M. Citron, C. Vreni Merriam, Yumi A. Warren, Kerin L. Tyrrell, and Helen T. Fernandez

R. M. Alden Research Laboratory, Santa Monica-University of California, Los Angeles, Medical Center, Santa Monica, California 90404, and University of California, Los Angeles, School of Medicine, Los Angeles, California 90073

Received 12 November 2001/Returned for modification 28 March 2002/Accepted 21 May 2002

The in vitro susceptibilities of 170 clinical isolates plus 12 American Type Culture Collection strains of *Pasteurella* species comprising nine species and three *Pasteurella multocida* subspecies were studied by an agar dilution method. Garenoxacin (BMS-284756), a new des-fluoro(6) quinolone, was active at ≤0.06 μg/ml against all isolates, including four β-lactamase-producing strains, with >90% of the strains susceptible to ≤0.008 μg/ml. Garenoxacin was generally 1 to 2 dilutions more active than levofloxacin and moxifloxacin and was the most active agent tested. Cefoxitin required 1 μg/ml for inhibition of 51 of 182 (29%) of strains, and 3 strains (also β-lactamase producers) were resistant to doxycycline.

Approximately 245,000 to 725,000 (5 to 15% of the 4.5 million people who suffer animal-bite wounds in the United States annually seek medical attention for infection, including 30,000 patients who visit an emergency department for medical treatment and 10,000 who are hospitalized with a bite wound infection (5, 16). Bite wound infections involve a complex polymicrobial flora (5, 6, 16), among which *Pasteurella multocida* is considered an important pathogen by clinicians. Recent advances in molecular methods have suggested a more complex taxonomic structure of the genus *Pasteurella*, with 11 genetically closely related taxa, the establishment of three subspecies of *P. multocida*, and a reevaluation of 11 taxa with low levels of genetic relatedness (4, 13). Many of the genetically closely related species have different ecological niches, different pathogenic potentials, and different propensities for specific tissue invasion. A recent prospective study (16) demonstrated that *Pasteurella* species are the most common aerobic isolates from both dog bites (50%) and cat bites (75%). However, *P. canis* is the predominant isolate present in 26% of dog-bite wounds, followed by *P. stomatis* (12%) and *P. multocida* subsp. *multocida* (12%). In addition, *P. dagmatis* (4%) and *P. multocida* subsp. *gallicida* (2%) and *P. multocida* subsp. *septica* (10%) are also encountered in dog-bite wounds. In contrast, *P. multocida* subsp. *multocida* and *P. multocida* subsp. *septica* are more prevalent in cat-bite wound infections (present in 54 and 28% of such wound infections, respectively). Most studies of the antimicrobial susceptibilities of members of the family *Pasteurellaceae* are often limited because veterinary and human isolates are combined or the full spectrum of *Pasteurella* species causing human infections is not tested.

Pasteurella species are associated with a variety of human infections, most but not all of which are associated with animal contact, especially dog and cat bites. While many patients with bite wounds receive a β-lactam agent such as amoxicillin-clavulanate, approximately 20% will report a history of an adverse reaction to penicillin or other β-lactam antibiotics and require an appropriate alternative agent. Fluoroquinolones have been suggested as alternative agents for the treatment of bite wounds. Garenoxacin {BMS-284756; T-3811ME; 1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-1-methyl-2,3-dihydro-1*H*-5-isoindolyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid methanesulfonate monohydrate} is a new des-fluoro(6) quinolone that lacks the 6-position fluorine that characterizes the previous class of fluoroquinolones. Preliminary data (2, 10, 15) indicate that this drug has a broad spectrum of activity against most gram-positive and gram-negative aerobes including certain strains that are resistant to other fluoroquinolones.

We therefore studied the antimicrobial susceptibilities of 170 clinical isolates and 12 American Type Culture Collection (ATCC) strains of the family *Pasteurellaceae* consisting of the nine species and three *P. multocida* subspecies most often associated with human infections.

Almost all of the strains had previously been isolated from bite wounds (almost all of the strains were isolated from 1995 to 2002) and were identified according to standard criteria (3, 12, 13). The sources of the isolates were as follows: dog bites, 60 strains; cat bites, 81 strains; tiger bites, 2 strains; bites caused by other animals, 15 strains; and bovine respiratory sources, 12 strains. The three isolates of *P. caballi* and six of the eight strains of *P. haemolytica* were from bovine respiratory sources. For comparative purposes, 12 ATCC strains of 10 species (*P. aerogenes* ATCC 12192, *P. canis* ATCC 43326, *P. dagmatis* ATCC 43325, *P. haemolytica* ATCC 33396, *P. multocida* subsp. *gallicida* ATCC 51689 and ATCC 51696, *P. multocida* subsp. *multocida* ATCC 7228 and ATCC 12947, *P. multocida* subsp. *septica* ATCC 51688, *P. pneumotropica* ATCC 35149, *P. stomatis* ATCC 43327, and *P. testudinis* ATCC 3368) were also tested.

Standard laboratory powders were supplied, as follows: garenoxacin, Bristol-Myers Squibb Co., Princeton, N.J.; amoxicillin-clavulanate, SmithKline Beecham Pharmaceuticals, Philadelphia, Pa.; ampicillin-sulbactam, Pfizer Inc., New York, N.Y.; levofloxacin, Ortho-McNeil Pharmaceuticals, Raritan, N.J.; moxifloxacin, Bayer Corp., West Haven, Conn.; cefoxitin,

* Corresponding author. Mailing address: 2021 Santa Monica Blvd., Suite 740 East, Santa Monica, CA 90404. Phone: (310) 315-1511. Fax: (310) 315-3662. E-mail: EJCGMD@aol.com.

Merck & Co., West Point, Pa.; and doxycycline and penicillin G, Sigma Chemical Co., St. Louis, Mo. Antimicrobial agents were reconstituted according to the instructions of the manufacturers. Serial twofold dilutions were added to the media on the day of testing.

Frozen cultures were transferred twice to ensure purity and good growth. Susceptibility testing was performed according to NCCLS standards (14). The basal medium used was Mueller-Hinton agar supplemented with 5% sheep blood. The agar plates were inoculated with a Steers replicator (Craft Machine Inc., Chester, Pa.) with an inoculum of 10^4 CFU per spot. Control plates without antimicrobial agents were inoculated before and after the inoculation of each set of drug-containing plates. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were included as controls. Plates were incubated at 35°C in an aerobic environment for 18 to 20 h and were then examined; the MIC was defined as the lowest concentration of an agent that yielded no growth or a marked change in growth compared to the growth on the control plate.

The results of the present study, in which we tested a large number of clinical isolates of nine *Pasteurella* species, including the three *P. multocida* subspecies, are presented in Table 1. Garenoxacin was active at <0.06 μg/ml against all isolates, including four β-lactamase-producing strains (*P. aerogenes*, $n = 1$; *P. caballi*, $n = 1$; *P. haemolytica*, $n = 2$), with >90% of the strains susceptible to <0.008 μg/ml. Three of these four strains, including the two strains of *P. haemolytica* and the single strain of *P. aerogenes*, were also resistant to doxycycline. Cefoxitin required 1 to 2 μg/ml for inhibition of 51 of 182 (29%) strains. Garenoxacin was generally 1 to 2 dilutions more active than levofloxacin and moxifloxacin on a weight basis and was the most active agent tested.

Pasteurella species are normal inhabitants of 12 to 92% of dogs and 52 to 99% of cats (9), and most human infections are associated with bites or animal exposure. Many publications do not differentiate or identify the different *Pasteurella* species, sometimes due to technical difficulties with the interpretation of biochemical tests (3). Our laboratory has recently reported (2) on the use of PCR fingerprinting and α-glucosidase activity as a means of differentiating *P. multocida* subsp. *multocida* from *P. multocida* subsp. *septica.* Some investigators (1, 11) have suggested different pathogenic potentials and ecological niches for the different species, which increases the clinical importance of differentiating these species in reports of studies. For example, *P. multocida* subsp. *multocida* and *P.* multocida subsp. *septica* are more frequently recovered from "more serious" infections (11), including bacteremias, and *P. multocida* subsp. *septica* is more frequently isolated from cat-bite wounds and has an affinity for the central nervous system (1).

In a prior study from our laboratory (8a), in which the same methodology described here but a limited number of isolates was used, we found that all *Pasteurella multocida* subsp. *multocida* and *P. multocida* subsp. *septica* isolates were susceptible to ≤0.015 μg of garenoxacin per ml. In general, the quinolones, including gatifloxacin (7), moxifloxacin (7), trovafloxacin (8), and levofloxacin (8, 8a), have consistently been active against *P. multocida* and other *Pasteurella* species.

On the basis of our in vitro data, garenoxacin offers an alternative for the treatment of human infections associated with all *Pasteurella* species.

TABLE 1. Comparative in vitro activities of the des-quinolone garenoxacin (BMS-284756) and seven other agents against 182 clinical isolates of *Pasteurella* species

Species (no. of isolates) and antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
P. canis (24)			
Garenoxacin	0.001–0.015	0.004	0.008
Moxifloxacin	0.008–0.015	0.008	0.015
Levofloxacin	0.004–0.015	0.015	0.015
Penicillin G	0.02–0.125	0.06	0.125
Amoxicillin-clavulanate	0.02–0.25	0.125	0.25
Ampicillin-sulbactam	0.02–0.25	0.125	0.125
Cefoxitin	0.02–1	0.5	0.5
Doxycycline	0.05–0.25	0.125	0.125
P. dagmatis (12)			
Garenoxacin	0.004–0.015	0.008	0.015
Moxifloxacin	0.008–0.03	0.015	0.03
Levofloxacin	0.008–0.015	0.015	0.015
Penicillin G	0.02–0.125	0.06	0.125
Amoxicillin-clavulanate	0.02–0.25	0.125	0.25
Ampicillin-sulbactam	0.06–0.125	0.125	0.125
Cefoxitin	0.125–0.5	0.25	0.5
Doxycycline	0.125–0.25	0.125	0.125
P. multocida subsp. *multocida* (61)			
Garenoxacin	0.001–0.015	0.008	0.008
Moxifloxacin	0.008–0.03	0.015	0.015
Levofloxacin	0.008–0.03	0.015	0.015
Penicillin G	0.03–0.125	0.125	0.125
Amoxicillin-clavulanate	0.125–0.25	0.25	0.25
Ampicillin-sulbactam	0.06–0.25	0.25	0.25
Cefoxitin	0.25–1	0.5	1
Doxycycline	0.05–0.25	0.125	0.125
P. multocida subsp. *septica* [45]			
Garenoxacin	0.001–0.015	0.008	0.008
Moxifloxacin	0.008–0.03	0.015	0.03
Levofloxacin	0.008–0.03	0.015	0.03
Penicillin G	0.02–0.125	0.125	0.125
Amoxicillin-clavulanate	0.06–0.25	0.25	0.25
Ampicillin-sulbactam	0.06–0.25	0.25	0.25
Cefoxitin	0.25–1	1	1
Doxycycline	0.06–0.25	0.125	0.125
P. stomatis (21)			
Garenoxacin	0.001–0.008	0.004	0.008
Moxifloxacin	0.008–0.015	0.008	0.015
Levofloxacin	0.008–0.015	0.008	0.015
Penicillin G	0.02–0.125	0.06	0.06
Amoxicillin-clavulanate	0.02–0.25	0.125	0.25
Ampicillin-sulbactam	0.02–0.25	0.06	0.125
Cefoxitin	0.25–1	0.5	0.5
Doxycycline	0.125–0.25	0.125	0.25
Other *P.* species[b] (19)			
Garenoxacin	0.004–0.06	0.015	0.06
Moxifloxacin	0.003–0.125	0.015	0.06
Levofloxacin	0.008–0.006	0.03	0.06
Penicillin G	0.06–>8	0.125	>8
Amoxicillin-clavulanate	0.02–0.25	0.125	0.25
Ampicillin-sulbactam	0.02–2	0.125	0.25
Cefoxitin	0.25–2	0.5	1
Doxycycline	0.125–>8	0.5	>8

[a] 50% and 90% MICs at which 50 and 90% of isolates tested, respectively, were inhibited.

[b] Includes the following species: *P. aerogenes* ($n = 1$), *P. caballi* ($n = 3$), *P. haemolytica* ($n = 8$), *P. multocida* subsp. *gallicida*, ($n = 4$), *P. pneumotropica* ($n = 2$), and *P. testudinis* ($n = 1$).

This study was supported, in part, by a grant from Bristol-Myers Squibb Co.

We thank Judee H. Knight and Alice E. Goldstein for assistance.

REFERENCES

1. **Biberstein, E. L., S. J. Spenser, P. H. Kass, and D. C. Hirsh.** 1990. Distribution of indole-producing urease-negative pasteurellas in animals. J. Vet. Diagn. Investig. **3:**319–323.
2. **Fung-Tomc, J. C., B. Minassian, B. Kolek, E. Huczko, L. Aleksunes, T. Stickle, T. Washo, E. Gradelski, L. Vallera, and D. P. Bonner.** 2000. Antibacterial spectrum of a novel des-fluoro(6) quinolone, BMS 284756. Antimicrob. Agents Chemother. **44:**3351–3356.
3. **Gerardo, S. H., M. C. Claros, D. M. Citron, and E. J. C. Goldstein.** 2001. Identification of *Pasteurella multocida* subspecies *multocida* and subspecies *septica*: polymerase chain reaction fingerprinting and α-glucosidase activity. J. Clin. Microbiol. **39:**2258–2264.
4. **Gerardo, S. H., and E. J. C. Goldstein.** 1998. *Pasteurella multocida* and other species, p. 326–335. *In* V. Yu and T. Merrigan (ed.), Antimicrobial therapy & vaccines. The Williams & Wilkins Co., Baltimore, Md.
5. **Goldstein, E. J. C.** 1992. Bite wounds and infection. Clin. Infect. Dis. **14:** 633–640.
6. **Goldstein, E. J. C.** 1998. New horizons in the bacteriology, antimicrobial susceptibility and therapy of animal bite wounds. J. Med. Microbiol. **47:**1–3.
7. **Goldstein, E. J. C., D. M. Citron, M. Hudspeth, S. H. Gerardo, and C. V. Merriam.** 1997. In vitro activity of Bay 12–8039, a new 8-methoxy quinolone, compared to 11 other oral antimicrobial agents against 390 aerobic and anaerobic bacteria isolated from human and animal bite wound skin and soft tissue infections in humans. Antimicrob. Agents Chemother. **41:**1552–1557.
8. **Goldstein, E. J. C., D. M. Citron, M. Hudspeth, S. H. Gerardo, and C. V. Merriam.** 1998. Trovafloxacin compared with levofloxacin, ofloxacin, ciprofloxacin, azithromycin and clarithromycin against unusual aerobic and anaerobic human and animal bite-wound pathogens. J. Antimicrob. Chemother. **41:**391–396.

8a. **Goldstein, E. J. C., et al.** 2002. Antimicrob. Agents Chemother. **46:**866–870.

9. **Greene, C. E., E. J. C. Goldstein, and J. C. Wright.**1998. Bite wound infections: dog and cat bite infections; rat bite infections, p. 330–337. *In* Infectious diseases of the dog and cat, 2nd ed. The W. B. Saunders Co., Philadelphia, Pa.
10. **Hoellman, D. B., L. M. Kelly, M. R. Jacobs, and P. C. Appelbaum.** 2001. Comparative antianaerobic activity of BMS 284756. Antimicrob. Agents Chemother. **45:**589–592.
11. **Holst, E., J. Rollof, L. Larsson, and J. P. Neilsen.** 1992. Characterization and distribution of *Pasteurella* species recovered from infected humans. J. Clin. Microbiol. **30:**2984–2987.
12. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1998. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
13. **Mutters, R., P. Ihm, S. Pohl, W. Frederiksen, and W. Mannheim.** 1985. Reclassification of the genus *Pasteurella* Trevisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, *Pasteurella anatis*, and *Pasteurella langaa*. Int. J. Syst. Bacteriol. **35:**309–322.
14. **National Committee for Clinical Laboratory Standards.** 1998. Method for dilution antimicrobial susceptibility testing for bacteria that grow aerobically: approved standard, 4th ed., NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
15. **Takahata, M., J. Mitsuyama, Y. Yamashiro, M. Yonezawa, H. Araki, Y. Todo, S. Minami, Y. Watanabe, and H. Narita.** 1999. In vitro and in vivo antimicrobial activity of T-3811ME, a novel des-F(6)-quinolone. Antimicrob. Agents Chemother. **43:**1077–1084.
16. **Talan, D. A., D. M. Citron, F. A. Abrahamian, G. J. Moran, E. J. C. Goldstein, and the Emergency Medicine Animal Bite Infection Study Group.** 1999. The bacteriology and management of dog and cat bite wound infections presenting to emergency departments. N. Engl. J. Med. **340:**85–92.