ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 2135–2139
0066-4804/91/102135-05$02.00/0

Vol. 35, No. 10

Susceptibility of *Staphylococcus* Species and Subspecies to Fleroxacin

TAMMY L. BANNERMAN,[1]* DIANE L. WADIAK,[1] AND WESLEY E. KLOOS[2]

Departments of Microbiology[1] and Genetics,[2] North Carolina State University, Raleigh, North Carolina 27695-7614

Received 4 April 1991/Accepted 24 July 1991

Twenty-four *Staphylococcus* species or subspecies were examined for their susceptibilities to the fluoroquinolone fleroxacin (Ro 23-6240) by disk diffusion (5-μg disk) and by agar dilution for the determination of MICs. Resistant strains were further tested for their susceptibilities to oxacillin and the fluoroquinolone ciprofloxacin. Reference strains of the novobiocin-resistant species (*Staphylococcus saprophyticus*, *Staphylococcus cohnii*, *Staphylococcus xylosus*, *Staphylococcus arlettae*, and *Staphylococcus gallinarum*) had an intrinsic intermediate susceptibility (MIC, 4 μg/ml) to fleroxacin. Fleroxacin resistance was not observed in the reference strains of the novobiocin-susceptible species (MIC, 0.5 to 2.0 μg/ml). Clinical isolates of coagulase-negative species were generally less susceptible to fleroxacin than were reference strains. Seven percent of the *Staphylococcus epidermidis* clinical strains were resistant (MIC, ≥8 μg/ml) to fleroxacin. Of these strains, 77% were resistant to oxacillin and 50% were resistant to ciprofloxacin. Thirty-four percent of the *Staphylococcus haemolyticus* clinical strains were resistant to fleroxacin, and 9% had intermediate susceptibility. Of the resistant strains, 95% were resistant to oxacillin and 77% were resistant to ciprofloxacin, while 23% had intermediate susceptibility to ciprofloxacin. Fleroxacin is an effective antimicrobial agent against most staphylococci.

Fleroxacin (Ro 23-6240) is a new orally and intravenously administered trifluoroquinolone with a half-life of 9 to 10 h in humans and peak levels of 2.3 μg/ml in serum after a single 200-mg oral dose (12). The drug has shown excellent activity against most members of the family *Enterobacteriaceae*, inhibiting 90% of isolates per species at concentrations of <1 μg/ml (2, 3). Because of its broad spectrum of activity, fleroxacin may also have use for treating staphylococcal infections; a previous report has shown the in vitro effectiveness of fleroxacin against two species of staphylococci (9). In the current study, 24 *Staphylococcus* species or subspecies were tested for their susceptibilities to fleroxacin by disk diffusion and by agar dilution for determination of MICs. The resistant strains were further tested for their susceptibilities to oxacillin and to the fluoroquinolone ciprofloxacin.

(A preliminary report of this work was presented previously [1].)

Reference strains of the 24 *Staphylococcus* species or subspecies were obtained from our laboratory's collection of isolates from the skin of healthy human volunteers or from animals. Clinical strains used in this study were isolated from April 1989 to February 1990 from a variety of patients at Rex Hospital, Raleigh, N.C. All the clinical strains were identified by using the criteria of Kloos and coworkers (4, 5). The reference and clinical strains were maintained on P agar (11) at 4°C for up to 2 months and preserved by desiccation on steatite fish spine insulator beads at 4°C. These storage conditions do not alter the antibiograms or plasmid compositions of strains tested in this laboratory.

Fleroxacin as powder and in 5-μg disks was kindly provided by Hoffmann-La Roche, Inc. (Nutley, N.J.). Oxacillin as powder was obtained from Sigma (St. Louis, Mo.), and oxacillin in 1-μg disks and ciprofloxacin in 5-μg disks were obtained from BBL Microbiological Systems (Cockeysville, Md.). Disk diffusion susceptibility testing was performed by the procedure outlined by the National Committee for Clinical Laboratory Standards (7). Agar dilution susceptibility tests for fleroxacin and oxacillin were performed by the procedure outlined by the National Committee for Clinical Laboratory Standards (8) with Mueller-Hinton agar. Oxacillin plates were supplemented with 4% NaCl. The plates were inoculated by using the Steers replicator (10). Ciprofloxacin MICs were obtained from the results of the Vitek GPS-SA card (Vitek Systems, Hazelwood, Mo.).

For the purpose of this study, the criteria for the determination of susceptibility, intermediate susceptibility, and resistance to fleroxacin were the following: susceptible, MIC of ≤2 μg/ml or zone of inhibition of ≥19 mm; intermediate, MIC of 4 μg/ml or zone of inhibition of 16 to 18 mm; resistant, MIC of ≥8 μg/ml or zone of inhibition of ≤15 mm (3). Criteria for susceptibilities to oxacillin and ciprofloxacin were those recommended by the National Committee for Clinical Laboratory Standards (7, 8).

A total of 338 reference strains of staphylococci were analyzed for their susceptibilities to fleroxacin (Table 1). The novobiocin-resistant species *Staphylococcus saprophyticus*, *S. xylosus*, *S. cohnii*, *S. arlettae*, *S. kloosii*, and *S. gallinarum* demonstrated an intrinsic intermediate susceptibility (MIC, 4 μg/ml; zone of inhibition, 16 to 19 mm) and were less susceptible to fleroxacin than were the novobiocin-susceptible species (MIC, 0.5 to 2.0 μg/ml; zone of inhibition, 21 to 27 mm). The novobiocin-resistant species were also more resistant to the fluoroquinolone ciprofloxacin than were the novobiocin-susceptible species; however, on the basis of the recommended cutoff points, they were just included in the susceptible category. DNA gyrase is a common target for novobiocin and the fluoroquinolones; this fact may account for the related responses of these species.

A total of 478 clinical strains of staphylococci were analyzed for their susceptibilities to fleroxacin (Table 1). However, six isolates of *S. haemolyticus* were found to be members of three different strains on the basis of antibio-

* Corresponding author.

TABLE 1. Fleroxacin susceptibilities of *Staphylococcus* species

Species or subspecies[a]	Source[b]	No. of strains	MIC (μg/ml)[c]		Zone of inhibition (diam, mm)[d]	
			Mean	Range	Mean	Range
S. epidermidis	S	20	0.5	≤0.25–1.0	24 ± 2	21–26
	C	315	1.0	≤0.25–32	24 ± 2	12–30
S. capitis subsp. *capitis*	S	20	2.0	≤0.25–2.0	21 ± 1	19–24
	C	7	2.0	1.0–4.0	23 ± 2	21–26
S. capitis subsp. *ureolyticus*	S	10	1.0	1.0	22 ± 1	20–24
	C	10	2.0	1.0–4.0	24 ± 3	20–30
S. caprae	S	10	1.0	0.5–1.0	25 ± 1	23–28
S. warneri	S	16	1.0	0.5–1.0	23 ± 2	20–26
	C	18	2.0	1.0–8.0	23 ± 3	15–29
S. warneri subsp. 2	S	4	1.0	0.5–1.0	23 ± 2	20–25
S. hominis	S	20	0.5	≤0.25–0.5	27 ± 2	23–30
	C	35	0.5	0.5–16	26 ± 2	9–30
S. haemolyticus	S	15	0.5	≤0.25–0.5	27 ± 3	24–35
	C	64	1, 32[e]	≤0.25–32	23 ± 3, 6[e]	5–29
S. haemolyticus subsp. 2	S	5	0.5	0.5	24 ± 2	22–26
S. lugdunensis	S	20	1.0	0.5–1.0	23 ± 2	21–26
	C	14	1.0	0.5–2.0	25 ± 3	19–29
S. aureus	S	20	0.5	≤0.25–1.0	23 ± 2	18–26
S. auricularis	S	8	1.0	0.5–1.0	24 ± 2	21–27
S. saprophyticus	S	20	4.0	2.0–4.0	16 ± 1	14–17
	C	3	4.0	4.0	16 ± 0	16
S. cohnii subsp. *cohnii*	S	18	2.0	1.0–4.0	19 ± 3	15–24
S. cohnii subsp. *urealyticum*	S	10	4.0	2.0–4.0	17 ± 2	14–22
S. xylosus	S	9	2.0	2.0–4.0	16 ± 1	15–18
S. arlettae	S	4	1.0	1.0–4.0	18 ± 1	17–19
S. kloosii	S	10	2.0	1.0–2.0	19 ± 2	17–24
S. gallinarum	S	7	4.0	1.0–4.0	16 ± 2	14–20
S. simulans	S	20	0.5	0.5	25 ± 2	22–28
	C	12	0.5–1.0	0.5–1.0	24 ± 1	22–26
S. intermedius	S	20	1.0	0.5–1.0	22 ± 2	15–25
S. schleiferi	S	4	1.0	1.0–2.0	21 ± 1	20–21
S. hyicus	S	20	1.0	0.5–1.0	22 ± 2	20–27
S. chromogenes	S	10	0.5	0.5–2.0	22 ± 2	20–25
S. sciuri	S	10	1.0	1.0–2.0	19 ± 1	17–21

[a] *S. equorum* and *S. lentus* strains grew poorly on Mueller-Hinton agar and produced variable results, but all were susceptible to fleroxacin except one strain of *S. lentus*, which was intermediate by MIC and disk diffusion (MIC, 4 μg/ml; zone of inhibition, 17 mm). The one strain of *S. caseolyticus* tested was susceptible to fleroxacin.

[b] S, standard reference strains isolated from healthy human or animal skin or from veterinary clinical specimens; C, strains from human clinical specimens taken at Rex Hospital (1989 to 1990).

[c] Determined by agar dilution. Resistant, ≥8 μg/ml; intermediate, 4 μg/ml; susceptible, ≤2 μg/ml.

[d] Determined by disk diffusion. Resistant, ≤15 mm; intermediate, 16 to 18 mm; susceptible ≥19 mm.

[e] *S. haemolyticus* clinical strains had two populations, susceptible and resistant.

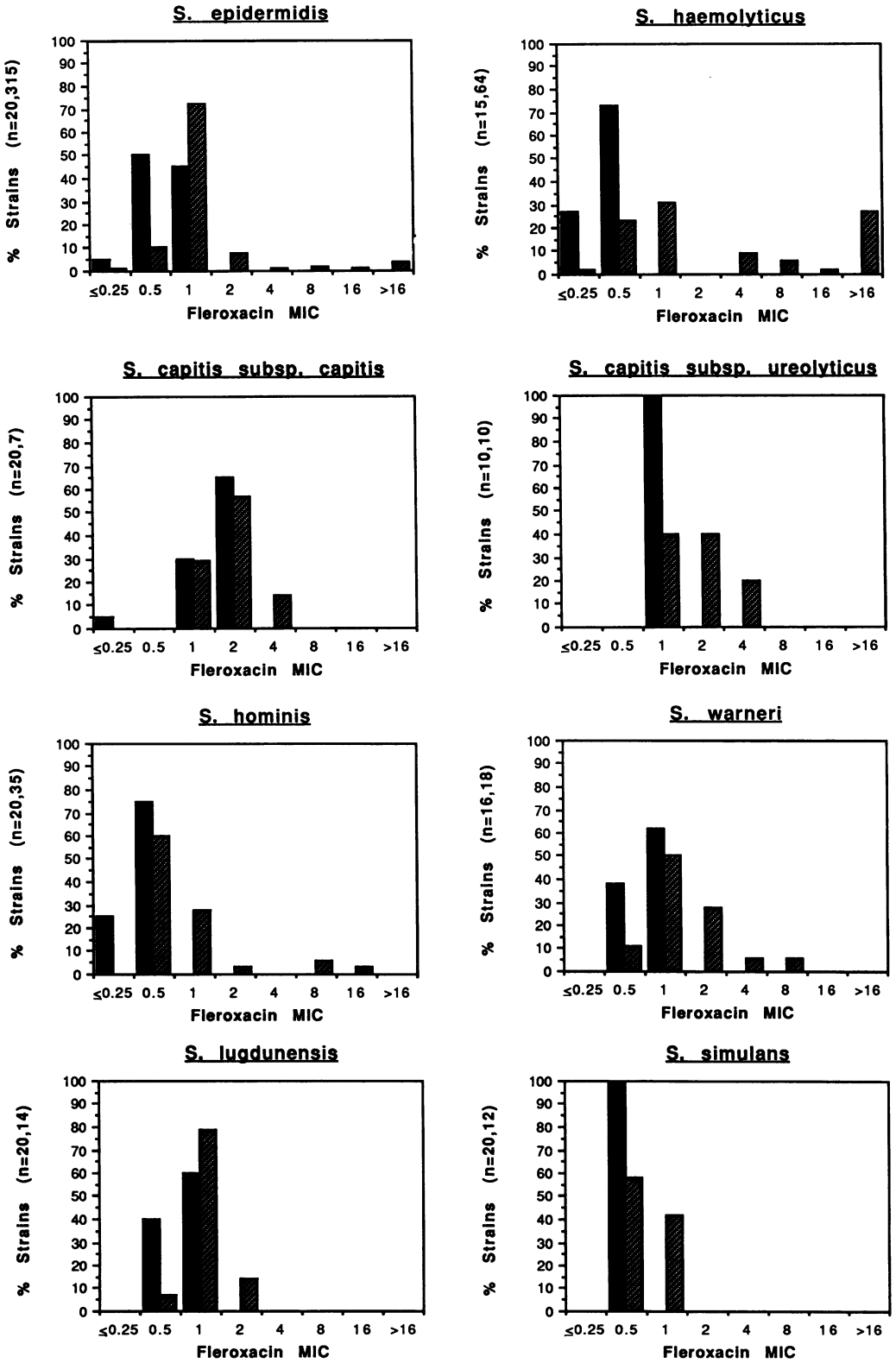

FIG. 1. Comparison of fleroxacin MICs for reference and clinical *Staphylococcus* strains. The numbers of strains analyzed are indicated in parentheses; the number of reference strains is shown first, followed by the number of clinical strains. The percentages of reference and clinical strains for which specific MICs were determined are indicated by solid and striped bars, respectively.

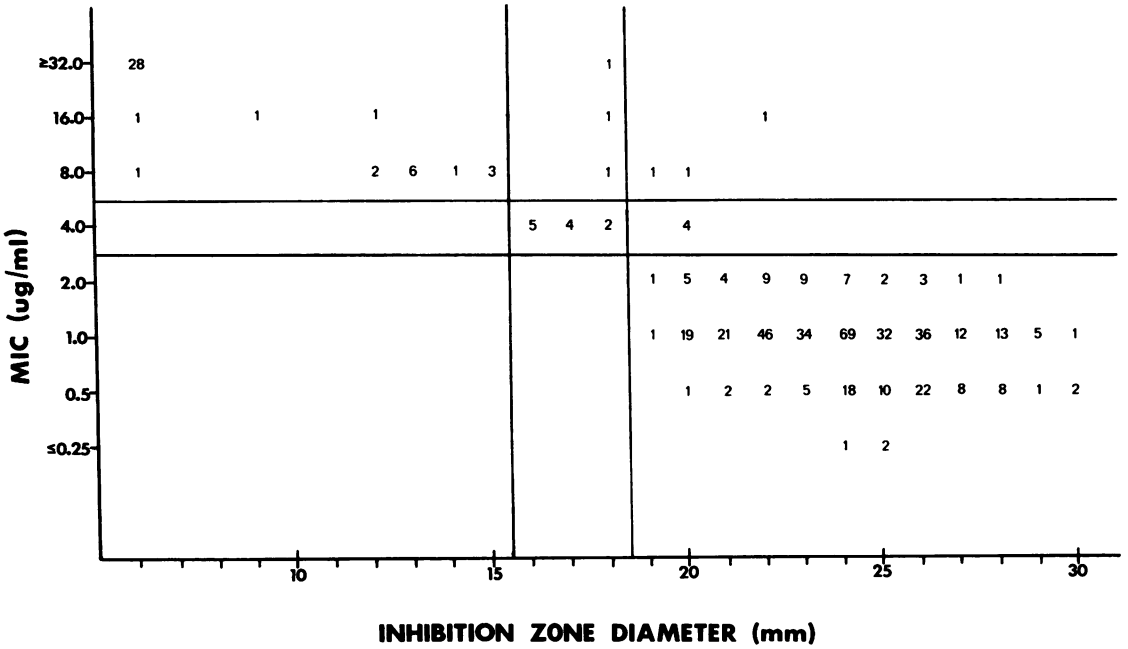

FIG. 2. Scattergram showing correlations among fleroxacin MICs and zone diameters around 5-μg fleroxacin disks for 478 isolates. Numbers represent the number of datum points at each location.

grams, biochemical reactions, colony morphologies, and plasmid compositions. The clinical strains were generally less susceptible to fleroxacin than were reference strains isolated from the skin of healthy volunteers. The MIC range for clinical strains was ≤0.25 to 32 μg/ml, whereas that for reference strains was ≤0.25 to 4 μg/ml. The differences in MICs are shown in Fig. 1. The largest difference in mean MIC was for clinical and reference strains of *S. haemolyticus*. There were two populations of clinical strains of *S. haemolyticus*, susceptible and resistant. Some clinical strains of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* either had intermediate susceptibility or were resistant to fleroxacin. Of 315 strains of *S. epidermidis*, 23 strains (7.3%) were resistant and 2 strains (0.6%) were intermediate by MIC. Of the resistant *S. epidermidis* strains, 17 were resistant and 3 were intermediate by disk diffusion. Both intermediate strains were intermediate by disk diffusion. Of 64 strains of *S. haemolyticus*, 22 strains (34.4%) were resistant and 6 strains (9.4%) were intermediate by MIC. Of the 22 resistant strains, all were resistant by disk diffusion. Five of the six strains intermediate by MIC were intermediate by disk diffusion. Three strains (8.6%) of *S. hominis* were resistant by MIC, and the disk diffusion data supported this finding. One strain of *S. warneri* showed resistance by MIC, and this was supported by disk diffusion. The correlation between zones of inhibition and MICs is shown in Fig. 2. Strains of *S. epidermidis* accounted for the major errors in the correlation between disk diffusion and MIC results. One likely source of error may be due to the difficulty in suspending adherent strains of *S. epidermidis* in aqueous solutions. The isolates resistant to fleroxacin were well distributed between inpatients and outpatients, and there were no clusters of infection noted within the hospital.

The resistant strains of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* were analyzed for their susceptibilities to oxacillin and ciprofloxacin. Of the 23 fleroxacin-resistant strains of *S. epidermidis*, 17 strains (77%) were oxacillin resistant and 11 strains (50%) were ciprofloxacin resistant. Of the 22 fleroxacin-resistant strains of *S. haemolyticus*, 21 strains (95%) were oxacillin resistant and 17 strains (77%) were ciprofloxacin resistant, while 23% had intermediate susceptibility to ciprofloxacin. All three fleroxacin-resistant strains of *S. hominis* were oxacillin susceptible, and one strain showed resistance to ciprofloxacin. The fleroxacin-resistant strain of *S. warneri* was susceptible to both oxacillin and ciprofloxacin. The fleroxacin-resistant strains of *S. epidermidis* and *S. haemolyticus* made up 16.5 and 56.8% of the total number of oxacillin-resistant strains of that species, respectively.

We conclude that fleroxacin is an effective antimicrobial agent against most staphylococci. However, in vitro tests indicate that in a clinical setting resistant strains of *S. haemolyticus* and *S. epidermidis* are being selected, and in several other species strains with reduced susceptibilities are being selected.

This work was supported by a grant from Hoffmann-La Roche, Inc.

We are grateful to Barbara Leasure for typing the manuscript.

REFERENCES

1. **Bannerman, T., D. Wadiak, and W. Kloos.** 1990. Program Abstr. 3rd Int. Symp. New Quinolones, abstr. no. 64.
2. **Chin, N.-X., D. C. Brittain, and H. C. Neu.** 1986. In vitro activity of Ro 23-6240, a new fluorinated 4-quinolone. Antimicrob. Agents Chemother. **29**:675–680.
3. **Fuchs, P. C., R. N. Jones, A. L. Barry, L. W. Ayers, T. L. Gavan, E. H. Gerlach, and C. Thornsberry.** 1987. Ro 23-6240 (AM-833), a new fluoroquinolone: in vitro antimicrobial activity and tentative disk diffusion interpretive criteria. Diag. Microbiol. Infect. Dis. **7**:29–35.
4. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified schemes for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–87.
5. **Kloos, W. E., and J. F. Wolfshohl.** 1982. Identification of *Staphylococcus* species with the API STAPH-IDENT system.

J. Clin. Microbiol. **16**:509–516.
6. **Manek, N., J. M. Andrews, and R. Wise.** 1986. In vitro activity of Ro 23-6240, a new difluoroquinolone derivative, compared with that of other antimicrobial agents. Antimicrob. Agents. Chemother. **30**:330–332.
7. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M2-A4. Performance standards for antimicrobic disk tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **Pohlod, D. J., L. D. Saravolatz, and M. M. Somervolle.** 1988. In-vitro susceptibility of staphylococci to fleroxacin in comparison with six other quinolones. J. Antimicrob. Chemother. **22**(Suppl. D):35–41.
10. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **9**:307–311.
11. **Vera, H. D., and D. A. Powers.** 1980. Culture media, p. 965–999. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
12. **Weidekamm, E., R. Pertman, K. Suter, C. Partos, D. Dell, and P. W. Lücker.** 1987. Single- and multiple-dose pharmacokinetics of fleroxacin, a trifluorinated quinolone, in humans. Antimicrob. Agents Chemother. **31**:1909–1914.