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In Vitro Activity of Ciprofloxacin against Aerobic Bacteria Isolated in a Southern European Hospital

GIUSEPPE CORNAGLIA,[1]† * RAFFAELLO POMPEI,[1] BENEDETTO DAINELLI,[2] AND GIUSEPPE SATTA[1]

Istituto di Microbiologia, Università degli Studi di Cagliari, Cagliari,[1] and Dipartimento di Medicina Sperimentale e Scienze Biochimiche, II Università degli Studi di Roma, Rome,[2] Italy

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The activity of ciprofloxacin was evaluated against 1,204 isolates freshly isolated in Southern Europe, including 193 isolates of 10 species never studied before. Ciprofloxacin proved more active than other quinolones and very active in absolute terms against the 10 new species and showed against the other species an activity close to that reported for isolates from other geographic areas.

Although many studies have already investigated the in vitro antimicrobial activity of ciprofloxacin (2, 3, 5, 10, 23), the information concerning its activity against some bacterial groups is still incomplete. For instance, both the *Providencia* genus and enterococci species were fully identified in only one study each (7, 12), and the susceptibility of all of the coagulase-negative staphylococcal species has not been previously reported (6, 11). Moreover, the activity of ciprofloxacin against strains isolated from Southern Europe has been scarcely evaluated (18).

In this work, we examined 1,204 isolates of human origin, freshly isolated and examined by standard procedures (14) at the Clinical Microbiology Laboratory of the University of Cagliari. We accurately identified all the isolates at the species level, dividing larger bacterial groups for the first time into individual taxa and including isolates of 10 species whose susceptibilities have not been specifically tested before, namely, *Enterobacter agglomerans*, *Pseudomonas putrefaciens*, *Staphylococcus simulans* (lyogroup II), *Staphylococcus capitis* (lyogroup III), *Staphylococcus xylosus* and *Staphylococcus cohnii* (lyogroup IV), *Staphylococcus warneri* (lyogroup VI), *Streptococcus mitis*, *Streptococcus salivarius*, and *Streptococcus sanguis*.

Strains of the family *Enterobacteriaceae* were identified with the API 20E system. Because an acceptable identification of the species belonging to the *Providencia* genus relies on fermentation of carbohydrates not included in the API 20E strips, the identification of these strains was completed as proposed by Brenner et al. (4) and Farmer et al. (9) by testing the fermentation of adonitol, D-arabitol, trehalose, and D-galactose in phenol red broth base (Difco Laboratories) with the addition of individual carbohydrates (17).

Staphylococci were assigned to the different lyogroups (20, 21), which were demonstrated to correspond to the species proposed by Kloos and Schleifer (16) as indicated in Table 2 and whose identification was found to be more reliable than commercial systems of identification (19).

Enterococci were identified according to the method advocated by Facklam (8), on the basis of 27 biochemical and physiological tests.

The antimicrobial susceptibility tests were generally done by using a standard broth macrodilution technique with Mueller-Hinton broth, which was supplemented with 5% defibrinated sheep blood for the testing of streptococci (15). Inocula of 10^6 CFU/ml in tubes containing 10 ml of broth were obtained from an overnight culture in Todd-Hewitt broth (for streptococci) or Mueller-Hinton broth (for all other microorganisms). Susceptibility of *Haemophilus* spp. and *Branhamella* spp. was tested by an agar dilution procedure, as described for fastidious organisms (22).

Tables 1 and 2 show the MICs for the strains examined. Ciprofloxacin was clearly the most active of the quinolones tested against all the bacterial species, followed by norfloxacin.

In addition, ciprofloxacin showed a very wide spectrum of activity, since, in all of the 52 taxa considered, except for one (*Providencia stuartii*), at least 90% of the strains tested turned out to be either susceptible or intermediate, according to the interpretive breakpoints proposed by Barry et al. (1).

With regard to the comparison of the activity of ciprofloxacin against strains in different geographic areas, major differences were observed in the *Providencia* group, of which the *P. stuartii* strains turned out to be the least susceptible microorganisms, with MICs significantly higher than those reported in previous works (2, 5, 10) and 32-fold higher than those for *Providencia rettgeri*.

Differences were also observed among the *Pseudomonas* isolates, of which *Pseudomonas cepacia* turned out to be more susceptible than previously reported (5, 10, 13), and among beta-hemolytic streptococci, of which group C strains were fivefold more resistant.

Finally, the *Streptococcus faecium* strains, reported by others as being eightfold more resistant than *Streptococcus faecalis* strains (7), here showed the same susceptibility level.

As regards the groups divided for the first time into separate taxa, the various staphylococcal lyogroups showed susceptibilities not very different from those reported by others for coagulase-negative staphylococci not separated into species. On the contrary, the various alpha-hemolytic streptococcal species demonstrated different degrees of susceptibility to ciprofloxacin ranging from the relatively low MICs for 90% of strains tested (MIC_{90}s) obtained for *S.*

* Corresponding author.
† Present address: Istituto di Microbiologia, 37134 Verona, Italy.

TABLE 1. MICs of four antimicrobial agents for various organisms

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Escherichia coli (35)	Ciprofloxacin	0.03–0.25	0.015	0.6
	Norfloxacin	0.06–1	0.125	0.25
	Oxolinic acid	0.06–16	4	8
	Nalidixic acid	0.5–16	2	8
Citrobacter freundii (26)	Ciprofloxacin	0.015–0.5	0.015	0.125
	Norfloxacin	0.06–1	0.125	0.5
	Oxolinic acid	0.5–16	1	2
	Nalidixic acid	1–32	4	8
Citrobacter diversus (20)	Ciprofloxacin	0.015–0.125	0.015	0.03
	Norfloxacin	0.008–0.5	0.03	0.25
	Oxolinic acid	0.25–8	0.5	8
	Nalidixic acid	0.5–8	2	8
Enterobacter aerogenes (20)	Ciprofloxacin	0.008–0.5	0.03	0.06
	Norfloxacin	0.03–2	0.25	0.5
	Oxolinic acid	0.06–8	0.5	1
	Nalidixic acid	2–16	2	8
Enterobacter agglomerans (23)	Ciprofloxacin	0.015–0.5	0.03	0.125
	Norfloxacin	0.03–2	0.125	0.5
	Oxolinic acid	0.5–16	1	2
	Nalidixic acid	2–8	2	8
Enterobacter cloacae (21)	Ciprofloxacin	0.015–0.25	0.015	0.125
	Norfloxacin	0.06–1	0.25	1
	Oxolinic acid	0.125–16	0.5	2
	Nalidixic acid	1–16	2	8
Klebsiella pneumoniae (23)	Ciprofloxacin	0.015–0.25	0.03	0.06
	Norfloxacin	0.125–1	0.015	0.125
	Oxolinic acid	0.25–8	0.5	8
	Nalidixic acid	1–16	2	16
Klebsiella oxytoca (25)	Ciprofloxacin	0.008–0.25	0.015	0.125
	Norfloxacin	0.06–2	0.25	1
	Oxolinic acid	0.5–4	0.5	2
	Nalidixic acid	1–8	2	8
Serratia marcescens (21)	Ciprofloxacin	0.03–1	0.125	0.5
	Norfloxacin	0.25–2	0.5	1
	Oxolinic acid	0.5–4	0.5	2
	Nalidixic acid	0.5–16	1	4
Proteus mirabilis (23)	Ciprofloxacin	0.008–0.25	0.06	0.125
	Norfloxacin	0.03–1	0.125	0.5
	Oxolinic acid	0.25–4	0.5	1
	Nalidixic acid	1–16	2	8
Proteus vulgaris (20)	Ciprofloxacin	0.008–0.125	0.015	0.06
	Norfloxacin	0.06–1	0.125	0.5
	Oxolinic acid	0.125–4	0.5	1
	Nalidixic acid	1–16	4	8
Morganella morganii (23)	Ciprofloxacin	0.015–0.06	0.015	0.03
	Norfloxacin	0.03–0.5	0.015	0.06
	Oxolinic acid	0.06–1	0.06	0.25
	Nalidixic acid	1–16	4	8
Providencia rettgeri (25)	Ciprofloxacin	0.06–0.5	0.125	0.25
	Norfloxacin	0.125–2	0.5	1
	Oxolinic acid	0.125–4	0.5	4
	Nalidixic acid	1–16	8	16
Providencia stuartii (34)	Ciprofloxacin	0.25–8	2	8
	Norfloxacin	0.5–16	8	16
	Oxolinic acid	1–16	16	16
	Nalidixic acid	2–>64	16	32
Providencia alcalifaciens (20)	Ciprofloxacin	0.015–0.25	0.03	0.06
	Norfloxacin	0.03–1	0.06	0.5
	Oxolinic acid	0.125–4	0.5	4
	Nalidixic acid	1–16	4	16
Salmonella sp. (O) group A (27)	Ciprofloxacin	0.03–0.25	0.125	0.125
	Norfloxacin	0.06–0.5	0.25	0.5
	Oxolinic acid	1–4	2	4
	Nalidixic acid	1–8	4	8
Salmonella sp. (O) group B (23)	Ciprofloxacin	0.03–0.125	0.06	0.06
	Norfloxacin	0.06–0.5	0.125	0.5
	Oxolinic acid	0.125–2	0.25	1
	Nalidixic acid	1–64	4	32
Salmonella sp. (O) group C (20)	Ciprofloxacin	0.06–0.25	0.06	0.125
	Norfloxacin	0.03–1	0.125	0.5
	Oxolinic acid	0.25–4	0.5	2
	Nalidixic acid	4–8	4	8
Salmonella sp. (O) group D (26)	Ciprofloxacin	0.125–0.5	0.25	0.25
	Norfloxacin	0.06–1	0.25	0.5
	Oxolinic acid	0.5–4	0.5	4
	Nalidixic acid	2–16	4	16
Salmonella sp. (O) group E (21)	Ciprofloxacin	0.06–0.125	0.125	0.125
	Norfloxacin	0.125–2	0.25	1
	Oxolinic acid	0.25–4	0.5	2
	Nalidixic acid	4–16	4	8
Shigella dysenteriae (24)	Ciprofloxacin	0.015–0.06	0.03	0.03
	Norfloxacin	0.03–0.25	0.03	0.125
	Oxolinic acid	1–4	4	4
	Nalidixic acid	2–8	4	4
Shigella flexneri (21)	Ciprofloxacin	0.015–0.06	0.015	0.03
	Norfloxacin	0.03–0.125	0.125	0.125
	Oxolinic acid	0.5–1	1	1
	Nalidixic acid	2–4	4	4
Shigella boydii (22)	Ciprofloxacin	0.015–0.06	0.03	0.03
	Norfloxacin	0.06–0.5	0.125	0.25
	Oxolinic acid	1–4	1	2
	Nalidixic acid	2–4	2	4
Shigella sonnei (20)	Ciprofloxacin	0.015–0.03	0.015	0.015
	Norfloxacin	0.03–0.125	0.125	0.125
	Oxolinic acid	0.5–2	1	2
	Nalidixic acid	2–4	2	2
Pseudomonas aeruginosa (29)	Ciprofloxacin	0.03–2	0.5	1
	Norfloxacin	1–4	1	2
	Oxolinic acid	2–16	2	16
	Nalidixic acid	8–16	16	16
Pseudomonas maltophilia (24)	Ciprofloxacin	0.03–2	0.25	1
	Norfloxacin	0.125–8	1	4
	Oxolinic acid	0.5–16	2	8
	Nalidixic acid	1–16	8	16
Pseudomonas cepacia (22)	Ciprofloxacin	0.125–4	1	2
	Norfloxacin	1–8	4	8

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TABLE 1—*Continued*

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TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
	Oxolinic acid	2–16	8	16
	Nalidixic acid	4–32	16	16
Pseudomonas fluorescens (21)	Ciprofloxacin	0.03–1	0.25	1
	Norfloxacin	0.25–4	0.5	4
	Oxolinic acid	0.25–16	2	16
	Nalidixic acid	4–16	16	16
Pseudomonas putrefaciens (23)	Ciprofloxacin	0.03–1	0.125	1
	Norfloxacin	0.25–8	0.5	4
	Oxolinic acid	1–16	2	8
	Nalidixic acid	2–16	4	16
Acinetobacter calcoaceticus (25)	Ciprofloxacin	0.125–0.5	0.25	0.5
	Norfloxacin	0.125–16	2	8
	Oxolinic acid	2–16	8	16
	Nalidixic acid	2–32	8	32
Yersinia enterocolitica (22)	Ciprofloxacin	0.008–0.06	0.015	0.03
	Norfloxacin	0.03–1	0.06	0.5
	Oxolinic acid	0.5–2	1	2
	Nalidixic acid	0.5–4	1	4
Haemophilus influenzae (26)	Ciprofloxacin	0.008–0.03	0.015	0.03
	Norfloxacin	0.03–0.125	0.03	0.06
	Oxolinic acid	1–4	2	4
	Nalidixic acid	0.5–4	1	4
Branhamella catarrhalis (20)	Ciprofloxacin	0.015–0.03	0.015	0.03
	Norfloxacin	0.06–0.5	0.5	0.5
	Oxolinic acid	0.5–1	1	1
	Nalidixic acid	0.5–2	1	2
Neisseria meningitidis (20)	Ciprofloxacin	0.008–0.015	0.015	0.015
	Norfloxacin	0.03–0.5	0.5	0.5
	Oxolinic acid	0.25–1	1	1
	Nalidixic acid	0.25–2	1	2

sanguis (0.25 μg/ml) to the eightfold-higher MIC_{90}s for *S. mitis* and *S. salivarius*. Differences in susceptibility were found among the various serological groups of *Salmonella* spp., whose MIC_{90}s ranged from 0.06 (O group B) to 0.25 (O group D) μg/ml (the latter value being higher than any ever reported for the genus as a whole), whereas the different *Shigella* species showed a more uniform pattern of susceptibility, with MIC_{90}s ranging from 0.015 to 0.03 μg/ml.

This study, in which, to the best of our knowledge, the antimicrobial activity of ciprofloxacin has been evaluated against the largest number of different microbial species ever tested in a single work, has not only confirmed the broad spectrum of activity of this antibiotic but, through analysis of 10 species whose susceptibility has not been evaluated before, has also shown it to be even broader than was known. In fact, of all the additional species tested, at least 90% of the strains turned out to be fully susceptible. What is more, ciprofloxacin also proved to be the most active of the antibiotics tested against strains of these species.

Our data also demonstrated that although the strains isolated in our country were, on the whole, no less susceptible than those previously described, some individual differences did exist, the most important of which were the lower susceptibility of *P. stuartii* (eightfold) and, to a lesser extent, of group C beta-hemolytic streptococci (fivefold) and, on the other hand, the higher susceptibility of *Pseudomonas cepacia* (threefold) and *S. faecium* (eightfold). Be-

TABLE 2. MICs of four antimicrobial agents for organisms identified by lyogroup

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Lyogroup I				
Staphylococcus aureus (30)	Ciprofloxacin	0.125–1	0.5	1
	Norfloxacin	0.25–8	0.25	4
	Oxolinic acid	0.5–8	2	8
	Nalidixic acid	16–32	32	32
Staphylococcus aureus, methicillin resistant (22)	Ciprofloxacin	0.5–1	0.5	1
	Norfloxacin	0.5–8	0.5	4
	Oxolinic acid	1–16	2	16
	Nalidixic acid	16	16	16
Lyogroup II				
Staphylococcus simulans (24)	Ciprofloxacin	0.125–1	0.25	0.5
	Norfloxacin	0.25–4	0.5	2
	Oxolinic acid	2–32	4	8
	Nalidixic acid	16–64	32	32
Lyogroup III				
Staphylococcus capitis (20)	Ciprofloxacin	0.06–0.5	0.125	0.5
	Norfloxacin	0.125–2	0.5	2
	Oxolinic acid	1–32	4	8
	Nalidixic acid	16–32	32	32
Lyogroup IV				
Staphylococcus saprophyticus, *S. xylosus*, *S. cohnii* (21)	Ciprofloxacin	0.06–1	0.125	1
	Norfloxacin	0.5–8	1	8
	Oxolinic acid	2–32	4	16
	Nalidixic acid	32–64	32	64
Lyogroup V				
Staphylococcus epidermidis (31)	Ciprofloxacin	0.125–1	0.25	1
	Norfloxacin	0.25–4	1	4
	Oxolinic acid	1–64	4	64
	Nalidixic acid	32–64	32	64
Lyogroup VI				
Staphylococcus hominis, *S. haemolyticus*, *S. warneri* (22)	Ciprofloxacin	0.125–1	0.125	1
	Norfloxacin	0.25–2	0.5	1
	Oxolinic acid	1–16	4	16
	Nalidixic acid	2–32	8	32
Streptococcus mitis (20)	Ciprofloxacin	0.5–2	1	2
	Norfloxacin	1–4	2	2
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus salivarius (20)	Ciprofloxacin	1–2	1	2
	Norfloxacin	1–4	2	2
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus sanguis (20)	Ciprofloxacin	0.25–0.25	0.25	0.25
	Norfloxacin	0.5–1	0.5	1
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus sp. group A (21)	Ciprofloxacin	0.5–4	0.5	2
	Norfloxacin	2–>64	4	8
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus sp. group B (21)	Ciprofloxacin	0.25–1	0.5	1
	Norfloxacin	1–8	1	8
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64

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TABLE 2—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Streptococcus sp. group C (21)	Ciprofloxacin	0.25–2	0.5	2
	Norfloxacin	2–8	4	8
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus faecalis (30)	Ciprofloxacin	0.5–8	1	2
	Norfloxacin	2–8	4	8
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus faecium (25)	Ciprofloxacin	0.5–8	1	2
	Norfloxacin	4–8	4	8
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus sp. group G (20)	Ciprofloxacin	0.25–1	0.25	1
	Norfloxacin	0.5–8	2	4
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Streptococcus pneumoniae (22)	Ciprofloxacin	0.5–2	1	1
	Norfloxacin	2–8	2	16
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64
Listeria monocytogenes (20)	Ciprofloxacin	0.25–0.5	0.5	0.5
	Norfloxacin	1–4	2	4
	Oxolinic acid	>64	>64	>64
	Nalidixic acid	>64	>64	>64

cause of differences between identification systems used in our study and those used in previous studies, we cannot rule out the possibility that at least some of the differences in susceptibilities observed depend, at least partially, on more accurate species identification. This seems likely, particularly for the apparent lower susceptibility of the *P. stuartii* strains. Along with the low susceptibility of this species, we also found a higher susceptibility for *P. rettgeri*, and the combination of these two findings suggests the possibility that lower susceptibility to quinolones is a property peculiar to *P. stuartii* strains and not to *P. rettgeri* strains; in the previous studies some of the *P. stuartii* strains may have been erroneously identified as *P. rettgeri* and vice versa. As a possible confirmation of this, it is worth mentioning that a similar, albeit less evident, trend of susceptibility to quinolones among *Providencia* strains has been reported only once (12), in the only work that took into account, as we did, virtually all the main *Providencia* species, identified by the same up-to-date criteria that we used (4, 9). We stress that these data are consistent with the well-known low susceptibility of *P. stuartii* strains to several other antimicrobial agents, as opposed to the other strains of the genus *Providencia*.

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