Journal of Antimicrobial Chemotherapy (1991) **27**, 199–208

Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine

Hubert Ph. Endtz[a]*, Gijs J. Ruijs[b], Bert van Klingeren[c], Wim H. Jansen[c], Tanny van der Reyden[a] and R. Peter Mouton[a]

[a]Department of Medical Microbiology, University Hospital, Leiden, [b]Regional Public Health Laboratory, Leyenburg Hospital, The Hague; [c]Departments of Chemotherapy and Bacteriology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands

Eight hundred and eighty-three strains of *Campylobacter* spp. isolated between 1982 and 1989 from human stools and poultry products were screened for quinolone resistance. In this period the prevalence of resistant strains isolated from poultry products increased from 0% to 14%. During the same period the prevalence in man increased from 0% to 11%. The emergence of quinolone resistance has implications for the identification of campylobacter up to species level: the susceptibility for nalidixic acid can no longer be used as a criterion for identification in the laboratory. The rapid emergence of resistant campylobacter may also have important implications for the treatment and prophylaxis of diarrhoeal disease. The increase of quinolone resistance coincides with the increasing use of fluoroquinolones in human and veterinary medicine. Extensive use of enrofloxacin in poultry and the almost exclusive transmission route of campylobacter from chicken to man, in The Netherlands, suggests that the resistance observed is mainly due to the use of enrofloxacin in the poultry industry.

Introduction

Campylobacter spp. have been recognized as a cause of diarrhoea in man since 1972 (Dekeyser *et al.*, 1972; Butzler *et al.*, 1973; Skirrow, 1977). *Campylobacter* spp. are more frequently isolated from stool specimens from patients with diarrhoea than salmonella and shigella together (Blaser *et al.*, 1983; Skirrow, 1987). Data from epidemiological studies performed in The Netherlands (Severin, 1978; Gezondheidsraad, 1988) do not differ greatly from the results of surveys in Great Britain (Skirrow, 1987) and the United States (Blaser *et al.*, 1983). Campylobacter infection is mainly a food-borne disease in which poultry play a particularly important rôle in the Netherlands (Gezondheidsraad, 1988).

Fluoroquinolones are a new class of potent, orally absorbed antimicrobial agents. The current quinolones have good activity against members of the Enterobacteriaceae, including *Salmonella* spp., *Shigella* spp., and *Yersinia* spp., and against *Campylobacter* spp. (Goossens *et al.*, 1985; Segreti *et al.*, 1989). Bacterial gastroenteritis has been

*Corresponding author: Department of Clinical Microbiology, University Hospital Rotterdam-Dijkzigt, 3015 GD Rotterdam, The Netherlands.

0305-7453/91/020199+10 $02.00/0

mentioned as an infection likely to respond to fluoroquinolone therapy because of high local drug concentrations and highly susceptible pathogens (Wolfson & Hooper, 1989).

Several authors have reported on the prophylaxis and treatment of acute bacterial diarrhoea with fluoroquinolones and suggested that these antimicrobial agents are effective for the treatment and prevention of such enteric infections (Dupont, Corrado & Sabbaj, 1987; Pichler *et al.*, 1987; Rademaker *et al.*, 1989; Wiström *et al.*, 1989).

In 1989 we noticed an increase of ciprofloxacin-resistant strains of *Campylobacter* spp. in our laboratory. This led us to screen campylobacter strains collected from humans between 1982 and 1989 for ciprofloxacin resistance. We suspected that the introduction of quinolone antimicrobial agents into both human and veterinary medicine may have been responsible for the appearance of ciprofloxacin resistance in *Campylobacter* spp. in The Netherlands. Furthermore, because of the important role of poultry in the epidemiology of campylobacter-associated diarrhoea, we also screened campylobacter strains isolated from poultry products between 1982 and 1989.

Materials and methods

Bacterial strains and identification

Eighty-seven strains isolated from poultry in 1982 were obtained from the Regional Public Health Laboratory, Rotterdam (Dr J. Bänffer), and 47 isolated in 1987 from the National Institute of Public Health and Environmental Protection, Bilthoven (Dr A. Hoogenboom-Verdegaal). All 273 strains isolated from poultry in 1988 and 1989 were obtained from the Food Inspection Service, Zutphen, The Netherlands (Dr E. de Boer). Two hundred and thirty-one campylobacter strains were isolated from patients with diarrhoeal disease between 1982 and 1989 at the University Hospital in Leiden. Fifty-nine human strains isolated in 1989 came from the Regional Public Health Laboratory in The Hague and a further 86 from the Regional Public Health Laboratory, Zwolle (Dr J. Frik). One hundred human strains were isolated in 1985 at the Antonius Hospital in Nieuwegein (Dr J. E. Landheer).

Under microaerophilic conditions all campylobacter cultures showed abundant growth at 42°C but not at 25°C. They were catalase and oxidase positive, resistant to cephalothin, and, except for the quinolone-resistant strains, susceptible to nalidixic acid. Hippurate hydrolysis, DNA hydrolysis and a rapid H_2S test were performed according to Lior (1984) and Lior & Patel (1987). *Campylobacter* species and biotypes were defined as proposed by Lior (1984). Since *C. lari* (formerly *C. laridis*) (von Graevenitz, 1990) can easily be misidentified as a quinolone-resistant *C. coli*, the following additional tests were performed on all resistant isolates: tolerance to $1\frac{1}{2}$% NaCl, anaerobic growth in the presence of 1 g/l trimethylamine-*N*-oxide hydrochloride (TMAO) (Benjamin *et al.*, 1983) and the rapid H_2S test.

Antimicrobial susceptibility tests

Disc diffusion tests were performed on Columbia agar (Oxoid) containing 5% sheep blood. Approximately 10^8 cfu were inoculated on each plate. The discs (BBL) contained 5 μg ciprofloxacin, 30 μg nalidixic acid, or 30 μg cephalothin. The broth dilution tests were performed in microtitre trays with doubling dilutions of ciprofloxacin (Bayer), enrofloxacin (Bayer), and flumequine (Riker-3M) in Mueller–Hinton broth (BBL). The inocula ($2–3 \times 10^6$ cfu/well) were prepared in Mueller–Hinton broth,

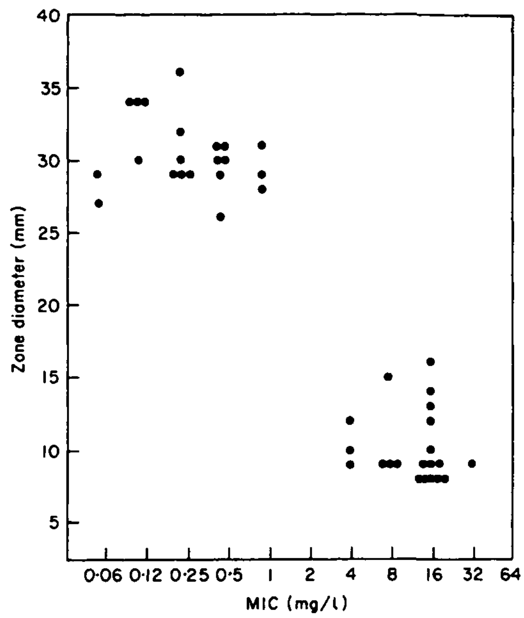

Figure 1. Relation between the MICs for ciprofloxacin and the zone diameters of 42 *Campylobacter* strains. Discs contained 5 μg of ciprofloxacin.

and the total volume per well was 0·1 ml. The microaerophilic atmosphere was obtained with a gas generating envelope (GasPak, BBL) without catalyst. All strains were incubated in a microaerophilic atmosphere at 37°C and read after 24 h. In the broth test the minimum inhibitory concentration (MIC) was defined as the lowest concentration giving no visible growth. The following breakpoints of ciprofloxacin were used: MIC $\geqslant$ 4 mg/l: resistant; MIC $\leqslant$ 1 mg/l: susceptible. According to the results of the dilution tests and the breakpoints mentioned, the strains were assigned to susceptible and resistant categories. All susceptible isolates had a zone diameter > 26 mm. All resistant isolates had one < 17 mm (Figure 1). Therefore, for initial screening of new isolates, a critical zone diameter of 25 mm was chosen. Subsequently, the MIC was performed for those strains with a zone diameter < 25 mm.

Serotyping

Serotyping was performed according to the method described by Penner *et al.* (1983) for heat stable antigens. All standard strains were obtained for the culture collection of the University of Göteborg, Sweden (CGUG).

Statistics

The Chi-square tests with the Yates' correction were used to establish differences in the distribution of species, serogroups and the frequency of resistances.

Results

The MICs of ciprofloxacin for susceptible isolates ranged from 0·06 to 1 mg/l. The MICs for resistant isolates ranging from 4 to 32 mg/l. Complete cross-resistance was

Table I. MICs of 21 quinolone susceptible campylobacter strains

	Number of strains with MIC (mg/l) 8	4	2	1	0·5	0·25	0·12	0·06
Enrofloxacin	—	—	—	1	3	6	6	5
Ciprofloxacin	—	—	—	3	4	6	7	1
Flumequine	3	5	5	6	2	—	—	—

Table II. MICs of 33 quinolone resistant campylobacter strains

	Number of strains with MIC (mg/l) 128	64	32	16	8	4	2
Enrofloxacin	—	—	—	1	5	22	5
Ciprofloxacin	—	—	1	16	13	3	—
Flumequine	17	7	8	16	—	—	—

observed between ciprofloxacin, enrofloxacin and flumequine (Tables I and II). Figure 2 shows the total number of campylobacter isolates tested and the percentages of ciprofloxacin-resistant strains. In 1982–83 none of the 165 human and chicken strains was resistant to ciprofloxacin. Comparing 1982 to 1989 the prevalence of resistant strains increased from 0% to 14% in isolates from poultry products ($P < 0{\cdot}01$). The prevalence in man increased from 0% to 11% ($P < 0{\cdot}01$). In 1989 the frequency of resistance in strains isolated at three geographically different locations (Leiden, The Hague, and Zwolle) was 10·8%, 10·2% and 12·8%, respectively.

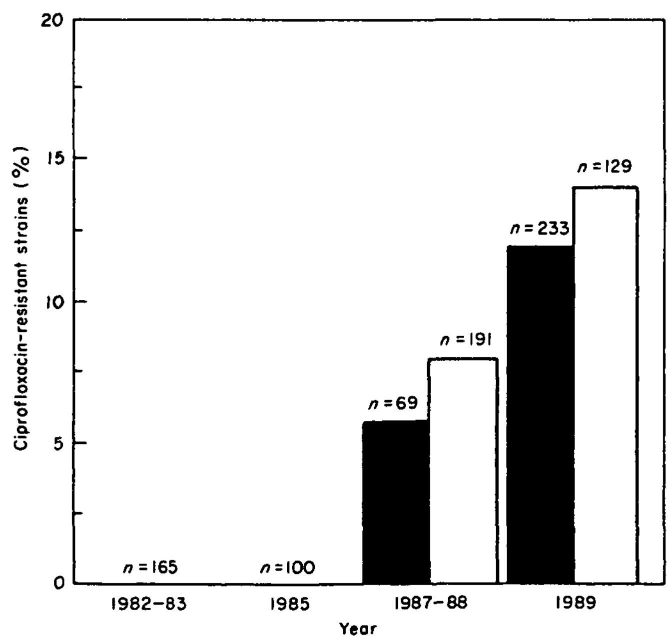

Figure 2. Ciprofloxacin resistance in campylobacter isolated from human stools (■) and poultry products (□).

Table III. Identification of 407 poultry and 290 human campylobacter isolates

Origin	Year	Number of strains	*C. jejuni* (%)	*C. coli* (%)
Human	1982	78	77 (99)	1 (1)
	1987–88	69	62 (90)	7 (10)
	1989	143	129 (90)	14 (10)
Poultry	1982	87	55 (63)	32 (37)
	1987–88	191	144 (75)	47 (25)
	1989	129	93 (72)	36 (28)

The results of identification to species level of all 407 poultry isolates and of 290 human isolates are shown in Table III. *C. jejuni* and *C. coli* were the only species isolated. However, when the campylobacter populations of human and poultry origin were compared, the species distribution was found to be different ($P < 0{\cdot}001$). *C. coli* accounted for only 8% of the human strains and 28% of the strains isolated from poultry.

Thirty-four of 560 *C. jejuni* (6%) and 19 of 137 *C. coli* (14%) were resistant to the fluoroquinolones ($P < 0{\cdot}01$). Additional tests for identification were performed, because quinolone resistant *C. coli* could not be distinguished from *C. lari* purely on the basis of nalidixic acid resistance. However, none of the resistant *C. coli* strains was tolerant to 1·5% NaCl or was able to grow anaerobically in a medium containing TMAO. All were H_2S negative. We therefore concluded that these strains were indeed quinolone-resistant *C. coli* and not *C. lari*.

Biotyping of the quinolone-resistant strains showed that 16 of 17 campylobacter strains isolated from poultry in 1989 were identified as *C. coli* biotype I and one strain as *C. jejuni* biotype I. The results of identification and biotyping were confirmed by the WHO Collaborating Centre for Campylobacter Infections, Brussels, Belgium (Dr H. Goossens). The 16 resistant *C. coli* biotype I strains were, however, distributed amongst four different serogroups (data not shown). The poultry products from which they were isolated were obtained from three different slaughterhouses.

The results of serotyping of all 229 strains of human origin isolated in 1989 are summarized in Table IV. A total of 29 serogroups were identified. Fifty-four (24%) strains were non-typable according to the Penner scheme. Our data show that O-serogroups 2, 11, 3, 44, 1 : 44, and 8 accounted for 41% of the isolates. Quinolone resistance was not equally distributed in the different serogroups ($P < 0{\cdot}01$), resistance was particularly common in serogroup O : 3 ($P < 0{\cdot}05$).

The 1989 sales of quinolone antibiotics in The Netherlands are summarized in Table V. In 1989 the veterinary sales of enrofloxacin alone amounted to about 50% of the sales of all fluoroquinolones for human medical use in The Netherlands.

Discussion

Our findings indicate a rise in the prevalence in The Netherlands of *Campylobacter* spp. resistant to the new fluoroquinolones. This phenomenon has been noted in campylobacter of human origin as well as in strains isolated from poultry products. Complete

Table IV. The distribution of quinolone resistance and O-serogroup amongst campylobacter isolated from human faeces in 1989 in The Netherlands

O-serogroup	Number susceptible	Number resistant	Total
2	29	4	33
11	15	2	17
3	7	5[a]	12
37	8	—	8
50	9	—	9
5	6	—	6
1	7	—	7
59	7	2	9
44	8	3	11
4	6	–	6
8	8	2	10
1:44	8	2	10
6:7	4	—	4
10	3	—	3
53	3	1	4
46	3	—	3
18	3	—	3
29	3	—	3
30	2	1	3
24	2	—	2
60	2	—	2
40:42	2	—	2
23:36	2	—	2
48	1	—	1
36	1	—	1
56	1	—	1
23	1	—	1
21	1	—	1
7	1	—	1
Non-typable	50	4	54
Total	203	26	229

[a] $P < 0{\cdot}05$.

Table V. Dutch sales of antimicrobials in 1989 expressed in million guilders

Human	total anti-infectious agents	203
	antibiotics	175
	total quinolones	11
	norfloxacin	5
	ciprofloxacin	5
Animal	enrofloxacin	6

Source: IMS Nederland.

cross-resistance to nalidixic acid was observed. In a previous report on resistance to fluoroquinolones, cross-resistance to nalidixic acid was also found (Taylor, Ng & Lior, 1985). The occurrence of this pattern of quinolone resistance may have important consequences: in the first place, it may create problems with the identification of campylobacter strains. Second, the origin of quinolone resistance in campylobacter may indicate the potential for resistance to appear in other bacterial species. Third, quinolone resistance will have important implications for the treatment of diarrhoea with these agents.

Problems with identification

Since both *C. jejuni* and *C. coli* are the species predominantly associated with human intestinal campylobacter infection and have been found to be almost invariably susceptible to nalidixic acid (Taylor *et al.*, 1985; Altwegg *et al.*, 1987), this characteristic has been widely used for the identification of these bacteria in the microbiology laboratory. The emergence of resistance to nalidixic acid could lead to problems with respect to the identification of campylobacter to the species level. *C. lari* is a *Campylobacter* species that is naturally resistant to nalidixic acid and has also been found to be intrinsically resistant to the new quinolones, in contrast to various other *Campylobacter* species (*C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis and C. hyointestinalis*), which are all intrinsically resistant to nalidixic acid but are all uniformly susceptible to the fluoroquinolones (Taylor *et al.*, 1985; Taylor & Courvalin, 1988). *C. lari* is very seldom isolated from human stools or poultry products and has been predominantly found in sea gulls. Since we were unable to distinguish between quinolone-resistant *C. coli* and *C. lari* using routine methods, further subtyping of our quinolone-resistant strains was performed. However, none of them were identified as *C. lari*.

Source and reservoir of resistant strains

Since our findings indicating the absence of resistance to quinolones in 1982–83 and 1985 are in accordance with the results of studies performed in the early eighties (Goodman *et al.*, 1984), it seems likely that quinolone resistance was introduced between 1985 and 1987. Flumequine was the only quinolone available in veterinary medicine before 1985, but since the amount of this drug sold in The Netherlands has not risen in the last ten years (L. H. P. Walenbergh, personal communication), the use of flumequine in animals is therefore unlikely to have caused resistance problems in man and animals. Ciprofloxacin, pefloxacin, and ofloxacin were introduced in The Netherlands in October 1988, April 1989, and November 1989, respectively. It is unlikely that these agents could have been responsible for the introduction of quinolone resistance between 1985 and 1987. However, two other fluoroquinolones were introduced in The Netherlands during this period: norfloxacin, for human use in 1985, and enrofloxacin for veterinary use in 1987. The former is used mainly in urinary tract infections, and the latter is used extensively for the treatment of diarrhoeal disease caused by *E. coli* and infections with *Mycoplasma* spp. in poultry and to a lesser extent in pigs. No other antimicrobial agent which could have selected for quinolone resistance are being prescribed in the poultry industry in The Netherlands.

Person-to-person transmission of campylobacter is rare and probably of no epidemiological importance. In The Netherlands the most important foods of animal origin are chicken, pork, beef, and milk, and among these probably only chicken plays an important role in the transmission of campylobacter to man (Gezondheidsraad, 1988). It is unlikely that the use of norfloxacin alone in human medicine may have led to the development of quinolone resistance in campylobacter isolated from man, it seems more probable that the introduction of enrofloxacin into veterinary medicine, i.e., in the treatment of poultry, contributed significantly to the appearance of quinolone resistance campylobacter causing infections in man. Since *C. coli* was isolated more often from poultry products than from human faeces ($P < 0{\cdot}01$) and quinolone resistance was more often associated with *C. coli* than with *C. jejuni* ($P < 0{\cdot}01$), the higher resistance rate in *C. coli* may also reflect an extensive use of quinolone antibiotics in veterinary medicine. The emergence of quinolone resistance in strains isolated from poultry must be the result of the use of these antibiotics in veterinary medicine, in the case of poultry, the route of transmission of campylobacter being from chicken to man and not from man to chicken.

Transfer of antimicrobial-resistant bacteria from animal to man is almost impossible to reconstruct accurately in the case of poultry where the chain of transmission is often complex. The difficulty encountered in documenting this sequence of events has contributed to the continuing controversy about the use of antimicrobial agents in veterinary medicine. Discussion of this problem was resumed in The Netherlands after the publication of recommendations made by a Dutch Working Party at the request of the National Institute of Public Health and Environmental Protection (1989).

Implications for treatment

Two conclusions may be made as follows: first, the increasing quinolone resistance of campylobacter in the animal reservoir could lead to failure of therapy if treatment of diarrhoeal disease with fluoroquinolones is used. Second, we could learn a lesson in human medicine from the experience in veterinary medicine: fluoroquinolones should not be used on a large scale (i.e., for prophylactic purposes) in diarrhoeal disease, because resistance in campylobacter is rapidly emerging under this selective pressure in animals. Monitoring of future changes in susceptibility to quinolones is important. Meanwhile, we must express serious concern about the large-scale use of quinolones in veterinary as well as human medicine. Medical practitioners and other health workers should be alert to the possibility of quinolone resistance in campylobacter and subsequent failure of quinolone treatment.

Acknowledgements

We thank Dr H. Goossens and Prof. J. W. M. van der Meer for their critical reading of this paper, Mrs I. Seeger for reading the English version, M. Biever for technical assistance and A. H. Zwinderman for statistical advice. We gratefully acknowledge the kindness of Dr J. Bänffer, Dr A. Hoogenboom-Verdegaal, Dr E. de Boer, Dr J. Frik and Dr J. E. Landheer for providing campylobacter strains. Preliminary results of parts of this study have been reported previously in a letter (Endtz *et al.*, 1990).

References

Altwegg, M., Burnens, A., Zollinger-Iten, J. & Penner, J. L. (1987). Problems in identification of *Campylobacter jejuni* associated with acquisition of resistance to nalidixic acid. *Journal of Clinical Microbiology* **25**, 1807–8.

Benjamin, J. L., Leaper, S., Owen, R. J. & Skirrow, M. B. (1983). Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) group. *Current Microbiology* **8**, 231–8.

Blaser, M. J., Wells, J. G., Feldman, R. A., Pollard, R. A., Allen, J. R. & the Collaborative Diarrheal Disease Study Group. (1983). Campylobacter enteritis in the United States. A multicenter study. *Annals of Internal Medicine* **98**, 360–5.

Butzler, J. P., Dekeyser, P. J., Detrain, M. & Dehaen, F. (1973). Related vibrio in stools. *Journal of Pediatrics* **82**, 493–5.

Dekeyser, P., Gossuin-Detrain, M., Butzler, J. P. & Sternon, J. (1972). Acute enteritis due to related vibrio: first positive stool cultures. *Journal of Infectious Diseases* **125**, 390–2.

Dupont, H. L., Corrado, M. L. & Sabbaj, J. (1987). Use of norfloxacin in the treatment of acute diarrheal disease. *American Journal of Medicine* **82**, *Suppl. 6B*, 79–83.

Endtz, H. Ph., Mouton, R. P., van der Reyden, T., Ruijs, G. J. & Biever, M. (1990). Fluoroquinolone resistance in *Campylobacter* spp isolated from human stools and poultry products. *Lancet* **335**, 787.

Gezondheidsraad. (1988). Advies inzake *Campylobacter jejuni* infecties in Nederland. Gezondheidsraad, rapport nummer 1988/13, The Hague.

Goodman, L. J., Fliegelman, R. M., Trenholme, G. M. & Kaplan, R. L. (1984). Comparative in vitro activity of ciprofloxacin against *Campylobacter* spp. and other bacterial enteric pathogens. *Antimicrobial Agents and Chemotherapy* **25**, 504–6.

Goossens, H., De Mol, P., Coignau, H., Levy, J., Grados, O., Ghysels, G. *et al.* (1985). Comparative in vitro activities of aztreonam, ciprofloxacin, norfloxacin, ofloxacin, HR 810 (a new cephalosporin), RU 28965 (a new macrolide), and other agents against enteropathogens. *Antimicrobial Agents and Chemotherapy* **27**, 388–92.

Lior, H. (1984). New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter coli*, and "*Campylobacter laridis*". *Journal of Clinical Microbiology* **20**, 636–40.

Lior, H. & Patel, A. (1987). Improved toluidine blue-DNA agar for detection of DNA hydrolysis by campylobacters. *Journal of Clinical Microbiology* **25**, 2030–1.

National Institute of Public Health and Environmental Protection, Netherlands (1989). Advice on limiting the arsenal of antimicrobial drugs for veterinary use, RIVM, rapportnummer 358471003, Bilthoven.

Penner, J. L., Hennessy, J. N. & Congi, R. V. (1983). Serotyping of *Campylobacter jejuni* and *Campylobacter coli* on the basis of thermostable antigens. *European Journal of Clinical Microbiology* **2**, 378–83.

Pichler, H. E. T., Dirdl, G., Stickler, K. & Wolf, D. (1987). Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. *American Journal of Medicine* **82**, *Suppl. 4A*, 329–32.

Rademaker, C. M. A., Hoepelman, I. M., Wolfhagen, M. J. H. M., Beumer, H., Rozenberg-Arska, M. & Verhoef, J. (1989). Results of a double-blind placebo-controlled study using ciprofloxacin for prevention of travelers' diarrhea. *European Journal of Clinical Microbiology and Infectious Diseases* **8**, 690–4.

Segreti, J., Nelson, J. A., Goodman, L. J., Kaplan, R. A. & Trenholme, G. M. (1989). In vitro activities of lomefloxacin and temafloxacin against pathogens causing diarrhea. *Antimicrobial Agents and Chemotherapy* **33**, 1385–7.

Severin, W. P. J. (1978). Campylobacter en enteritis. *Nederlands Tijdschrift voor Geneeskunde* **122**, 499–504.

Skirrow, M. B. (1977). Campylobacter enteritis: a "new" disease. *British Medical Journal ii*, 9–11.

Skirrow, M. B. (1987). A demographic survey of campylobacter, salmonella and shigella infections in England. A Public Health Laboratory Service Survey. *Epidemiology and Infection* **99**, 647–57.

Taylor, D. E. & Courvalin, P. (1988). Mechanisms of antibiotic resistance in *Campylobacter* species. *Antimicrobial Agents and Chemotherapy* **32**, 1107–12.

Taylor, D. E., Ng, L. K. & Lior, H. (1985). Susceptibility of *Campylobacter* species to nalidixic acid, enoxacin, and other DNA gyrase inhibitors. *Antimicrobial Agents and Chemotherapy* **28**, 708–10.

von Graevenitz, A. (1990). Revised nomenclature of *Campylobacter laridis, Enterobacter intermedium*, and *"Flavobacterium branchiophila"*. *International Journal of Systematic Bacteriology* **40**, 211.

Wiström, J., Jertborn, M., Hedström, S. A., Alestig, K., Englund, G., Jellheden, B. *et al.* (1989). Short-term self-treatment of travellers' diarrhoea with norfloxacin: a placebo-controlled study. *Journal of Antimicrobial Chemotherapy* **23**, 905–13.

Wolfson, J. S. & Hooper, D. C. (1989). Fluoroquinolone antimicrobial agents. *Clinical Microbiology Reviews* **2**, 378–424.

(*Received 22 June 1990; accepted 22 September 1990*)