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Campylobacter jejuni isolated from poultry and humans in Styria, Austria: epidemiology and ciprofloxacin resistance

I. HEIN[1]*, C. SCHNECK[1], M. KNÖGLER[1], G. FEIERL[2], P. PLESS[3], J. KÖFER[3], R. ACHMANN[4] AND M. WAGNER[1]

[1] *Institute of Milk Hygiene, Milk Technology, and Food Science, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria*
[2] *Institute of Hygiene, Karl-Franzens University Graz, Universitaetsplatz 1, A-8010 Graz, Austria*
[3] *Department of Veterinary Administration in Styria, Zimmerplatzgasse 15, A-8010 Graz, Austria*
[4] *Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Veterinaerplatz 1, A-1210 Vienna, Austria*

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SUMMARY

Sixty-six broiler flocks were sampled to determine the presence of *Campylobacter* spp. at slaughter in 1998. Thirty flocks (45%) tested positive and *C. jejuni* was identified in all isolates. Combined pulsed-field gel electrophoresis/amplified fragment length polymorphism (PFGE/AFLP) subtyping of 177 isolates from 24 positive flocks revealed 62 subtypes; 16 flocks harboured more than one subtype. When subtyping 101 clinical *C. jejuni* isolates collected in the same time period and area, 60 PFGE/AFLP types were identified. Comparison of subtypes from poultry and human isolates revealed three shared PFGE/AFLP types, which were present in 11 human isolates. Fifty per cent of all poultry isolates and 39% of all human isolates were resistant to ciprofloxacin. The present study confirms the increase in ciprofloxacin resistance in both human and poultry *C. jejuni* isolates in Austria, as observed in several countries worldwide. A small number of human isolates shared PFGE/AFLP types with poultry isolates, however, further studies should also focus on the identification of other sources of *C. jejuni* infection in humans.

INTRODUCTION

Thermophilic *Campylobacter* spp., mainly *Campylobacter jejuni*, have been recognized as a major cause of human gastroenteritis throughout the world. In Austria, the incidence of campylobacter infections, based on laboratory-confirmed cases, was 70 per 100 000 inhabitants, as determined in a national survey in the federal province of Styria (1·2 million inhabitants) in 2000 [1]. In Austria, following *Salmonella* spp., *C. jejuni* is the second most common foodborne bacterial pathogen that is known to cause diarrhoea. Contaminated raw milk, drinking water and poultry are considered to be the main sources of infection, both in outbreaks and in sporadic cases [2]. *Campylobacter* spp. have been isolated from up to 82% of broiler flocks at slaughter [3–6].

Quinolones are frequently used for the treatment of campylobacteriosis in humans but the prevalence of resistance of *Campylobacter* spp. to these antibiotics has increased in several countries. For instance, in Austria the resistance of clinical *Campylobacter* spp. isolates to ciprofloxacin increased from 0% in 1988 to 34·1% in 1997 [7–9]. Several researchers attribute this pattern to the introduction of quinolones in poultry production [10, 11].

* Author for correspondence.

For epidemiological purposes, several molecular typing methods of *Campylobacter* spp., such as the random amplified polymorphic DNA (RAPD) technique, the restriction fragment length polymorphism (RFLP) technique, and pulsed-field gel electrophoresis (PFGE) have been used [12–18]. PFGE was shown to be a highly discriminatory and reproducible technique, especially when two or more different restriction enzymes were applied [19]. The amplified fragment length polymorphism (AFLP) technique is also a highly discriminatory method of subtyping *Campylobacter* spp. [20–22].

The aims of the present study were (*a*) to assess the prevalence of *Campylobacter* spp. resistant and sensitive to ciprofloxacin in poultry flocks at slaughter in the Austrian district of Styria over a period of 3 months; (*b*) to investigate the percentage of ciprofloxacin-resistant and ciprofloxacin-sensitive clinical *Campylobacter* spp. isolates collected in the same area and time period; and (*c*) to compare the PFGE and AFLP types of poultry and clinical isolates in order to estimate their genetic similarity.

METHODS

Sample collection

Sixty-six broiler flocks of the eastern and southern parts of Styria, Austria, which were slaughtered from October 1998 to December 1998 were sampled at slaughter by the Department of Veterinary Administration in Graz (Styria), Austria, to determine the presence of *Campylobacter* spp. The average flock size was 20 000 chickens per flock. In regular time intervals during the slaughtering process the intestines of a total of 10 chickens per flock were collected from the eviscerating line. The samples were placed into sterile plastic bags, stored at 4 °C, transported to the laboratory in chilled boxes, and analysed within 24 h. Caecal contents were removed aseptically, cultured on modified Preston Agar (mCCDA, Oxoid, Basingstoke, UK) supplemented with CCDA Selective Supplement Code SR 155E (Oxoid), and incubated under microaerobic conditions (GENbox Microaer, BioMérieux, Marcy l'Etoile, France) at 42 °C for 48 h. Cultures suspected to contain *Campylobacter* spp. were sent on mCCDA to the Institute of Hygiene, University of Graz, Austria, for further confirmation. Generally, one colony per plate was picked for further confirmation. In parallel, 239 *C. jejuni* strains were included in the study; the strains were isolated from human faecal samples collected in the same time period and area by local practitioners and in hospitals.

Genus and species identification

Strains were subcultured on blood agar (BioMérieux) under microaerobic conditions at 42 °C for 48 h. Genus and species identity was confirmed by inspection of colony morphology on mCCDA, Gram stain, detection of catalase activity, cytochrome oxidase production and hydrolysis of hippurate. Strains that did not hydrolyse hippurate were subjected to PCR targeting the *flaA* gene [23] and the *hip* gene [24].

Antimicrobial susceptibility testing

Susceptibility testing to ciprofloxacin (5 μg disk) was performed by using the agar disk diffusion method [25] on Mueller–Hinton agar supplemented with 5% defibrinated sheep blood (BioMérieux). The plates were inoculated with bacterial cultures adjusted to a 0.5 McFarland standard under microaerobic conditions at 42 °C for 24–48 h. Isolates showing an inhibition zone diameter $\leqslant 15$ mm were designated as resistant, and $\geqslant 21$ mm as sensitive. Ciprofloxacin sensitive *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 as well as a ciprofloxacin resistant *C. jejuni* CIP 105890 were used as control strains.

Subtyping of *C. jejuni* isolates by PFGE

Subtyping of *C. jejuni* isolates by PFGE was performed as published previously [16], using the restriction enzymes *Sma*I and *Sal*I (New England Biolabs, Beverly, MA, USA). Macrorestriction profiles were visualized after gels were stained with ethidium bromide under UV light and stored as digitized images in TIFF format.

To facilitate the visual analysis of the profiles, computerized distance estimation and cluster analysis was performed. Normalization according to molecular weight standards on the outermost lanes of each gel and conversion of band patterns to binary data were done using RFLPscan™ software (Scanalytics, Billerica, MA, USA). For estimation of distance according to the coefficient of Link et al. [26] and cluster analysis using the unweighted-pair group method (UPGMA), the TREECON software package (version 1.2, Yves van de Peer, Royal University of Antwerp, Belgium) was applied. Two isolates shared one PFGE type if the combined *Sma*I/*Sal*I patterns were completely identical.

Table 1. *Sequences and modifications of adapter and primer oligonucleotides used for AFLP*

Oligonucleotide	Sequence and modification
*Hin*dIII-specific adapter	5′-CTC GTA GAC TGC GTA CC-NH_2-3′
	5′-P-AGC TGG TAC GCA GTC-3′
*Hha*I-specific adapter	5′-GAC GAT GAG TCC TGA TCG-3′
	5′-ATC AGG ACT CAT CG-NH_2-3′
*Hin*dIII + 0	5′-GAC TGC GTA CCA GCT T-3′
*Hin*dIII + A	5′-FAM-GAC TGC GTA CCA GCT TA-3′
*Hha*I + 0	5′-GAT GAG TCC TGA TCG C-3′
*Hha*I + A	5′-GAT GAG TCC TGA TCG CA-3′

Subtyping of *C. jejuni* isolates by AFLP

Restriction and ligation

AFLP was performed as published previously [20]. Genomic DNA was extracted from undigested PFGE agarose plugs using the QIAquick™ Gel Extraction Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. A total of 25 ng of DNA were digested and ligated in a volume of 14 μl containing 5 U of *Hin*dIII, 5 U of *Hha*I, and 1 U T4 DNA ligase (New England Biolabs), 570 nM *Hin*dIII- and 5·7 μM *Hha*I-restriction site-specific adapter, 36 mM NaCl, 54 mM Tris–HCl (pH 7·8), 11 mM $MgCl_2$, 11 mM DTT, 1 mM ATP, and 63 ng/μl BSA for 2 h at 37 °C. The adapters were modified to allow exponential amplification of heterosite fragments only (Table 1) [27]. A total of 186 μl distilled H_2O were added to the restriction ligation mixture.

PCR

Primers used for preselective and selective PCR are listed in Table 1. Preselective PCR was performed in a 20 μl volume containing 10 mM Tris–HCl, 50 mM KCl, 2·5 mM $MgCl_2$, 0·001% (w/v) gelatin, 250 nM adapter-specific primer *Hin*dIII + 0, 2·5 μM adapter-specific primer *Hha*I + 0, 200 μM each of dATP, dTTP, dGTP and dCTP, 1·25 U Ampli*Taq* Gold™ polymerase (Applied Biosystems, Foster City, CA, USA) and 4 μl diluted restriction-ligation mixture by using an initial step of 10 min at 94 °C, 20 cycles of 20 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C. The amplicons were diluted 1:20 in distilled H_2O and 3 μl were subjected to selective PCR using a mastermix of the same concentration as that used for preselective PCR, and adapter-specific primers *Hin*dIII + A and *Hha*I + A, containing an additional A nucleotide at the 3′ end. An initial denaturation for 10 min at 94 °C was followed by a touchdown PCR protocol consisting of (*a*) 11 cycles of 20 s each at 94 °C, 30 s annealing starting at 66 °C and decreasing 1 °C every cycle until 56 °C was achieved, and 2 min each at 72 °C and (*b*) 19 cycles of 20 s each at 94 °C, 30 s each at 56 °C, and 2 min each at 72 °C. A final elongation step was performed for 30 min at 60 °C, after which the samples were cooled to 4 °C. Preselective and selective PCR was performed in a GeneAmp® PCR System 9700 (Applied Biosystems) with the ramp rate set at 90%.

Capillary electrophoresis

Amplified DNA fragments were separated by capillary electrophoresis on an ABI-310 Genetic Analyzer (Applied Biosystems) with POP4 polymer and GeneScan TAMRA-500 as the internal standard for each sample (Applied Biosystems). After capillary electrophoresis, the ABI Genescan software (Applied Biosystems) was applied. The detection threshold was set at 200 fluorescence units. The AFLP patterns consisted of fragments ranging in size from 50 to 400 bases and were manually edited using the ABI Genotyper software (Applied Biosystems). The TREECON software package (version 1.2, Yves van de Peer, Royal University of Antwerp, Belgium) was applied for distance estimation according to the coefficient of Nei and Li [28] and cluster analysis using the unweighted-pair group method (UPGMA). In accordance with Duim et al. [20], AFLP patterns with at least 90% similarity were considered to be closely related and were designated as an AFLP type.

Table 2. *PFGE, AFLP, and combined PFGE/AFLP types of* C. jejuni *isolates from poultry flocks and clinical isolates*

Combined PFGE/ AFLP type	Flock	Proportion of isolates per flock (%)	Number of human isolates	PFGE type	AFLP type	Combined PFGE/ AFLP type	Flock	Proportion of isolates per flock (%)	Number of human isolates	PFGE type	AFLP type
1	1	88	1	1	1	60	24	33	—	17	53
2	1	12	—	2	1	61	24	33	—	17	54
3	2	72	9	3	2	62	24	33	—	17	55
4	2	14	—	3	3	63	—	—	11	3	56
5	2	14	—	3	4	64*	—	—	7	37	39
6	3	100	—	4	5	65	—	—	5	38	57
7	4	100	—	5	6	66	—	—	4	39	58
8	5	33	—	6	7	67	—	—	3	4	59
9	5	33	—	7	8	68	—	—	3	40	60
10	5	33	—	8	9	69	—	—	2	41	2
11	6	10	—	9	10	70	—	—	2	42	61
12	6	30	—	10	11	71	—	—	2	43	59
13	6	10	—	10	12	72†	—	—	2	44	62
14	6	10	—	9	13	73†	—	—	2	45	63
15	6	10	—	10	14	74†	—	—	2	46	57
16	6	10	—	9	15	75	—	—	1	3	64
17	6	20	—	10	16	76	—	—	1	17	65
18	7	29	—	11	17	77	—	—	1	38	66
19	7	14	—	12	17	78	—	—	1	44	61
20	7	14	—	12	18	79	—	—	1	47	67
21	7	29	—	12	19	80	—	—	1	48	68
22	7	14	—	11	19	81	—	—	1	49	69
23	8	30	—	8	20	82	—	—	1	50	62
24	8	10	—	8	21	83	—	—	1	51	70
25	8	30	—	8	22	84	—	—	1	52	62
26	8	10	—	13	22	85	—	—	1	53	62
27	8	10	—	14	23	86	—	—	1	54	72
28	8	10	—	8	24	87	—	—	1	55	72
29	9	13	—	15	25	88	—	—	1	56	73
30	9	13	—	16	26	89	—	—	1	57	41
31	9	63	1	17	8	90	—	—	1	58	74
32	9	13	—	18	27	91	—	—	1	59	75
33	10	100	—	19	28	92	—	—	1	60	62
34	11	100	—	20	29	93	—	—	1	61	76
35	12	100	—	21	30	94	—	—	1	62	62
36	13	60	—	22	31	95	—	—	1	63	62
37	13	20	—	23	32	96	—	—	1	64	77
38	13	10	—	22	33	97	—	—	1	65	71
39	13	10	—	24	34	98	—	—	1	66	78
40	14	100	—	25	35	99	—	—	1	67	41
3	15	14	—	3	2	100	—	—	1	68	39
41	15	57	—	26	36	101	—	—	1	69	57
42	15	29	—	3	37	102	—	—	1	70	79
43	16	78	—	9	38	103	—	—	1	71	80
44	16	11	—	27	39	104	—	—	1	72	39
45	16	11	—	28	40	105	—	—	1	73	81
46	17	83	—	29	41	106	—	—	1	74	82
47	17	17	—	30	41	107	—	—	1	75	83
1	18	83	—	1	1	108	—	—	1	76	59
48	18	17	—	31	42	109	—	—	1	77	84
49	19	88	—	32	41	110	—	—	1	78	85
50	19	13	—	33	43	111	—	—	1	79	62

Table 2. (*cont.*)

Combined PFGE/ AFLP type	Flock	Proportion of isolates per flock (%)	Number of human isolates	PFGE type	AFLP type	Combined PFGE/ AFLP type	Flock	Proportion of isolates per flock (%)	Number of human isolates	PFGE type	AFLP type
51	20	100	—	34	44	112	—	—	1	80	41
52	21	91	—	35	45	113	—	—	1	81	2
53	21	9	—	35	46	114	—	—	1	82	62
54	22	50	—	36	47	115	—	—	1	83	86
55	22	13	—	17	48	116	—	—	1	84	87
56	22	13	—	36	49	117	—	—	1	85	41
57	22	13	—	17	50	118	—	—	1	86	62
58	22	13	—	17	51	119	—	—	1	87	88
59	23	100	—	10	52						

* Including a set of three epidemiologically related strains.
† Including a set of two epidemiologically related strains each.

RESULTS

Prevalence of *Campylobacter* spp. and species identification in poultry flocks at slaughter

Thirty of 66 poultry flocks (45%) tested positive for *Campylobacter* spp. at slaughter. Positive hydrolysis of hippurate in almost all isolates identified them as *C. jejuni*. All isolates of 3 flocks as well as 4 isolates of 1 flock failed to hydrolyse hippurate. However, PCR detection of the *hip* gene identified these isolates as *C. jejuni* as well.

In 21 flocks, *C. jejuni* was isolated from all samples; in 3 flocks from 90%, in 1 flock from 80% and in 2 flocks from 50% of the samples. Totals of 40, 30, and 10% of the samples tested positive for *C. jejuni* in 1 flock each.

Subtyping of *C. jejuni* isolated from poultry

A total of 177 isolates from 24 flocks were subtyped by both PFGE and AFLP (Table 2).

PFGE

When combining *Sma*I and *Sal*I profiles, 36 distinct PFGE types were distinguished. Isolates from 11 flocks (46%) had only one PFGE type. In 8 (33%), 4 (17%), and 1 (4%) flock(s) 2, 3, and 4 PFGE types, respectively, were observed. Five types were shared by 2 different flocks each, and 1 type by 3 flocks.

AFLP

Fifty-five different AFLP types were observed. Ten flocks (42%) had only one AFLP type. Multiple AFLP types were distributed as follows: 3 flocks (13%) had 2, 6 flocks (25%) had 3, 2 flocks (8%) each had 4 and 5, and 1 flock (4%) had 7 AFLP types. Four genotypes were shared by 2 flocks each.

Combination of PFGE and AFLP

When combining PFGE and AFLP types, a total of 62 different types were identified. Eight flocks (33%) contained isolates of only one combined PFGE/AFLP type. Multiple PFGE/AFLP types were distributed as follows: 5 flocks (21%) each had 2 and 3, 2 flocks (8%) each had 4 and 5, and 1 flock (4%) each had 6 and 7 PFGE/AFLP types. Two PFGE/AFLP types were shared by 2 flocks each.

In some cases, identical AFLP types were further differentiated by PFGE. However, when the criteria for genetic relatedness according to Tenover et al. [29] were applied, they never showed entirely unrelated PFGE profiles. On the other hand, identical PFGE types could more often be further differentiated by AFLP than vice versa, and in some cases did not even cluster together.

Ciprofloxacin resistance of *C. jejuni* isolated from poultry

One hundred and thirty-nine of 274 (50%) *C. jejuni* isolates of 30 flocks were resistant to ciprofloxacin. All *C. jejuni* isolates of 13 flocks (43%) were sensitive to ciprofloxacin and 13 flocks (43%) contained resistant isolates only. Four flocks (14%) had sensitive as well as resistant isolates, which were clearly identified as different PFGE/AFLP types.

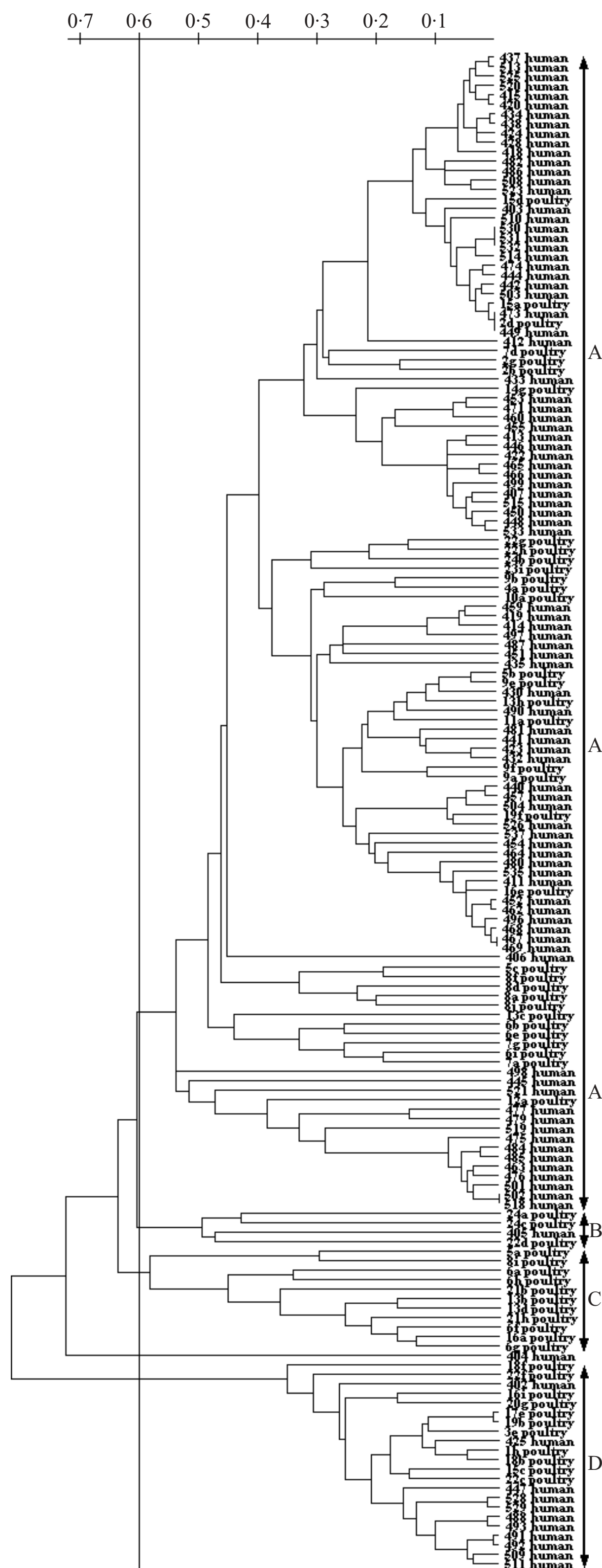

Fig. 1. UPGMA dendrogram of AFLP fingerprints from all human *C. jejuni* isolates included in the study and one poultry isolate per different AFLP type each. At 60% dissimilarity the isolates are separated in 4 distinct clusters (A–D).

Subtyping of human *C. jejuni* isolates

One hundred and one clinical *C. jejuni* isolates were subtyped by PFGE and AFLP (Table 2).

PFGE

A total of 55 combined *Sma*I/*Sal*I types were identified. Thirteen PFGE types were found in more than one isolate. Type 3 was the predominant type, being observed in 21 isolates (21%).

AFLP

Thirty-eight different AFLP types were found. Thirteen AFLP types were present in more than one isolate. The predominant AFLP type 2 was observed in 12 (12%), and types 56 and 62 in 11 (11%) isolates, respectively.

Combination of PFGE and AFLP

When combining PFGE and AFLP subtyping data, a total of 60 different PFGE/AFLP types were identified. Thirteen types were shared by different isolates. The predominant PFGE/AFLP types 63 and 3 occurred in 11 (11%) and 9 (9%) isolates, respectively.

Three sets of two and one set of three epidemiologically related isolates were included in the study and could not be distinguished by either subtyping method. One suspected epidemiologically related set of two strains revealed different subtypes with the two methods. Identical AFLP types had completely unrelated PFGE types in many cases. Identical PFGE types yielded, in most cases, either identical AFLP types or AFLP types that clustered together. However, these AFLP clusters also contained isolates with entirely unrelated PFGE types.

Ciprofloxacin resistance of human *C. jejuni* isolates

Ninety-four of 239 (39%) of clinical *C. jejuni* isolates were resistant to ciprofloxacin. Of 101 clinical *C. jejuni* isolates PFGE and AFLP data were available. With both subtyping methods, some types comprised resistant as well as susceptible isolates. Fifty per cent of the isolates of the PFGE/AFLP types 70 and 71, 40% of the isolates of type 64 and 33% of the isolates of types 3 and 67 were resistant to ciprofloxacin.

Comparison of subtyping data of *C. jejuni* isolates from poultry and humans

Cluster analysis of AFLP data revealed four distinct clusters (A–D) with 60% dissimilarity (Fig. 1). One

human isolate (404) did not concur with any cluster group. Human and poultry isolates clustered together throughout the dendrogram, with the exception of cluster C, which contained poultry isolates only.

Four PFGE types and five AFLP types were shared by human and poultry isolates. When combining the results of both subtyping methods, three PFGE/AFLP types observed in 11 human isolates (11%) were shared. PFGE/AFLP type 3 comprised nine human isolates (6 of them sensitive and 3 resistant to ciprofloxacin) and ciprofloxacin-resistant isolates of poultry flocks 2 and 15. PFGE/AFLP type 1 consisted of one human isolate and isolates of poultry flocks 1 and 18, all of them being resistant to ciprofloxacin. PFGE/AFLP type 31 comprised ciprofloxacin-sensitive isolates and was shared by one human isolate and isolates from poultry flock 9.

DISCUSSION

Campylobacter spp. were found in 45% of poultry flocks at slaughter in Austria. This rate is lower than that registered in The Netherlands (>82%) and the United Kingdom (76%), but higher than that in Sweden and Norway (27 and 18%, respectively) [3–6]. Probably, the performance of a pre-enrichment step could have increased the isolation rate. However, an increase in the prevalence of *Campylobacter* spp. to 56·8% during the last 2 years, within flocks sampled in the same area, was reported recently [30]. Both, the predominance of the *Campylobacter* species *C. jejuni* observed in the present study and the high percentage of positive isolates per contaminated flock are in accordance with previously published data [3, 4, 31, 32].

With respect to the individual chickens sampled, a single colony was picked for further investigation if the morphology of the colonies was uniform and typical for the expected species. In a few cases two different types of suspected *Campylobacter* spp. colonies were observed and then a colony of each type was picked. Further analysis of these isolates revealed that one chicken could simultaneously harbour a ciprofloxacin-resistant and a ciprofloxacin-sensitive *C. jejuni* strain. As only one isolate per chicken was further analysed by PFGE/AFLP it is not known, whether the different types of isolates per chicken observed by ciprofloxacin resistance testing belong to a different PFGE/AFLP type as well.

The combined PFGE/AFLP data revealed multiple infections of flocks with different strains of *C. jejuni* as well as different flocks being infected with strains comprising the same PFGE/AFLP type. Similar results have been obtained with RFLP typing of the *fla*A and B genes and serotyping [3, 32–35].

Fifty per cent of *C. jejuni* strains isolated from poultry in Austria were found to be resistant to ciprofloxacin. This figure lies within the range reported in other European countries: 3·2–45·9% of *Campylobacter* spp. and 98·7% of *C. jejuni* isolated from poultry tested resistant to ciprofloxacin in Ireland, Germany, and Spain, respectively [36–38]. In The Netherlands, 29% of *Campylobacter* spp. isolates from poultry were resistant to quinolone [39].

Of the clinical *C. jejuni* isolates tested in the presented study, 39% were resistant to ciprofloxacin, compared with 22·1% of clinical isolates of *Campylobacter* spp. in 1993. In 2000 there was a further rise in resistance to 40·2% [40], the increase being much the same in several countries worldwide [10, 11, 37, 38, 41–44]. In many countries, the prevalence of resistance in clinical strains parallels that in poultry strains. In countries such as Finland, where no quinolones were used in poultry production until 1998 [45], the proportion of human *C. jejuni* isolates resistant to ciprofloxacin was 2·8% in 1997 [13]. On the other hand, in countries like Spain, where enrofloxacin was licensed for use in poultry production in 1986 [45], 75% of clinical and 98·7% of poultry *C. jejuni* isolates were ciprofloxacin resistant in 1997 [38]. The government of Austria has permitted the use of enrofloxacin in poultry since 1989. Unfortunately, there were no data available concerning the use of enrofloxacin in the flocks sampled, but it is commonly used in Austria. Therefore the resistance data revealed in the present study are concordant with this general trend.

When comparing PFGE and AFLP data, we encountered major differences in the ability to subtype *C. jejuni* isolates of human and poultry origin. With AFLP, many poultry isolates of the same as well as of different flocks showing the same PFGE types were further differentiated, whereas several human isolates representing the same AFLP types showed different PFGE types. DeBoer et al. [46] applied AFLP using the restriction enzymes *Hin*dIII and *Hha*I for subtyping poultry isolates. Their comparison of AFLP data with *Sma*I-derived PFGE data also showed AFLP to be slightly more discriminative; however, the number of subtyped isolates may have been too small to confirm this trend.

In the present study, comparison of subtyping data revealed three combined PFGE/AFLP types that were

shared by poultry and clinical isolates. A total of 11 human isolates (11 %) belonged to this category. With regard to ciprofloxacin resistance, type 3 comprised resistant poultry isolates and resistant as well as sensitive clinical isolates. These data suggest either (*a*) conversion of a ciprofloxacin-resistant *C. jejuni* strain into a susceptible one during passage in the slaughter house or in the human intestine, which seems unlikely, (*b*) the presence of resistant and susceptible strains belonging to the same PFGE/AFLP type in poultry flocks, which might have been detected when examining more than 10 chickens per flock, or (*c*) an unknown common source of infection for both poultry and humans. The small number of subtypes shared by clinical and poultry isolates is in accordance with previously published studies, in which a variety of subtyping techniques were used [13, 19, 20, 22, 36, 47].

Cluster analysis of AFLP data in the present study supports previous reports to the effect that no distinct subpopulations of *C. jejuni* isolates associated with humans or poultry exist [36, 48, 49]. Duim et al. [48] performed AFLP analysis of poultry and human *C. jejuni* isolates using the same restriction enzymes as those used in the present study. Cluster analysis revealed two subgroups with 40 % genetic homology. In our study, four subgroups containing poultry as well as human isolates were observed. One human isolate was clearly distinguished from these groups.

In conclusion, the present study confirms an increase in ciprofloxacin resistance in *C. jejuni* isolates of human and poultry origin, as observed in several countries worldwide. A small number of human isolates shared PFGE/AFLP types with poultry isolates. However, further studies should also focus on the identification of other sources for *C. jejuni* infection in humans.

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