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Genotypic Identification of Erythromycin-Resistant *Campylobacter* Isolates as *Helicobacter* Species and Analysis of Resistance Mechanism

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The correct identification of *Campylobacter* species remains cumbersome, especially when conventional biochemical tests and antimicrobial susceptibility patterns are used for a phenotypical identification. Correct identification is important for epidemiological purposes and for studying changes in antimicrobial resistance patterns. Six erythromycin-resistant campylobacter strains were investigated by 16S ribosomal DNA (rDNA) sequencing, 23S rDNA sequencing, and restriction fragment length polymorphism analysis of a putative heme-copper oxidase domain described as being specific for thermophilic *Campylobacter* species. Three erythromycin-resistant isolates from feces of human immunodeficiency virus (HIV)-seropositive patients with diarrhea and one blood isolate of from HIV-seropositive patient with cellulitis were identified by 16S rDNA analysis as *Helicobacter cinaedi*, whereas 23S rDNA sequencing suggested *Wolinella succinogenes*. The 16S rDNA sequence data of fecal isolates of two patients with travelers diarrhea revealed *Helicobacter pullorum* and were also in contrast with 23S rDNA sequencing. Of 4 *H. cinaedi* isolates, 1 contained the putative heme-copper oxidase gene thought to be specific for thermophilic species. The six erythromycin-resistant *Helicobacter* species had a similar point mutation A2143G in 23S rDNA resembling the macrolides resistance in *Helicobacter pylori*. We conclude that 16S rDNA sequencing should be preferred to 23S rDNA analysis and that macrolide-resistant campylobacter strains should be investigated by this approach for a correct identification.

The family *Campylobacteriaceae* includes 18 species and subspecies within the genus *Campylobacter* and 4 species in genus *Arcobacter* (13, 25). The clinically most important enteropathogenic *Campylobacter* species, encompassing *C. jejuni*, *C. coli*, *C. upsaliensis*, and *C. lari*, belong to the thermophilic group. The genus *Helicobacter* contains 19 species and is characterized by the presence of multiple bipolar sheathed flagella, except for *H. pylori* and *H. pullorum* (3, 4, 13, 16). Of enteric helicobacters, *H. canis*, *H. cinaedi*, *H. fennelliae*, and *H. pullorum* have been isolated from fecal specimens of humans with diarrhea, whereas *H. cinaedi* and *H. westmaedii* have also been recovered from human blood cultures.

Identification of campylobacters and helicobacters to the exact species level using phenotypic tests is difficult, since these criteria are still insufficient for identification of all species adequately (7, 15). The use of nalidixic acid susceptibility in the identification schedule of campylobacters is hampered by acquired resistance to fluoroquinolones. Difficulties with correct identification are illustrated in literature by the use of terms as "atypical *Campylobacter* strain" and "*Campylobacter*-like organisms" in cases where phenotypic characteristics are insufficient for conclusive identification (6, 14). Several species that were formerly classified as *Campylobacter* have been reclassified as *Helicobacter* and *Arcobacter* (6, 13, 14, 22). Additionally, helicobacters are frequently misidentified as campylobacters (26).

Several molecular methods have been reported to reliably identify the clinically important species of *Campylobacter* and *Helicobacter*. In recent years, most published work has relied on 16S rRNA gene sequencing to identify the species and a few studies report on the usefulness of 23S rRNA sequences (2, 5, 7, 11, 27). In a previous study to prevalence of campylobacters among human immunodeficiency virus (HIV)-seropositive patients with diarrhea, we found variations of the region between helices 43 and 69 of the 23S ribosomal DNA (rDNA) gene and restriction patterns of a putative heme-copper oxidase domain useful for a correct identification of *Campylobacter* species (15). Restriction patterns of the heme-copper oxidase domain were specific for identification of the thermophilic species (10). Subsequently, these methods were introduced in our routine laboratory practice for identification of campylobacters that are difficult to identify with phenotypical tests.

The finding of a *C. lari*-like isolate resistant to erythromycin from the blood of a HIV-seropositive patient and the difficulties we encountered with a correct genetic identification of this isolate, prompted us to study other erythromycin-resistant campylobacter strains from our collection. Macrolides are the drugs of first choice for the treatment of *Campylobacter* infections. Macrolide resistance is rare in *Campylobacter* species but not in *Helicobacter pylori.* Five erythromycin-resistant fecal isolates were present in our collection of campylobacters stored at −70°C in the period 1995 to 2001. We subsequently genotyped the isolates by nucleotide sequence analysis of parts of the 23S and 16S rDNA to determine the species of the isolates. Fur-

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TABLE 1. Reference strains and clinical isolates used in this study

Strain or isolate	Source[a]	Clinical symptom(s)	Phenotypical identification
7543	LMG		*H. cinaedi*
C. jejuni subsp. *jejuni* Lior 2	LMG		*C. jejuni*
Z6	CCUG		*H. fennelliae*
C. lari Lior 34	CCUG		*C. lari*
59C1133	LMA		*S. pyogenes*
59C1139	LMA		*S. pyogenes*
D19B1	HIV-infected patient	Diarrhea	Unidentified
D19G1	HIV-infected patient	Diarrhea	Unidentified
D23G3	HIV-infected patient	Diarrhea	Unidentified
C14D7	Non-HIV-infected patient	Diarrhea	Atypical *C. lari*
C14E3	Non-HIV-infected patient	Diarrhea	Atypical *C. lari*
208445J	HIV-infected patient	Bacteremia and cellulitis	Atypical *C. lari*

[a] Abbreviations: LMG, Laboratory of Medical Microbiology, University of Ghent; CCUG, Culture collection of the University of Goteborg; LMA, Laboratory of Medical Microbiology, University of Antwerp.

thermore, we performed PCR for a putative heme-copper oxidase domain and we analyzed the mechanisms underlying erythromycin resistance in these isolates.

MATERIALS AND METHODS

Reference strains and clinical isolates. Reference strains of *C. jejuni* subspecies *jejuni* (Lior serotype 2) and *H. cinaedi* (LMG 543) were obtained from the culture collection of the Laboratory for Microbiology of the University of Ghent, Ghent, Belgium (P. Vandamme). Reference strains of *C. lari* (Lior 34) and *H. fennelliae* were obtained from the culture collection of the University of Goteborg, Goteborg, Sweden. Two erythromycin-resistant control strains of *Streptococcus pyogenes* [59C133, *erm*(B) positive; 59C139, *mef*(A) positive] were donated by H. Goossens (University of Antwerp, Antwerp, Belgium). See Table 1 for a list of strains and isolates used in this study.

The five fecal isolates were selected on the basis of erythromycin resistance from a collection of campylobacters stored at −70°C at the Medical Microbiology Laboratory at the Amsterdam Medical Center. All fecal isolates were recovered from fecal samples by using the membrane filtration method (14) in the period 1995 to 2001. Three strains were associated with diarrhea in HIV-seropositive patients and two strains were found as a cause of travelers diarrhea. One strain was found in the blood of a HIV-seropositive patient who was admitted with fever and cellulitis.

Phenotypical identification. *Campylobacter* species were identified on the basis of colony morphology, the presence of curved gram-negative rods in a Gram stain and a positive oxidase reaction. All isolates were further tested using nitrate reduction; production of urease and H_2S; hydrolysis of hippurate and indoxyl acetate; growth at 25, 35, and 42°C; and susceptibility to nalidixic acid and cephalothin (13, 23, 25).

Antimicrobial susceptibility testing. To determine susceptibility to nalidixic acid and cephalothin for identification purposes, a standard disk diffusion technique on 5% sheep blood agar plates was used. MICs of erythromycin were determined on Mueller-Hinton agar supplemented with 5% sheep blood by E-test (AB Biodisk, Solna, Sweden) in a microaerobic environment for 24 to 28 h at 37°C. Strains were considered resistant to erythromycin when the MIC of erythromycin was ≥8 μg/ml (24).

Genetic characterization. (i) DNA isolation. DNA was isolated from suspensions of bacterial cultures from solid media using the QIAmp DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

(ii) PCR amplification. (a) 23S rDNA amplification. A specific part of the 23S rDNA gene for identification of thermophilic *Campylobacter* sp. was amplified using Therm1 and Therm2 (2, 5, 14), and Bob1 and Bob2 primers (E. J. Kuijper, B. de Wever, F. Snijders, S. A. Danner, J. Dankert, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. C-254, p. 173, 1998) (Table 2). PCR amplification was carried out in Qiagen PCR Mastermix with 10 pmol of each primer and using 5 μl of bacterial DNA. After an initial denaturation step of 3 min at 95°C, the protocol consisted of 35 cycles of 1 min at 95°C (denaturation), 1 min at 54°C (annealing), and 2 min at 72°C (elongation). The Bob primers and Therm primers yielded an amplified fragment of 290 and 330 bp, respectively. PCR products were investigated by nucleotides sequence analysis.

(b) 16S rDNA amplification. The 16S rDNA gene was amplified by broad-range PCR using primers in conserved regions of the gene. Parts of the 16S rRNA genes were amplified using primer pairs P1-P2 and P3-P4, which were selected from conserved parts of this gene (18). The first pair amplified a 300-bp fragment from the 5′ end of the 16S rRNA gene, which is the most discriminatory part. The second pair amplified 330 bp from the second half of the gene. Amplification was carried out using Qiagen PCR Mastermix, with 10 pmol of each primer and 5 μl of DNA. The cycling protocol was identical as for the 23S rRNA genes. Reactions were performed in a total volume of 50 μl containing 25 μl of HotStar PCR Mastermix (Qiagen), 10 pmol of each primer, and 5 μl of DNA isolate. Subsequently PCR products were analyzed by nucleotide sequence analysis.

(c) 23S rDNA codon 714 mutation analysis. Single nucleotide polymorphisms at positions 2142 and 2143 of the 23S rDNA were investigated by amplification of adjacent 23S rDNA sequences using primer pair ar69fw and ery23sr (Table 2). PCR was performed in a volume of 50 μl containing 25 μl of HotStar PCR Mastermix (Qiagen), 25 pmol of each primer, and 5 μl of DNA isolate. Subsequently PCR products were sequenced (see below).

(d) PCR for the putative heme-copper oxidase domain of *Campylobacter* spp. A PCR specific for thermophilic *Campylobacter* spp. was performed according to the method of Jackson et al. (10), targeting a gene encoding a putative heme-copper oxidase domain, downstream from a *Campylobacter* two-component regulator gene (Table 2 shows the oligonucleotide sequences). After digestion of the PCR products with *Alu*I, *Dde*I, and *Dra*I, the restriction fragment length polymorphisms were analyzed by electrophoresis on 2% NuSieve agarose.

(e) *erm*(B) and *mef*(A) analysis. PCR assays for the *erm*(B) gene, encoding a 23S rDNA methylase and the *mef*(A) gene, encoding a macrolide efflux protein were performed as described previously (1, 12, 17) (Table 2 shows oligonucleotide sequences).

(iii) Nucleotide sequence analysis. Nucleotide sequence analysis of PCR products was performed using an ABI PRISM BigDye Terminator Cycle Sequencing kit (version 3; Applied Biosystems). PCR products were sequenced in forward and reverse orientation using the same primers as used for PCR amplification.

TABLE 2. Sequences of oligonucleotides used in this study

Primer	Sequence (5′-3′)	Position (nucleotides) or reference
P1	TAACACATGCAAGTCGAACG	49–67[a]
P2	CCCATTGTGCAATATTCCCC	378–359[a]
P3	GGATTAGATACCCTGGTAGTCC	785–806[a]
P4	TCGTTGCGGGACTTAACCCAAC	1197–1176[a]
Ar69fw	GTAACTATAACGGTCCTAAG	1994–2013[b]
Ery23sr	GACCGCCCCAGTCAAACT	2342–2312[b]
Bob1	TCGTCATCGTAGGGTTAGT	1429–1447[b]
Bob2	CGCGCCTTAGAATACTCATC	1758–1739[b]
Therm1	TATTCCAATACCAACATTAGT	Snijders et al. (15)
Therm2	CGGTACGGGCAACATTAG	Snijders et al. (15)
BO4263	AGAACACGCGGACCTATATA	Jackson et al. (10)
BO4264	CGATGCATCCAGGTAATGTAT	Jackson et al. (10)

[a] Based on *Escherichia coli* numbering in the work of Suzuki et al. (18).
[b] Based on 23S rRNA numbering in the work of Taylor et al. (19).

TABLE 3. Results of sequence analysis of 23S and 16S rDNA fragments

Strain	Phenotype	Result of 23S rDNA sequence analysis (similarity data) using GenBank primers Bob1-Bob2	Result of 16S rDNA sequence analysis (similarity data) using GenBank primers:	
			P1–P2	P3–P4
LMG7543	*H. cinaedi*	*Wolinella succinogenes* (323/330; 97.9%)	*H. cinaedi* (350/352; 99.4%)	*F. rappini* (367/369; 99.4%), *H. cinaedi* (366/369; 99.2%)
C. lari Lior 34	*C. lari*	*C. lari* (332/332; 100%)	*C. lari* (382/387; 98.7%)	*C. lari* (409/412; 99.3%)
Z6	*H. fennelliae*	*W. succinogenes* (321/331; 97%)	*H. fennelliae* (593/593; 100%)	*H. fennelliae* (470/474; 99.2%)
D19B1[a]	Unidentified	*W. succinogenes* (320/327; 97.9%)	*H. cinaedi* (376/379; 99.2%), *Flexispira rappini* (376/379; 99.2%)	*H. cinaedi* (447/451; 99.1%), *F. rappini* (448/451; 99.3%)
D19G1[a]	Unidentified	*W. succinogenes* (307/314; 97.8%) *C. lari* (304/314; 96.8%)	*H. cinaedi* (341/341; 100%)	*H. cinaedi* (419/423; 99.2%), *F. rappini* (420/423; 99.3%)
D23G[a]	Unidentified	*W. succinogenes* (303/311; 97.4%)	*H. cinaedi* (231/231; 100%)	*H. cinaedi* (474/477; 99.4%), *F. rappini* (476/477; 99.8%)
C14D7[b]	Atypical *C. lari*	*W. succinogenes* (257/309; 83.2%), *C. lari* (255/309; 82.7%)	*H. pullorum* (350/350; 100%), *H. cinaedi* (347/350; 99.1%)	*H. pullorum* (498/499; 99.8%), *H. cinaedi* (487/496; 98.2%)
C14E3[b]	Atypical *C. lari*	*W. succinogenes* (292/305; 96%)	*H. pullorum* (398/399; 99.7%), *H. cinadedi* (394/400; 98.5%)	*H. pullorum* (425/428; 99.3%), *H. cinaedi* (419/429; 97.6%)
208445J[a]	Atypical *C. lari*	*W. succinogenes* (320/325; 98%), *H. pylori* (201/208; 96%)	*H. cinaedi* (351/351; 100%)	*H. cinaedi* (494/497; 99.4%), *F. rappini* (496/497; 99.8%)
C. jejuni Lior 2	*C. jejuni*	*C. jejuni* (315/315; 100%)	*C. jejuni* (374/374; 100%)	ND[c]

[a] The final identification of isolates D19B1, D19G1, D23G3, and 208445J was *H. cinaedi.*
[b] Strains C14D7 and C14E3 were identified as *H. pullorum*
[c] ND, not done.
[d] Similarity data are presented as the number of similar nucleotides/the number of nucleotides analyzed and the resulting percentage.

Sequence reaction mixtures were applied to an ABI PRISM 310 Genetic Analyzer. Nucleotide sequences were edited and aligned using Vector NTI Suite 6 (InforMax Inc.) and compared against GenBank DNA sequences using Basic Logic Alignment Tool (BLAST), which is available at http://www.ncbi.nlm.nih.gov.

RESULTS

Phenotypic characterization. Three strains (D19B1, D19G1, and D23G3) could not be identified to the species level but had similar biochemical reactions. All three isolates showed positive reactions for catalase and nitrate reduction, failed to produce H_2S, did not grow at 42°C and were unable to hydrolyze of hippurate and indoxyl acetate. They were susceptible to nalidixic acid and cephalothin but resistant to erythromycin (MIC $\geq$ 256 mg/liter). Strains C14D7, C14E3, and 208445J were phenotypically identified as nalidixic acid susceptible variants of *C. lari.* Strains C14D7 and C14E3 showed resistance to cephalothin and erythromycin (MIC $\geq$ 256 and 16 mg/liter, respectively), whereas strain 208445J was susceptible to cephalothin and resistant to erythromycin (MIC $\geq$ 256 mg/liter).

Genotypic identification by 23S and 16S rDNA sequence analysis. All six clinical isolates reacted with the Therm1-Therm2 primer pair. Of the reference strains, *C. jejuni*, *C. lari*, and *H. cinaedi* (LMG 7543) reacted with Therm1-Therm2, but *H. fennelliae* (Z6) showed a negative reaction. 23S rDNA sequence analysis of PCR products obtained with primer pair Bob1-Bob2 showed strongest homology of all isolates with *Wolinella succinogenes* (Table 3). Reference strains *C. lari* Lior 34 and *C. jejuni* were correctly identified based on their 23S rDNA sequence (similarity with GenBank sequences of *C. lari* and *C. jejuni* of 100%), but *H. cinaedi* reference strain LMG 7543 was incorrectly identified as *W. succinogenes* with 97.9% similarity. Since 23S rDNA sequence analysis was poorly informative and homology with *Campylobacter* species was low, we sequenced 16S rDNA using primer pairs P1-P2 and P3-P4, covering more than 40% of the gene, including the most discriminative 5′ end. Sequence analysis using P1-P2 enabled identification of isolates D19B1, D19G1, D23G3, and 208445J as *Helicobacter cinaedi*, with similarity percentages of 99.2 to 100%. Strains C14D7 and C14E3 showed strongest homology with *H. pullorum.* Sequence analysis using primer pair P3-P4 confirmed the identification of these strains as *H. pullorum* with a similarity of 99.8 and 99.3%, respectively, whereas the similarity to *H. cinaedi* was 98.2 and 97.6% (Table 3).

Detection of the putative heme-copper oxidase domain. *C. jejuni* and *C. lari* Lior 34 reference strains were positive for the putative heme-copper oxidase gene PCR. Furthermore, isolate 208445J, identified as *H. cinaedi* based on 16S rDNA sequencing, was positive. All other clinical isolates were negative with this PCR.

Genetic mechanisms underlying erythromycin resistance in *H. cinaedi* and *H. pullorum*. The putative mechanisms that may lead to erythromycin resistance in the *Helicobacter* strains was investigated by a PCR for the *erm*(B) and *mef*(A) genes. None of the isolates were positive in *erm*(B) PCR. Control *S. pyogenes* strain 59C1133 was positive for *erm*(B) DNA in this experiment. All isolates were negative for the *mef*(A) gene as well, while control *S. pyogenes* strains 59C1139 was positive for *mef*(A) DNA (Table 4).

Subsequently, we analyzed the nucleotide sequences at positions 2142 and 2143 of the 23S rRNA gene of the *H. cinaedi* and *H. pullorum* isolates identified above. Point mutations (substitution of A to G) at these positions of the 23S rRNA gene are related to macrolide-lincosamide-streptogramin B antibiotics resistance in *H. pylori* (9, 19). In all isolates that were resistant to erythromycin, a G was observed at position 2142 (codon 714: AGA). In the reference *H. cinaedi* strain, which is sensitive to erythromycin, the codon was AAA. The erythro-

TABLE 4. Phenotypic and genotypic analysis of erythromycin resistance

Strain	MIC (μg/ml) of erythromycin	Erythromycin susceptibility	Result of PCR with:		Sequence (nucleotides 2142–2144)
			ErmB	MefA	
LMG 7543	0.016	S[a]	−	ND[b]	AAA
Z6	0.094	S	ND	ND	AAA
C. lari	0.5	S	ND	ND	AAA
C. jejuni	0.5	S	ND	ND	AAA
S. pyogenes [*erm* (B) positive]	>256	R[c]	+	−	ND
S. pyogenes [*mef* (A) positive]	8	R	−	+	ND
D19B1	>256	R	−	−	AGA
D19G1	>256	R	−	−	AGA
D23G3	>256	R	−	−	AGA
C14D7	>256	R	−	−	AGA
C14E3	16	R	−	−	AGA
208445J	>256	R	−	−	AGA

[a] S, sensitive.
[b] ND, not done.
[c] R, resistant.

mycin-sensitive *C. lari* Lior 34 and *C. jejuni* reference strains also showed the wild-type AAA codon at this position.

DISCUSSION

This study describes the difficulties with identification of six erythromycin-resistant *Campylobacter* isolates from five patients with diarrhea and from one patient with septicemia and cellulitis. In contrast with cephalothin and nalidixic acid susceptibilities, erythromycin susceptibility is used for patient treatment options and does not contribute to the phenotypical identification. Phenotypical identification of the erythromycin-resistant isolates was inconclusive, and therefore a molecular biological approach was used to identify the isolates. All six isolates gave a PCR product in 23S rDNA using primers Therm1 and Therm2 specific for thermophilic *Campylobacter* species (5). However, sequence analysis of 23S rDNA of the isolates showed poor homology to thermophilic *Campylobacter* sequences from GenBank. This is mainly due to the fact that only a limited number of 23S rDNA sequences are currently available in this database. For instance, no sequence data of 23S rDNA are available for *H. pullorum* and *H. cinaedi* and thus no complete match can be achieved. The next step was to sequence the most informative part of 16S rDNA, as recently recommended by the International Subcommittee on the Taxonomy of *Campylobacter* and *Helicobacter* (4). Subsequently, the six erythromycin-resistant *Campylobacter* isolates were identified as *H. cinaedi* ($n = 4$) and *H. pullorum* ($n = 2$).

The nucleotide sequences of 23S rDNA fragment between helices 42 and 63 in 23S rDNA have been described as one of the most variable regions of the 23S rDNA of campylobacters. This fragment has been used to develop specific primers for thermophilic *Campylobacter* species (5). Although the primer set was used on a large number of reference strains (including *H. cinaedi* but not *H. pullorum*) and clinical isolates, only visible bands corresponding to 290 bp were observed for the thermophilic *Campylobacter* species. In contrast with these findings, we found a reference strain of *H. cinaedi* (LMG 7543) and clinical erythromycin-resistant isolates of *H. pullorum* and *H. cinaedi* also positive with these primers. This finding has important implications, since we introduced this method in our routine laboratory practice and misidentified clinical isolates as erythromycin-resistant *C. lari*-like strains (15).

Restiction patterns of the putative heme-copper oxidase domain PCR product has also been reported as a tool for accurate specification of thermophilic *Campylobacter* spp. (10). Unfortunately, we found this gene also present in an *H. cinaedi* isolate. *H. cinaedi* was not included in the manuscript of Jackson et al. in which the specificity of the primers for the heme-copper oxidase domain were tested on all campylobacters of RNA homology group 1, all *Arcobacter* species and *Helicobacter pylori* species (10). The target sequence of the PCR is highly conserved and under conditions of low stringency, specific 256-bp products can be amplified from DNA of all members of the genus *Campylobacter*. However, we applied similar stringent criteria as described by Jackson et al. and found other *Helicobacter cinaedi* isolates, *H. pullorum* and *H. pylori* strains negative.

Of six clinical isolates, four showed homology of 99.2 to 99.8% to *Flexispira rappini* in 16S rDNA. *F. rappini* is a provisional name to a helicobacter with spiral periplasmic fibers and bipolar tufts of sheathed flagella (27). Considerable diversity in the 16S rRNA genes of 35 *F. rappini* strains has been observed so that the International Subcommittee on the Taxonomy of *Campylobacter* and *Helicobacter* decided that *F. rappini* is not a well-defined species but encompasses multiple *Helicobacter* species (3, 27). Therefore, the several interesting reports of bacteremia due to *Helicobacter* (*Flexispira*) *rappini* in HIV-seropositive patients and in a patient with X-linked hypogammaglobulinemia should be interpreted with care (8, 20, 21, 30).

Macrolides are the drugs of first choice for the treatment of *Campylobacter* and *Helicobacter* infections, except for *H. pylori.* Three point mutations in the 23S rRNA gene of *H. pylori* were found to be associated with macrolide resistance (19). The mechanism of erythromycin resistance we found in four *H. cinaedi* and two *H. pullorum* strains seems to resemble macrolides resistance in *H. pylori* and is due to due to an mutation of one of two adenines (A2142G or A2143G) in the 23S rRNA at the erythromycin-binding site (28, 29). Recently, sequencing of the 23S rRNA genes from 22 erythromycin-resistant *Campylobacter* spp. identified mutations at these same sites, which are most probably responsible for resistance (24; C. A. Trieber and D. E. Taylor, Abstr. Final Prog. 10th Int. Workshop *Campylobacter Helicobacter* Rel. Organisms, abstr. CA6, p. 3, 1999). In one study high levels of erythromycin resistance in 34 *H. cinaedi* strains (MIC $\geq$ 128 mg/liter) has been reported, but the mechanism of erythromycin resistance was not investigated further (11). Identification of these 34 *H. cinaedi* strains was performed by total DNA-DNA hybridization but discrepancies of DNA-DNA hybridization have been observed with 16S rRNA sequencing (30). Neither in vitro susceptibility testing data nor treatment recommendations are available for infections caused by *H. pullorum*, but it seems reasonable to follow the same guidelines as for other helicobacters and to take macrolides resistance into account. Of special interest is the observation of Tee et al. that two strains fulfilling the morphological criteria of *Helicobacter* (*Flexispira*) *rappini* were also resistant to erythromycin (20). Whether erythromycin-resistant *Helicobacter* species other than *H. pylori* are an increasing

clinical problem should be the scope of a larger study on this subject.

This study clearly demonstrates the shortcomings of identification of erythromycin-resistant *Campylobacter*-like strains using phenotypical tests, available 23S rRNA sequence data and the gene encoding a putative heme-copper oxidase domain. The strains were finally identified using the sequence of the most informative part of 16S rDNA, as recently recommended by the International Subcommittee on the Taxonomy of *Campylobacter* and *Helicobacter* (4). Therefore, we recommend that erythromycin-resistant campylobacter-like strains and campylobacter-like strains isolated from blood cultures be investigated further by sequencing of 16S rDNA. We realize that this technology is not available for routine microbiology laboratories, but reference laboratories should overcome this problem until commercial available reagents and procedures are introduced in routine practice. Additionally, this is also the first report of *H. cinaedi* and *H. pullorum* strains resistant to erythromycin with mutations in the 23S rDNA gene similar to those observed in *H. pylori*.

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