Campylobacters are the most common bacterial cause of human gastrointestinal infection worldwide. The species most frequently isolated from humans are \textit{Campylobacter jejuni} and \textit{Campylobacter coli} (8). Other species, such as \textit{Campylobacter fetus}, \textit{Campylobacter upsaliensis}, and \textit{Campylobacter lari}, are occasionally found in clinical isolates (7). Macrolides are the drugs of choice for treating \textit{Campylobacter} infections (15). \textit{Campylobacter} resistance to macrolides is mainly found in strains of animal origin, especially \textit{C. coli} from pigs but also from chickens (1, 2, 18). Such strains can be transmitted to humans. Furthermore, macrolide resistance may develop during the course of antibiotic treatment in humans (5).

In a closely related bacterium, \textit{Helicobacter pylori}, three major point mutations occurring in the peptidyl-encoding region in domain V of the 23S rRNA gene were found to be associated with macrolide resistance (A2142G, A2143G, and less frequently A2142C) (19). Their susceptibility to erythromycin (Sigma, St. Louis, Mo.) was detected by disk diffusion and confirmed by the standard agar dilution method in accordance with the recommendations of the Comité de l’Antibiogramme de la Société Française de Microbiologie (3). Strains were considered resistant to erythromycin with a MIC of \(\geq 8\) \(\mu\)g/ml. The bacterial cells were harvested and then subjected to DNA extraction with the QIAamp DNA mini kit (Qiagen SA, Courtaboeuf, France).

Since the genetic information for domain V of the 23S rRNA gene of some campylobacters was available in the GenBank database, sequences were aligned by using multiple sequence alignment with hierarchical clustering (4) and a search was carried out to identify conserved regions flanking the sequence that supposedly presented mutations associated with erythromycin resistance. As a result, we selected a set of primers, \textit{F1-campy-23S} (5’-AAGAGGATGTTAGGTTGTCAC G-3’) and \textit{R1-campy-23S} (5’-AACGTATTCCAACCGTCT TGC-3’), designed to amplify a 508-bp sequence corresponding to nucleotides 1825 to 1847 and 2313 to 2332 of the three copies of the 23S rRNA gene of \textit{C. jejuni} NCTC 11168, respectively (GenBank accession numbers AL139074, AL139075, and AL139076) (13). The amplification reaction was carried out in a final volume of 50 \(\mu\)l containing 1\(\times\) buffer, 1.5 mM MgCl\(_2\), 200 \(\mu\)M deoxynucleoside triphosphates, 0.2 \(\mu\)M each of the primers, 1 U of \textit{Taq} polymerase (Eurobio, Les Ulis, France), and 2 \(\mu\)l of extracted DNA. Amplification was performed in a Perkin-Elmer GeneAmp 9700 thermocycler under the following conditions: 1 cycle of 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 1 cycle of 72°C for 7 min. PCR amplicons were purified by using MicroSpin S-400 HR columns in accordance with the manufacturer’s (Amerham Pharmacia Biotech Inc., Uppsala, Sweden) instructions. Sequencing of both strands of the amplified fragments was achieved by using an Applied Biosystems Prism 377 automated sequencer with dRhodamine-labeled terminators (PE Applied Biosystems, Foster City, Calif.).

On the basis of the sequence alignment of the 23S rRNA genes obtained with \textit{F1-campy-23S} and \textit{R1-campy-23S}, we designed a new set of primers, \textit{F2-campy-23S} (5’-AATTGATGGTATTAGGCATTAGC-3’) and \textit{R2-campy-23S} (5’-CAACAA

\section*{PCR-Restriction Fragment Length Polymorphism Analysis for Detection of Point Mutations Associated with Macrolide Resistance in \textit{Campylobacter} spp.}

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A 23S rRNA gene fragment in domain V was sequenced from 30 clinical isolates of \textit{Campylobacter} spp., including 22 resistant to macrolides. Two point mutations associated with erythromycin resistance were identified at positions 2074 and 2075 on the 23S rRNA gene (homologous to A2142C and A2143G mutations in \textit{Helicobacter pylori}) in which an adenine residue is also replaced with a cytosine and a guanine residue, respectively. A combined PCR-restriction fragment length polymorphism technique was developed to detect these mutations by using the \textit{BsaI} and \textit{BceAI} enzymes.

\textit{Campylobacter} sp. isolates were cultured on Trypticase soy medium containing 5% horse blood under microaerobic conditions at 37°C. Their susceptibility to erythromycin (Sigma, St. Louis, Mo.) was detected by disk diffusion and confirmed by the standard agar dilution method in accordance with the rec
TGGCTCATATACACTGG-3'), corresponding to nucleotides 1869 to 1890 and 2184 to 2161 of the 23S rRNA gene of C. jejuni NCTC 11168, respectively (13). Amplification with these primers resulted in a 316-bp PCR product. The conditions of amplification were the same as those described above.

After successful amplification of the 316-bp PCR products, amplicons were precipitated and suspended in 15 μl of H₂O and 5 μl of the amplicons was digested with the restriction enzymes BsaI (5 U) and BceAI (1 U) (New England Biolabs, Beverly, Mass.) as previously reported for H. pylori (9). The fragments were incubated overnight at 50°C for BsaI and 37°C for BceAI, separated by electrophoresis on a 10% acrylamide gel, and visualized under UV light after ethidium bromide staining.

Thirty Campylobacter strains isolated in different areas of France were selected from the collection of the National Reference Center for Campylobacters on the basis of the disk diffusion test results; 22 were erythromycin-resistant (MIC, ≥128 μg/ml) strains of different species, and 8 were susceptible strains. They were chosen randomly from among the different species isolated in humans. The C. jejuni reference strain, NCTC 11168, which is susceptible to erythromycin, was also included. DNA sequences of an internal area of the 23S rRNA gene were determined for the 30 strains. Representative sequences are presented in Fig. 1. At least one mutation was detected in all of the macrolide-resistant strains but in none of the strains susceptible to this group of antibiotics. Mutations were found at positions 2074 and 2075, which are homologous to positions 2142 and 2143 in H. pylori, respectively (16). The predominant mutation was the transition mutation A2075G (20 cases). The transversion mutation A2074C was found in three resistant strains. It was noted that one strain (C. jejuni 00039) carried both the A2075G and A2074C mutations. Finally, none of the strains contained the mutation homologous to the A2142G mutation commonly described in H. pylori.

As previously described, each of these two mutations created an additional site for digestion by the restriction enzymes BsaI (for the A2075G mutation) and BceAI (for the A2074C mutation) (9, 20). PCR amplification with the F2-campy-23S and R2-campy-23S primers was performed on the 30 clinical isolates and the reference strain. A 316-bp amplicon was obtained from 17 selected Campylobacter sp. strains and C. jejuni reference strain NCTC 11168 (GenBank accession numbers AL139074, AL139075, and AL139076). The strain numbers and the MICs of erythromycin are indicated. M, position of a point mutation associated with resistance to erythromycin; *, spontaneous mutation.
producing three fragments of 41, 24, and 251 bp (Fig. 2, lanes 6 and 7). Mutation A2074C created an additional recognition site, leading to the occurrence of another mutation, the transversion A2074C, as well as the presence of both mutations in one strain. A technique that is easy to carry out in most laboratories is also described. This is an alternative to the line probe assay that has already been described, which detects only half of the mutations occurring in Japanese strains (11). While the rate of macrolide resistance in clinical isolates (≤5%) is not yet alarming (8, 10, 14), a trend toward an increase has been noted both in animal strains that are potentially transmissible to humans via food and in human strains during macrolide treatments. Therefore, the study and monitoring of this resistance have, in turn, become increasingly necessary.

**Nucleotide sequence accession numbers.** The partial 23S rRNA gene sequences of strains 98054 (464 bp), 00251 (457 bp), 98178 (464 bp), 00264 (457 bp), 01190 (461 bp), 89556 (451 bp), 98010 (410 bp), 00072 (456 bp), 00123 (464 bp), 00222 (464 bp), 00135 (464 bp), 95089 (344 bp), 99252 (421 bp), 98212 (420 bp), 01206 (394 bp), 95064 (384 bp), and 00039 (423 bp) have been submitted to the GenBank database and assigned accession numbers AY190985 to AY191001, respectively.

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