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A Highly Macrolide-Resistant *Campylobacter jejuni* Strain with Rare A2074T Mutations in 23S rRNA Genes

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The main molecular mechanisms underlying high-level macrolide resistance in *Campylobacter* species are multiple mutations such as A2074G, A2074C, and A2075G, in 23S rRNA genes (1). *Campylobacter* possesses 3 copies of 23S rRNA genes (2), and the occurrence of two such mutations among 3 copies of 23S rRNA genes has been reported to confer macrolide resistance (3–5). The association between A2074T mutations and high-level macrolide resistance in *Campylobacter* has not been fully elucidated because isolates with these mutations in only some copies of the 23S rRNA genes have been recovered (6). It was reported that the A2074T mutation might confer only a low level of macrolide resistance (1, 6). Here, we characterized a highly macrolide-resistant *Campylobacter jejuni* clinical isolate containing A2074T mutations in all 3 copies of 23S rRNA genes.

The *C. jejuni* strain NC05-27 was isolated from a patient who had diarrhea and demonstrated high-level macrolide resistance (Table 1) (7). Classical PCR using primers that amplify each of the three 23S rRNA genes (see Table S1 in the supplemental material) and Sanger sequencing analyses in addition to whole-genome sequence data (accession no. BCNK01000000) from MiSeq platforms revealed that this strain possessed a A2074T mutation in all 3 copies (4). However, this strain possessed no other known resistance factors, such as the 23S rRNA methyltransferase gene *erm*(B), or amino acid changes in L4/L22 ribosomal proteins (8–10). Therefore, it is strongly suggested that the A2074T mutations in all 3 copies of 23S rRNA genes were mainly responsible for high-level macrolide resistance in NC05-27.

To further clarify the role of A2074T mutations in macrolide resistance, we PCR amplified the A2074T mutation-containing 23S rRNA genes from NC05-27 (using primers 23SunivF and 23SunivR [see Table S1 in the supplemental material]) and introduced them into a macrolide-susceptible *C. jejuni* NCTC11168 strain (Ery^s) by natural transformation. The transformants were selected on Mueller-Hinton agar plates supplemented with 64 μg/ml erythromycin. One representative transformant (Ery^r), confirmed to carry A2074T mutations in all 3 copies of the 23S rRNA gene, was subjected to susceptibility testing. The Ery^r transformant was found to acquire a high level of macrolide resistance, comparable to that of the parent strain NC05-27 (Table 1). Our results indicated that A2074T mutations conferred high-level macrolide resistance on *Campylobacter* when present in all 3 copies of 23S rRNA genes, along with A2074G, A2074C, and A2075G mutations.

The A2074G, A2074C, and A2075G mutations in 23S rRNA genes have been associated with a growth disadvantage, the so-called fitness cost (8, 11, 12). To understand the influence of A2074T mutations on growth kinetics, isogenic Ery^r and Ery^s strains were grown separately and their proliferation was monitored (Fig. 1A). There was no considerable difference in the growth rates between Ery^r and Ery^s strains. However, when the strains were evaluated in a mixed-culture medium (12), the Ery^r/Ery^s ratio dramatically reduced with increasing number of passages (Fig. 1B). After three sequential passages, the ratio was below 3%. Consequently, the A2074T mutations resulted in a growth disadvantage in *Campylobacter*. Although the reason for the fitness cost caused by the A2074T mutations in 23S rRNA genes is unclear, it is speculated that the mutations could affect protein synthesis, leading to a fitness burden under antibiotic-free conditions.

We concluded that the A2074T mutations conferred high-level macrolide resistance when present in all 3 copies of 23S rRNA genes; however, they imposed a fitness cost on the bacteria.

TABLE 1 Testing of susceptibility of *C. jejuni* strains to various antibiotics

Antimicrobial agent	MIC (μg/ml) against isolate:		
	C. jejuni NC05-27	*C. jejuni* NCTC11168 Ery^r	*C. jejuni* NCTC11168 Ery^s
Erythromycin	>512	>512	2
Clarithromycin	512	512	4
Azithromycin	>512	>512	0.25
Spiramycin	256	256	4
Leucomycin	64	32	0.25
Tylosin	>512	>512	8
Clindamycin	512	512	1
Ciprofloxacin	4	≤0.13	≤0.13
Tetracycline	1	≤0.13	≤0.13
Chloramphenicol	8	2	2

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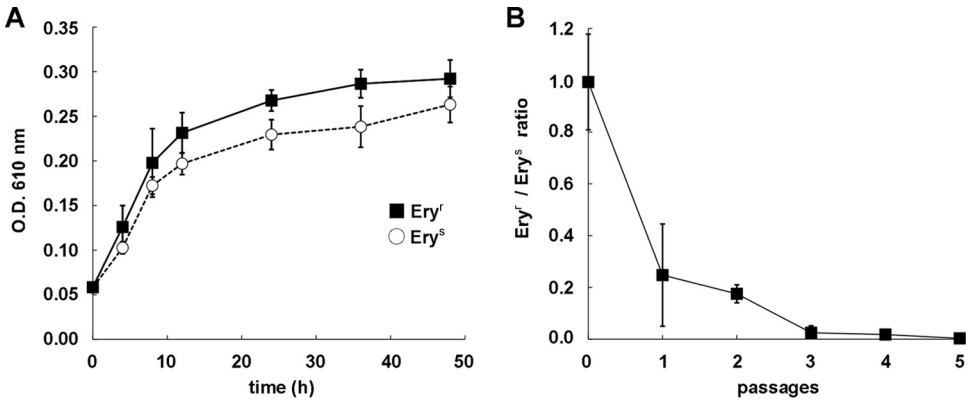

FIG 1 (A) Growth kinetics of Ery^r and Ery^s strains. The experiment was repeated three times. O.D., optical density. (B) Growth competition assay of Ery^r and Ery^s strains in a mixed culture. The Ery^r/Ery^s ratio was initially adjusted to approximately 1:1. At every passage, an aliquot of cultured broth was diluted and spread on a Mueller-Hinton agar plate with and without 16 μg/ml erythromycin. The number of colonies was counted to estimate the ratio of Ery^s and Ery^r strains. The experiment was repeated four times.

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