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Interspecies Transfer of Antibiotic Resistance between *Helicobacter pylori* and *Helicobacter acinonychis*

Helicobacter pylori is a gram-negative microaerophilic bacterium that causes chronic gastritis and peptic ulcer disease in humans and is associated with gastric cancer. Antibiotic resistance in *H. pylori* is a major cause of therapy failure. In general, bacteria can acquire antibiotic resistance either by spontaneous mutation or by horizontal transfer of resistance genes. A possible mechanism for acquisition of antibiotic resistance by *H. pylori* is genetic transformation (7). Recently, the mechanism for transformation of *H. pylori* was suggested to be specific for DNA of members of the genus *Helicobacter* (6). This proposed genus-specific DNA uptake led us to investigate whether interspecies transfer of antibiotic resistance can take place in *Helicobacter*.

Previously, metronidazole resistance was reported for *Helicobacter acinonychis* isolates from captive tigers with ulcers (1). We observed that the *H. acinonychis* reference isolate (NCTC12686) and *H. acinonychis* isolate Sheeba (2) both consisted of a large metronidazole-sensitive subpopulation and a small metronidazole-resistant subpopulation. By repetitive subculture of colonies of each subpopulation, both homogeneous metronidazole-sensitive isolates (designated NCTC12686 MtzS and Sheeba MtzS) and fully metronidazole-resistant isolates (NCTC12686 MtzR and Sheeba MtzR; MICs for both were >256 μg/ml) were obtained. DNA was isolated from the metronidazole-resistant strains Sheeba MtzR and NCTC12686 MtzR and used for transformation of two metronidazole-sensitive *H. pylori* strains through natural transformation, essentially as described by Wang et al. (9). Metronidazole-resistant transformants were obtained for both *H. pylori* strains (Table 1). In contrast, no metronidazole-resistant colonies were obtained in a mock transformation with phosphate-buffered saline (Table 1).

TABLE 1. Numbers of antibiotic-resistant colonies obtained upon transformation[a]

Recipient strain and donor DNA	Selecting antibiotic[b]	No. of colonies
H. pylori 26695		
H. acinonychis NCTC12686 MtzR	Metronidazole	>1,000
H. acinonychis Sheeba MtzR	Metronidazole	>1,000
Saline (no DNA)	Metronidazole	0
H. pylori J99		
H. acinonychis NCTC12686 MtzR	Metronidazole	40
H. acinonychis Sheeba MtzR	Metronidazole	29
Saline (no DNA)	Metronidazole	0
H. acinonychis Sheeba MtzS		
H. pylori 1061 MtzR	Metronidazole	>1,000
H. pylori NCTC11637	Metronidazole	>1,000
H. pylori pRdxA	Metronidazole	>1,000
Saline (no DNA)	Metronidazole	0
H. pylori 1061 MtzR/ClaR	Clarithromycin	140
23S rDNA PCR product of 1061 MtzR/ClaR	Clarithromycin	>1,000
Saline (no DNA)	Clarithromycin	0
H. acinonychis NCTC12686 MtzS		
H. pylori 1061 MtzR	Metronidazole	500
H. pylori NCTC11637	Metronidazole	57
Saline (no DNA)	Metronidazole	0
H. pylori 1061 MtzR/ClaR	Clarithromycin	374
Saline (no DNA)	Clarithromycin	0

[a] Bacteria were transformed with approximately 1 μg of DNA as described previously (8).
[b] Metronidazole, 20 μg/ml; clarithromycin, 2 μg/ml.

Conversely, the metronidazole-sensitive *H. acinonychis* strains Sheeba MtzS and NCTC12686 MtzS could be transformed to metronidazole resistance with chromosomal DNA isolated from two *H. pylori* strains which are metronidazole resistant due to known mutations that inactivate the *rdxA* nitroreductase gene (Table 1). Type strain NCTC11637 is resistant due to a transposon-induced deletion in *rdxA* (4); the metronidazole-resistant variant of strain 1061 (1061 MtzR) contains the null mutation containing *rdxA* of strain 439 (5). Transformation with this DNA resulted in high numbers of transformants, in contrast to control transformations (phosphate-buffered saline without DNA), which did not yield any metronidazole-resistant colonies. Additional evidence for interspecies DNA transfer comes from transformation with plasmid pRdxA, which contains a disrupted *H. pylori*-derived *rdxA* gene (8). Transformation of *H. acynonychis* with pRdxA resulted in similar numbers of transformants (Table 1).

Interspecies transformation was not limited to metronidazole resistance, as both *H. acinonychis* strains could be readily transformed to clarithromycin resistance. Both chromosomal DNA of the clarithromycin-resistant variant of strain 1061 (1061 MtzR/ClaR) (8), which is clarithromycin resistant due to a single base pair mutation (A_{2142}-G) in the 23S ribosomal RNA gene (rDNA) (3), and an 850-bp PCR product of the 23S rDNA of this strain transformed both *H. acinonychis* strains to a clarithromycin-resistant phenotype (Table 1).

In conclusion, we provide evidence that (i) *H. acinonychis* is competent for natural transformation and (ii) *H. pylori* can acquire antibiotic resistance by uptake of DNA from other *Helicobacter* species and vice versa. To what extent interspecies gene transfer contributes to antibiotic resistance in the genus *Helicobacter* depends on the relative frequencies of intraspecies transfer, interspecies transfer, and mutation rate in vivo, which remain to be investigated.

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