

## Furazolidone- and Nitrofurantoin-Resistant *Helicobacter pylori*: Prevalence and Role of Genes Involved in Metronidazole Resistance

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**The prevalence of furazolidone, nitrofurantoin, and metronidazole resistance among *Helicobacter pylori* strains was assessed with 431 clinical isolates. Fifty-two percent were metronidazole resistant, compared to 2% (7 of 431) with resistance to furazolidone and nitrofurantoin. All seven furazolidone- and nitrofurantoin-resistant isolates were also metronidazole resistant. *rdxA*, *frxA*, and *fdxB* knockouts did not result in furazolidone or nitrofurantoin resistance. These data suggest that furazolidone and nitrofurantoin may be good alternatives to metronidazole for treating *H. pylori* infection.**

*H. pylori* infection is one of the most common infections worldwide and is etiologically related to chronic gastritis, duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and primary gastric lymphoma (3, 17, 18). Approximately one in six *H. pylori*-infected persons develop peptic ulcer disease. Patients with peptic ulcer disease experience pain and reduced quality of life and risk ulcer complications (1, 19). Cure of *H. pylori* infection prevents ulcer recurrence, heals gastritis, and also prevents progression from mild superficial gastritis to chronic atrophic gastritis (the precursor lesion to gastric cancer), which may reduce the risk, or prevent, gastric cancer (1, 15). Clinical experience has demonstrated that cure of *H. pylori* infection is difficult due to lack of compliance with the drug regimens and development of antibiotic-resistant *H. pylori* (8, 15). Metronidazole has been widely used as a critical component of combination therapies for *H. pylori* infection. Monotherapy with metronidazole results in more than 50% of *H. pylori* isolates acquiring resistance (13), and current metronidazole-containing triple therapies are being undermined by development of resistance (4). Because of the high rate of metronidazole resistance in *H. pylori*, furazolidone and nitrofurantoin have been recommended as alternative agents (2, 9, 21).

Although furazolidone, nitrofurantoin, and metronidazole are classified as nitroheterocyclic and nitroaromatic compounds, it is not known whether the drug actions and the resistance mechanisms are similar. Metronidazole resistance among *H. pylori* strains has been reported worldwide with variable frequencies (6, 13, 16). The mechanism of metronidazole resistance among *H. pylori* strains has been related to alterations in gene products having metronidazole nitroreductase activity, including oxygen-insensitive NAD(P)H nitroreductase (RdxA), NAD(P)H flavin oxidoreductase (FrxA), and

ferredoxin-like protein (FdxB) (7, 12). This study asked whether furazolidone or nitrofurantoin susceptibility and resistance shared common features with metronidazole susceptibility and resistance.

We evaluated the prevalence of metronidazole, furazolidone, and nitrofurantoin resistance among *H. pylori* strains isolated from 431 patients, including 297 males and 134 females (median age, 45 years; range, 16 to 82 years) presenting at Guro Hospital in Seoul, Korea, between September 1993 and September 1999. The endoscopic diagnoses were chronic gastritis (101 patients), peptic ulcer diseases (85 patients with gastric ulcer disease and 128 patients with duodenal ulcer disease), and gastric cancer (117 patients). *H. pylori* was isolated from gastric mucosal biopsies that were plated onto selective (containing 1% nalidixic acid, 0.5% trimethoprim, 0.3% vancomycin, and 0.2% amphotericin) and nonselective brain heart infusion (BHI) agar plates containing 5% defibrinated horse blood. All plates were incubated under microaerobic conditions at 37°C for up to 14 days. *H. pylori* was identified by colony morphology, Gram staining, and catalase, urease, and oxidase reactions. MIC measurements were performed by agar dilution as described previously (12). Only 48% of the strains were susceptible to metronidazole. The MICs for 224 resistant strains ranged from  $\geq 8$  to 256  $\mu\text{g}$  of metronidazole per ml; those for 172 strains (40% of the total) ranged from  $\geq 16$  to 256  $\mu\text{g}$  of metronidazole per ml. Only 7 of the 431 strains (1.6%) required a MIC of 4  $\mu\text{g}$  of furazolidone per ml. The same seven strains also required a MIC of 4  $\mu\text{g}$  of nitrofurantoin per ml, and all seven strains with furazolidone and nitrofurantoin resistance also showed low-level metronidazole resistance (MICs of 8 or 16  $\mu\text{g}/\text{ml}$ ) (Table 1). All other strains (424 of 431) required MICs of  $\leq 0.5$   $\mu\text{g}$  of furazolidone or nitrofurantoin per ml. Similar findings (i.e., low frequency with low-level MICs and cross-resistance of furazolidone and metronidazole) have been recently reported for clinical *H. pylori* isolates from Brazil (14).

We evaluated the rate of spontaneous development of low-

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TABLE 1. Comparisons of MICs of metronidazole, furazolidone, and nitrofurantoin among 431 clinical *H. pylori* isolates

<i>H. pylori</i> strain(s) <sup>b</sup>	Disease <sup>c</sup>	Sex of patient <sup>d</sup>	Age of patient (yr)	MIC <sup>a</sup> (μg/ml)		
				Metronidazole	Furazolidone	Nitrofurantoin
KH33A	GU	M	40	16	4	4
KH134A	GU	M	65	16	4	4
KH273A	DU	M	44	8	4	4
KH359A	CA	M	68	16	4	4
KH368B	GA	F	36	16	4	4
KH373A	CA	F	61	16	4	4
KH392B	GA	M	32	8	4	4
All others ( <i>n</i> = 424)	All	M and F	30–70	0.5–256	≤0.5	≤0.5

<sup>a</sup> MICs were determined by growth on 5% horse blood–BHI agar plates supplemented with 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg of metronidazole, furazolidone, or nitrofurantoin per ml for 4 days. The results were confirmed twice by measuring all MICs at the same time.

<sup>b</sup> A, antrum; B, corpus.

<sup>c</sup> CA, cancer associated; DU, duodenal ulcer associated; GA, gastritis associated; GU, gastric ulcer associated.

<sup>d</sup> M, male; F, female.

level furazolidone or nitrofurantoin resistance (MIC of 4 μg/ml) using *H. pylori* ATCC 700392. Two-day-old *H. pylori* cells from two 89-mm-diameter plates were harvested and suspended in 4 ml of 5% horse serum–BHI broth. The cells were adjusted to approximately 10<sup>9</sup> cells and spread onto 5% horse blood–BHI agar plates supplemented with 2 μg of furazolidone or nitrofurantoin per ml or 8 μg of metronidazole per ml. The plates containing the cells were incubated at 37°C under microaerobic conditions for 6 days. No colonies grew on plates supplemented with furazolidone or nitrofurantoin. However, 253 colonies appeared on plates supplemented with metronidazole. These results suggest that furazolidone and nitrofurantoin resistance occurs spontaneously at a much lower frequency than metronidazole resistance. The ease of selection of spontaneous metronidazole-resistant colonies and the difficulty of selecting spontaneous furazolidone- or nitrofurantoin-resistant colonies could be related to clinical observations of high frequencies of metronidazole resistance but low furazolidone or nitrofurantoin resistance among *H. pylori* strains.

It was already established that the resistance mechanism for metronidazole is different from that for furazolidone and nitrofurantoin (7, 12, 20). To confirm that the resistance mechanisms for metronidazole and for furazolidone and nitrofurantoin are indeed different, susceptibilities to these drugs were measured in *rdxA*-, *frxA*-, and *fdxB*-inactivated (knockout) *H. pylori* strains. We used 92 single and/or dual knockout *H. pylori* strains, including a positive control (furazolidone- and nitrofurantoin-resistant clinical isolate requiring a MIC of 4 μg/ml,

as mentioned above) and a negative control (*H. pylori ureB* knockout strain) to measure MICs of furazolidone and nitrofurantoin. The 92 strains were isolated in the United States and were also used to measure MICs of metronidazole as reported previously (12). None of the parental, knockout, or negative control strains grew on 5% horse blood–BHI plates containing 2 μg of either furazolidone or nitrofurantoin per ml, while the positive controls grew on the same plates. These results were confirmed using five additional clinical isolates from Korea, which are susceptible to furazolidone and nitrofurantoin (MIC of 0.5 μg/ml), including *H. pylori* ATCC 43629, in which *rdxA*, *frxA*, and *fdxB* were knocked out as described previously (12). The MICs of metronidazole, furazolidone, and nitrofurantoin in these strains were evaluated as described previously (12). As shown in Table 2, the metronidazole MICs for *rdxA* and *frxA* knockout strains increased from 1 to 32 μg/ml (ATCC 43629, KH220, and KH278) and from 8 to 128 μg/ml (KH259 and KH261). The metronidazole MICs for *fdxB* knockout strains, however, were unchanged for strains ATCC 43629, KH220, KH278 but increased from 8 to 64 μg/ml for KH259 and from 8 to 32 μg/ml for KH261. In contrast, the furazolidone and nitrofurantoin MICs for all the knockout strains were unchanged (0.5 μg/ml) from those for the parental strains. These results are consistent with those from the 92 knockout strains and confirm the previous data regarding the involvement of these genes in metronidazole resistance. In addition, these results indicate that the genes (*rdxA*, *frxA*, and *fdxB*) are un-

TABLE 2. Effects of the *rdxA*, *frxA*, and *fdxB* genes on metronidazole, furazolidone, and nitrofurantoin susceptibilities in *H. pylori*

<i>H. pylori</i> strain	MIC (μg/ml) <sup>a</sup>											
	Metronidazole				Furazolidone				Nitrofurantoin			
	Clinical isolate	<i>rdxA</i> ::cat mutant	<i>frxA</i> ::cat mutant	<i>fdxB</i> ::cat mutant	Clinical isolate	<i>rdxA</i> ::cat mutant	<i>frxA</i> ::cat mutant	<i>fdxB</i> ::cat mutant	Clinical isolate	<i>rdxA</i> ::cat mutant	<i>frxA</i> ::cat mutant	<i>fdxB</i> ::cat mutant
ATCC 43629	1	32	32	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KH220	1	32	32	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KH278	1	32	32	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KH259	8	128	128	64	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
KH261	8	128	128	32	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

<sup>a</sup> MICs were determined by growth on 5% horse blood–BHI agar plates supplemented with 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg of metronidazole, furazolidone, or nitrofurantoin per ml for 4 days. The results were confirmed twice by measuring all MICs at the same time.

likely to be directly involved in furazolidone and nitrofurantoin resistance in *H. pylori*.

Although the modes of drug action of all three antibiotics are similar and nitroreduction is required for activation, the mechanisms of resistance to metronidazole and to furazolidone and nitrofurantoin may not be the same (20). Nitroreduction of furazolidone and nitrofurantoin may be exerted by nitroreductases other than RdxA, FrxA, and FdxB in *H. pylori* as described by Whiteway et al. (20) for *E. coli*, in which nitroreduction of 5-nitrofurans is exerted by the *nsfA* and *nsfB* products. It is possible that the nitroreductase for furazolidone and nitrofurantoin may be essential for *H. pylori* survival and that resistance may be acquired by partial inactivation of the nitroreductase. Candidates for furazolidone and nitrofurantoin nitroreductases include pyruvate:flavodoxin oxidoreductase (PorCDAB) and 2-oxoglutarate oxidoreductase (OorDABC). The lethal effect and possible metronidazole nitroreductase activity of the *porCDAB* and *oorDABC* products have been shown for *H. pylori* (10, 11). In this view, we postulate that furazolidone and nitrofurantoin may be more specific to PorCDAB and/or OorDABC, while metronidazole may be more specific to RdxA, FrxA, and FdxB, as substrate molecules. Therefore, strains with knocked-out *rdxA*, *frxA*, or *fdxB* were still sensitive to furazolidone and nitrofurantoin because of the existence of fully functional *porCDAB* and *oorDABC* genes. In contrast, partially functional *porCDAB* and *oorDABC* genes that confer low-level furazolidone and nitrofurantoin resistance also confer low-level metronidazole resistance, as shown in this study. We are currently testing this hypothesis by purifying PorCDAB and OorDABC from *H. pylori* and measuring the nitroreductase activities for furazolidone and nitrofurantoin.

Furazolidone- or nitrofurantoin-containing therapeutic regimens for *H. pylori* infection have been suggested for use instead of metronidazole to overcome the high frequency of metronidazole resistance among *H. pylori* strains. The ideal antibiotic for *H. pylori* therapy would have high efficacy without the development of antibiotic resistance. Several reports published recently indicate that furazolidone or nitrofurantoin is efficacious in *H. pylori* therapy, although details of the best therapy have not yet been defined (2, 9, 21). The results reported here suggest that furazolidone and nitrofurantoin may be good alternatives, especially in areas where metronidazole resistance is common.

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#### REFERENCES

- Buckley, J. M., and M. Deltenre. 1997. Therapy of *Helicobacter pylori* infection. *Curr. Opin. Gastroenterol.* **13**:56–62.
- Coudron, P. E., and C. W. Stratton. 1998. *In-vitro* evaluation of nitrofurantoin as an alternative agent for metronidazole in combination antimicrobial therapy against *Helicobacter pylori*. *J. Antimicrob. Chemother.* **42**:657–660.
- Crump, M., M. Gospodarowicz, and F. A. Shepherd. 1999. Lymphoma of the gastrointestinal tract. *Semin. Oncol.* **26**:324–337.
- de Boer, W. A., and G. N. Tytgat. 1995. The best therapy for *Helicobacter pylori* infection: should efficacy or side-effect profile determine our choice? *Scand. J. Gastroenterol.* **30**:401–407.
- Dore, M. P., M. S. Osato, D. H. Kwon, D. Y. Graham, and F. A. K. El-Zaatari. 1998. Demonstration of unexpected antibiotic resistance of genotypically identical *Helicobacter pylori* isolates. *Clin. Infect. Dis.* **27**:84–89.
- Glupczynski, Y. 1998. Antimicrobial resistance in *Helicobacter pylori*: a global overview. *Acta Gastroenterol. Belg.* **61**:357–366.
- Goodwin, A., D. Kersulyte, G. Sisson, S. J. O. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman. 1998. Metronidazole-resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NAD(P)H nitroreductase. *Mol. Microbiol.* **28**:383–393.
- Graham, D. Y., W. A. de Boer, and G. N. Tytgat. 1996. Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. *Am. J. Gastroenterol.* **91**:1072–1076.
- Graham, D. Y., M. S. Osato, J. Hoffman, A. R. Opekun, S. Anderson, and H. M. El-Zimaty. 2000. Furazolidone combination therapies for *Helicobacter pylori* infection in the United States. *Aliment. Pharmacol. Ther.* **14**:211–215.
- Hughes, N. J., C. L. Clayton, P. A. Chalk, and D. J. Kelly. 1998. *Helicobacter pylori porCDAB* and *oorDABC* genes encode distinct pyruvate:flavodoxin and 2-oxoglutarate:acceptor oxidoreductases which mediate electron transport to NADP. *J. Bacteriol.* **180**:1119–1128.
- Kaihoavaara, P., J. Hook-Nikanne, M. Uusi-Oukari, T. U. Kosunen, and M. Salaspuro. 1998. Flavodoxin-dependent pyruvate oxidation, acetate production and metronidazole reduction by *Helicobacter pylori*. *J. Antimicrob. Chemother.* **41**:171–177.
- Kwon, D.-H., F. A. K. El-Zaatari, M. Kato, M. S. Osato, R. Reddy, Y. Yamaoka, and D. Y. Graham. 2000. Analysis of an *rdxA* gene and involvement of additional genes encoding NADPH flavin oxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in metronidazole resistance of *Helicobacter pylori*. *Antimicrob. Agents Chemother.* **44**:2133–2142.
- Megraud, F. 1994. *Helicobacter pylori* resistance to antibiotics, p. 570–583. In R. H. Hunt and G. N. Tytgat (ed.), *Helicobacter pylori*: basic mechanisms to clinical cure. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Mendonca, S., C. Ecclissato, M. S. Sartori, A. P. Godoy, R. A. Guerzoni, M. Degger, and J. Pedrazzoli, Jr. 2000. Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil. *Helicobacter* **5**:79–83.
- National Cancer Institute. 1995. Cancer statistics review, 1973–1992, p. 2789. National Institutes of Health, Bethesda, Md.
- Osato, M. S., R. Reddy, and D. Y. Graham. 1999. Metronidazole and clarithromycin resistance amongst *Helicobacter pylori* isolates from a large metropolitan hospital in the United States. *Int. J. Antimicrob. Agents* **12**:341–347.
- Parsonnet, J. 1998. *Helicobacter pylori*. *Infect. Dis. Clin. N. Am.* **12**:185–197.
- Parsonnet, J. 1998b. *Helicobacter pylori*: the size of the problem. *Gut* **43**(Suppl. 1):S6–S9.
- Soll, A. H. 1993. Gastric, duodenal, and stress ulcer, p. 580–679. In M. Sleisenger and J. Fordtran (ed.), *Gastrointestinal disease*, 5th ed. W. B. Saunders, Philadelphia, Pa.
- Whiteway, J., P. Koziarz, J. Veall, N. Sandhu, P. Kumar, B. Hoecher, and I. B. Lambert. 1998. Oxygen-insensitive nitroreductases: analysis of the roles of *nsfA* and *nsfB* in development of resistance to 5-nitrofurans derivatives in *Escherichia coli*. *J. Bacteriol.* **180**:5529–5539.
- Xiao, S. D., W. Z. Liu, P. J. Hu, D. H. Xia, and G. N. Tytgat. 1999. High cure rate of *Helicobacter pylori* infection using tripotassium dicitrato bismuthate, furazolidone and clarithromycin triple therapy for 1 week. *Aliment. Pharmacol. Ther.* **13**:311–315.