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Fast-Track Communication

Stable and Unstable Amoxicillin Resistance in *Helicobacter pylori*: Should Antibiotic Resistance Testing Be Performed Prior to Eradication Therapy?

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Amoxicillin is often implemented in *Helicobacter pylori* treatment protocols. To date, amoxicillin-resistant *H. pylori* strains have rarely been detected, and only a total of 14 have been reported in the literature (1). Conspicuously, complete loss of the resistant phenotype was observed after these strains were stored at −80°C. Only one amoxicillin-resistant *H. pylori* strain has been isolated, in The Netherlands, in which in contrast, the amoxicillin resistance remained stable after repeated cycles of freezing and culture (5). The MIC for this strain was 8 μg/ml, which is relatively low.

Since 1996, we have isolated seven *H. pylori* strains exhibiting high-level amoxicillin resistance (MIC > 256 μg/ml) (Table 1). Four of these exhibited a stable resistance phenotype (strains ACR3, -4, -5, and -7). Strains ACR1, -2, and -4 were isolated from two 11-year-old girls and one 15-year-old girl with recurrent abdominal pain. They received triple therapy in accordance with the results of antibiotic susceptibility testing, and symptoms subsided. The *H. pylori* strains ACR3 (stable amoxicillin resistance) and ACR6 (unstable resistance) were isolated from an 8-year-old boy and an 11-year-old girl, respectively. Eradication therapy with amoxicillin, clarithromycin, and omeprazole was empirically initiated but remained without effect. Similarly, *H. pylori* strains ACR5 and ACR7 (stable amoxicillin resistance) were isolated from a 17-year-old girl and a 40-year-old woman with recurrent duodenal ulcers who had been unsuccessfully treated with triple combinations containing amoxicillin. Following treatment failure, the patients were examined by gastrointestinal endoscopy and *H. pylori* was isolated from both antral and corpus biopsy specimens. Identification was confirmed by 16S rRNA gene sequence analysis, and antibiotic resistance determinations revealed both strains to be highly resistant to amoxicillin.

TABLE 1. MICs of antibiotics for seven *H. pylori* strains with stable and unstable amoxicillin resistance

H. pylori strain and condition	MIC (μg/ml)[a]								
	Amoxicillin	Amoxicillin-clavulanic acid	Penicillin G	Cefuroxime	Cefotaxime	Azithromycin	Clarithromycin	Tetracycline	Metronidazole
ACR1									
Before storage	>256	ND	ND	0.016	ND	0.094	<0.016	0.5	>32
After storage	<0.016[b]	<0.016	0.016	0.125	0.25	0.19	<0.016	0.25	32
ACR2									
Before storage	>256	ND	ND	0.19	ND	0.25	<0.016	0.064	0.094
After storage	<0.016[b]	<0.016	0.125	0.016	0.016	0.064	<0.016	0.094	0.094
ACR3									
Before storage	>256	ND	ND	>256	ND	>256	>256	0.25	1.0
After storage	>256[c]	>256	>32	>256	>32	>256	>256	0.75	>256
ACR4									
Before storage	>256	ND	ND	0.094	ND	>256	>256	0.25	0.125
After storage	>256[c]	0.38	>32	>256	0.023	>256	>256	0.047	0.047
ACR5									
Before storage	>256	0.064	ND	>256	ND	>256	>256	0.38	0.064
After storage	>256[c]	ND	ND	>256	ND	>256	>256	<0.016	<0.016
ACR6									
Before storage	>256	1.5	ND	>256	ND	>256	>256	>256	1.0
After storage	<0.016[b]	<0.016	ND	<0.016	0.002	>256	>256	>256	<0.016
ACR7									
Before storage	>256	>256	ND	>256	ND	>256	>256	0.75	>256
After storage	>256[c]	>256	>256	>256	>256	>256	>256	0.50	>256

[a] As determined by the E-test method (AB BIODISK, Solna, Sweden). MICs were read after 3 to 4 days of incubation. ND, not determined.
[b] Not stable.
[c] Stable.

After storage at −70°C and reculture, strains ACR1, -2, and -6 lost their amoxicillin resistance, whereas strains ACR3, -4, -5, and -7 remained stable despite repetitive subculture or freeze-thaw cycles (Table 1). Although β-lactamase activity could not be detected by the nitrocefin assay, amoxicillin resistance was overcome by clavulanic acid in three strains (ACR4, -5, and -6). In contrast, strains ACR3 and -7 were also resistant to amoxicillin-clavulanic acid. All four strains with stable amoxicillin resistance were also resistant to cefuroxime.

The antibiotic resistance phenotypes suggest the existence of multiple resistance mechanisms in *H. pylori*. Antibiotic resistance testing of these bacteria may become increasingly necessary in patients experiencing treatment failures. In four of our cases, amoxicillin resistance correlated with treatment failure. There are general causes for concern. (i) The amoxicillin-resistant phenotype is transferable in vitro to amoxicillin-susceptible strains (5), presumably due to DNA exchange by transformation or a conjugation-like mechanism (2). Colonization of the stomach with other β-lactam-resistant bacteria may also lead to transfer of amoxicillin resistance to *H. pylori*. (ii) Amoxicillin MICs have been observed to increase upon repeated exposure of *H. pylori* to the antibiotic (3, 4). To date, MICs for stable amoxicillin-resistant bacteria have been in the range of 8 μg/ml. However, we report the emergence of high-level amoxicillin resistance, the MICs for our strains being >256 μg/ml, which may represent a major threat regarding an effective *H. pylori* eradication therapy.

In conclusion, it may be prudent to perform antibiotic susceptibility testing of *H. pylori* at an early stage whenever a treatment failure becomes apparent.

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