

Sensitivity of Amoxicillin-Resistant *Helicobacter pylori* to Other Penicillins

MARIA P. DORE,^{1*} DAVID Y. GRAHAM,¹ ANTONIA R. SEPULVEDA,¹ GIUSEPPE REALDI,²
AND MICHAEL S. OSATO¹

Department of Medicine, VA Medical Center, and Baylor College of Medicine, Houston, Texas 77030,¹ and
Department of Internal Medicine, University of Sassari, Sassari, Italy 07100²

Received 24 November 1998/Returned for modification 8 March 1999/Accepted 7 May 1999

The sensitivities to penicillins and to a penicillin and β -lactamase inhibitor combination agent were determined for *Helicobacter pylori* strains that were sensitive, moderately resistant, or highly resistant to amoxicillin. All strains were resistant to nafcillin and oxacillin. Moderately resistant strains showed an intermediate zone of inhibition to ticarcillin, mezlocillin, piperacillin, and amoxicillin-clavulanic acid. High-level resistance was associated with the smallest zone size for all penicillins tested.

The reliable treatment of *Helicobacter pylori* infection has been difficult, and successful regimens generally require two or more antimicrobial drugs coupled with an acid inhibitor (3, 6). Results with the dual therapy that combined omeprazole with amoxicillin have varied widely (8–10). We have recently identified amoxicillin-resistant *H. pylori* bacteria from patients from both the United States and Italy (1). This study investigated the sensitivity patterns of these *H. pylori* isolates to different penicillins.

Isolates. Six amoxicillin-resistant *H. pylori* strains isolated from three patients with peptic ulcers from the United States and 14 amoxicillin-resistant strains isolated from dyspeptic patients from Sardinia, Italy, were used. The strains were stored at -80°C for 5 to 10 months in cysteine-Albimi broth medium containing 20% glycerol (4). Before and after storage at -80°C , the MIC of amoxicillin was assessed by the E-test method (4).

β -Lactamase assay. The chromogenic cephalosporin method was used to test for the production of β -lactamase. Cefinase disks (Becton Dickinson Microbiology Systems, Cockeysville, Md.) impregnated with nitrocefin, a chromogenic cephalosporin, were moistened with a drop of sterile distilled water. Several well-isolated colonies of *H. pylori* from the agar plate were selected and smeared over the disk surface. β -Lactamase activity was identified by a change in the color of the chromogenic cephalosporin after incubation at room temperature for up to 2 h. *Staphylococcus aureus* (ATCC 29213) served as the positive control (7).

Amoxicillin gradient plates. Frozen strains that were amoxicillin resistant prestorage, as well as strains that were never resistant to amoxicillin, were cultured onto brain heart infusion (Difco Laboratories, Detroit, Mich.) agar plates containing 7% defibrinated horse blood (Cocalico Biologicals, Reamstown, Pa.) (BHIB agar plates) and incubated for 3 to 5 days at 37°C in 12% CO_2 and 100% relative humidity. Amoxicillin resistance in amoxicillin-tolerant *H. pylori* bacteria was restored by using a modification of the method described by Kim and Anthony (5) as previously described (1).

Bacterial suspensions. The *H. pylori* strains used in this study were (i) five prestorage amoxicillin-resistant *H. pylori*

isolates (SS234, SS260, SS158, SS211, and BLMA) characterized by high levels of amoxicillin resistance (32 $\mu\text{g}/\text{ml}$ determined on gradient plates), (ii) 12 prestorage amoxicillin-resistant *H. pylori* isolates (SS47, SS70, SS128, SS130, SS93, SS96, SS263, BLMC, WLRA, WLRC, 93IAA, and 93IAC) for which MICs were 2 $\mu\text{g}/\text{ml}$, and (iii) three amoxicillin-sensitive strains (Mp4, SS204, and SS88) for which MICs were 0.016 $\mu\text{g}/\text{ml}$. All *H. pylori* isolates were grown on BHIB plates for 3 to 4 days at 37°C in 12% CO_2 and 100% relative humidity. Each plate was swabbed with a cell suspension in sterile saline equivalent to a 2.0 McFarland standard.

Antibiotics tested included ampicillin (10 μg), penicillin (2 IU), oxacillin (1 mg), ticarcillin (75 mg), mezlocillin (75 mg), nafcillin (1 mg), piperacillin (100 mg), and amoxicillin-clavulanic acid (10 and 20 mg) (BBL, Becton Dickinson Microbiology Systems). Mueller-Hinton agar plates (diameter, 150 mm) containing 5% sheep blood (BBL) were covered with each cell suspension to produce a lawn of bacterial growth. Disks were aseptically placed onto the dried surfaces of the inoculated plates. The diameters of the zones of inhibition were measured after incubation at 37°C for 72 to 96 h in an atmosphere with 100% relative humidity and 12% CO_2 . All disk diffusion tests were performed in duplicate.

There are no standard, published, disk zone diameter breakpoints for any antimicrobial agent for *H. pylori*. For each antibiotic, the mean zone diameters were taken as the inhibitory zone.

Twenty *H. pylori* strains obtained prior to antibiotic therapy from 17 patients were used in this study. Seventeen isolates were initially resistant to amoxicillin, with MICs for all strains greater than 256 $\mu\text{g}/\text{ml}$, as determined by the E test. The susceptibility to amoxicillin was also tested by using the agar dilution method, and similar levels of resistance were determined (unpublished data).

No β -lactamase activity was detected in any of the amoxicillin-resistant *H. pylori* strains by the nitrocefin assay. When we used an adaptation of the gradient agar plate method previously described (5), five resistant strains grew in media containing 32 μg of amoxicillin per ml and 12 strains grew in media containing 2 μg of amoxicillin per ml. All *H. pylori* strains regardless of their sensitivity to amoxicillin were resistant to nafcillin and oxacillin as determined by their almost complete lack of inhibition zones (≤ 7.6 mm) (Table 1). Isolates sensitive to amoxicillin showed the largest zones of inhibition with all other penicillins tested. All strains with a high level of amoxi-

* Corresponding author. Mailing address: Department of Medicine, VA Medical Center (111D), 2002 Holcombe Blvd., Houston, TX 77030. Phone: (713) 794-7233. Fax: (713) 795-4471. E-mail: mariap@bcm.tmc.edu.

TABLE 1. Sensitivities of 20 *H. pylori* isolates to penicillins, determined by the disk diffusion method

Resistance level	Amoxicillin MIC ($\mu\text{g/ml}$) ^a	Mean zone of inhibition (mm) ^b							
		Amp	Pen	Oxa	Ticar	Mez	Naf	Pip	Amox-Clav
Sensitive	0.016	27.1	23.8	6.4	39	38.5	6.4	39.4	42.8
Low	2	20.8	19	6.4	35.2	31.5	7.6	31.1	34.9
High	>32	19.4	18.3	6.4	27	26.5	6.4	25.7	29.2

^a Determined by agar dilution.

^b A value of 6.4 mm is the size of the disk; i.e., there is no zone of inhibition. Amp, ampicillin; Pen, penicillin; Oxa, oxacillin; Ticar, ticarcillin; Mez, mezlocillin; Naf, nafcillin; Pip, piperacillin; Amox-Clav, amoxicillin-clavulanic acid.

cillin resistance (≥ 32 $\mu\text{g/ml}$) showed the smallest inhibition zone diameters against all penicillins tested (Table 1). The 12 strains for which amoxicillin MICs were 2 $\mu\text{g/ml}$ demonstrated zone diameters with ticarcillin, mezlocillin, piperacillin, and amoxicillin-clavulanic acid that were of intermediate size.

Of 121 treatment studies of omeprazole plus amoxicillin (4,137 patients) the intention-to-treat analysis showed that the cure of *H. pylori* infection averaged only 59% (95% confidence interval, 58 to 61%). The overall eradication rate (total of 5,725 patients) was 61% (95% confidence interval, 59 to 62%) (8). Resistance to penicillins occurs by the decreased activity of β -lactamases, the binding of the antibiotic to target penicillin-binding proteins (PBPs), possible efflux mechanisms, or outer membrane permeability, especially in gram-negative bacteria. The resistance of these *H. pylori* isolates was not due to β -lactamase production. Amoxicillin resistance was associated with minimal bactericidal concentration/MIC ratios of ≥ 32 , indicating that *H. pylori* bacteria resistant to amoxicillin demonstrated a tolerance to the antibiotic (1).

PBPs are a set of enzymes responsible for the terminal stages of peptidoglycan biosynthesis. PBPs are also a group of target enzymes for the β -lactam antibiotic family. Amoxicillin-tolerant *H. pylori* strains with high levels of amoxicillin resistance showed cross-resistance to all the other penicillins tested, consistent with our observation that penicillin-resistant *H. pylori* bacteria lack a PBP with a molecular weight of 30,000 to 32,000, termed PBP D (2). It appears likely that alterations in PBPs might affect the sensitivity to all penicillins tested, not just amoxicillin or penicillin, such that cross-resistance to other penicillins excludes the possibility of replacing amoxicillin with other penicillins. Amoxicillin resistance among *H. pylori* strains may reflect the indiscriminate use of β -lactam antibiotics.

This work was supported by the Department of Veterans Affairs and by the generous support of Hilda Schwartz.

We are thankful for the contribution of Siddarta Reddy, VAMC and Baylor College of Medicine, Houston, Texas.

REFERENCES

- Dore, M. P., M. S. Osato, G. Realdi, I. Mura, D. Y. Graham, and A. R. Sepulveda. 1999. Amoxicillin tolerance in *Helicobacter pylori*. *J. Antimicrob. Chemother.* **43**:47–54.
- Dore, M. P., D. Y. Graham, and A. R. Sepulveda. 1998. PBP-D, a novel penicillin-binding protein is involved in amoxicillin resistance in *Helicobacter pylori*. *Gastroenterology* **114**:A-109. (Abstract.)
- 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.
- Hachem, C. Y., J. E. Clarridge, R. Reddy, R. Flamm, D. G. Evans, S. K. Tanaka, and D. Y. Graham. 1996. Antimicrobial susceptibility testing of *Helicobacter pylori*. Comparison of E-test, broth microdilution, and disk diffusion for ampicillin, clarithromycin, and metronidazole. *Diagn. Microbiol. Infect. Dis.* **24**:37–41.
- Kim, K. S., and B. F. Anthony. 1983. Use of penicillin-gradient and replicate plates for the demonstration of tolerance to penicillin in streptococci. *J. Infect. Dis.* **148**:488–491.
- Megraud, F. 1997. Resistance of *Helicobacter pylori* to antibiotics. *Aliment. Pharmacol. Ther.* **11**(Suppl. 1):43–53.
- O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler. 1972. Novel method for detection of β -lactamases by using a chromogenic cephalosporin substrate. *Antimicrob. Agents Chemother.* **1**:283–288.
- Penston, J. G., and K. E. L. McColl. 1997. Eradication of *Helicobacter pylori*: an objective assessment of current therapies. *Br. J. Clin. Pharmacol.* **43**:223–243.
- Unge, P. 1996. Review of *Helicobacter pylori* eradication regimens. *Scand. J. Gastroenterol.* **31**(Suppl. 215):74–81.
- van der Hulst, R. W. M., J. J. Keller, E. A. J. Raws, and G. N. J. Tytgat. 1996. Treatment of *Helicobacter pylori* infection in humans: a review of the world literature. *Helicobacter* **1**:6–19.