

## Susceptibility of *Campylobacter pyloridis* to 20 Antimicrobial Agents

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**The susceptibility of 50 strains of *Campylobacter pyloridis*, isolated from human gastric biopsies, to 20 antimicrobial agents representing the different families of antibiotics was tested. The pattern of susceptibility was similar to that of the *C. jejuni-C. coli* group except for ampicillin, cephalothin, and rifampin. The relative resistance to cefsulodin was also noted.**

Histologically proven gastritis is a very common disease with which *Campylobacter pyloridis* is associated in most cases (9, 13, 15). The other arguments for the pathogenicity of this bacterial species are (i) an increase in antibody levels against *C. pyloridis* antigens in patients with *C. pyloridis* (2, 6, 11) and (ii) the development of the disease after an experimental inoculation with *C. pyloridis* of a volunteer (10). Further proof of this association could be generated from a clinical trial with antibiotics versus placebo. At present, a better knowledge of the spectrum of susceptibility of this bacterial species is needed. As a result of this type of work, a more adapted selective medium could also be developed.

In this study we tested the susceptibility of 50 strains of *C. pyloridis* to 20 antimicrobial agents representing the different families of antibiotics. The MICs were determined by the standard dilution technique in solid medium. The following antibiotics were tested: penicillin G (Specia RP, Paris, France), ampicillin and kanamycin (Bristol, Paris, France), clavulanic acid, amoxicillin plus clavulanic acid (Beecham-Séviné, Paris, France), cephalothin, tobramycin, and vancomycin (Eli Lilly, Saint Cloud, France), cefotaxime, chloramphenicol, erythromycin, and streptomycin (Roussel-UCLAF, Paris, France), cefsulodin (Ciba-Geigy, Ruëil Malmaison, France), gentamicin (Unilabo, Levallois, France), josamycin (Pharmuka, Gennevilliers, France), lincomycin (Upjohn, Paris, France), oxytetracycline hydrochloride (Pfizer, Orsay, France), rifampin (Le Petit, Neuilly sur Seine, France), pefloxacin, and colistin (Roger Bellon, Neuilly sur Seine, France). The plates were prepared the day before testing and stored at 4°C.

The strains were isolated from patients attending an endoscopic clinic in Bordeaux and presenting upper gastrointestinal symptoms (25 had ulcers and 25 had nonulcerous dyspepsia with histological lesions of gastritis). The gastric antrum biopsies were inoculated onto chocolate agar and brucella agar supplemented with 10% sheep blood and antibiotics as described by Skirrow (17). After an incubation period of 3 to 6 days at 37°C in a microaerobic atmosphere, the bacteria were identified as *C. pyloridis* by morphology, positive oxidase, catalase, urease, and  $\gamma$ -glutamyl transferase tests. Sixteen of these strains were studied more extensively (16). All the strains were adapted to growth in vitro and were passaged five times. They were maintained frozen at -70°C.

The strains were harvested from cultures grown for 2 days at 37°C on Mueller-Hinton agar (Diagnostics Pasteur, Marne la Coquette, France) supplemented with 5% horse blood. A bacterial suspension of approximately 10<sup>9</sup> CFU/ml was prepared in brain heart infusion broth (Diagnostics Pasteur). A multipoint inoculator derived from that of Steers and delivering 7  $\mu$ l was used to inoculate the strains onto the plates. *C. coli* BM2509 and *C. fetus* subsp. *fetus* CIP5396 were used as control organisms (8). The plates were incubated at 37°C in a microaerobic atmosphere (10% O<sub>2</sub>, 4% CO<sub>2</sub>, 86% H<sub>2</sub>) for 3 days. Readings were taken after 72 h. The MIC was defined as the lowest concentration of antibiotic inhibiting the visible growth of the strain.

The results are shown in Table 1. All the strains were very susceptible to the antibiotics tested except cefsulodin, lincomycin, pefloxacin, colistin, and vancomycin. Kasper and Dickgiesser tested 10 antibiotics against 30 strains (7). Six antibiotics were common to both studies, and the results obtained are similar. McNulty et al. tested nine antibiotics and two antiulcer drugs against 70 isolates (14). Three antibiotics were identical and gave similar results. *C. pyloridis* resembles the *C. jejuni-C. coli* group in that it was susceptible to clavulanic acid (1). These three bacterial species had a comparable spectrum of susceptibility to chloramphenicol, tetracycline, vancomycin, colistin, and the aminoglycosides, except for a single strain in the *C. jejuni-C. coli* group (8). There was also a similarity in the susceptibility patterns for erythromycin, josamycin, and lincomycin (3). Differences were found with ampicillin (40% resistance in the *C. jejuni-C. coli* group), cephalothin (100% resistance), and rifampin (100% resistance). However, rifampin is known to be active against "*C. cinaedi*" and "*C. fennelliae*" (4).

Since strains of this bacterial species are resistant to nalidixic acid (13), we tested a new quinolone which has been proposed to separate nalidixic acid-resistant strains (18). Pefloxacin showed lower activity than ciprofloxacin (14) tested in another study.

A selective medium is necessary to isolate *C. pyloridis* from biopsy specimens because of contamination, particularly by *Streptococcus* sp. and *Pseudomonas aeruginosa*. The selective medium which is currently used contains (per milliliter) vancomycin (5  $\mu$ g), trimethoprim (2.5  $\mu$ g), colistin (5  $\mu$ g), and, in some cases, nalidixic acid (10  $\mu$ g) (5, 12). This medium is unsuitable for the isolation of *C. pyloridis* since many strains are inhibited by colistin or nalidixic acid or both. In addition, overgrowth of *P. aeruginosa* occurred before the optimum incubation time for the *C. pyloridis*. We have tested cefsulodin in an attempt to find an alternative to

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TABLE 1. Susceptibilities of 50 isolates of *C. pyloridis* to 20 antimicrobial agents

Agent	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>		
	Range	50%	90%
Penicillin G	0.015-0.12	0.06	0.12
Ampicillin	<0.003-0.03	0.015	0.03
Clavulanic acid	<0.01-0.64	0.16	0.64
Amoxicillin + clavulanic acid	<0.01-0.02	<0.01	0.01
Cephalothin	0.025-0.4	0.2	0.2
Cefotaxime	0.01-0.16	0.04	0.08
Cefsulodin	5.12-41	20.5	41
Streptomycin	0.04-1.28	0.32	0.64
Kanamycin	0.04-0.64	0.16	0.32
Tobramycin	0.04-0.64	0.08	0.16
Gentamicin	0.04-0.32	0.08	0.16
Erythromycin	0.1-0.8	0.2	0.4
Josamycin	0.4-1.6	0.8	0.8
Lincomycin	3.2-12.8	6.4	12.8
Chloramphenicol	2.0-8.0	2.0	4.0
Tetracycline	0.01-0.16	0.08	0.16
Rifampin	0.5-2.0	1.0	1.0
Pefloxacin	1.0-8.0	4.0	8.0
Colistin	2.0-64	8.0	32.0
Vancomycin	50.0->100	>100	>100

<sup>a</sup> 50% and 90%, MIC for 50 and 90% of the strains, respectively.

colistin. The results are encouraging, and a selective medium containing cefsulodin is currently under study in our laboratory.

We did not find strains of *C. pyloridis*, in particular, resistant to the antibiotics (macrolides, tetracyclines, and ampicillin) for treating gastritis which could be used in control studies to resolve the question of the pathogenicity of these bacteria. However, all the strains must be screened systematically if a resistant strain is to be found.

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#### LITERATURE CITED

- Corbel, M. J., M. E. M. Ibrahim, and K. P. W. Cull. 1984. Sensitivity of campylobacter isolates to clavulanate-potentiated amoxicillin. *Vet. Rec.* **115**:465.
- Eldridge, J., A. N. Lessels, and D. M. Jones. 1984. Antibody to spiral organisms on gastric mucosa. *Lancet* **i**:1237.
- Elharrif, Z., F. Mégraud, and A.-M. Marchand. 1985. Susceptibility of *Campylobacter jejuni* and *Campylobacter coli* to macrolides and related compounds. *Antimicrob. Agents Chemother.* **28**:695-697.
- Flores, B. M., C. L. Fennell, K. K. Holmes, and W. E. Stamm. 1985. In vitro susceptibilities of *Campylobacter*-like organisms to twenty antimicrobial agents. *Antimicrob. Agents Chemother.* **28**:188-191.
- Goodwin, C. S., E. D. Blincow, J. R. Warren, T. E. Waters, C. R. Sanderson, and L. Easton. 1985. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. *J. Clin. Pathol.* **38**:1127-1131.
- Kaldor, J., W. Tee, P. McCarthy, J. Watson, and B. Dwyer. 1985. Immune response to *Campylobacter pyloridis* in patients with peptic ulceration. *Lancet* **i**:921.
- Kasper, G., and N. Dickgiesser. 1984. Antibiotic sensitivity of "*Campylobacter pylori*." *Eur. J. Clin. Microbiol.* **3**:444.
- Lambert, T., G. Gerbaud, P. Trieu-Cuot, and P. Courvalin. 1985. Structural relationship between the genes encoding 3'-aminoglycoside phosphotransferases in *Campylobacter* and in gram-positive cocci. *Ann. Inst. Pasteur Microbiol.* **136B**:135-150.
- Langenberg, M. L., G. N. J. Tytgat, M. E. I. Schipper, P. J. G. M. Rietra, and H. C. Zanen. 1984. *Campylobacter*-like organisms in the stomach of patients and healthy individuals. *Lancet* **i**:1348.
- Marshall, B. J., J. A. Armstrong, D. B. McGeachie, and R. J. Glancy. 1985. Attempt to fulfil Koch's postulates for pyloric campylobacter. *Med. J. Austr.* **142**:436-439.
- Marshall, B. J., D. B. McGeachie, G. J. Francis, and P. J. Utley. 1984. Pyloric campylobacter serology. *Lancet* **ii**:281.
- Marshall, B. J., D. B. McGeachie, P. A. Rogers, R. J. Glancy. 1985. Pyloric campylobacter infection and gastroduodenal disease. *Med. J. Austr.* **142**:439-444.
- Marshall, B. J., and J. R. Warren. 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* **i**:1311-1315.
- McNulty, C. A. M., J. Dent, and R. Wise. 1985. Susceptibility of clinical isolates of *Campylobacter pyloridis* to 11 antimicrobial agents. *Antimicrob. Agents Chemother.* **28**:837-838.
- McNulty, C. A. M., and D. M. Watson. 1984. Spiral bacteria of the gastric antrum. *Lancet* **i**:1068-1069.
- Mégraud, F., F. Bonnet, M. Garnier, and H. Lamouliatte. 1985. Characterization of "*Campylobacter pyloridis*" by culture, enzymatic profile, and protein content. *J. Clin. Microbiol.* **22**:1007-1010.
- Skirrow, M. B. 1977. *Campylobacter* enteritis: a "new" disease. *Br. Med. J.* **2**:9-11.
- Taylor, D. E., L.-K. Ng, and H. Lior. 1985. Susceptibility of *Campylobacter* species to nalidixic acid, enoxacin, and other DNA gyrase inhibitors. *Antimicrob. Agents Chemother.* **28**:708-710.