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Short Communication

Linezolid susceptibility in *Helicobacter pylori*, including strains with multidrug resistance

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ABSTRACT

Only a few studies have evaluated *Helicobacter pylori* susceptibility to linezolid. The aim of the present study was to assess linezolid susceptibility in *H. pylori*, including strains with double/multidrug resistance. The susceptibility of 53 *H. pylori* strains was evaluated by Etest and a breakpoint susceptibility testing method. *Helicobacter pylori* resistance rates were as follows: amoxicillin, 1.9%; metronidazole, 37.7%; clarithromycin, 17.0%; tetracycline, 1.9%; levofloxacin, 24.5%; and linezolid (>4 mg/L), 39.6%. The linezolid MIC_{50} value was 31.2-fold higher than that of clarithromycin and 10.5-fold higher than that of levofloxacin; however, 4 of 11 strains with double/multidrug resistance were linezolid-susceptible. The MIC range of the oxazolidinone agent was larger (0.125–64 mg/L) compared with those in the previous two reports. The linezolid resistance rate was 2.2-fold higher in metronidazole-resistant strains and in strains resistant to at least one antibiotic compared with the remaining strains. Briefly, linezolid was less active against *H. pylori* compared with clarithromycin and levofloxacin, and linezolid resistance was linked to resistance to metronidazole as well as to resistance to at least one antibiotic. However, linezolid activity against some strains with double/multidrug resistance may render the agent appropriate to treat some associated *H. pylori* infections following in vitro susceptibility testing of the strains. Clinical trials are required to confirm this suggestion.

1. Introduction

Linezolid is the first commercially available 1,3-oxazolidinone antibacterial agent. It interferes with formation of the *N*-formylmethionyl–tRNA–30S ribosome–mRNA initiation complex in bacterial translation, thus inhibiting protein synthesis [1,2].

Linezolid is active against most clinically important Gram-positive pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, vancomycin-resistant *Enterococcus* spp., *Mycobacterium tuberculosis*, anaerobes such as *Clostridium* spp. and Gram-positive anaerobic cocci, but it is not active against enteric bacteria and *Pseudomonas aeruginosa* [1,3]. The agent affects toxin production of clinically important species such as *S. aureus* and *S. pyogenes* and can modulate the inflammatory response of the host [1,2].

Linezolid resistance, mostly in Gram-positive bacteria, has been associated with mutations in 23S rRNA and in specific regions of ribosomal proteins L3 and L4 as well as with the *cfr* gene encoding Cfr methyltransferase [3].

Recently, increasing *Helicobacter pylori* resistance to clarithromycin and levofloxacin (mainly in developed countries) and to metronidazole (mostly in developing countries) has hindered successful eradication of the infection [4]. However, only scant data are available regarding the activity of linezolid against *H. pylori* [5,6].

The aim of this study was to assess the in vitro susceptibility of *H. pylori* clinical strains to linezolid, including strains with double and multidrug resistance.

2. Materials and methods

In total, 53 consecutive *H. pylori* strains were collected from 53 symptomatic patients (4 children and 49 adults; mean age, 50.7 years; 25 males and 28 females) during routine diagnostic examinations in 2012–2014. The strains were from 6 treated adults and 47 untreated patients.

Collection of gastric biopsy specimens and isolation of *H. pylori* strains were performed as described in our previous report [7]. Written informed consent was obtained from adult patients and

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Table 1
Linezolid resistance according to the susceptibility patterns to other antibiotics.

Antimicrobial agent	Susceptibility of strains	No. of strains	MIC (mg/L)			% linezolid-resistant	*P*-value[a]
			MIC_{50}	MIC_{90}	Range		
Linezolid[b]	All	53	2	48	0.125–64	N/A	N/A
Clarithromycin[b]	Resistant	9	24	256	0.75 to ≥256	66.7	0.132
	Susceptible	44	0.047	0.19	≤0.016–0.25	34.1	
Levofloxacin[b]	Resistant	13	32	>32	1.5 to ≥32	46.2	0.746
	Susceptible	40	0.125	0.5	≤0.002–1	37.5	
Metronidazole[c]	Resistant	20	≥32	≥32	16 to ≥32	60.0	0.024
	Susceptible	33	≤4	8	≤4–8	27.3	
Amoxicillin[c]	Resistant	1	N/A	N/A	0.25	0	1.000
	Susceptible	52	≤0.12	≤0.12	≤0.12	40.4	
Tetracycline[c]	Resistant	1	N/A	N/A	>2	0	1.000
	Susceptible	52	≤1	≤1	≤1	40.4	
≥2 drugs[b,c]	Resistant	11	N/A	N/A	N/A	63.6	0.090
	Susceptible	42	N/A	N/A	N/A	33.3	
≥1 drugs[b,c]	Resistant	28	N/A	N/A	N/A	53.6	0.048
	Susceptible	25	N/A	N/A	N/A	24.0	

MIC, minimum inhibitory concentration; $MIC_{50/90}$, MICs that inhibit 50% and 90% of the isolates, respectively; N/A, not applicable.
[a] Difference in linezolid resistance rates according to the susceptibility or resistance rates to other antibiotics.
[b] Etest.
[c] Breakpoint susceptibility testing.

from the parents of all paediatric patients. The study was approved by the Ethical Committee of the Medical University of Sofia (Sofia, Bulgaria).

Susceptibility of the strains to clarithromycin, levofloxacin and linezolid was assessed by Etest (M.I.C.Evaluator™; Oxoid Ltd., Basingstoke, UK; and Liofilchem, Roseto degli Abruzzi, Italy). Volumes of 0.5 mL of *H. pylori* suspensions (density of 2–3 McFarland standard) in Mueller–Hinton broth were inoculated onto Mueller–Hinton agar with 5% sheep blood [National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria]. Etest strips were placed on the dried plates (one strip per 90-mm diameter plate) and the plates were incubated microaerophilically (CampyGen™; Oxoid Ltd.) at 37 °C for 48–72 h. The results were read according to the supplier's recommendations.

Susceptibility of the strains to amoxicillin, metronidazole and tetracycline was assessed by a breakpoint susceptibility testing method using a two-fold dilution technique as previously described [7]. Approximately 30–60 μL of *H. pylori* suspensions in Mueller–Hinton broth (NCIPD; density of 2 McFarland standard) were inoculated onto blood Mueller–Hinton agar plates (NCIPD) containing 4, 8, 16 and 32 mg/L metronidazole, 0.12, 0.5, 1 and 2 mg/L amoxicillin, and 1 and 2 mg/L tetracycline. The antibiotics were obtained from Sigma–Aldrich (St Louis, MO). The plates were incubated microaerophilically (as above) at 37 °C for 2–3 days. *Helicobacter pylori* growth on the agar containing the lowest antibiotic concentration indicated resistance. Non-selective Mueller–Hinton blood agar plates were used for a control of strain viability.

The strains were deemed resistant if they showed minimum inhibitory concentrations (MICs) of higher than 8 mg/L metronidazole, 0.5 mg/L clarithromycin, 0.12 mg/L amoxicillin, 1 mg/L tetracycline and 1 mg/L levofloxacin according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Clinical breakpoints Version 5.0, 2015; http://www.eucast.org). For clarithromycin, the breakpoint for intermediately susceptible strains was >0.25–0.5 mg/L. MIC_{50} and MIC_{90} values were the lowest concentrations of the antibiotic at which growth of at least 50% and 90% of the strains were inhibited, respectively.

For linezolid, there are no recommendations for *H. pylori*, therefore the EUCAST-recommended non-species-related breakpoints of susceptibility ≤2 mg/L and resistance >4 mg/L of the agent were used according to pharmacodynamic data [8]. Strains with linezolid MICs >2 mg/L and ≤4 mg/L were deemed intermediately susceptible.

Multidrug resistance was defined as resistance to three or more antibiotics of different classes.

2.1. Statistical analysis

χ^2 with Fisher's exact test was used to compare the groups (http://graphpad.com/quickcalcs/contingency1.cfm).

3. Results

Helicobacter pylori resistance rates were as follows: amoxicillin, 1.9% ($n = 1$); metronidazole, 37.7% ($n = 20$); clarithromycin, 17.0% ($n = 9$); tetracycline, 1.9% ($n = 1$); levofloxacin, 24.5% ($n = 13$); and linezolid, 39.6% ($n = 21$) (Table 1). The $MIC_{50/90}$ values and MIC range were 0.064/8 mg/L and ≤0016 to ≥256 mg/L for clarithromycin, 0.19/32 mg/L and ≤0.002 to ≥32 mg/L for levofloxacin and 2/48 mg/L and 0.125–64 mg/L for linezolid. The MIC_{50} of linezolid was 31.2-fold higher than that of clarithromycin and 10.5-fold higher than that of levofloxacin.

Linezolid resistance was more frequent in metronidazole-resistant strains (12/20 strains; 60.0%) compared with susceptible strains (9/33; 27.3%) ($P = 0.024$) and in strains with resistance to at least one drug (15/28; 53.6%) compared with the reminder (6/25; 24.0%) ($P = 0.048$).

Of 11 strains with double or multidrug resistance, 4 were susceptible to linezolid (Table 2).

4. Discussion

Two methods were used to determine the susceptibility of *H. pylori* to antibiotics, namely the breakpoint susceptibility testing method for amoxicillin, metronidazole and tetracycline, and Etest for clarithromycin, levofloxacin and linezolid. However, in our previous study, category agreement between the breakpoint susceptibility testing method and Etest or agar dilution results was good (>93%) [7].

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