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Validation of in vitro Activity of Aminoglycosides Against Recently Isolated *Helicobacter pylori* for Commercialization of Gentamicin-intercalated Smectite Hybrid as A New Therapeutic Agent

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Background

The eradication rate of *Helicobacter pylori* (*H. pylori)* as a standard therapy based on amoxicillin and clarithromycin, exhibits a decreasing trend. Alternative approaches have been explored, but there is still controversy in the regimen change and these do not provide a satisfactory substitute to the existing standard therapy. Thus, a novel and efficient *H. pylori* eradication regimen should be developed.

Smectite can serve as a drug delivery system and gentamicin-intercalated smectite hybrids (S-GEN) are expected to supersede the standard therapy for *H. pylori* eradication. In the previous study, we synthesized S-GEN complexes as a novel therapeutic agent. In a murine model, S-GEN released gentamicin to the gastric wall stably and the therapeutic effect was not inferior to the conventional standard therapy.

The aim of this study was to confirm whether the minimum inhibitory concentration (MIC) of aminoglycosides applied as smectite hybrids remained low against recently isolated *H. pylori* strains.

Material and Methods

The *H. pylori* strains were collected via endoscopic biopsy from 1,422 patients at Gangnam Severance Hospital in Seoul, Korea, between March 2015 and February 2018. Antimicrobial susceptibility tests were performed, and the MICs of eight antibiotics were determined by using the Epsilometer test, following the EUCAST recommendations.

Results

Finally, 140 *H. pylori* strains were analyzed in this study. The resistance rate to clarithromycin was 30.7%, although it is a major antimicrobial agent used in standard therapy. The MIC_{50} and MIC_{90} of gentamicin (MIC_{50} 0.25mg/L, MIC_{90} 0.75mg/L) and netilmicin (MIC_{50} 0.19mg/L, MIC_{90} 0.75mg/L) were lower than that of metronidazole, tetracycline and levofloxacin, which are alternative therapies for *H. pylori* eradication. In clarithromycin-resistant strains, the MIC_{50} was 0.25 mg/L and the MIC_{90} was 1 mg/L for gentamicin; for netilmicin, the values were 0.25 mg/L and 0.75 mg/L, respectively.

The breakpoint for *H. pylori* in aminoglycosides has not been studied since it has not attempted to use aminoglycosides as *H. pylori* therapy. Therefore, we conservatively estimated the break point at 1 mg/L and compared the results with the other five antibiotics. The resistance rate was 3.6% and 2.1%

Table 1. Resistance rate, MIC_{50} and MIC_{90} of antibiotics against *Helicobacter pylori* strains (n=140).

Antibiotics	Resistance rate, n (%)	MIC_{50}	MIC_{90}	MIC range (mg/L)
Amoxicillin	12 (8.6)	0.023	0.125	0.016 - 32
Clarithromycin	43 (30.7)	0.064	256	0.016 - 256
Metronidazole	37 (26.4)	0.5	256	0.016 - 256
Tetracycline	13 (9.2)	0.38	1	0.016 - 6
Levofloxacin	53 (37.9)	0.5	32	0.002 - 32
Gentamicin	No standard	0.25	0.75	0.016 - 6
Netilmicin	No standard	0.19	0.75	0.016 - 4
Tobramycin	No standard	1	2	0.016 - 8

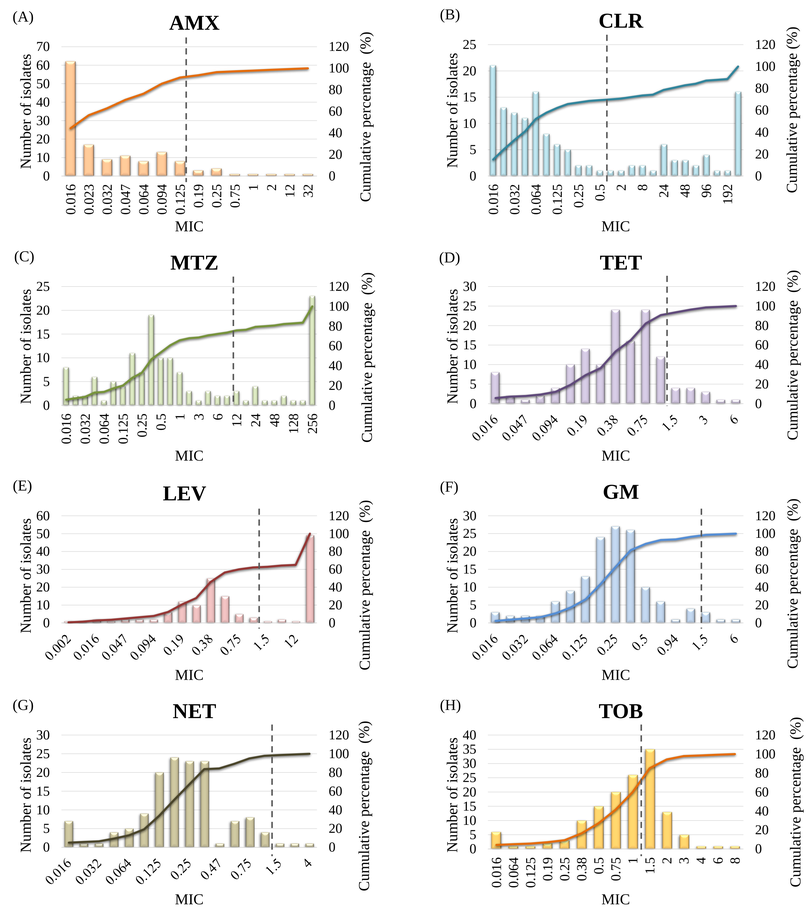
Table 2. Resistance rate, MIC_{50} and MIC_{90} of antibiotics against clarithromycin-resistant strains of *Helicobacter pylori* (n=43).

Antibiotics	Resistance rate, n (%)	MIC_{50}	MIC_{90}	MIC range (mg/L)
Amoxicillin	8 (18.6)	0.032	0.25	0.016 - 32
Metronidazole	15 (34.9)	1	256	0.016 - 256
Tetracycline	8 (18.6)	0.75	2	0.016 - 4
Levofloxacin	21 (48.8)	1	32	0.002 - 32
Gentamicin	No standard	0.25	1	0.047 - 6
Netilmicin	No standard	0.25	0.75	0.016 - 4
Tobramycin	No standard	1	2	0.016 - 8

for gentamicin and netilmicin respectively, and 40.0% for tobramycin. In 43 clarithromycin-resistant strains, the MICs of gentamicin and netilmicin were still low, with a 7% resistance rate.

The dotted line in Figure 1 represented the breakpoint according to the EUCAST recommendations for each antibiotic, and was also indicated as 1 mg/L for aminoglycosides antibiotics. The strain on the left side of the dotted line is susceptible strains to the corresponding antibiotic and the strain on the right from the break point dotted line is resistant to the corresponding antibiotic. Dividing the number of all strains below the specific MIC by the total number of H. pylori strains (the number of strains below specific MIC/140 *100) means cumulative susceptibility percentage in the corresponding MIC. The cross point with the break point line and cumulative percentage line is close to 100%, the more likely the antibiotic is effective as a therapeutic agent.

Figure 1. Minimum inhibitory concentration distributions of various antibiotics against *Helicobacter pylori* isolates.



Conclusion

Through the use of gentamicin and netilmicin, which have low MICs for *H. pylori*, aminoglycoside-intercalated smectite hybrids are expected to emerge as a new standard therapy for *H. pylori* eradication.