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Original Article

Can Aminoglycosides Be Used as a New Treatment for *Helicobacter pylori*? *In vitro* Activity of Recently Isolated isolated *Helicobacter pylori*

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ABSTRACT

Background: Smectite can serve as a drug delivery system and gentamicin-intercalated smectite hybrids are expected to supersede the standard therapy for *Helicobacter pylori* eradication. 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.
Materials and Methods: A total of 140 strains were collected for a minimum period of 3 years. Antimicrobial susceptibility tests were performed, and the MICs of eight antibiotics (amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin, gentamicin, netilmicin, and tobramycin) were determined by using the Epsilometer test and following the European Committee on Antimicrobial Susceptibility Testing recommendations.
Results: The resistance rate of clarithromycin was high, up to 30.7%, although it is a major antimicrobial agent used in standard therapy. The MIC_{50} and MIC_{90} of gentamicin (0.25 mg/L and 0.75 mg/L) and netilmicin (0.19 mg/L and 0.75 mg/L) were lower than other 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.
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.

Keywords: *Helicobacter pylori*; Clarithromycin; Aminoglycosides

INTRODUCTION

The global prevalence of *Helicobacter pylori* infection remains high; there were approximately 4.4 billion individuals with *H. pylori* infection worldwide in 2015, although the infection prevalence varies by country. In South Korea, one of every two healthy people is a carrier of *H. pylori* [1-3]. This gram-negative bacillus is associated with peptic ulcers, mucosa-associated

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Conflict of Interest
No conflicts of interest.

Author Contributions
Conceptualization: YGS. Data curation: SYP. Formal analysis: SJJ, DHJ, JHK. Funding acquisition: IMK, YGS. Methodology: SHJ. Supervision: IMK, YGS. Writing - original draft: KHL. Writing - review & editing: IMK, YGS.

lymphoid tissue lymphoma, and gastric cancer. Therefore, the eradication of *H. pylori* is a critical aspect of disease management and prevention [4, 5]. However, the eradication rate of *H. pylori* as a standard therapy based on amoxicillin and clarithromycin, exhibits a decreasing trend, in the range of 74.6%–75.8% in South Korea, which represents a high burden for the country of *H. pylori* [6, 7]. This failure of *H. pylori* eradication results from antimicrobial resistance, especially to clarithromycin [8-11]. Alternative approaches have been explored, such as sequential therapy, concomitant therapy, quinolone- or rifabutin-containing therapy, and a tailored therapy based on antimicrobial susceptibility have been introduced; however, there is still controversy in the regimen change and these do not provide a satisfactory substitute to the existing standard therapy [11-19]. Thus, a novel and efficient *H. pylori* eradication regimen should be developed.

Aminoglycosides not previously been considered for *H. pylori* eradication, even though they have been conventionally used for aerobic Gram-negative bacterial infections. Aminoglycosides cannot be absorbed by the gastrointestinal tract owing to their polar, water-soluble nature; they have very poor intestinal membrane permeability [20]. Thus, they are usually delivered through intravenous or intramuscular forms, which are not practical for *H. pylori* eradication. In contrast, because *H. pylori* adheres to the gastric epithelium and lives in the gastric mucosa layer [21], if aminoglycosides are applied as coating agents to the gastric wall, their poor absorption characteristics can prove to be an advantage for local therapy.

Therefore, in the previous study, we synthesised gentamicin-intercalated smectite hybrid (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 [22]. Although aminoglycosides have been used for decades, few studies have determined the MIC values of aminoglycosides against *H. pylori* over the last two decades [23, 24]. Therefore, for the clinical use of S-GEN, it is necessary to confirm the MIC of aminoglycosides against recently isolated *H. pylori*.

The aim of this study was to confirm whether the MIC of aminoglycosides remained low against recently isolated *H. pylori* strains. If the MIC remained sufficiently low, the results may indicate the possibility for the development of new aminoglycosides-based therapeutic agents against *H. pylori* through using a smectite hybrid complex.

MATERIALS AND METHODS

1. *Helicobacter pylori* strains in the study

We collected 222 strains of *H. pylori* which were isolated from 1,422 patients, at Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, between March 2015 and February 2018. If *H. pylori* infection was suspected during endoscopy, a gastric tissue biopsy and *H. pylori* culture were routinely performed by gastroenterologists and physicians of laboratory medicine in our hospital. We used only collected *H. pylori* strains without any patient-identifying information, and institutional review board approval was waived because the research did not involve human subjects. The isolated strains were subcultured with eight antimicrobials (amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin, gentamicin, netilmicin, and tobramycin) and susceptibility testing was conducted. Among 222 *H. pylori* strains, 37 strains did not grow at all in the subculture tests and 45 strains were excluded because some of the susceptibility tests were not reported. As the results of 82

strains could produce confounding factors in the data analysis, we excluded them all. Finally, 140 *H. pylori* strains, for which the results of the susceptibility subculture tests against eight antimicrobial agents were analyzed (**Supplementary Table 1**).

2. Isolation of *Helicobacter pylori*

H. pylori strains were cultured on egg yolk emulsion (EYE) agar plates (Yuhan LabTech, Seoul, Korea) and their growth was closely observed. The EYE agar contained 43.82 μg/mL Columbia agar, 112.36 μL/mL EYE, 11.23 μL/mL IsoVitaleX, and 45.0 μg/mL 2,3,5-triphenyltetrazolium chloride for colony staining [25]. The plates were stored in a multi-gas incubator (microaerophilic atmosphere: 10% CO_2, 5% O_2, and 85% N_2) at 37°C for 3–7 days.

The isolation of *H. pylori* was performed on the basis of colony morphology and was confirmed via matrix-assisted laser desorption/ionisation time-of-fight mass spectrometry (MALDI-TOF) using a Microflex LT system (Bruker Daltonics, Bremen, Germany). The measured profiles were compared with a database by using MALDI Biotyper 3.1 software (Bruker Daltonics, Bremen, Germany).

3. Antimicrobial susceptibility test

The *in vitro* MICs of five kinds of antibiotics: amoxicillin, clarithromycin, metronidazole, tetracycline, levofloxacin and three kinds of aminoglycosides: gentamicin, netilmicin and tobramycin against recent clinical isolates of *H. pylori* were tested. The MICs of these eight antibiotics against *H. pylori* were determined via the Epsilometer test (E-test) using an E-strip (BioMerieux SA, France) three times. E-test is reliable and shows excellent agreement with agar dilution and broth microdilution tests [26]. The MICs were determined in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [27]. Their clinical breakpoints for amoxicillin, clarithromycin, metronidazole, tetracycline, and levofloxacin are >0.125 mg/L, >0.5 mg/L, >8 mg/L, >1 mg/L, and >1 mg/L, respectively. Because breakpoints for aminoglycosides against *H. pylori* have not been provided, we compared the MIC_{50}, the MIC_{90}, and the MIC range. We performed quality control by using a standard strain, *H. pylori* ATCC 43504 (NCTC 11637, Manassas, VA, USA), with every batch.

4. Statistical analysis

Categorical variables were described as frequencies and percentages (%). McNemar's test was used to analyze the association of two different categorical variables. The resistance rates of two different antibiotic groups were compared. All *P*-values were two-tailed, and values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS Version 23 software (IBM Corp., Armonk, NY, USA).

RESULTS

1. Antimicrobial susceptibility of eight antimicrobial agents against *Helicobacter pylori*

Based on the results of the antimicrobial susceptibility test, twelve strains (8.6%) were resistant to amoxicillin when the breakpoint of 0.125 mg/L was applied according to the EUCAST recommendation. The resistance rate of clarithromycin, which is one of the standard treatment methods for *H. pylori* eradication, was 30.7%. When the first line

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

Antibiotics	Resistance rate, n (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	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

MIC, minimum inhibitory concentration; MIC_{50}, minimum concentration able to inhibit the growth of 50% of organisms; MIC_{90}, minimum concentration able to inhibit the growth of 90% of organisms.

standard therapy failed, the recommended second line of treatment was a combination of metronidazole and levofloxacin. Their resistance rates were also high at 26.4% and 37.9%, respectively.

The definition of resistance to aminoglycosides in *H. pylori* organisms has not been established yet. Thus, we cannot distinguish the number of *H. pylori* strains that are resistant to aminoglycosides. The MIC_{50} and MIC_{90} of gentamicin and netilmicin were 0.19-0.25 mg/L and 0.75 mg/L, respectively (**Table 1**). And the number of *H. pylori* isolates in each MIC and total MIC distributions of all 140 strains are shown in **Figure 1** for each of the eight antibiotics.

2. Clarithromycin-resistant *Helicobacter pylori*

As mentioned previously, the high resistance rate to clarithromycin in South Korea is a major cause of *H. pylori* eradication failure. The recently clinical isolated *H. pylori* strain has a 30% resistance rate of clarithromycin, as shown in this study. Therefore, we analyzed resistance patterns of other antimicrobial agents in clarithromycin-resistant *H. pylori* strains. Among the 43 clarithromycin-resistant strains, eight (18.6%) were resistant to amoxicillin. Metronidazole and levofloxacin showed a higher resistance rate of 34.9% and 48.8%, respectively. The resistance rate of tetracycline was also 18.6%. For the aminoglycosides, 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. Finally, for tobramycin, these values were 1 mg/L and 2 mg/L, respectively (**Table 2**).

3. Susceptibility of aminoglycosides and cumulative percentage

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% for gentamicin and netilmicin, respectively, and 40.0% for tobramycin. In the 43 clarithromycin-resistant strains, the MICs of gentamicin and netilmicin were still low, with a 7% resistance rate based on a resistance breakpoint of >1 mg/L (**Table 3**). 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

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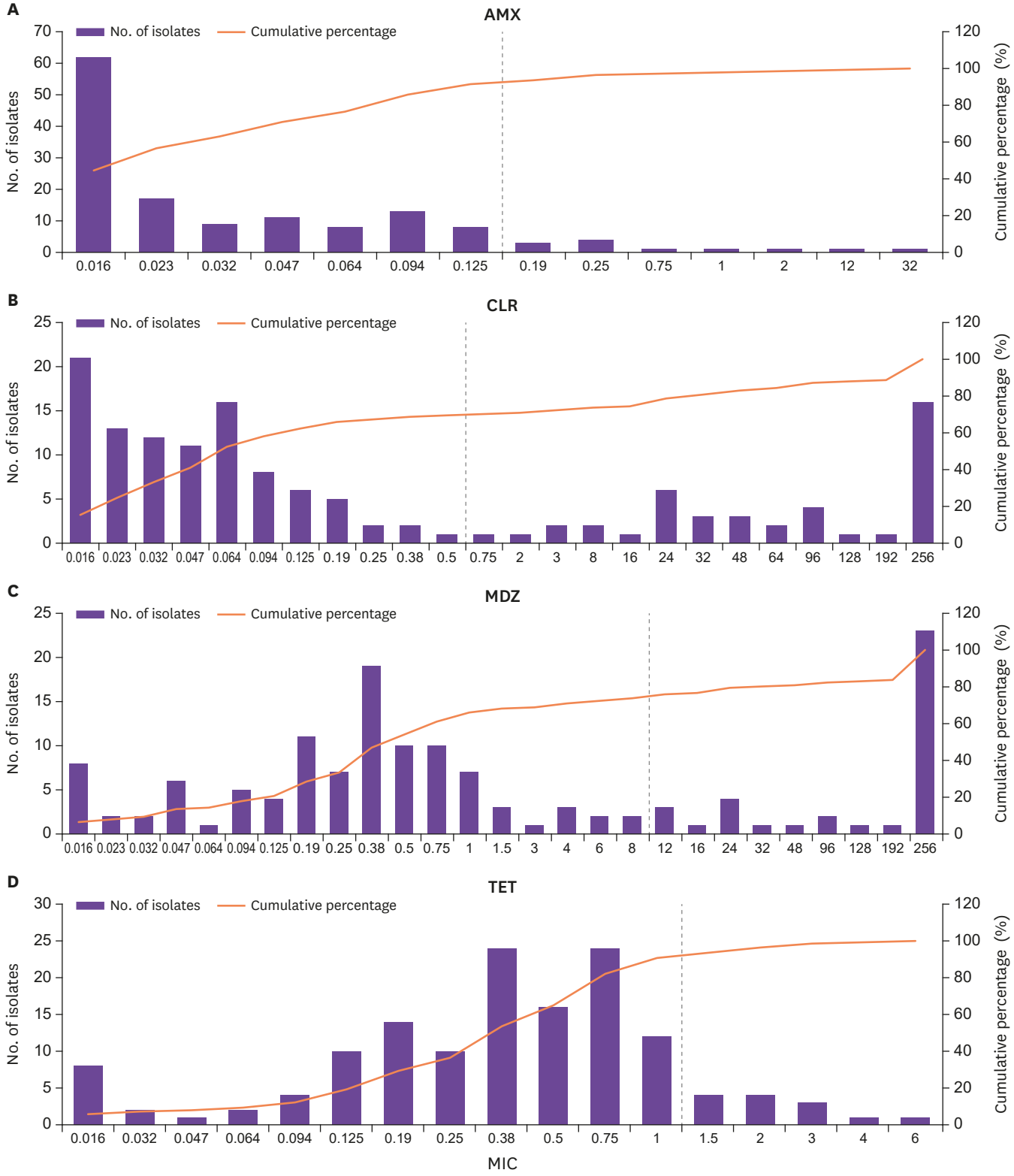

Figure 1. Minimum inhibitory concentration distributions of various antibiotics against *Helicobacter pylori* isolates.
The dotted line represents the breakpoint in each antibiotic. Resistance break points for MICs against *Helicobacter pylori* were defined based on the recommendations of the European Committee on Antimicrobial Susceptibility Testing. For aminoglycosides, breakpoints for *H. pylori* have not been previously studied; therefore, strict breakpoints >1 mg/L have been applied.
MIC, minimum inhibitory concentration; AMX, amoxicillin; CLR, clarithromycin; MDZ, metronidazole; TET, tetracycline; LVX, levofloxacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin.
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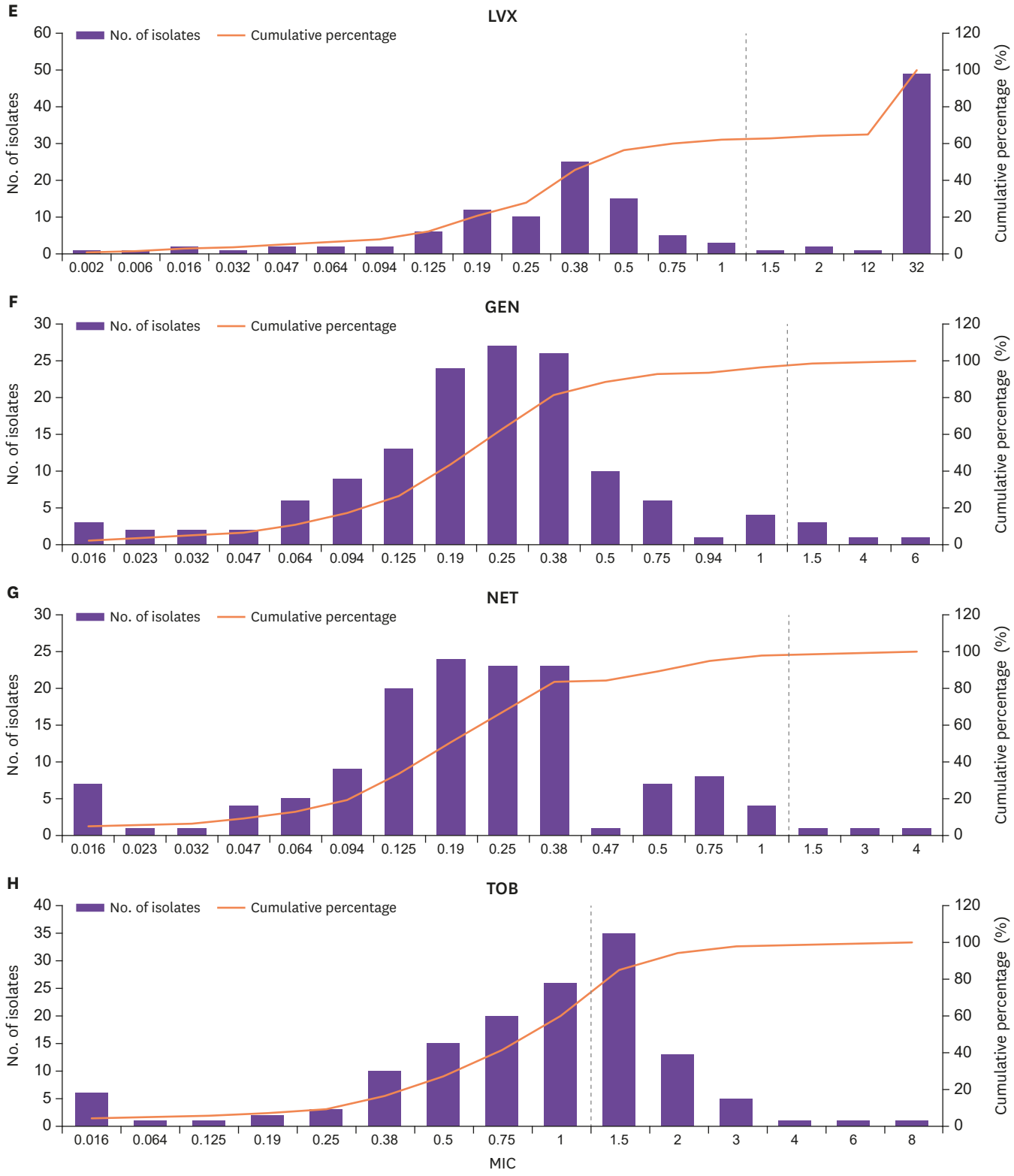

Figure 1. (Continued) Minimum inhibitory concentration distributions of various antibiotics against *Helicobacter pylori* isolates.
The dotted line represents the breakpoint in each antibiotic. Resistance break points for MICs against *Helicobacter pylori* were defined based on the recommendations of the European Committee on Antimicrobial Susceptibility Testing. For aminoglycosides, breakpoints for *H. pylori* have not been previously studied; therefore, strict breakpoints >1 mg/L have been applied.
MIC, minimum inhibitory concentration; AMX, amoxicillin; CLR, clarithromycin; MDZ, metronidazole; TET, tetracycline; LVX, levofloxacin; GEN, gentamicin; NET, netilmicin; TOB, tobramycin.

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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} (mg/L)	MIC_{90} (mg/L)	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

MIC, minimum inhibitory concentration; MIC_{50}, minimum concentration able to inhibit the growth of 50% of organisms; MIC_{90}, minimum concentration able to inhibit the growth of 90% of organisms.

Table 3. The resistance rate of aminoglycosides against *Helicobacter pylori* with a breakpoint of >1 mg/L

H. pylori strain	Antibiotics	Resistance break point[a] (mg/L)	Resistance rate, n (%)
All strains (n = 140)	Gentamicin	>1	5 (3.6)
	Netilmicin	>1	3 (2.1)
	Tobramycin	>1	56 (40.0)
Clarithromycin-resistant strains (n = 43)	Gentamicin	>1	3 (7.0)
	Netilmicin	>1	2 (4.7)
	Tobramycin	>1	19 (44.2)

[a]The breakpoints of aminoglycosides against *Helicobacter pylori* have not been studied before; therefore, we conservatively assumed a strict resistance breakpoint of >1 mg/L for estimation of the resistance rate.

cumulative percentage line is close to 100%, the more likely the antibiotic is effective as a therapeutic agent.

4. Comparison of resistance rate in statistical analysis

We compared the resistance rate of each antibiotic via pairing comparisons using McNemar's test (**Table 4**). Comparing the resistance rates of the eight antibiotics, netimicin was the least resistant and gentamicin was the next, but the difference between the two antibiotics was not significant (2.1% *vs.* 3.6%, $P = 0.625$). In the case of tobramycin, which is a kind of aminoglycosides, the resistance rate was 40% and different from other aminoglycosides. The difference in resistance rate between them was significant. (netilmicin vs tobramycin, 2.1% *vs.* 40.0%, $P < 0.001$; gentamicin vs tobramycin, 3.6% *vs.* 40.0%, $P < 0.001$). When the resistance rates of the standard therapies with amoxicillin and clarithromycin were checked, the amoxicillin had significantly higher resistance than that of netilimcin ($P = 0.012$), and the difference between amoxicillin and gentamicin was not significant ($P = 0.065$). Clarithromycin showed significantly higher resistance than netilimcin and gentamicin ($P < 0.001$). The resistance of tetracycline, metronidazole, and levofloxacin used as second-line therapy was significantly higher than that of netilmicin ($P < 0.001$), and there was a statistically significant difference with gentamicin ($P < 0.001$) except tetracycline ($P = 0.057$).

Table 4. Comparison of the resistance rates of various antibiotics against *Helicobacter pylori* strains

Antibiotics	Resistance rate (%)	NET	GEN	AMX	TET	MDZ	CLR	LVX	TOB
NET	2.1	-	0.625	0.012	0.006	<0.001	<0.001	<0.001	<0.001
GEN	3.6	0.625	-	0.065	0.057	<0.001	<0.001	<0.001	<0.001
AMX	8.6	0.012	0.065	-	>0.999	<0.001	<0.001	<0.001	<0.001
TET	9.3	0.006	0.057	>0.999	-	<0.001	<0.001	<0.001	<0.001
MDZ	26.4	<0.001	<0.001	<0.001	<0.001	-	0.480	0.036	0.032
CLR	30.7	<0.001	<0.001	<0.001	<0.001	0.480	-	0.220	0.124
LVX	37.9	<0.001	<0.001	<0.001	<0.001	0.036	0.220	-	0.810
TOB	40.0	<0.001	<0.001	<0.001	<0.001	0.032	0.124	0.810	-

Data represent the *p*-value of pairwise comparison by McNemar's test. The breakpoints of aminoglycosides against *H. pylori* have not been studied before; therefore, we conservatively assumed a strict resistance breakpoint of >1 mg/L for estimation of the resistance rate.
NET, netilmicin; GEN, gentamicin; AMX, amoxicillin; TET, tetracycline; MDZ, metronidazole; CLR, clarithromycin; LVX, levofloxacin; TOB, tobramycin.

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DISCUSSION

In our study, we reported the MIC and resistance rate of various antibiotics, including aminoglycosides, against recently isolated *H. pylori* strains. As expected, the resistance rate to clarithromycin was high (up to 30.7%), despite the prominence of clarithromycin as a major antimicrobial agent in standard therapy [19]. Resistance to metronidazole and levofloxacin, which are used in alternative *H. pylori* eradication therapies, was also high (above 26%). Against 43 clarithromycin-resistant strains, the MIC_{50} and MIC_{90} of gentamicin and netilmicin were lower than those of other antibiotics, except amoxicillin, which is a component of the standard therapy. The overall resistance rate of antimicrobial agents to the 43 clarithromycin-resistant strains was higher than that of those of the total *H. pylori* strains. Among aminoglycosides, gentamicin and netilmicin had a lower MIC than tobramycin. The MIC_{50} and MIC_{90} of gentamicin and netilmicin were lower than that of metronidazole, tetracycline, and levofloxacin, which are alternative therapies for *H. pylori* eradication [19].

Generally, the MIC against most gram-negative bacteria for aminoglycosides is not below 1–4 mg/. [27, 28]. The resistance breakpoint against *Enterobacteriaceae* and *Pseudomonas spp.* was >4–16 mg/L. The breakpoints of aminoglycosides against *H. pylori* have not been previously studied; therefore, we conservatively assumed a strict resistance breakpoint of >1 mg/L for the estimation of the resistance rate. At a strict resistance breakpoint of >1 mg/L, gentamicin and netilmicin had lower resistance rates than the other antimicrobial agents currently used in *H. pylori* therapies; amoxicillin had the second-lowest resistance rate and this results were statistically significant (**Fig. 1**, **Table 4**).

Currently, smectite is expected to play a role as a drug delivery system, in addition to its original role as an antidiarrheal agent [22, 29-31]. Thus, gentamicin and netilmicin, which have low MICs for *H. pylori*, may be considered as good candidates for a smectite hybrid complex aimed at *H. pylori* eradication. Our results present a novel alternative therapy for *H. pylori* eradication by reporting the latest MIC of aminoglycosides against recently identified *H. pylori*. Aminoglycoside-intercalated smectite hybrids are expected to emerge as a novel standard therapy for the eradication of *H. pylori*, which is resistant to conventional antibiotics and has a low eradication rate.

And the serum concentration is a very important factor in the use of aminoglycosides. The S-GEN formulation proposed in our previous study, is a novel method of topical application to the stomach wall by oral administration. Because the aminoglycoside is not absorbed, its serum concentration is not significant. This is the key point where hybrid formulation differs from conventional *H. pylori* eradication therapy.

We have already reported the results of animal experiments using *H. pylori* standard strains for in vivo activity at low pH conditions [22]. After confirming the in vivo activity of *H. pylori* standard strains, this paper tried to establish evidence that it is applicable not only to standard strains but also to recent clinical isolated strains. In other words, it was necessary to check whether the MIC for the recent clinical strains remained still low as in the past, and whether it could be commercialized in clinical practice.

This study has some limitations with respect to the progression to in vivo studies and applications in real practice. First, the microbiological activity of aminoglycosides is pH-dependent. In an in vitro study, for example, the MIC of aminoglycosides was increased at pH <6.5 [32]. Thus, they should be applied with antacid drugs to overcome gastric acidity.

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When we added PPI to S-GEN in our previous animal studies, we found therapeutic effects similar to those of the standard therapies [22]. Second, direct comparison of MIC values is controversial. It is necessary to consider the pharmacokinetics and pharmacodynamics of each antimicrobial agent in the interpretation of MICs; furthermore, clinical studies should be considered. Third, aminoglycosides can cause serious adverse effects such as nephrotoxicity and ototoxicity [20, 33]. Further research is needed to overcome these drawbacks and localised therapy in clinical studies with aminoglycosides-smectite hybrid complex needs to prove that they do not cause such adverse effects.

In conclusion, our data demonstrated that gentamicin and netilmicin still have low MIC values against recently isolated *H. pylori*, even though many intravenous and intramuscular aminoglycosides have been used for decades. These MIC values are believed to be sufficient to eradicate *H. pylori* by using smectite hybrid complex coating forms as localised therapy. Additional clinical studies should confirm that localised aminoglycoside treatment with a smectite drug delivery system is effective against *H. pylori*, as shown in the animal studies.

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SUPPLEMENTARY MATERIAL

Supplementary material can be found with this article on-line https://icjournal.org/src/sm/ic-51-10-s001.xls.

Supplementary Table 1
Minimum inhibitory concentrations of antibiotics for *Helicobacter pylori* strains (n = 222)

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REFERENCES

1. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, Malfertheiner P, Graham DY, Wong VWS, Wu JCY, Chan FK, Sung JJY, Kaplan GG, Ng SC. Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology 2017;153:420-9.
PUBMED | CROSSREF
2. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, Seo GS, Kim HU, Baik GH, Sin CS, Cho SH, Oh BH. Seroprevalence of *Helicobacter pylori* in South Korea. Helicobacter 2007;12:333-40.
PUBMED | CROSSREF
3. Lim SH, Kwon JW, Kim N, Kim GH, Kang JM, Park MJ, Yim JY, Kim HU, Baik GH, Seo GS, Shin JE, Joo YE, Kim JS, Jung HC. Prevalence and risk factors of Helicobacter pylori infection in Korea: nationwide multicenter study over 13 years. BMC Gastroenterol 2013;13:104.
PUBMED | CROSSREF
4. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784-9.
PUBMED | CROSSREF

5. Seta T, Takahashi Y, Noguchi Y, Shikata S, Sakai T, Sakai K, Yamashita Y, Nakayama T. Effectiveness of *Helicobacter pylori* eradication in the prevention of primary gastric cancer in healthy asymptomatic people: a systematic review and meta-analysis comparing risk ratio with risk difference. PLoS One 2017;12:e0183321.
PUBMED | CROSSREF
6. Heo J, Jeon SW. Changes in the eradication rate of conventional triple therapy for Helicobacter pylori infection in Korea. Korean J Gastroenterol 2014;63:141-5.
PUBMED | CROSSREF
7. Kim SE, Park MI, Park SJ, Moon W, Choi YJ, Cheon JH, Kwon HJ, Ku KH, Yoo CH, Kim JH, Lee GW, Song SE. Trends in *Helicobacter pylori* eradication rates by first-line triple therapy and related factors in eradication therapy. Korean J Intern Med 2015;30:801-7.
PUBMED | CROSSREF
8. Lee JH, Shin JH, Roe IH, Sohn SG, Lee JH, Kang GH, Lee HK, Jeong BC, Lee SH. Impact of clarithromycin resistance on eradication of *Helicobacter pylori* in infected adults. Antimicrob Agents Chemother 2005;49:1600-3.
PUBMED | CROSSREF
9. Boyanova L, Mitov I. Geographic map and evolution of primary *Helicobacter pylori* resistance to antibacterial agents. Expert Rev Anti Infect Ther 2010;8:59-70.
PUBMED | CROSSREF
10. Gasparetto M, Pescarin M, Guariso G. *Helicobacter pylori* eradication therapy: current availabilities. ISRN Gastroenterol 2012;2012:186734.
PUBMED | CROSSREF
11. Shin WG, Lee SW, Baik GH, Huh KC, Lee SI, Chung JW, Jung WT, Park MI, Jung HK, Kim HU, Kim JH, Seol SY, Yoon SM, Jeon SW, Hong SJ, Kim GH, Lee DH, Kim HS, Choi SC, Kang HM, Lee J, Kim JG, Kim JJ. Eradication rates of *Helicobacter pylori* in Korea over the past 10 years and correlation of the amount of antibiotics use: nationwide survey. Helicobacter 2016;21:266-78.
PUBMED | CROSSREF
12. Chey WD, Wong BC; Practice Parameters Committee of the American College of Gastroenterology. American college of gastroenterology guideline on the management of *Helicobacter pylori* infection. Am J Gastroenterol 2007;102:1808-25.
PUBMED | CROSSREF
13. Venerito M, Krieger T, Ecker T, Leandro G, Malfertheiner P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of *Helicobacter pylori* infection. Digestion 2013;88:33-45.
PUBMED | CROSSREF
14. Kim SG, Jung HK, Lee HL, Jang JY, Lee H, Kim CG, Shin WG, Shin ES, Lee YC; Korean College of Helicobacter and Upper Gastrointestinal Research. Guidelines for the diagnosis and treatment of Helicobacter pylori infection in Korea, 2013 revised edition. J Gastroenterol Hepatol 2014;29:1371-86.
PUBMED | CROSSREF
15. Graham DY, Lee YC, Wu MS. Rational Helicobacter pylori therapy: evidence-based medicine rather than medicine-based evidence. Clin Gastroenterol Hepatol 2014;12:177-86.e3; Discussion e12-3.
PUBMED | CROSSREF
16. Sugimoto M, Furuta T. Efficacy of tailored *Helicobacter pylori* eradication therapy based on antibiotic susceptibility and CYP2C19 genotype. World J Gastroenterol 2014;20:6400-11.
PUBMED | CROSSREF
17. Draeger S, Wüppenhorst N, Kist M, Glocker EO. Outcome of second- and third-line *Helicobacter pylori* eradication therapies based on antimicrobial susceptibility testing. J Antimicrob Chemother 2015;70:3141-5.
PUBMED | CROSSREF
18. Hu Y, Zhang M, Lu B, Dai J. *Helicobacter pylori* and antibiotic resistance, a continuing and intractable problem. Helicobacter 2016;21:349-63.
PUBMED | CROSSREF
19. Fallone CA, Chiba N, van Zanten SV, Fischbach L, Gisbert JP, Hunt RH, Jones NL, Render C, Leontiadis GI, Moayyedi P, Marshall JK. The Toronto consensus for the treatment of *Helicobacter pylori* infection in adults. Gastroenterology 2016;151:51-69.e14.
PUBMED | CROSSREF
20. Kumana CR, Yuen KY. Parenteral aminoglycoside therapy. Selection, administration and monitoring. Drugs 1994;47:902-13.
PUBMED | CROSSREF
21. Amieva MR, El-Omar EM. Host-bacterial interactions in *Helicobacter pylori* infection. Gastroenterology 2008;134:306-23.
PUBMED | CROSSREF

22. Jeong SJ, Kim JH, Jung DH, Lee KH, Park SY, Song Y, Kang IM, Song YG. Gentamicin-intercalated smectite as a new therapeutic option for *Helicobacter pylori* eradication. J Antimicrob Chemother 2018;73:1324-9.
PUBMED

23. Irie Y, Tateda K, Matsumoto T, Miyazaki S, Yamaguchi K. Antibiotic MICs and short time-killing against *Helicobacter pylori*: therapeutic potential of kanamycin. J Antimicrob Chemother 1997;40:235-40.
PUBMED | CROSSREF

24. Brenciaglia MI, Fornara AM, Scaltrito MM, Braga PC, Dubini F. Activity of amoxicillin, metronidazole, bismuth salicylate and six aminoglycosides against *Helicobacter pylori*. J Chemother 1996;8:52-4.
PUBMED | CROSSREF

25. Valdez Y, Velapatiño B, Gilman RH, Gutierrez V, León C. Antimicrobial susceptibility of *Helicobacter pylori* determined by the E test using tetrazolium egg yolk agar. J Clin Microbiol 1998;36:2784-5.
PUBMED

26. Piccolomini R, Di Bonaventura G, Catamo G, Carbone F, Neri M. Comparative evaluation of the E test, agar dilution, and broth microdilution for testing susceptibilities of *Helicobacter pylori* strains to 20 antimicrobial agents. J Clin Microbiol 1997;35:1842-6.
PUBMED

27. European Committee on Antimicrobial Susceptibility Testing. Breakpoint- bacteria (v 8.0). Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf. Accessed 2018 March 12.

28. Hosaka Y, Irinoda K, Nakano R, Kitasato H, Okamoto R, Saigenji K, Inoue M. Antibacterial activity of 16 antibiotics against *Helicobacter pylori*. Jpn J Antibiot 2000;53:623-30.
PUBMED

29. Wang C, Ding Y, Teppen BJ, Boyd SA, Song C, Li H. Role of interlayer hydration in lincomycin sorption by smectite clays. Environ Sci Technol 2009;43:6171-6.
PUBMED | CROSSREF

30. Li Z, Chang PH, Jean JS, Jiang WT, Wang CJ. Interaction between tetracycline and smectite in aqueous solution. J Colloid Interface Sci 2010;341:311-9.
PUBMED | CROSSREF

31. Trivedi V, Nandi U, Maniruzzaman M, Coleman NJ. Intercalated theophylline-smectite hybrid for pH-mediated delivery. Drug Deliv Transl Res 2018;8:1781-9.
PUBMED | CROSSREF

32. Nanavaty J, Mortensen JE, Shryock TR. The effects of environmental conditions on the *in vitro* activity of selected antimicrobial agents against *Escherichia coli*. Curr Microbiol 1998;36:212-5.
PUBMED | CROSSREF

33. Mattie H, Craig WA, Pechère JC. Determinants of efficacy and toxicity of aminoglycosides. J Antimicrob Chemother 1989;24:281-93.
PUBMED | CROSSREF