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ORIGINAL ARTICLE

Basic Study

Early genetic diagnosis of clarithromycin resistance in *Helicobacter pylori*

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Abstract

BACKGROUND

The drug resistance rate of clinical *Helicobacter pylori* (*H. pylori*) isolates has increased. However, the mechanism of drug resistance remains unclear. In this study, drug-resistant *H. pylori* strains were isolated from different areas and different populations of Chinese for genomic analysis.

AIM

To investigate drug-resistant genes in *H. pylori* and find the genes for the early diagnosis of clarithromycin resistance.

METHODS

Three drug-resistant *H. pylori* strains were isolated from patients with gastritis in Bama County, China. Minimal inhibitory concentrations of clarithromycin, metronidazole, and levofloxacin were determined and complete genome sequencing was performed with annotation. *Hp1181* and *hp1184* genes were found in these strains and then detected by reverse transcription polymerase chain reaction. The relationships between *hp1181* or *hp1184* and clarithromycin resistance were ascertained with gene mutant and drug-resistant strains. The homology of the strains with *hp26695* was assessed through complete genome detection and identification. Differences in genome sequences, gene quantity, and

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gene characteristics were detected amongst the three strains. Prediction and analysis of the function of drug-resistant genes indicated that the RNA expression of *hp1181* and *hp1184* increased in the three strains, which was the same in the artificially induced clarithromycin-resistant bacteria. After gene knockout, the drug sensitivity of the strains was assessed.

RESULTS

The strains showing a high degree of homology with *hp26695*, *hp1181*, and *hp1184* genes were found in these strains; the expression of the genes *hp1184* and *hp1181* was associated with clarithromycin resistance.

CONCLUSION

Hp1181 and *hp1184* mutations may be the earliest and most persistent response to clarithromycin resistance, and they may be the potential target genes for the diagnosis, prevention, and treatment of clarithromycin resistance.

Key Words: *Helicobacter pylori*; Clarithromycin-resistance; Diagnostic gene; Early genetic diagnosis; *Helicobacter pylori* strains

Core Tip: The World Health Organization designated clarithromycin-resistant *Helicobacter pylori* (*H. pylori*) a high priority among bacteria for antibiotic research and development, but the clarithromycin resistance mechanism remains unclear. We isolated and cultured clinical *H. pylori* strains, determined their minimal inhibitory concentrations, completed genome sequencing of *hp1181* and *hp1184* genes, analyzed their mutations, and found that the expression of the genes *hp1184* and *hp1181* was associated with clarithromycin resistance, which suggested that they can be used as genes for early diagnosis. This research may prove useful in the diagnosis, prevention, and treatment of clarithromycin-resistant *H. pylori*.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is recognized as an important human pathogen that colonizes the gastric mucus, resulting in superficial gastritis, atrophic gastritis, and gastric cancer[1-3]. Present treatments for *H. pylori* infection include proton pump inhibitors, bismuth in combination with amoxicillin, metronidazole and clarithromycin [4,5]. The rate of drug resistance is increasing because of the wide use of antibiotics and high resistance rates to clarithromycin, metronidazole, and levofloxacin are associated with the failure of *H. pylori* eradication[6-8]. The World Health Organization designated clarithromycin-resistant *H. pylori* a high priority bacterium for antibiotic research and development[9].

At present, the mechanism of antibiotic resistance of *H. pylori* is not completely understood[10,11]. It is widely accepted that the resistance to these antimicrobials is related to mutations in *H. pylori* genes, and clarithromycin-resistant strains present three point mutations in the region of domain V of 23S ribosomal RNA (rRNA): A2142G, A2142C, and A2143G[12,13]. In addition to the mutations, the efflux pump cluster is also involved in the development of resistance to clarithromycin[14,15]. However, there may be gene mutation sites that are not yet known, and the mechanism of drug resistance warrants further study.

We isolated and cultured *H. pylori* from the population in Bama County, which is a township known for the longevity of its residents in Guangxi, and randomly selected three strains of multiple drug-resistant *H. pylori* with resistance to clarithromycin.

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Complete genome sequences were analyzed to study the genomic characteristics of the strains and to elucidate the underlying mechanism of drug resistance in *H. pylori*.

MATERIALS AND METHODS

Isolation and culture of H. pylori

This study had received a strict medical ethics review from Youjiang Medical University for Nationalities. Written informed consent was obtained from each patient. Gastric mucosa tissue samples were collected from the People's Hospital of Bama Yao Autonomous County in patients' gastric body and pylorus with gastritis or gastric ulcers. Isolation and culture of *H. pylori* were performed at the Research Center for the Prevention and Treatment of Drug Resistant Microbial Infection, Youjiang Medical University for Nationalities. Patients investigated had not taken any antibiotics for at least 4 wk before examination. The isolation and identification of *H. pylori* were performed as previously described[16,17]. The bacteria were cultured on Columbia agar plates containing 5% fresh defibrinated sheep blood. The microaerophilic conditions included 5% O_2, 10% CO_2, and 85% N_2 at 37 °C for 3 to 5 days. Suspicious colonies were confirmed by Gram staining, urease, oxidase, and catalase activity testing, and urease gene polymerase chain reaction (PCR).

Antibiotic susceptibility testing

The antibiotic resistance of *H. pylori* was measured by dilution methods with reference to the protocols of the Clinical and Laboratory Standards Institute (Wayne, PA, United States)[18]. Briefly, the density of *H. pylori* was adjusted to be 1 × 10^6 CFU/mL and incubated at 37 °C for 3 to 5 d under microaerophilic conditions. After incubation, the plates were visually examined and the minimal inhibitory concentration (MIC) was determined to be the lowest concentration that resulted in no turbidity. Metronidazole (Aladdin, d1707126), amoxicillin (Xiansheng pharmaceutical, Co., Ltd, China), levofloxacin (Shandong Lukang Pharmaceutical Group Saite Co., Ltd, China), and clarithromycin (Yangzi River Pharmaceutical Group Co., Ltd, China) were also used.

Complete genome sequencing and analysis

Drug-resistant strains were selected and sent to the Shenzhen Huada Gene Co., Ltd (China) for complete genome analysis. After the DNA samples were delivered, the quality of the samples was tested and then used to construct a BSlibrary. The purified genomic DNA samples including genomic DNA, bacterial artificial chromosomes, or long-length PCR products were sheared into smaller fragments by CovarisS/E210 or using a Bioruptor. The overhangs resulting from fragmentation were converted into blunt ends using T4 DNA polymerase, Klenow fragment, and T4 polynucleotide kinase. After adding an 'A' base to the 3' end of the blunt phosphorylated DNA fragments, adapters were ligated to the ends of the DNA fragments. The desired fragments were purified though gel-electrophoresis, selectively enriched, and amplified by PCR. The index tag was introduced into the adapter at the PCR stage as appropriate and a library quality test was conducted. Finally, the qualified BSlibrary was used for sequencing. Genomic component and gene function analyses were performed, including gene prediction, tRNA, sRNA, and gene annotation, and prediction of open reading frames by GO.

Drug-resistant gene detection

Drug-resistant genes were predicted based on the results of the complete genome sequence analysis and selected for detection by reverse transcription PCR (RT-PCR). The reaction for cDNA synthesis was held at 25 °C for 10 min, 42 °C for 60 min, and then 99 °C for 5 min. The reaction consisted of 32 cycles with each cycle composed of 1 min at 95 °C, 4 min at 56 °C, and 7 min at 70 °C. After a final extension of 15 min at 72 °C, the RT-PCR products were visualized by electrophoresis on 1% agarose gel and 15% acrylamide gel with a 200-bp ladder size marker.

Knockout of mutant genes

Hp1181 and *hp1184* knockout mutants were constructed by insertion of the KAN resistance cassette. Double-knockout mutants were made by natural transformation of the KAN resistance cassette with pBSII KS (as presented by Bi HK, Laboratory of Nanjing Medical University, China) containing an internal fragment interrupted with a cat cassette from pAV35, with selection for both KAN- and CHL-resistant colonies.

Table 1 Drug resistance characteristics of three drug-resistant strains (minimal inhibitory concentration: μg/mL)

Strain	Metronidazole	Clarithromycin	Levofloxacin	Amoxicillin
Hpbs1	32	8	8	0.125
Hpbs2	16	8	0.125	0.125
Hpbs3	0.125	8	8	0.125

Table 2 Sequence information of three drug-resistant strains

Sample name	ID name	Sequence type	Sequence topology	Sequence number	Total length (bp)	GC content
Hpbs1	Chromosome1	Chromosome	Circular	1	1563701	38.90
	All	All	-	1	1563701	38.90
Hpbs2	Chromosome1	Chromosome	Circular	1	1534481	38.87
	All	All	-	1	1534481	38.87
Hpbs3	Chromosome1	Chromosome	Circular	1	1534930	38.90
	All	All	-	1	1534930	38.90

Sequence type: Chromosome or plasmid; Sequence topology: Circular or linear.

Table 3 Gene information of three drug-resistant strains

Sample name (#)	Genome size (#)	Total number (#)	Total length (bp)	Average length (#)	Length/genome length (%)	GC content (%)
Hpbs1	1563701	1571	1434202	912.92	91.72	39.49
Hpbs2	1534481	1792	1395399	778.68	90.94	39.44
Hpbs3	1534930	1732	1407495	812.64	91.70	39.49

Total number denotes the count of genes. Total length represents the total length of all genes. Average length refers to the average length of all genes. GC content is the content of G and C in a gene. Length/genome length is the proportion of gene length in the genome.

Table 4 Gene annotation statistics A

Sample name (#)	Total	P450 (#) (%)	VFDB (#) (%)	ARDB (#) (%)	CAZY (#)	SWISSPROT (#) (%)	NOG (#) (%)	COG (#) (%)	CARD (#) (%)	NR (#)
Hpbs1	1571	22 (1.4)	196 (12.47)	0 (0)	14 (0.89)	742 (47.23)	67 (4.26)	1084 (69)	14 (0.89)	1599 (99.23)
Hpbs2	1792	21 (1.17)	177 (9.87)	0 (0)	14 (0.78)	751 (41.9)	125 (6.97)	1111 (61.99)	13 (0.72)	1723 (96.14)
Hpbs3	1732	22 (1.27)	174 (10.04)	0 (0)	14 (0.75)	750 (43.3)	97 (5.6)	1113 (64.26)	15 (0.86)	1698 (98.03)

Insertion of the KAN and cat resistance cassette at the desired locations in the *H. pylori* putative efflux genes was validated by PCR.

Induction of drug resistance

The MIC of clarithromycin to hp26695 was detected. Drug resistance was induced by 1/4 MIC. The culture medium was changed every 2 d and MIC was detected every 4 d. The concentration of induced drug was changed with MIC.

Table 5 Gene annotation statistics B

Sample name (#)	DBCAN (#) (%)	T3SS (#) (%)	TREMBL (#) (%)	IPR (#)	PHI (#) (%)	KEGG (#) (%)	GO (#) (%)	KOG (#) (%)	Over all (#) (%)
Hpbs1	30 (1.9)	175 (11.13)	1557 (99.1)	1234 (78.54)	54 (3.43)	1026 (65.3)	957 (60.91)	142 (9.03)	1563 (99.49)
Hpbs2	29 (1.61)	197 (10.99)	1706 (95.2)	1372 (76.56)	52 (2.9)	1078 (60.15)	1056 (58.92)	144 (8.03)	1750 (97.65)
Hpbs3	30 (1.73)	209 (12.06)	1688 (97.45)	1340 (77.36)	51 (2.94)	1067 (61.6)	1030 (59.4)	139 (8.02)	1711 (98.78)

RESULTS

Bacterial resistance

Three drug-resistant strains were isolated and identified by Gram staining, urease, oxidase, catalase activity testing, and urease gene PCR. The drug resistance information of these strains is summarized in Table 1.

Bacterial sequence information

Based on the valid data from the previous sequencing platform, the CleanData could be assembled for each sample and the optimal assembly results were obtained after multiple adjustments. The assembly sequence was analyzed by correcting single base, circular judgment, and plasmid comparison. The results of the genome assembly statistics of each sample are displayed in Table 2. These three strains have been uploaded to the NCBI Biosample database: Hpbs1 (https://www.ncbi.nlm.nih.gov/biosample/?term=SAMN10461767). Hpbs2 (https://www.ncbi.nlm.nih.gov/biosample/?term=SAMN10663081), and Hpbs3 (https://www.ncbi.nlm.nih.gov/biosample/?term=SAMN10663175).

Gene information

Gene prediction was applied to determine gene composition. The statistics are shown in Table 3.

Circular genome analysis

GC skew analysis was carried out using (G-C)/(G+C) calculations based on genomic sequences of strains. The results of gene distribution, ncRNA distribution, and gene annotation are demonstrated in Figure 1. Hpbs1 had 835 genes, 26 tRNAs, 6 rRNAs, and 2 sRNAs in the positive chain. It also had 736 genes, 10 tRNAs, 0 rRNAs, and 5 sRNAs in the negative chain and 157 repeats without positive or negative chain. There are 943 genes, 26 tRNAs, 6 rRNAs, 3 sRNAs, 849 genes, 10 tRNAs, 0 rRNA, 3 sRNAs, and 153 repeats in Hpbs2; there are 869 genes, 26 tRNAs, 6 rRNAs, 3 sRNAs, 863 genes, 10 tRNAs, 0 rRNA, 3 sRNAs, and 155 repeats in Hpbs3.

Gene annotation

Functional annotation was accomplished by analysis of protein sequences. We aligned genes with databases to obtain their corresponding annotations. To demonstrate the biological meaning, the highest quality alignment result was chosen as a gene annotation. Functional annotation was completed by blast resistance genes with different databases. In this project we have finished annotations using 17 databases, including P450, VFDB, ARDB, CAZY, SWISSPROT, NOG, COG, CARD, NR, DBCAN, T3SS, TREMBL, IPR, PHI, KEGG, GO, and KOG. The annotation results are shown in Tables 4 and 5.

Analysis of drug-resistant gene database

The drug resistance gene numbers of three strains were different in the CARD (Comprehensive Antibiotic Resistance Database), which are 14, 13, and 15 genes, respectively. However, after sorting, it was found that some genes were repetitive. The specific numbers and characteristics of genes are presented in the Tables 6 and 7. NP_207975.1 and NP_207972.1 were efflux pump genes of 26695 strain, *i.e.*, *hp1181* and *hp1184* genes. Their drug resistance was verified by RT-PCR, as illustrated in Figure 2. After knocking out the drug-resistant genes, drug sensitivity was significantly improved, as shown in Figure 3.

Table 6 Analysis of gene resistance in CARD

Gene ID	Subject ID	Align length	Mismatch	Gap	Gene start	Gene end	Subject start	Subject end	*E* value
GL000175	YP_208874.1	97	39	0	2	98	4	100	6.00E-40
GL000286	YP_006374661.1	398	88	2	1	397	29	421	0
GL000295	NP_312937.1	1389	658	21	8	1371	8	1339	0
GL000296	AAK44936.1	124	35	0	1	124	1	124	4.00E-63
GL000306	NP_207975.1	459	16	0	1	459	1	459	0
GL000309	NP_207972.1	443	10	0	1	443	1	443	0
GL000772	AIL15701	421	220	3	1	420	1	417	4.00E-126
GL000822	YP_002344422.1	853	293	6	3	818	2	851	0
GL000911	NP_415611.1	247	130	2	1	247	1	243	2.00E-66
GL000972	WP_005768149.1	810	390	18	3	773	12	809	0
GL001063	AJF83452.1	287	164	2	1	283	2	288	1.00E-71
GL001265	NP_415804.1	262	141	1	1	261	1	262	2.00E-80
GL001295	YP_001332362.1	222	123	4	1	221	1	216	7.00E-51
GL001455	AJF82049.1	254	141	2	4	255	7	260	2.00E-62

Identification of 23S rRNA gene mutations

As three strains were resistant to clarithromycin, so we analyzed and identified the sites of clarithromycin-resistant mutations. We found that three strains had mutations in A2142G, A2143G, G2144T, and some had mutations in other sites, as shown in Table 8.

Gene mutation induced in drug-resistant strains

After induction with clarithromycin, hp26695 drug resistance was enhanced on the 12th day, reached the highest level on day 16, and increased to 8 μg/mL on the 24th day. The expression of *hp1181* and *hp1184* was also increased with increasing clarithromycin resistance, especially *hp1184*, as shown in Figure 4. Only A2142G and A 2143G mutations were detected in 23S RNA, with no other mutation sites being found, as shown in Table 9. These data indicated that these two genes may be involved early in the regulation of clarithromycin resistance.

DISCUSSION

The treatment of *H. pylori* infection remains reliant on bismuth tetralogy at present. *H. pylori* is eradicated clinically using common antibiotics including clarithromycin, amoxicillin, metronidazole, tetracycline and levofloxacin. However, in recent years, the growing rate of antibiotic resistance has resulted in the failure of *H. pylori* eradication[19,20]. The most serious resistance has developed to drugs including metronidazole, clarithromycin, and levofloxacin star. The common mechanisms of bacterial resistance involve the production of inactivated enzymes, change in the target position of antibacterial drugs, change in the permeability of bacterial outer membrane, effects on the active outflow system, and formation of bacterial biofilm and cross resistance[21-23]. There are some differences in the mechanisms of drug resistance of each kind of bacteria; however, the same kind of bacteria still have different resistances to the same antibiotic in different areas[24]. The mechanism of drug resistance of *H. pylori* remains unclear and needs further study.

We selected drug-resistant strains using metronidazole, clarithromycin, and levofloxacin for genome sequencing analysis. We found that there were no significant differences in the number of drug-resistant genes in the CARD database. This may be because two kinds of antibiotic resistance can develop and the drug-resistant genes in *H. pylori* are mainly *hp1181* and *hp1184*. *Hp1181* encodes a putative NDA translocase that is related to the major facilitator superfamily and is an integral membrane protein; *hp1184* encodes another translocase that belongs to the MATE family, resulting in the aforementioned susceptibility. These can contribute to resistance *via* a multidrug-

Table 7 Characteristics of drug-resistant genes in CARD

Subject ID	ARO number	Definition of term
YP_208874.1	*Neisseria gonorrhoeae* FA 1090	rpsJ is a tetracycline resistance protein identified in *Neisseria gonorrhoeae*. Tetracycline resistance is conferred by binding to the ribosome as a 30S ribosomal protection protein[27]
YP_006374661.1	*Enterococcus faecium* DO	Sequence variants of *Enterococcus faecium* elongation factor Tu that can confer resistance to GE2270A[28]
NP_312937.1	*Escherichia coli* O157•H7 str. Sakai	Point mutation that occurs in *Escherichia coli* rpoB resulting in resistance to rifampicin[29]
AAK44936.1	*Mycobacterium tuberculosis* CDC1551	Ribosomal protein S12 stabilizes the highly conserved pseudoknot structure formed by 16S rRNA. Amino acid substitutions in RpsL affect the higher-order structure of 16S rRNA and confer streptomycin resistance by disrupting interactions between 16S rRNA and streptomycin[30-35]
NP_207975.1	*Helicobacter pylori* 26695	hp1184 is a translocase that belongs to the MATE efflux pump family. It is found in *H. pylori* and is involved in the active efflux of antibiotics[25,26]
NP_207972.1	*Helicobacter pylori* 26695	hp1181 is a translocase that is part of the MFS efflux pump family. It is found in *H. pylori* and plays a role in the active efflux of antibiotics[25]
AIL15701	*Escherichia coli* ATCC25922	murA or UDP-N-acetylglucosamine enolpyruvy1 transferase catalyzes the initial step in peptidoglycan biosynthesis and is inhibited by Fosfomycin. Over-expression of murA through mutations such as Asp369Asn and Leu370I1e confers fosfomycin resistance. Extensive evidence has shown the significance of C115 mutations in conferring fosfomycin resistance since this residue represents a primary binding site for the antibiotic across many species[36-39]
YP_002344422.1	*Campylobacter jejuni* subsp. *jejuni* NCTC 11168	*Campylobacter jejuni* is a major bacterial infectious agent associated with gastroenteritis. Quinolone resistance is reportedly conferred by a single C-257-T nucleotide substitution in the gyrA gene[40]
NP_415611.1	*Escherichia coli* str. K-12 substr. MG1655	Fab G is a 3-oxoacyl-acyl carrier protein reductase involved in lipid metabolism and fatty acid biosynthesis. The bacterial biocide Triclosan blocks the final reduction step in fatty acid elongation, inhibiting biosynthesis. Point mutations in fabG can confer resistance to Triclosan[41]
WP_005768149.1	*Bartonella bacilliformis* KC583	Point mutation in *Bartonella bacilliformis* results in amino coumarin resistance[42]
AJF83452.1	*Acinetobacter baumannii*	The LpxC gene is widely known to be involved in the biosynthesis of lipid A in Gram-negative bacteria and mutations to this gene may cause resistance to antimicrobial peptides that target the outer membrane[43,44]
NP_415804.1	*Escherichia coli* str. K-12 substr. MG1655	fabI is an enoyl-acy1 carrier reductase used in lipid metabolism and fatty acid biosynthesis. The bacterial biocide Triclosan blocks the final reduction step in fatty acid elongation, inhibiting biosynthesis. Point mutations in fabI can confer resistance to Triclosan and Isoniazid[41]
YP_001332362.1	*Staphylococcus aureus* subsp. *aureus* str. Newman	Ar1R is a response regulator that binds to the norA promoter to activate expression. Ar1R must first be phosphorylated by Ar1S[45]
AJF82049.1	*Acinetobacter baumannii*	The *LpxA* gene is widely known to be involved in the biosynthesis of lipid A in Gram-negative bacteria and mutations to this gene may cause resistance to antimicrobial peptides that target the outer membrane[43,44]

H. pylori: *Helicobacter pylori*.

resistant efflux protein, active-efflux of antibiotics, and other efflux pump genes, such as *HefA*. After knockout of these two genes, the MICs of the drugs were significantly decreased and the sensitivity was increased. It is noteworthy that in addition to these two genes, the *GE2270A* gene of *Enterococcus* and *MurA* gene of *Escherichia coli* also show a correlation. It is likely that the drug-resistant plasmids of other strains invade *H. pylori* through transformation or other mechanisms. Bacteria other than *H. pylori* in

Table 8 Mutations in the 23S rRNA genes of *Helicobacter pylori* strains

Nucleotide position	Ref	Mutation	Hpbs1	Hpbs2	Hpbs3
2143	A	G	+	+	+
2142	A	G	+	+	+
2144	G	T	+	+	+
2302	A	G	-	-	+
2182	T	C	-	+	-
2173	C	T	+	+	+
1513	G	A	-	+	+
2196	C	T	+	-	-
1280	A	G	+	-	-
1023	G	A	-	-	+

Table 9 23S rRNA mutations of *Helicobacter pylori* strains

Nucleotide position	Ref	Mutation	26695(S)	26695(R)
2142	A	G	-	+
2143	A	G	-	+

the gastric mucosa of patients can indirectly confirm this view. The main reason for this may be long-term acid resistant treatment, gastric erosion, or intestinal bacterial reflux. This will lead to drug resistance becoming more difficult to prevent and control. In addition, all three strains have clarithromycin resistance. The mechanism of resistance to clarithromycin is mainly reflected in the mutations A2142G, A2143G, and G2144T. In addition, it is common that there are several mutations in the same strain.

Hp1181 and *hp1184* are related to multidrug resistance and to clarithromycin resistance, which has been previously reported in the literature[25,26]. The RNA expression of *hp1181* and *hp1184* were increased with the emergence of clarithromycin resistance, with *hp1184* showing the fastest increase. Therefore, these genes are also involved in the regulation of drug resistance and may be one of the mechanisms of *H. pylori* resistance to clarithromycin. Compared with the clinical isolates, 23S RNA mutation sites of *H. pylori* were less frequent in artificially induced strains that had only A2142G and A2143G mutations. These may be attributed to the single factor of artificial induction that is not as complex as human stomach environment. More importantly, *hp1181* and *hp1184* mutations may be the earliest and most persistent response to clarithromycin resistance, and they may be the main target genes for the diagnosis, prevention, and treatment of clarithromycin resistance.

The genetic characteristics of multidrug-resistant strains in this area were preliminarily identified: The relationship between *hp1181* or *hp1184* and clarithromycin resistance was ascertained through genome sequencing analysis and gene function identification of drug-resistant *H. pylori* from Bama County, Guangxi Province. Our study further provided an improved experimental basis for the prevention and treatment of drug resistance of *H. pylori*.

CONCLUSION

Hp1181 and *hp1184* mutations may be the earliest and most persistent response to clarithromycin resistance, and they may be the main target genes for the diagnosis, prevention, and treatment of clarithromycin resistance.

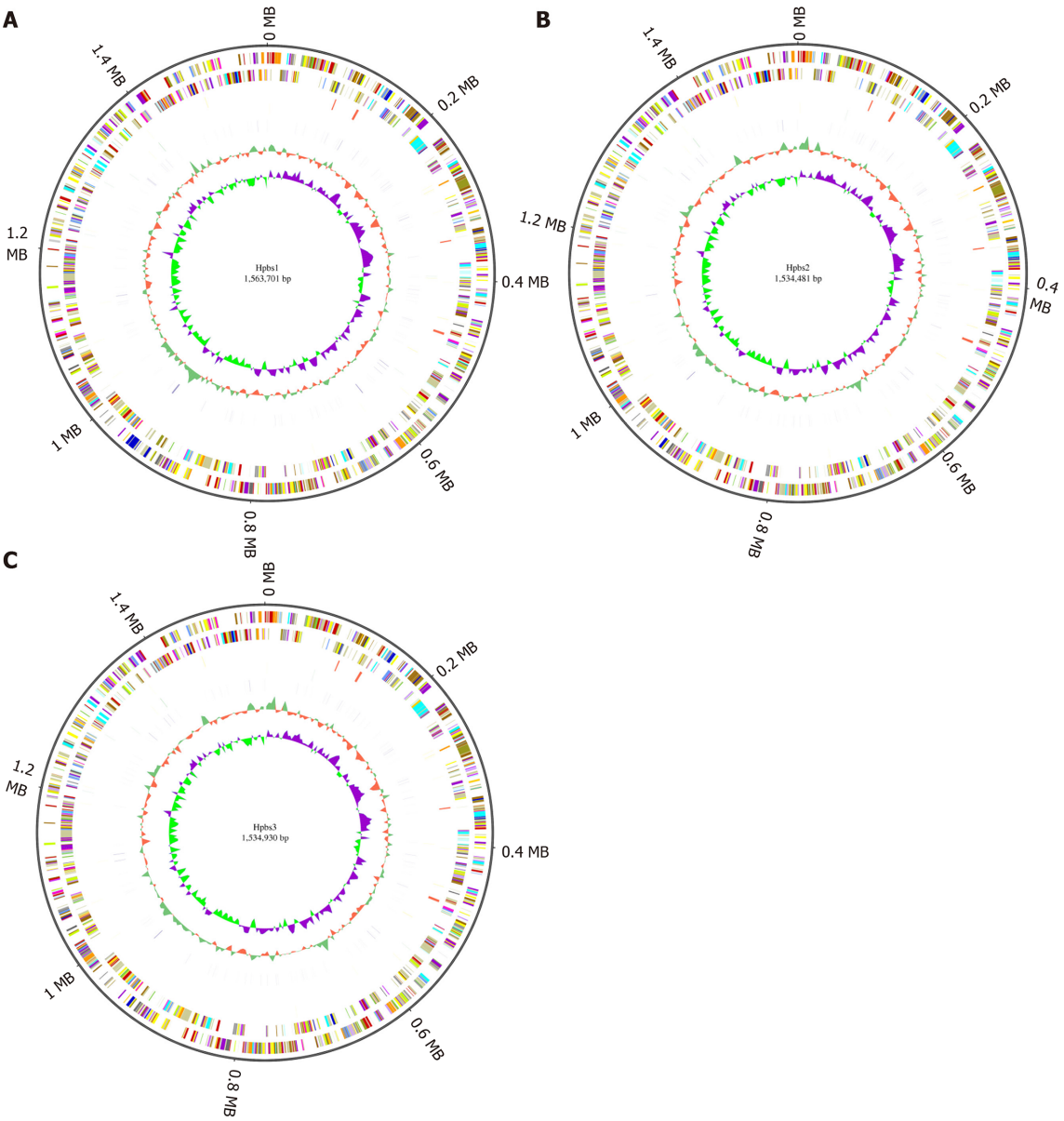

Figure 1 Circular genome analysis of three drug-resistant strains. A: Hpbs1; B: Hpbs2; C: Hpbs3.

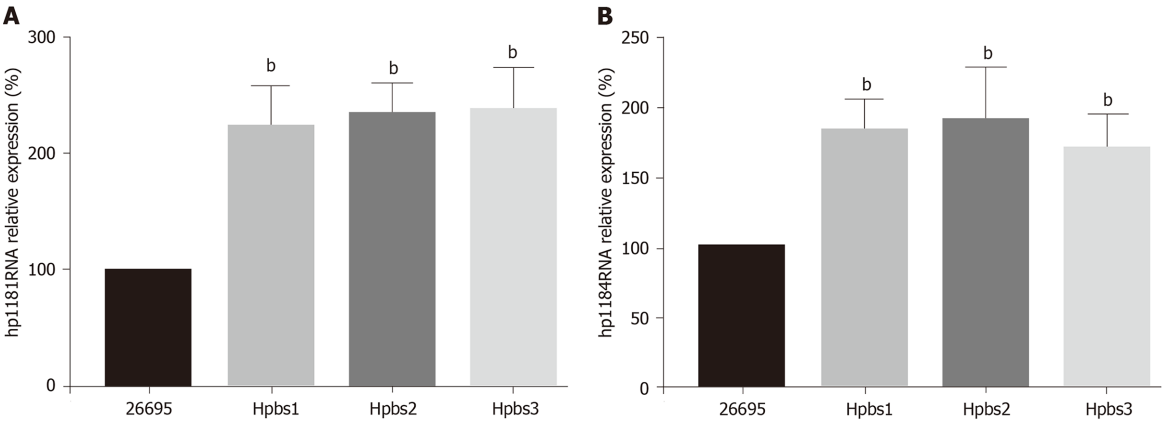

Figure 2 *Hp1181* and *hp1184* gene expression in drug-resistant strains. A: *Hp1181*; B: *Hp1184*. $^{b}P < 0.01$.

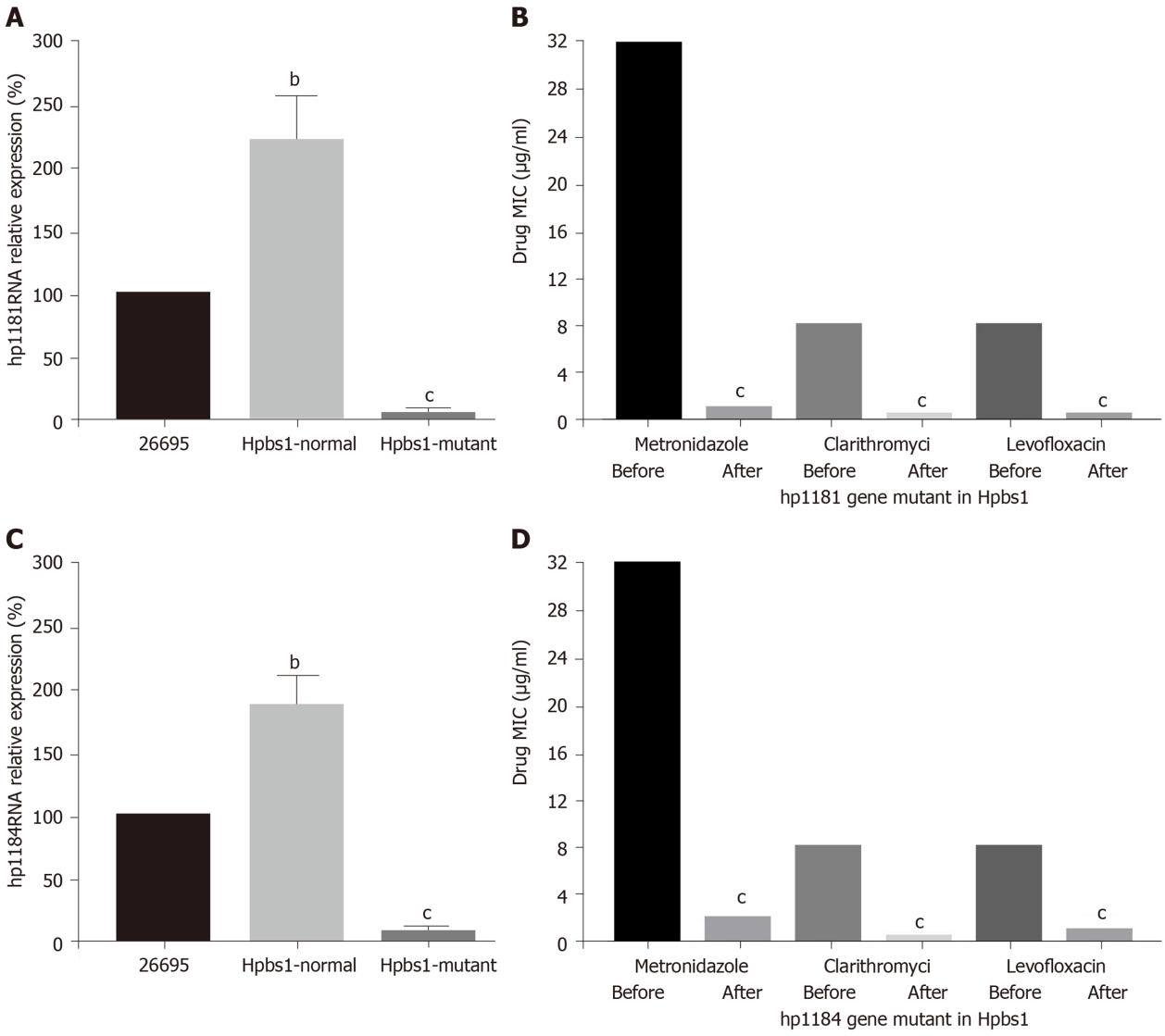

Figure 3 Drug sensitivity is improved after knockout of the drug-resistant genes. A: *Hp1181* knockout; B: Minimal inhibitory concentration (MIC) after *hp1181* knockout; C: *Hp1184* knockout; D: MIC after *hp1184* knockout. MIC: Minimal inhibitory concentration. $^{b}P < 0.01$; $^{c}P < 0.001$.

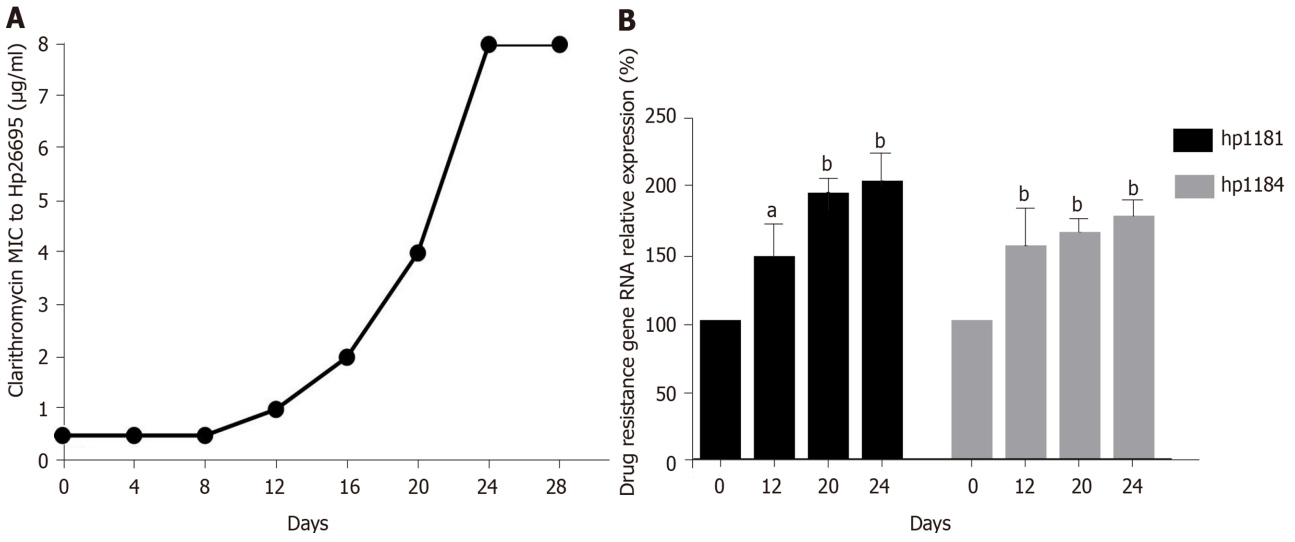

Figure 4 Induction of resistance to clarithromycin and expression of drug-resistant genes in *Helicobacter pylori*. A: Induction of clarithromycin resistance; B: Expression of drug-resistant genes. $^{a}P < 0.05$; $^{b}P < 0.01$.

ARTICLE HIGHLIGHTS

Research background

Helicobacter pylori (*H. pylori*) is recognized as an important human pathogen associated with superficial gastritis, atrophic gastritis, gastric cancer, *etc*., each of which has become a serious threat to human health and survival. The rate of drug resistance is increasing due to the wide use of antibiotics and high rates of resistance to clarithromycin, metronidazole, and levofloxacin are associated with the failure of *H. pylori* eradication. At present, the mechanism of antibiotic resistance of *H. pylori* is not completely understood. It is very difficult to prevent drug resistance and improve the rate of eradication of the target, thus warranting exploration of the mechanism of drug resistance to *H. pylori*, and provision of an experimental basis for the prevention and treatment of drug resistance.

Research motivation

Clarithromycin-resistant *H. pylori* urgently needs new antibiotics; however, antibiotic research and development are very difficult. If we can detect drug resistance by detecting drug-resistant genes in a timeous manner, this may help to alleviate the problem of clarithromycin resistance.

Research objectives

The objectives of this study were to investigate drug-resistant genes in *H. pylori*, and find a gene suited to early diagnosis of clarithromycin resistance, thereby rationalizing the rate of use of the drug.

Research methods

H. pylori strains were isolated and cultured, minimal inhibitory concentrations were measured, and complete genome sequence was determined. Prediction and analysis of the function of drug-resistant genes indicated that the RNA expression of *hp1181* and *hp1184* increased in the *H. pylori* strains, which was the same in the artificially induced clarithromycin-resistant bacteria. The relationships between *hp1181* or *hp1184* and clarithromycin resistance were confirmed with gene mutant and drug-resistant strains.

Research results

Hp1181 and *hp1184* genes were found in these *H. pylori* strains. Their expression was associated with clarithromycin resistance.

Research conclusions

Hp1181 and *hp1184* mutations may be the earliest and most persistent response to clarithromycin resistance, and they may be the main target genes for the diagnosis, prevention, and treatment of clarithromycin resistance.

Research perspectives

The relationship between *hp1181* or *hp1184* and clarithromycin resistance was demonstrated, providing an improved experimental basis for early diagnosis of clarithromycin resistance in *H. pylori*.

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REFERENCES

1 **Yoon K**, Kim N, Lee JW, Yoon H, Shin CM, Park YS, Lee DH. Annual eradication rate of bismuth-containing quadruple therapy as second-line treatment for Helicobacter pylori infection: A 15-year prospective study at a tertiary hospital in Korea. *Helicobacter* 2020; **25**: e12685 [PMID: 32141173 DOI: 10.1111/hel.12685]

2 **Fischbach W**, Malfertheiner P. Helicobacter Pylori Infection. *Dtsch Arztebl Int* 2018; **115**: 429-436 [PMID: 29999489 DOI: 10.3238/arztebl.2018.0429]

3 **Diaconu S**, Predescu A, Moldoveanu A, Pop CS, Fierbințeanu-Braticevici C. Helicobacter pylori infection: old and new. *J Med Life* 2017; **10**: 112-117 [PMID: 28616085]

4 **Lahner E**, Carabotti M, Annibale B. Treatment of *Helicobacter pylori* infection in atrophic gastritis. *World J Gastroenterol* 2018; **24**: 2373-2380 [PMID: 29904244 DOI: 10.3748/wjg.v24.i22.2373]

5 **Suzuki S**, Esaki M, Kusano C, Ikehara H, Gotoda T. Development of *Helicobacter pylori* treatment: How do we manage antimicrobial resistance? *World J Gastroenterol* 2019; **25**: 1907-1912 [PMID: 31086459 DOI: 10.3748/wjg.v25.i16.1907]

6 **Malfertheiner P**, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study Group and Consensus panel. Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017; **66**: 6-30 [PMID: 27707777 DOI: 10.1136/gutjnl-2016-312288]

7 **Savoldi A**, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. *Gastroenterology* 2018; **155**: 1372-1382. e17 [PMID: 29990487 DOI: 10.1053/j.gastro.2018.07.007]

8 **Liu DS**, Wang YH, Zeng ZR, Zhang ZY, Lu H, Xu JM, Du YQ, Li Y, Wang JB, Xu SP, Chen Y, Lan CH, Cheng H, Jiang MD, Zhang LX, Huo LJ, Chen SY, Zhang GX, Wu KC, Zhu X, Chen YX, Zhu Y, Shu X, Xie Y, Lu NH. Primary antibiotic resistance of Helicobacter pylori in Chinese patients: a multiregion prospective 7-year study. *Clin Microbiol Infect* 2018; **24**: 780.e5-780. e8 [PMID: 29138101 DOI: 10.1016/j.cmi.2017.11.010]

9 **Ge X**, Cai Y, Chen Z, Gao S, Geng X, Li Y, Jia J, Sun Y. Bifunctional Enzyme SpoT Is Involved in Biofilm Formation of Helicobacter pylori with Multidrug Resistance by Upregulating Efflux Pump Hp1174 (*gluP*). *Antimicrob Agents Chemother* 2018; **62** [PMID: 30181372 DOI: 10.1128/AAC.00957-18]

10 **Bińkowska A**, Biernat MM, Łaczmański Ł, Gościniak G. Molecular Patterns of Resistance Among *Helicobacter pylori* Strains in South-Western Poland. *Front Microbiol* 2018; **9**: 3154 [PMID: 30619218 DOI: 10.3389/fmicb.2018.03154]

11 **Zhang XY**, Shen WX, Chen CF, Sheng HH, Cheng H, Li J, Hu F, Lu DR, Gao HJ. Detection of the clarithromycin resistance of Helicobacter pylori in gastric mucosa by the amplification refractory mutation system combined with quantitative real-time PCR. *Cancer Med* 2019; **8**: 1633-1640 [PMID: 30864275 DOI: 10.1002/cam4.1986]

12 **Matta AJ**, Zambrano DC, Pazos AJ. Punctual mutations in *23S rRNA* gene of clarithromycin-resistant *Helicobacter pylori* in Colombian populations. *World J Gastroenterol* 2018; **24**: 1531-1539 [PMID: 29662291 DOI: 10.3748/wjg.v24.i14.1531]

13 **Alarcón-Millán J**, Fernández-Tilapa G, Cortés-Malagón EM, Castañón-Sánchez CA, De Sampedro-Reyes J, Cruz-Del Carmen I, Betancourt-Linares R, Román-Román A. Clarithromycin resistance and prevalence of Helicobacter pylori virulent genotypes in patients from Southern México with chronic gastritis. *Infect Genet Evol* 2016; **44**: 190-198 [PMID: 27355861 DOI: 10.1016/j.meegid.2016.06.044]

14 **Kouitcheu Mabeku LB**, Eyoum Bille B, Tepap Zemnou C, Tali Nguefack LD, Leundji H. Broad spectrum resistance in Helicobacter pylori isolated from gastric biopsies of patients with dyspepsia in Cameroon and efflux-mediated multiresistance detection in MDR isolates. *BMC Infect Dis* 2019; **19**: 880 [PMID: 31640588 DOI: 10.1186/s12879-019-4536-8]

15 **Attaran B**, Falsafi T, Ghorbanmehr N. Effect of biofilm formation by clinical isolates of *Helicobacter pylori* on the efflux-mediated resistance to commonly used antibiotics. *World J Gastroenterol* 2017; **23**: 1163-1170 [PMID: 28275296 DOI: 10.3748/wjg.v23.i7.1163]

16 **Su P**, Li Y, Li H, Zhang J, Lin L, Wang Q, Guo F, Ji Z, Mao J, Tang W, Shi Z, Shao W, Zhu X, Zhang X, Tong Y, Tu H, Jiang M, Wang Z, Jin F, Yang N. Antibiotic resistance of Helicobacter pylori isolated in the Southeast Coastal Region of China. *Helicobacter* 2013; **18**: 274-279 [PMID: 23418857 DOI: 10.1111/hel.12046]

17 **Ranjbar R**, Farsani FY, Dehkordi FS. Phenotypic analysis of antibiotic resistance and genotypic study of the *vacA*, *cagA*, *iceA*, *oipA* and *babA* genotypes of the *Helicobacter pylori* strains isolated from raw milk. *Antimicrob Resist Infect Control* 2018; **7**: 115 [PMID: 30288255 DOI: 10.1186/s13756-018-0409-y]

18 **Humphries RM**, Kircher S, Ferrell A, Krause KM, Malherbe R, Hsiung A, Burnham CA. The Continued Value of Disk Diffusion for Assessing Antimicrobial Susceptibility in Clinical Laboratories: Report from the Clinical and Laboratory Standards Institute Methods Development and Standardization Working Group. *J Clin Microbiol* 2018; **56** [PMID: 29743302 DOI: 10.1128/JCM.00437-18]

19 **Goderska K**, Agudo Pena S, Alarcon T. Helicobacter pylori treatment: antibiotics or probiotics. *Appl Microbiol Biotechnol* 2018; **102**: 1-7 [PMID: 29075827 DOI: 10.1007/s00253-017-8535-7]

20 **Thung I**, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, Valasek MA. Review article: the global emergence of Helicobacter pylori antibiotic resistance. *Aliment Pharmacol Ther* 2016; **43**: 514-533 [PMID: 26694080 DOI: 10.1111/apt.13497]

21 **Munita JM**, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiol Spectr* 2016; **4** [PMID: 27227291 DOI: 10.1128/microbiolspec.VMBF-0016-2015]

22 **Lee CR**, Lee JH, Park M, Park KS, Bae IK, Kim YB, Cha CJ, Jeong BC, Lee SH. Biology of *Acinetobacter baumannii*: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. *Front Cell Infect Microbiol* 2017; **7**: 55 [PMID: 28348979 DOI:

10.3389/fcimb.2017.00055]
23 **Pang Z**, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnol Adv* 2019; **37**: 177-192 [PMID: 30500353 DOI: 10.1016/j.biotechadv.2018.11.013]
24 **Chung The H**, Baker S. Out of Asia: the independent rise and global spread of fluoroquinolone-resistant Shigella. *Microb Genom* 2018; **4** [PMID: 29595412 DOI: 10.1099/mgen.0.000171]
25 **Falsafi T**, Ehsani A, Attaran B, Niknam V. Association of hp1181 and hp1184 Genes With the Active Efflux Phenotype in Multidrug-Resistant Isolates of Helicobacter pylori. *Jundishapur J Microbiol* 2016; **9**: e30726 [PMID: 27303615 DOI: 10.5812/jjm.30726]
26 **van Amsterdam K**, Bart A, van der Ende A. A Helicobacter pylori TolC efflux pump confers resistance to metronidazole. *Antimicrob Agents Chemother* 2005; **49**: 1477-1482 [PMID: 15793129 DOI: 10.1128/AAC.49.4.1477-1482.2005]
27 **Kubanov A**, Vorobyev D, Chestkov A, Leinsoo A, Shaskolskiy B, Dementieva E, Solomka V, Plakhova X, Gryadunov D, Deryabin D. Molecular epidemiology of drug-resistant Neisseria gonorrhoeae in Russia (Current Status, 2015). *BMC Infect Dis* 2016; **16**: 389 [PMID: 27506605 DOI: 10.1186/s12879-016-1688-7]
28 **Miele A**, Goldstein BP, Bandera M, Jarvis C, Resconi A, Williams RJ. Differential susceptibilities of enterococcal species to elfamycin antibiotics. *J Clin Microbiol* 1994; **32**: 2016-2018 [PMID: 7989561 DOI: 10.1128/JCM.32.8.2016-2018.1994]
29 **Jin DJ**, Gross CA. Mapping and sequencing of mutations in the Escherichia coli rpoB gene that lead to rifampicin resistance. *J Mol Biol* 1988; **202**: 45-58 [PMID: 3050121 DOI: 10.1016/0022-2836(88)90517-7]
30 **Ballif M**, Harino P, Ley S, Coscolla M, Niemann S, Carter R, Coulter C, Borrell S, Siba P, Phuanukoonnon S, Gagneux S, Beck HP. Drug resistance-conferring mutations in Mycobacterium tuberculosis from Madang, Papua New Guinea. *BMC Microbiol* 2012; **12**: 191 [PMID: 22943573 DOI: 10.1186/1471-2180-12-191]
31 **Finken M**, Kirschner P, Meier A, Wrede A, Böttger EC. Molecular basis of streptomycin resistance in Mycobacterium tuberculosis: alterations of the ribosomal protein S12 gene and point mutations within a functional 16S ribosomal RNA pseudoknot. *Mol Microbiol* 1993; **9**: 1239-1246 [PMID: 7934937 DOI: 10.1111/j.1365-2958.1993.tb01253.x]
32 **Okamoto S**, Tamaru A, Nakajima C, Nishimura K, Tanaka Y, Tokuyama S, Suzuki Y, Ochi K. Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. *Mol Microbiol* 2007; **63**: 1096-1106 [PMID: 17238915 DOI: 10.1111/j.1365-2958.2006.05585.x]
33 **Sreevatsan S**, Pan X, Stockbauer KE, Williams DL, Kreiswirth BN, Musser JM. Characterization of rpsL and rrs mutations in streptomycin-resistant Mycobacterium tuberculosis isolates from diverse geographic localities. *Antimicrob Agents Chemother* 1996; **40**: 1024-1026 [PMID: 8849220 DOI: 10.1128/AAC.40.4.1024]
34 **Nair J**, Rouse DA, Bai GH, Morris SL. The rpsL gene and streptomycin resistance in single and multiple drug-resistant strains of Mycobacterium tuberculosis. *Mol Microbiol* 1993; **10**: 521-527 [PMID: 7968530 DOI: 10.1111/j.1365-2958.1993.tb00924.x]
35 **Brzostek A**, Sajduda A, Sliwiński T, Augustynowicz-Kopeć E, Jaworski A, Zwolska Z, Dziadek J. Molecular characterisation of streptomycin-resistant Mycobacterium tuberculosis strains isolated in Poland. *Int J Tuberc Lung Dis* 2004; **8**: 1032-1035 [PMID: 15305490]
36 **Wanke C**, Amrhein N. Evidence that the reaction of the UDP-N-acetylglucosamine 1-carboxyvinyltransferase proceeds through the O-phosphothioketal of pyruvic acid bound to Cys115 of the enzyme. *Eur J Biochem* 1993; **218**: 861-870 [PMID: 8281938 DOI: 10.1111/j.1432-1033.1993.tb18442.x]
37 **Kim DH**, Lees WJ, Kempsell KE, Lane WS, Duncan K, Walsh CT. Characterization of a Cys115 to Asp substitution in the Escherichia coli cell wall biosynthetic enzyme UDP-GlcNAc enolpyruvyl transferase (MurA) that confers resistance to inactivation by the antibiotic fosfomycin. *Biochemistry* 1996; **35**: 4923-4928 [PMID: 8664284 DOI: 10.1021/bi952937w]
38 **Cheng G**, Hu Y, Lu N, Li J, Wang Z, Chen Q, Zhu B. Identification of a novel fosfomycin-resistant UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) from a soil metagenome. *Biotechnol Lett* 2013; **35**: 273-278 [PMID: 23143172 DOI: 10.1007/s10529-012-1074-5]
39 **Takahata S**, Ida T, Hiraishi T, Sakakibara S, Maebashi K, Terada S, Muratani T, Matsumoto T, Nakahama C, Tomono K. Molecular mechanisms of fosfomycin resistance in clinical isolates of Escherichia coli. *Int J Antimicrob Agents* 2010; **35**: 333-337 [PMID: 20071153 DOI: 10.1016/j.ijantimicag.2009.11.011]
40 **Hormeño L**, Palomo G, Ugarte-Ruiz M, Porrero MC, Borge C, Vadillo S, Píriz S, Domínguez L, Campos MJ, Quesada A. Identification of the main quinolone resistance determinant in Campylobacter jejuni and Campylobacter coli by MAMA-DEG PCR. *Diagn Microbiol Infect Dis* 2016; **84**: 236-239 [PMID: 26658311 DOI: 10.1016/j.diagmicrobio.2015.11.002]
41 **Khan R**, Kong HG, Jung YH, Choi J, Baek KY, Hwang EC, Lee SW. Triclosan Resistome from Metagenome Reveals Diverse Enoyl Acyl Carrier Protein Reductases and Selective Enrichment of Triclosan Resistance Genes. *Sci Rep* 2016; **6**: 32322 [PMID: 27577999 DOI: 10.1038/srep32322]
42 **Battisti JM**, Smitherman LS, Samuels DS, Minnick MF. Mutations in Bartonella bacilliformis gyrB confer resistance to coumermycin A1. *Antimicrob Agents Chemother* 1998; **42**: 2906-2913 [PMID: 9797224 DOI: 10.1128/AAC.42.11.2906]

43 **Moffatt JH**, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, St Michael F, Cox AD, Adler B, Nation RL, Li J, Boyce JD. Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. *Antimicrob Agents Chemother* 2010; **54**: 4971-4977 [PMID: 20855724 DOI: 10.1128/AAC.00834-10]

44 **Beceiro A**, Moreno A, Fernández N, Vallejo JA, Aranda J, Adler B, Harper M, Boyce JD, Bou G. Biological cost of different mechanisms of colistin resistance and their impact on virulence in Acinetobacter baumannii. *Antimicrob Agents Chemother* 2014; **58**: 518-526 [PMID: 24189257 DOI: 10.1128/AAC.01597-13]

45 **Fournier B**, Aras R, Hooper DC. Expression of the multidrug resistance transporter NorA from Staphylococcus aureus is modified by a two-component regulatory system. *J Bacteriol* 2000; **182**: 664-671 [PMID: 10633099 DOI: 10.1128/jb.182.3.664-671.2000]

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