

Role of AlgC and GalU in the Intrinsic Antibiotic Resistance of *Helicobacter pylori*

Shunhang Feng^{1,2,*}, Jiansheng Lin^{1,2,*}, Xiaoyan Zhang^{1,2}, Xin Hong^{1,2}, Wanyin Xu^{1,2},
Yancheng Wen^{1,2}, Feifei She^{1,2}

¹Key Laboratory of Gastrointestinal Cancer (Fujian Medical University), Ministry of Education, Fuzhou, People's Republic of China; ²Fujian Key Laboratory of Tumor Microbiology, Department of Medical Microbiology, School for Basic Medical Sciences, Fujian Medical University, Fuzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Feifei She, Fujian Medical University, Xueyuan Road 1, Minhou County, Fuzhou, Fujian Province, People's Republic of China, Tel +86-135-1406-3583, Email Shefeifei@yeah.net; Yancheng Wen, Fujian Medical University, Xueyuan Road 1, Minhou County, Fuzhou, Fujian Province, People's Republic of China, Tel +86-157-0593-5209, Email hitwyc@qq.com

Purpose: *Helicobacter pylori* is associated with the development of gastrointestinal diseases. However, its eradication is challenged by an increased rate of drug resistance. AlgC and GalU are important for the synthesis of UDP-glucose, which is a substrate for the synthesis of lipopolysaccharide (LPS) in *H. pylori*. In this study, we investigated the role of UDP-glucose in the intrinsic drug resistance in *H. pylori*.

Methods: Gene knockout strains or complementation strains, including $\Delta algC$, $\Delta galU$, $\Delta galE$, $\Delta hp0045$, $\Delta algC/algC^*$ and $\Delta galU/galU^*$ were constructed in *Hp26695*; and $\Delta algC$ and $\Delta galU$ were also constructed in two clinical drug-resistant strains, *Hp008* and *Hp135*. The minimum inhibitory concentrations (MIC) of *H. pylori* to amoxicillin (AMO), tetracycline (TET), clarithromycin (CLA), metronidazole (MNZ), levofloxacin (LEV), and rifampicin (RIF) were measured using MIC Test Strips. Silver staining was performed to examine the role of AlgC and GalU in LPS synthesis. Ethidium bromide (EB) accumulation assay was performed to assess the outer membrane permeability of *H. pylori* strains.

Results: Knockout of *algC* and *galU* in *H. pylori* resulted in increased drug sensitivity to AMO, MNZ, CLA, LEV, and RIF; whereas knockout of *hp0045* and *galE*, which are involved in GDP-fucose and UDP-galactose synthesis, respectively, did not significantly alter the drug sensitivity of *H. pylori*. Knockout of *algC* and *galU* in clinically drug-resistant strains resulted in significantly increased drug sensitivity to all the antibiotics, except MNZ. The lipid A-core structure was altered in $\Delta algC$ and $\Delta galU$ when their EB accumulation was higher than that in the wild type and complementation strains.

Conclusion: UDP-glucose may play an important role in increasing drug resistance to AMO, MNZ, CLA, LEV, TET, and RIF by maintaining the lipid A-core structure and decreasing membrane permeability. AlgC and GalU may serve as potential drug targets for decreasing antibiotic resistance in clinical isolates.

Keywords: *H. pylori*, UDP-glucose, *algC*, *galU*, intrinsic multidrug resistance

Introduction

Helicobacter pylori is a gram-negative, spiral-shaped, microaerophilic bacterium that infects more than half of the world's population.¹ *H. pylori* infection is characterized by the development of gastrointestinal diseases, including chronic atrophic gastritis, peptic ulcer, gastric cancer, and gastric mucosa-associated lymphoma.² Furthermore, a *H. pylori* infection lasts a lifetime, unless eradicated with antibiotics.³ *H. pylori* infection can be eradicated by administering triple or quadruple therapy using at least two different antibiotics, clarithromycin (CLA) and amoxicillin (AMO).⁴ However, the eradication rate has decreased to < 80% due to bacterial drug resistance, particularly to CLA.⁵

Lipopolysaccharide (LPS), one of the main components of the bacterial outer membrane, is typically composed of lipid A, core oligosaccharides, and O antigens. The distribution of LPS on the outer leaf of the outer membrane provides

a potent barrier to antibiotics.⁶ UDP-glucose is one of the major building blocks of polysaccharide.⁷ Notably, the UDP-glucose synthetic pathway is highly conserved in gram-negative bacteria. The *de novo* biosynthesis of UDP-glucose involves three steps: (a) generation of α -D-glucose-6-phosphate by a glucokinase, (b) conversion of glucose-6-phosphate into glucose-1-phosphate by phosphoglucomutase (PGM), and (c) transformation of α -D-glucose-1-phosphate and UTP into UDP-glucose by UDP-Glc pyrophosphorylase (UGP), also known as GalU. PGM plays an important role in the synthesis of LPS and in the antibiotic resistance of various pathogens, such as *Salmonella* Typhimurium, *Yersinia pestis*, and *Stenotrophomonas maltophilia*.^{8–10} UGP is associated with LPS synthesis, drug resistance, and bacterial virulence in pathogens, such as *Haemophilus parasuis*, *Proteus mirabilis*, and *Vibrio cholerae*.^{11–13} Therefore, UDP-glucose may play an important role in the intrinsic drug resistance of gram-negative bacteria. However, the role of UDP-glucose in the drug resistance of *H. pylori* has not been elucidated yet.

H. pylori harbors two ortholog genes, *hp1275(algC)* and *hp0646(galU)* that encode PGM and UGP, respectively. In *H. pylori*, AlgC was suggested to have phosphomannomutase (PMM) activity, in addition to PGM activity (Figure 1). Furthermore, *algC* inactivation can lead to the disruption of UDP-galactose and GDP-fucose synthesis, both of which are active glycosyl donors for LPS. Moreover, the disruption of GDP-fucose synthesis resulted in LPS truncation, thereby increasing the susceptibility of *H. pylori* to SDS and novobiocin.¹⁴ In this study, we constructed $\Delta algC$ and $\Delta galU$ mutants of *H. pylori* and investigated their drug sensitivity, LPS structure, and outer membrane permeability. We found that AlgC and GalU were critical for the intrinsic resistance of *H. pylori*. Knockout of either *algC* or *galU* resulted in the alteration of the lipid A-core structure and higher outer membrane permeability. Furthermore, knockout of either *algC* or *galU* in two clinically drug-resistant strains significantly increased the antibiotic sensitivity of *H. pylori*, especially to CLA, rifampicin (RIF), and levofloxacin (LEV).

Materials and Methods

Bacterial Strains and Growth Conditions

The *H. pylori* strains *Hp26695*, *Hp008*, *Hp135*, and their isogenic mutant strains were cultured on Columbia blood agar (OXOID, Basingstoke, UK) plates supplemented with 5% sheep blood or in Brucella broth (BD, Sparks, MD, USA) supplemented with 10% fetal bovine serum (PAN Seratech, Aidenbach, Germany) (BB+FBS) with gentle agitation (120 rpm) at 37 °C under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). Kanamycin (5 μ g/mL; MP Biomedicals LLC, Solon, OH, USA) or chloramphenicol (4 μ g/mL; Inalco S.p.A., Milano, Italy) was added to *H. pylori* isogenic or complemented mutant strains when constructing and screening positive mutants.

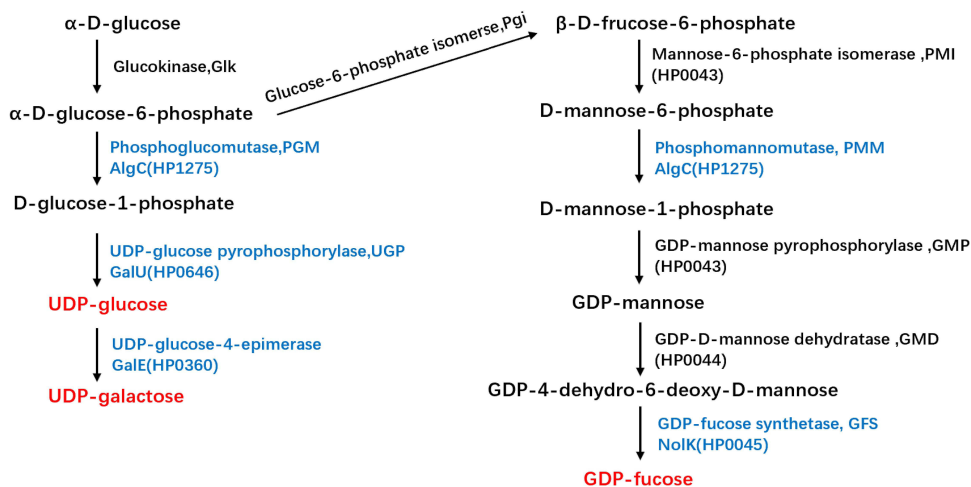


Figure 1 Biosynthetic pathways for UDP-glucose, UDP-galactose, and GDP-fucose synthesis in *H. pylori*. The three sugar nucleotides are marked in red and the enzymes inactivated in this study are marked in blue.

Construction of *algC*, *galU*, *hp0045*, or *galE* Knockout Mutants in *Hp26695*, *Hp008* and *Hp135*, and Complementation of *algC* or *galU* in $\Delta algC$ and $\Delta galU$

To knockout *algC* from the chromosomes of *Hp26695*, *Hp008*, and *Hp135*, the upstream sequence of *algC* was amplified using the primers *algC*-up-FI and *algC*-up-RI, or *algC*-up-FII and *algC*-up-RII; downstream sequence of *algC* was amplified using *algC*-down-FI and *algC*-down-RI, or *algC*-down-FII and *algC*-down-RII; and a nonpolar kanamycin resistance cassette was amplified using *aphA*-F and *aphA*-R (primer sequences are listed in [Supplementary Table 1](#)). The plasmid pBluescript SK II (-) was ligated with the upstream and downstream sequence of *algC* flanked via a nonpolar kanamycin resistance cassette using ClonExpress[®] Ultra One Step Cloning Kit (C115, Vazyme Biotech, Nanjing, China). The resulting plasmid, pBluescript-*algCKO*, was transformed into *E. coli DH5 α* , and plasmid construction was confirmed via DNA sequencing. $\Delta algC$ strains were obtained by transforming pBluescript-*algCKO* into *Hp26695*, *Hp008*, and *Hp135* through electroporation, as described previously.¹⁵ The construction of $\Delta galU$, $\Delta hp0045$, and $\Delta galE$ was performed using a similar method, except for the primers used ([Supplementary Table 1](#)).

To complement *algC* in $\Delta algC$, a pBlcom-hp0203-hp0204-cat vector constructed previously was used as template and amplified using the primers *algC*-com-inverse-F and *algC*-com-inverse-R.¹⁶ The coding sequence of *algC* was amplified using the *algC*-COM-F and *algC*-COM-R. Subsequently, these fragments were ligated, generating pBlue-Com-*AlgC*, which was further transformed into *E. coli DH5 α* . The complementation strain $\Delta algC/algC^*$ was obtained by transforming pBlue-Com-*AlgC* into $\Delta algC$, as described previously.¹⁶ $\Delta galU/galU^*$ was obtained using a similar method except for the primers listed in [Supplementary Table 1](#).

Antibiotic Susceptibility Test

The minimum inhibitory concentrations (MIC) of *H. pylori* against amoxicillin (AMO), tetracycline (TET), clarithromycin (CLA), metronidazole (MNZ), levofloxacin (LEV), and rifampicin (RIF) for all strains were determined using the diffusion MIC test strips (Liofilchem, Roseto degli Abruzzi, Italy). MIC values were measured as previously described,¹⁷ with slight modifications. Briefly, *H. pylori* strains were cultured on Columbia agar plates for 3 d, and colonies were collected and resuspended in Brucella broth to a turbidity value of 2 McFarland units measured using a DensiCHEK[™] plus Instrument (BioMerieux Inc, Loveland, CO, USA). Subsequently, the samples were spread evenly on Mueller–Hinton blood agar (OXOID, Basingstoke, UK) using sterile cotton swabs, and MIC Test Strips were placed on the surface of the agar. MIC was determined after 3 d of incubation under 37 °C according to the concentration of the strips at the intersection of the growth inhibition zone. Each experiment was repeated at least thrice.

Growth Curves and Growth Inhibition Curves

To monitor the growth curve, *H. pylori* strains were cultured in Brucella broth supplemented with 10% fetal bovine serum, with an initial OD₆₀₀ of 0.05 in the presence or absence of antibiotics at the indicated concentrations, and cell density was measured every 12 h. The results are the average means of at least three biological replicates.

LPS Extraction and Silver Staining

LPS extraction was performed as described previously.¹⁸ Briefly, bacterial cells harvested from Columbia agar plates were harvested and resuspended in 1 mL of Brucella broth with OD₆₀₀ of approximately 3.0; and subsequently washed by PBS (137mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 2mM KH₂PO₄; pH 7.4), and resuspended in of 100 μ L LPS lysis buffer (2% SDS, 4% β -mercaptoethanol, 0.1% bromophenol blue, 10% glycerol, and 1 M Tris-HCl; pH 6.8). Each sample was then heated at 100 °C for 10 min. The cooled samples were incubated overnight at 55 °C with 5 μ L of proteinase K (20 mg/mL). The samples were then subjected to 15.5% Tricine-SDS-PAGE. The LPS profile was visualized using a Fast Silver Stain Kit (Beyotime, Shanghai, China), according to the manufacturer's instructions.

Ethidium Bromide Accumulation Assay

H. pylori cultured on Columbia blood agar was harvested, and the bacterial cells were washed twice and resuspended in PBS (137mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 2mM KH₂PO₄; pH 7.4) at a density of OD₆₀₀~0.4. Subsequently, 10 μ M of

carbonyl cyanidem-chlorophenylhydrazine (CCCP; C2759, Sigma-Aldrich, St-Louis, Missouri, USA) was added, and the samples were incubated for 30 min at 37 °C to deplete metabolic energy. Subsequently, 2 µM of EB was added and EB fluorescence intensity was measured for 30 min at an interval of 60s using an EnSight™ multimode plate reader (Perkin Elmer, Waltham, MA, USA), with an excitation wavelength of 545 nm and an emission wavelength of 590 nm.

Statistical Analysis

All experiments were performed at least three times, and the results were analyzed using GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, United States). Student's *t*-test was used to evaluate the significance of the differences. A *P*-value < 0.05 was considered statistically significant.

Results

Knockout of Either *algC* or *galU* Results in Higher Antibiotic Susceptibility in *H. pylori*

To determine whether UDP-glucose synthesis is involved in the antibiotic susceptibility in *H. pylori*, we constructed *Hp26695ΔalgC* and *Hp26695ΔgalU* and the complementation strains *Hp26695ΔalgC/algC** and *Hp26695ΔgalU/galU**. The MIC values of each strain for AMO, MNZ, CLA, LEV, TET, and RIF were determined using the MIC Test Strip. The MIC values of both *ΔalgC* and *ΔgalU* for AMO, MNZ, CLA, LEV, TET, and RIF were 2-, 2.7-, 16.5-, 6-, 4-, and 4-fold, respectively, lower than those in the wild-type *Hp26695* (Table 1). Furthermore, the complementation strains *Hp26695ΔalgC/algC** and *Hp26695ΔgalU/galU** showed MIC values similar to those of the wild-type strain. These results suggest that AlgC and GalU contribute to the intrinsic drug resistance of *H. pylori*.

Furthermore, AlgC participates in the synthesis of GDP-fucose, which is a substrate for the O antigen.^{19,20} While GalE is a UDP-glucose-4-epimerase that transfers UDP-glucose to UDP-galactose, another substrate incorporated into the O-side chain of LPS (Figure 1).²¹ Therefore, we constructed *Hp26695Δhp0045* and *Hp26695ΔgalE* to disrupt the synthesis of GDP-fucose and UDP-galactose, respectively. We then investigated the role of GalE and HP0045 in the drug sensitivity of *H. pylori* (Table 1). The results showed that the MIC values of *ΔgalE* and *Δhp0045* for AMO, MNZ, CLA, LEV, TET, and RIF were significantly higher than those for *ΔalgC* and *ΔgalU*, suggesting that UDP-glucose synthesis plays a more vital role in the intrinsic resistance of *H. pylori* to antibiotics, compared to UDP-galactose or GDP-fucose.

We also compared the growth curves of *H. pylori* wild type, *ΔalgC*, and *ΔgalU* in the presence or absence of antibiotics to confirm the role of UDP-glucose synthesis in the antibiotic resistance of *H. pylori* (Figure 2). In the presence of antibiotics, the growth of *ΔgalU* and *ΔalgC* was completely inhibited by CLA, RIF, TET, and AMO, whereas it was significantly reduced by LEV and MNZ, compared to that of the wild-type and complementation strains. These results suggest that UDP-glucose is critical for the intrinsic bacterial resistance of *H. pylori* to various antibiotics.

Table 1 MICs (µg/mL) of Hp26695 Strains to Antibiotics

Strain	Hp26695						
	WT	Δ <i>algC</i>	Δ <i>algC/algC*</i>	Δ <i>galU</i>	Δ <i>galU/galU*</i>	Δ <i>galE</i>	Δ <i>hp0045</i>
Amoxicillin	0.125	0.064	0.125	0.064	0.125	0.094	0.094
Metronidazole	4	1.5	4	1.5	4	3	4
Clarithromycin	0.38	0.023	0.38	0.023	0.38	0.125	0.094
Levofloxacin	0.25	0.047	0.25	0.047	0.25	0.19	0.19
Tetracycline	0.25	0.064	0.25	0.064	0.25	0.25	0.25
Rifampicin	0.38	0.094	0.38	0.094	0.38	0.19	0.25

Abbreviations: WT, wild type strain; Δ*algC*, *algC* knockout strain; Δ*algC/algC**, *algC* complementation strain; Δ*galU*, *galU* knockout strain; Δ*galU/galU**, *galU* complementation strain; Δ*galE*, *galE* knockout strain; Δ*hp0045*, *hp0045* knockout strain.

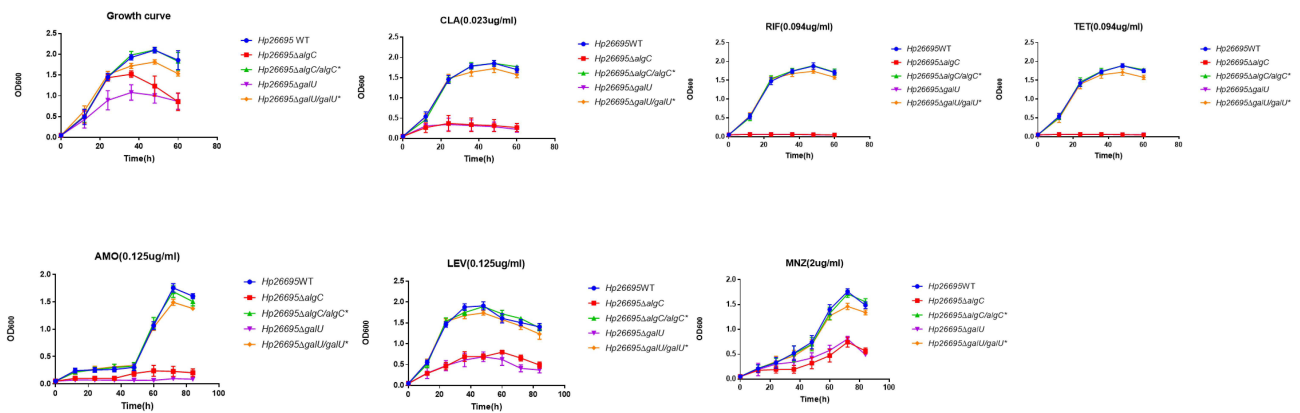


Figure 2 Influence of AlgC and GalU in drug resistance of *H. pylori*. Growth curves of *Hp26695*, $\Delta algC$, $\Delta algC/algC^*$, $\Delta galU$ and $\Delta galU/galU^*$ with CLA (0.023 $\mu\text{g}/\text{mL}$), RIF (0.094 $\mu\text{g}/\text{mL}$), TET (0.094 $\mu\text{g}/\text{mL}$), AMO (0.125 $\mu\text{g}/\text{mL}$), LEV (0.125 $\mu\text{g}/\text{mL}$) and MNZ (2 $\mu\text{g}/\text{mL}$). The data represents the mean \pm SEM of three independent experiments.

Disruption of AlgC or GalU Increases Drug Sensitivity of Clinical Drug-Resistant Isolates

We also investigated whether the disruption of UDP-glucose synthesis restores the drug sensitivity of clinical drug-resistant isolates. Two clinical drug-resistant *H. pylori* isolates, *Hp135* and *Hp008*, were used in this study. *Hp008* is resistant to MNZ, CLA, LEV, and RIF; and *Hp135* is resistant to CLA and MNZ. Resistance of *Hp135* and *Hp008* to antibiotics was due to drug resistance-associated point mutations, which were confirmed via DNA sequencing (data not shown). We constructed a knockout mutation of *algC* and *galU* in *Hp135* and *Hp008*, and investigated the drug sensitivity of these strains using MIC test strips (Table 2). Except for MNZ, knockout of either *algC* or *galU* reduced the MIC of *Hp008* to CLA from >256 to 8 $\mu\text{g}/\text{mL}$, and that of *Hp135* to CLA from >256 to 0.016 $\mu\text{g}/\text{mL}$. The MICs of $\Delta algC$ and $\Delta galU$ against AMO, LEV, TET, and RIF were 2-, 8-, 2.9-, and 32-fold, respectively, lower than those of the wild-type strain in *Hp008*, whereas the MICs of $\Delta algC$ and $\Delta galU$ against AMO, LEV, TET, and RIF were 2-, 4-, 4.1-, and 8.1-fold, respectively, lower than those of the wild-type strain in *Hp135*. These results suggest that the inactivation of UDP-glucose synthesis resulted in a significant increase in the drug sensitivity of the clinical isolates to CLA, TET, RIF, LEV, and AMO.

Knockout of Either *algC* or *galU* Altered LPS Structure

The structure of LPS is important for maintaining drug sensitivity. Therefore, we verified whether loss of *algC* and *galU* affected LPS structure. LPS from wild-type, $\Delta algC$, $\Delta algC/algC^*\Delta galU$, and $\Delta galU/galU^*$ strains was isolated and visualized via silver staining (Figure 3). The results showed that the lipid A-core in $\Delta algC$ and $\Delta galU$ were significantly different from that in the wild-type strains. Moreover, complementation of either *algC* or *galU* in the cognate mutant strain restored the lipid A-core profile, thereby confirming that AlgC and GalU participated in the synthesis or structure of the lipid A-core.

Table 2 MICs ($\mu\text{g}/\text{mL}$) of Two Clinical Drug-Resistant *H. pylori* Isolates

Antibiotic \ Strain	<i>Hp008</i>			<i>Hp135</i>		
	WT	$\Delta algC$	$\Delta galU$	WT	$\Delta algC$	$\Delta galU$
Amoxicillin	0.047	0.023	0.023	0.032	<0.016	<0.016
Metronidazole	>256	>256	>256	>256	>256	>256
Clarithromycin	>256	8	8	>256	0.016	0.016
Levofloxacin	>32	4	4	0.5	0.125	0.125
Tetracycline	0.047	0.016	0.016	0.094	0.023	0.023
Rifampicin	4	0.125	0.125	0.38	0.047	0.047

Abbreviations: WT, wild type strain; $\Delta algC$, *algC* knockout strain; $\Delta galU$, *galU* knockout strain.

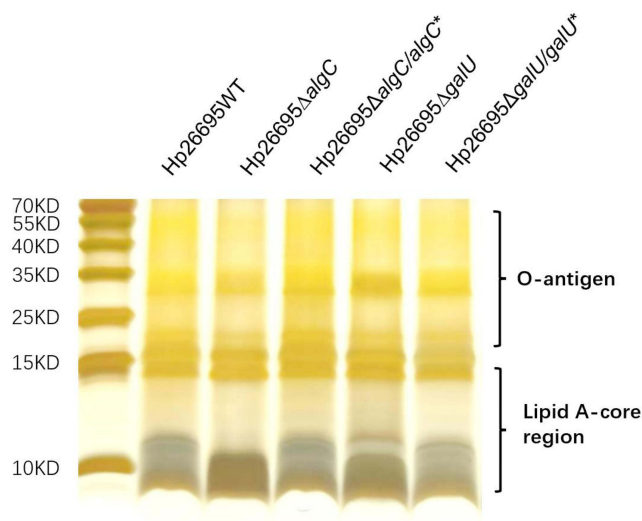


Figure 3 LPS profile of *H. pylori*. Silver-stained Tricine-SDS-PAGE gel (15.5%) depicting the LPS profiles from wild type, *algC* knockout, *algC* complementation, *galU* knockout, and *galU* complementation in the strain *Hp26695*. This experiment has been repeated independently for at least three times with similar results.

Knockout of *algC* and *galU* Increases Bacterial Outer Membrane Permeability

LPS establishes a membrane barrier for the entry of several antibiotics.^{22,23} Therefore, we further investigated whether AlgC and GalU influenced outer membrane permeability by comparing the ethidium bromide (EB) accumulation among the strains wild type, $\Delta algC$, $\Delta galU$, $\Delta algC/algC^*$ and $\Delta galU/galU^*$ (Figure 4). CCCP, an efflux inhibitor, was added to eliminate the effects of efflux pumps harbored by *H. pylori*. In the presence of EB, the fluorescence of the wild type increased at a significantly slower rate than that of $\Delta algC$ or $\Delta galU$, suggesting $\Delta algC$ or $\Delta galU$ harbors a higher outer membrane permeability comparing to wild-type strain. Complementation of *algC* or *galU* reduced the fluorescence of $\Delta algC$ or $\Delta galU$ to a level similar to that of the wild type, suggesting that AlgC and GalU are important for outer membrane permeability of *H. pylori*.

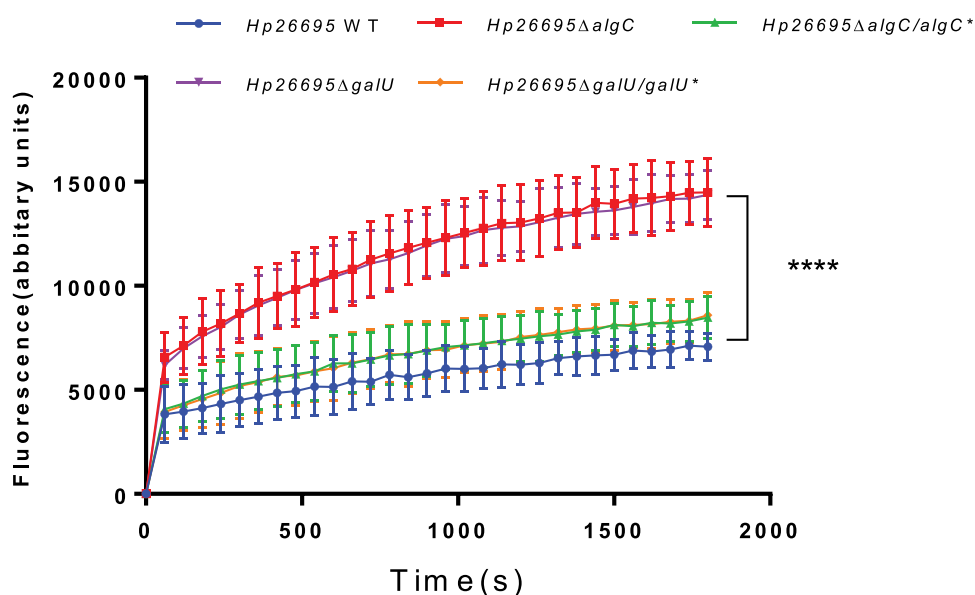


Figure 4 Comparison of EB accumulation in various types of *H. pylori* strains. The fluorescence intensity was recorded at excitation and emission wavelengths of 545 and 590 nm, respectively, over a 30-min incubation period. The data represents the mean \pm SEM of three independent experiments. A paired Student's *t* test was performed for comparing the EB accumulation between the WT and gene knockout strains ($\Delta algC$ and $\Delta galU$). **** $P < 0.0001$.

Discussion

The rise in antibiotic resistance in *H. pylori* has led to a declining eradication rate, thereby necessitating new antimicrobial strategies.^{4,24} LPS is a major component of the outer leaflet of the outer membrane, and is considered to be of great importance in the intrinsic resistance of gram-negative bacteria.²⁵ An outer membrane-penetrating agent to disrupt the outer membrane can be used to increase antimicrobial activity and broaden antimicrobial spectrum, even in clinical drug-resistant strains.²⁶ Targeting the enzymes involved in LPS synthesis is also a prospective strategy to increase bacterial sensitivity to antibiotics.

UDP-glucose is involved in cell envelope biosynthesis, and its role in LPS synthesis has been confirmed in gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Bordetella bronchiseptica*, *Stenotrophomonas maltophilia*, and *Proteus mirabilis* etc.^{10,12,27–29} In this study, we found that AlgC and GalU are important for maintaining LPS structure and intrinsic drug sensitivity. This result is in agreement with previous studies on other gram-negative bacteria. In *Bordetella bronchiseptica*, loss of PGM caused apparent physical changes in the electrophoretic profiles of LPS, making bacteria more vulnerable to treatment with antimicrobial peptides and oxidative stress.²⁹ In *Stenotrophomonas maltophilia*, PGM mutant strains displayed shorter O-polysaccharide chains and higher sensitivity to nalidixic acid, gentamicin, polymyxin B, and polymyxin E than the wild-type strains.¹⁰ Similarly, disruption of GalU in *Vibrio cholerae* and *Proteus mirabilis* increased the sensitivity to novobiocin, SDS, bile salts etc.^{12,13}

We also found that the disruption of GalE (encoding UDP-galactose 4-epimerase) and HP0045 (encoding GDP-l-fucose synthetase) had no significant effect on the regulation of bacterial drug sensitivity in *H. pylori* (Table 1). These results suggest that GDP-fucose and UDP-galactose play less important roles in intrinsic drug resistance of *H. pylori*. A similar result was reported in a study on *Vibrio cholerae*, where the disruption of *galU* increased sensitivity to antibiotics, such as novobiocin, polymyxin, and the hydrophilic agent SDS, whereas the disruption of *galE* played no significant role.¹³

CLA-resistant *H. pylori* has been listed as a high-priority pathogen for the development of new drugs.³⁰ In the present study, the knockout of either *algC* or *galU*, in the two clinical antibiotic-resistant isolates, greatly improved antibiotic sensitivity, especially for CLA (Table 2). *Hp008* carries the drug resistance point mutations 2143A>G and 2182T>C, whereas *Hp135* carries 2182T>C in 23S rRNA, as confirmed by sequencing.^{31,32} The loss of either *algC* or *galU* leads to an increase in the membrane permeability of bacteria, thereby increasing the bactericidal efficacy of CLA. However, unlike CLA, knockout of *algC* and *galU* did not change high resistance to MNZ in these clinical isolates (Table 2). MNZ resistance in *Hp008* and *Hp135* was due to mutations in *rdxA*, which was also confirmed via sequencing. *Hp008* carries R16C, H53R, and V144A point mutations, whereas *Hp135* carries a Q50-stop mutation. MNZ is a prodrug that requires a nitroreductase, encoded by *rdxA*, to convert MNZ to a bactericidal agent.^{33,34} This explains why a higher outer membrane permeability in *algC* and *galU* mutants did not increase bacterial sensitivity to MNZ.

Lipid A, the anchor for LPS molecules on the outer membrane, directly influences membrane properties.³⁵ Inhibition of lipid A biosynthesis is lethal to most gram-negative bacteria, whereas reduced lipid A biosynthesis leads to hypersensitivity to a wide range of antibiotics.^{36,37} In this study, LPS structural analysis, via silver staining, indicated that the loss of *algC* or *galU* caused changes in the lipid A-core region, while not altering the O-antigen (Figure 3). This result is also consistent with reports on other gram-negative bacteria. In *Pseudomonas aeruginosa*, loss of *algC* altered the LPS core region.^{10,27} Jutta et al reported that LPS in the mutant of *galU* showed altered core oligosaccharide, but with intact O antigen in *Vibrio cholerae*.¹³ Similar to our results from a recent study, *algC* knockout also led to an aberrant core region in *H. pylori*.¹⁴ However, mutations in LPS synthesis-related genes do not always increase membrane permeability. Our previous study showed that the loss of *rfaF* encoding lipopolysaccharide heptosyltransferase II, which transfers a second heptose residue into the LPS core, reduced membrane permeability.³⁸ Therefore, we conclude that the structure of lipid A-core region plays an important role in the outer membrane permeability and intrinsic resistance of *H. pylori*, and the UDP-glucose synthesis pathway can serve as a good drug target to increase the sensitivity of clinical drug-resistant strains.

In addition to LPS synthesis, AlgC and GalU are also involved in bacterial adaptability, growth, and metabolism.^{7,39} In *H. pylori*, UDP-glucose is also required for the synthesis of cholesterol glucosides, which are vital components of the cell envelope

involved in host immune escape and resistance to toxic compounds.^{40,41} In addition, bacterial PGM showed low homology with eukaryotes.^{39,42} Bacterial UGPs, encoded by *galU*, are unrelated to their eukaryotic counterparts.⁴³ AlgC and GalU are potential targets for developing bacteria-targeted drugs that can reduce the drug resistance of clinical multidrug-resistant *H. pylori* strains. This could be a useful strategy as an antibiotic adjuvant to overcome the challenge of drug resistance. However, the limitation of this study is that the findings rely on in vitro experiments. Since the host environment is complex, whether similar results can be obtained in vivo requires further investigation.

Conclusion

Taken together, our results suggest that AlgC and GalU might influence LPS structure and outer membrane permeability through the synthesis of UDP-glucose. Since we have confirmed that the disruption of the synthesis of UDP-galactose and GDP-fucose exhibited no significant effect on antibiotic resistance, we speculate that UDP-glucose is important for the intrinsic drug resistance of *H. pylori*. AlgC and GalU might serve as drug targets for clinical multidrug-resistant strains of *H. pylori*.

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Disclosure

The authors declare no competing interests.

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