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ORIGINAL ARTICLE

Correlation Analysis of Pathways and Operons of *Helicobacter pylori* Resistance Genes Using Bibliometrics

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SUMMARY

Background: ***Helicobacter pylori*** **is a gram-negative bacterium infecting approximately 50% of the world's population. Antibiotic resistance in** ***H. pylori*** **has significantly increased due to the overuse and misuse of common antibiotics. Mutations in the** ***23S rRNA gene*** **and other** ***H. pylori*** **genetic mutations have been identified as significant drivers in the emergence of resistance. Therefore, there is an urgent need for genetic analysis of** ***H. pylori*** **antibiotic** ***resistance*****.**

Methods: **Published** ***H. pylori*** **resistance genes were collected from PubMed websites by bibliometrics. Pathway analysis and operon analysis were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Database for Annotation, Visualization and Integrated Discovery (DAVID), and Database of prOkaryotic OpeRons (DOOR) databases to investigate pathways and biological functions of resistance genes and statistically discover novel resistance genes.**

Results: **A total of 148 genes were mined from the literature using bibliometrics, and 46 enriched pathways were identified using KEGG. Subsequently, 7 novel resistant genes of** ***H. pylor,*** **including HP0776, HP0192, HP0193, HP0475, HP1057, HP0632, and HP0633, were identified and predicted by functional enrichment analysis of pathways and operons.**

Conclusions: **The discovery of these novel** ***H. pylori*** **resistance genes is of great significance to treat** ***H. pylori*****-induced diseases and develop optimal treatment regimens. They also provide theoretical fundamentals for epidemiological prevention and strengthen our understanding of the molecular mechanism of** ***H. pylori*** **resistance to antibiotics.**

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KEY WORDS

Helicobacter pylori, resistance genes, bibliometrics, pathway analysis, operon

INTRODUCTION

Helicobacter pylori is a gram-negative, microaerophilic bacteria that infects approximately half of the global population, with an average incidence rate of 40% to 80%. Infection with *H. pylori* causes gastric mucosal inflammation, and although it is frequently asymptomatic early in life, it may progress to more serious outcomes including chronic active gastritis, gastric duodenal ulcers, gastric mucosal-related lymphatic tissue lymphoma, and gastric cancer [1,2]. The only effective evidence-based strategy for timely eradication of *H. pylori* infection is antimicrobial therapy that reduces the risk of *H. pyl*ori-related diseases and subsequent morbidity and mortality [3]. The numerous antibiotics increases the risk of antimicrobial resistance, resulting in treatment failure in many cases [4].

Significant progress in understanding the molecular mechanisms of *H. pylori* resistance to antimicrobials has been made in recent years [5]. Two categories of *H. pylori* resistance to antibiotics, primary resistance and secondary (acquired) resistance, have been proposed by the World Health Organization [6]. Primary resistance is mainly derived from heredity changes in *H. pylori* through selection, whereas secondary resistance is mostly caused by the overuse of antibiotics [7]. Commonly used antibiotics for the eradication of *H. pylori* include imidazole (metronidazole or tinidazol), macrolide (clarithromycin or azithromycin), amoxicillin, quinolones, tetracycline, and furazolidone [8]. Previous studies revealed that point mutations on the 23S rRNA gene and other genes including gyrA, PBP1, and 16S rRNA result in significant resistance of *H. pylori* to these antibiotics [9]. However, the interaction and biological functions of these relevant resistance genes remain to be elucidated.

The objectives of the present study were to identify novel *H. pylori* resistance genes and analyze the associations of *H. pylori* resistance genes in pathway, gene function, and operon analyses through data mining and functional enrichment analysis. In the present study, a set of published *H. pylori* resistance genes were mined by bibliometrics and then functionally analyzed with the following databases: KEGG (Kyoto Encyclopedia of Genes and Genomes), DAVID (Database for Annotation, Visualization and Integrated Discovery), and DOOR (Database of prOkaryotic OpeRons). Our study provides theoretical fundamentals to aid our understanding of the molecular mechanisms of *H. pylori* resistance to antibiotics, and may provide insight for novel drug development for *H. pylori*-related diseases.

MATERIALS AND METHODS

Bibliometric method

Resistance genes published from 2003 to 2018 were searched in PubMed using a bibliometric method as previously described [10], with the keywords "*Helicobacter pylori*", "resistance", and "gene". Using Epidata 3.1, duplicated publications and unrelated literature were deleted by parallel entry and logical error test. A total of 582 publications were retrieved from PubMed, of which 326 were used to analyze the resistance gene modules [10,11].

KEGG analysis, data sources, and pretreatment

The KEGG pathway database contains a collection of manually curated pathway maps representing the molecular interaction, reaction, and relation networks for genomes, biological pathways, diseases, drugs, and chemical substances. In this study, a total of 46 relevant KEGG pathways were downloaded from the KEGG database (http://ftp.genome.jp/pub/kegg/) in May 2018 [12].

Gene function annotation analysis

The functional analysis and pathway classification of the genes were performed using the DAVID (http://david.abcc.ncifcrf.gov/) and KEGG databases, respectively [13].

Operon analysis

The operon database was downloaded from DOOR2 (Database of prOkaryotic OpeRons, Version 2.0) (http://csbl.bmb.uga.edu/DOOR/index.php) and was developed using the Computational Systems Biology Lab (CSBL) at the University of Georgia [14]. The operons in this database were predicted based on essential genomic features. The operon database algorithm is a data-mining classifier, featuring intergenic distance, neighborhood conservation, phylogenetic distance, information from short DNA motifs, similarity scores between GO terms of gene pairs, and length ratios between a pair of genes.

RESULTS AND DISCUSSION

Correlation analysis of H. pylori resistance genes and pathways

A total of 148 *H. pylori* resistance genes that have been confirmed were identified through bibliometrics (Table S1 and Table 1) and account for approximately 9.5% of the entire Hp26695 genome (1,555 genes in total for *H. pylori* strain 26,695). Forty-six pathways related to these genes (Table S2 and Table 2) were identified using the KEGG database. Of the 148 resistance genes, 77 genes (52.03%) were enriched in relative pathways (Table S3). As shown in Table 1, three resistance genes (HP1111, HP1110 and HP0191) were involved in 10 pathways, and 14 genes were related to at least five

Table 1. Drug resistant genes of *H. pylori* that were distributed in at least three pathways.

Gene	Pathway	Pathway number
HP1110	hpy00010/hpy00020/hpy00620/hpy00640/hpy00650/hpy00680/hpy01100/hpy01120/hpy01130/hpy01200	10
HP1111	hpy00010/hpy00020/hpy00620/hpy00640/hpy00650/hpy00680/hpy01100/hpy01120/hpy01130/hpy01200	10
HP0191	hpy00020/hpy00190/hpy00620/hpy00650/hpy01100/hpy01110/hpy01120/hpy01130/hpy01200/hpy02020	10
HP0154	hpy00010/hpy00680/hpy01100/hpy01110/hpy01120/hpy01130/hpy01200/hpy01230/hpy03018	9
HP1108	hpy00010/hpy00020/hpy00620/hpy00640/hpy00650/hpy00680/hpy01100/hpy01120/hpy01130	9
HP1109	hpy00010/hpy00020/hpy00620/hpy00640/hpy00650/hpy00680/hpy01100/hpy01120/hpy01130	9
HP0589	hpy00010/hpy00020/hpy00620/hpy00650/hpy01100/hpy01120/hpy01130/hpy01200	8
HP0590	hpy00010/hpy00020/hpy00620/hpy00650/hpy01100/hpy01120/hpy01130/hpy01200	8
HP1470	hpy00230/hpy00240/hpy01100/hpy03030/hpy03410/hpy03420/hpy03440	7
HP1022	hpy00230/hpy00240/hpy01100/hpy03030/hpy03410/hpy03420/hpy03440	7
HP1277	hpy00260/hpy00400/hpy01100/hpy01110/hpy01130/hpy01230	6
HP0072	hpy00220/hpy00230/hpy01100/hpy01120/hpy05120	5
HP0073	hpy00220/hpy00230/hpy01100/hpy01120/hpy05120	5
HP0294	hpy00330/hpy00360/hpy00380/hpy00643/hpy01120	5
HP0615	hpy03030/hpy03410/hpy03420/hpy03430	4
HP0588	hpy00020/hpy01100/hpy01100/hpy01200	4
HP0591	hpy00020/hpy01100/hpy01100/hpy01200	4
HP1198	hpy00230/hpy00240/hpy01100/hpy03020	4
HP1293	hpy00230/hpy00240/hpy01100/hpy03020	4
HP0075	hpy00520/hpy01100/hpy01130	3
HP0220	hpy00730/hpy01100/hpy04122	3
HP0237	hpy00860/hpy01100/hpy01110	3

Table 2. Pathways with more than five resistance genes *in H. pylori.*

Pathway	Gene	Gene No.
hpy01100	HP1110/HP0588/HP1111/HP0154/HP1108/HP1109/HP0589/HP0590/HP1277/HP0072/HP0073/HP0591/HP1198/HP1293/ HP0075/HP0220/HP0237/HP0005/HP1583/HP0191/HP1022/HP1470	22
hpy02010	HP0298/HP0251/HP0473/HP0818/HP0889/HP0939/HP1251/HP1561/HP0299/HP0300/HP0474/HP0940/ HP1252/HP1562/HP1564	15
hpy05120	HP0072/HP0887/HP0009/HP0532/HP0725/HP0912/HP1243/HP0073/HP0071/HP0913/HP0537/HP0541/HP0544/HP0547	14
hpy01120	HP1110/HP0072/HP1111/HP0154/HP1108/HP1109/HP0589/HP0590/HP0591/HP0588/HP0294/HP0073/HP0191	13
hpy01130	HP1110/HP0075/HP1111/HP0154/HP1108/HP1109/HP0589/HP0590/HP1277/HP0191	10
hpy02020	HP1329/HP0019/HP0115/HP0714/HP0391/HP0392/HP0393/HP0601/HP0616/HP0191	10
hpy03420	HP0911/HP1478/HP0705/HP0821/HP1114/HP0615/HP1022/HP1470/HP1541	9
hpy00020	HP1110/HP1108/HP0588/HP1111/HP1109/HP0591/HP0589/HP0590/HP0191	9
hpy01200	HP1110/HP0589/HP0588/HP0154/HP1111/HP0590/HP0591/HP0191	8
hpy00010	HP1110/HP1111/HP0154/HP1108/HP1109/HP0589/HP0590	7
hpy02030	HP0019/HP0298/HP0616/HP1030/HP0391/HP0392/HP0393	7
hpy00620	HP1110/HP1108/HP1111/HP1109/HP0589/HP0590/HP0191	7
hpy00650	HP1110/HP1108/HP1111/HP1109/HP0589/HP0590/HP0191	7
hpy00230	HP0072/HP1198/HP0073/HP1293/HP1022/HP1470	6
hpy00640	HP1110/HP1108/HP1111/HP1109/HP0589/HP0590	6
hpy00680	HP1110/HP1111/HP0154/HP1108/HP1109	5
hpy00240	HP1198/HP0005/HP1293/HP1470/HP1022	5
Hpy01110	HP0154/HP1277/HP0237/HP0152/HP0191	5

Table 3. Pathway statistics: the proportion of resistance genes is higher than or equal to unknown genes.

Pathway	Gene		Percentage of drug resistance genes
	Drug-resistance genes	Unknown Drug-resistance genes	
hpy03020	HP1198/HP1293	HP0776	66.7%
hpy03420	HP0911/HP1478/HP0705/HP0821/HP1114/ HP1541/ HP1470/HP1022/ HP0615		100%
hpy00020	HP1110/HP1108/HP0588/HP1111/HP1109/ HP0591/HP0589/HP0590/HP0191	HP0026/HP0027/HP0086/ HP0192/HP0193/ HP0779 /HP1325/	56%
hpy00650	HP1110/HP1108/HP1111/HP1109/HP0589/ HP0590/HP0191	HP0690/ HP0192 / HP0193/HP0691/ HP0692	58%
hpy02010	HP0251/P0298/HP0299/HP0300/HP0473/ HP0474/HP0818/HP0889/HP0939/HP0940/ HP1251/HP1252/HP1561/HP1562/HP1564	HP0250/HP0301/HP0302/HP0362/HP0475/HP0715/HP0748/HP0749/HP0819/HP0888/HP1082/HP1464/HP1465/HP1466/HP1498/HP1576/HP1577/	46.9%

Table 4. Classification of drug resistant genes *in H. pylori.*

ABC transporters	Penicillin-binding proteins (PBPs)	Permeability of membrane protein	rdxA mutations	Polypeptide transferase	Unknown genes
Gene (18)	Gene (2)	Gene (63)	Gene (2)	Gene (7)	Gene (58)
HP0251/HP0298/ HP0299/HP0300/ HP0473/HP0474/ HP0818/HP0889/ HP0939/HP0940/ HP1251/HP1252/ HP1564/HP0807/ HP0887/HP1114/ HP1169/HP1170	HP1565/ HP1556/	HP0009/HP0912/HP0913/HP1243/ HP0873/HP0876/HP0896/HP0914/ HP0923/HP1055/HP1056/HP1066/ HP1083/HP1107/HP1113/HP1156/ HP1157/HP1167/HP1177/HP1342/ HP1395/HP1453/HP1467/HP1469/ HP1501/HP1525/HP0009/HP0912/ HP0913/HP1243/HP0025/HP0079/ HP0101/HP0115/HP0127/HP0165/ HP0209/HP0227/HP0229/HP0252/ HP0289/HP0317/HP0324/HP0373/ HP0389/HP0472/HP0477/HP0486/ HP0487/HP0547/HP0582/HP0601/ HP0608/HP0638/HP0671/HP0725/ HP0687/HP0706/HP0710/HP0714/ HP0782/HP0788/HP0796	HP1379/ HP0642	HP0955/HP1428/ HP0017/HP0441/ HP0154/HP0402/ HP0459	HP0821/HP0874/HP0911/ HP0916/HP0958/HP1027/ HP1089/HP1109/HP1110/ HP1111/HP1130/HP1149/ HP1161/HP1165/HP1183/ HP1186/HP1198/HP1209/ HP1320/HP1339/HP1340/ HP1341/HP1372/HP1400/ HP1445/HP1446/HP1459/ HP1478/HP1508/HP1512/ HP1553/HP0004/HP0005/ HP0070/HP0071/HP0072/ HP0073/HP0075/HP0152/ HP0153/HP0223/HP0237/ HP0243/HP0275/HP0501/ HP0532/HP0537/HP0544/ HP0582/HP0686/HP0701/ HP0705/HP0722/HP0191/ HP0615/HP1022/HP1470/ HP1541
Pathway (5)	Pathway (2)	Pathway (3)	Pathway (0)	Pathway (11)	Pathway (26)
hpy02010/hpy02030/ hpy03070/hpy03420/ hpy05120	hpy00550/ hpy01501	hpy02020/hpy02040/ hpy05120		hpy00010/ hpy00680/ hpy00970/ hpy01100/ hpy01110/ hpy01120/ hpy01130/ hpy01200/ hpy01230/ hpy03018/ hpy03070/	hpy00010/hpy00020/ hpy00130/hpy00220/ hpy00230/hpy00240/ hpy00520/hpy00550/ hpy00620/hpy00640/ hpy00650/hpy00680/ hpy00860/hpy00910/ hpy01100/hpy01110/ hpy01120/hpy01130/ hpy01200/hpy01501/ hpy03010/hpy03020/ hpy03420/hpy03430/ hpy03440/hpy05120

Table 5. Operons with at least two *H. pylori* resistance genes.

Operon	Gene number	Resistance genes
4062	4	HP0588/ HP0589/ HP0590/ HP0591
4167	4	HP1108/ HP1109/ HP1110/ HP1111
4023	3	HP0391/ HP0392/ HP0393
3999	3	HP0298/ HP0299/ HP0300/
4073	3	HP0631/HP0634/ HP0635
4208	3	HP1339/ HP1340/ HP1341
4170	2	HP1129/ HP1130
4139	2	HP0955/ HP0958/
3986	2	HP0237/HP0243
4055	2	HP0541/ HP0544
4196	2	HP1251/ HP1252/
3969	2	HP0153/ HP0154
4136	2	HP0939/HP0940/
4229	2	HP1445/HP1446
4176	2	HP1156/ HP1157/
4178	2	HP1169/ HP1170/
4039	2	HP0473/ HP0474
4158	2	HP1055/HP1056/
3948	2	HP0072/ HP0073
4041	2	HP0486/HP0487
4132	2	HP0912/ HP0913

Table 6. Operons with *H. pylori* resistance genes more than unknown genes.

Operon	Gene	
	Resistance genes	Unknown resistanc genes
4062	HP0588/ HP0589/ HP0590/ HP0591	
4167	HP1108/ HP1109/ HP1110/ HP1111	
4208	HP1339/ HP1340/ HP1341	
4073	HP0631/HP0634/ HP0635	HP0632/ HP0633
3999	HP0298/ HP0299/ HP0300	HP0301/ HP0302/ HP0303/ HP0304
4023	HP0391/ HP0392/ HP0393	HP0394/ HP0395/ HP0396/ HP0397/ HP0398/ HP0399
4039	HP0473/ HP0474	HP0475
4158	HP1055/HP1056	HP1057

pathways.

The pathway analysis of *H. pylori* drug resistance genes revealed that four pathways (hpy01100, hpy02010, hpy05120, hpy01120) contained at least 13 drug resistance genes, and one pathway (hpy01100) harbored 22 resistance genes. The pathway hpy01100 was related to glycan biosynthesis and metabolism that is the most common pathway for drug resistance genes [15].

Pathway 02010 is associated with ATP-binding cassette (ABC) transporters in the periplasmic binding protein-dependent transport system of *H. pylori*, representing important components of drug excretion. As shown in Table 3, pathway 02010 included 32 genes, among which 15 genes were previously identified drug resistance genes [16], and 17 genes were of unknown drug resistance function. The ABC transporters are a large

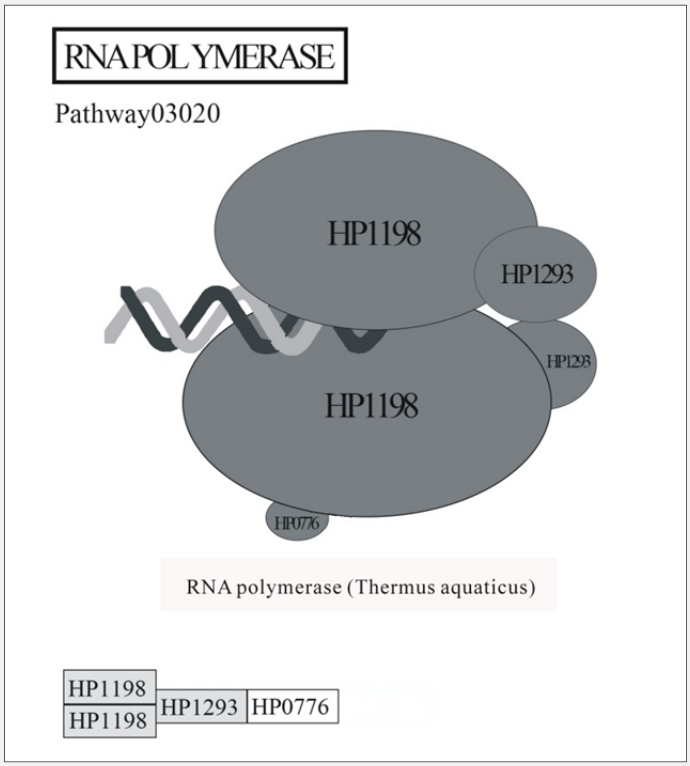

Figure 1. The hpy03020 pathway and its function in genetic information processing.

The grey boxes indicate resistance genes of *H. pylori*, and the white boxes indicate other genes.

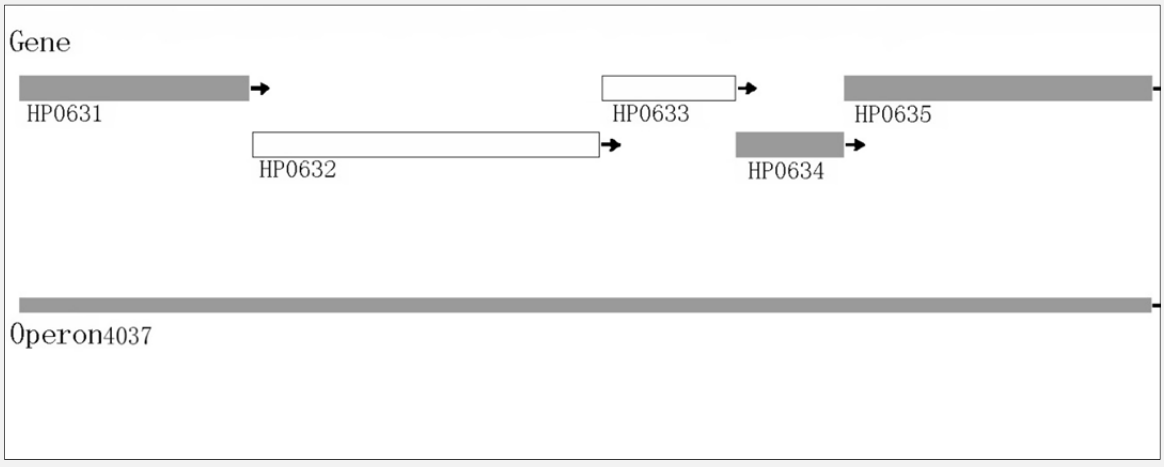

Figure 2. The *H. pylori* operon4073.

The upper part denotes the genes, and the lower part denotes the operon. Resistance genes were marked dark, while unknown genes are marked light.

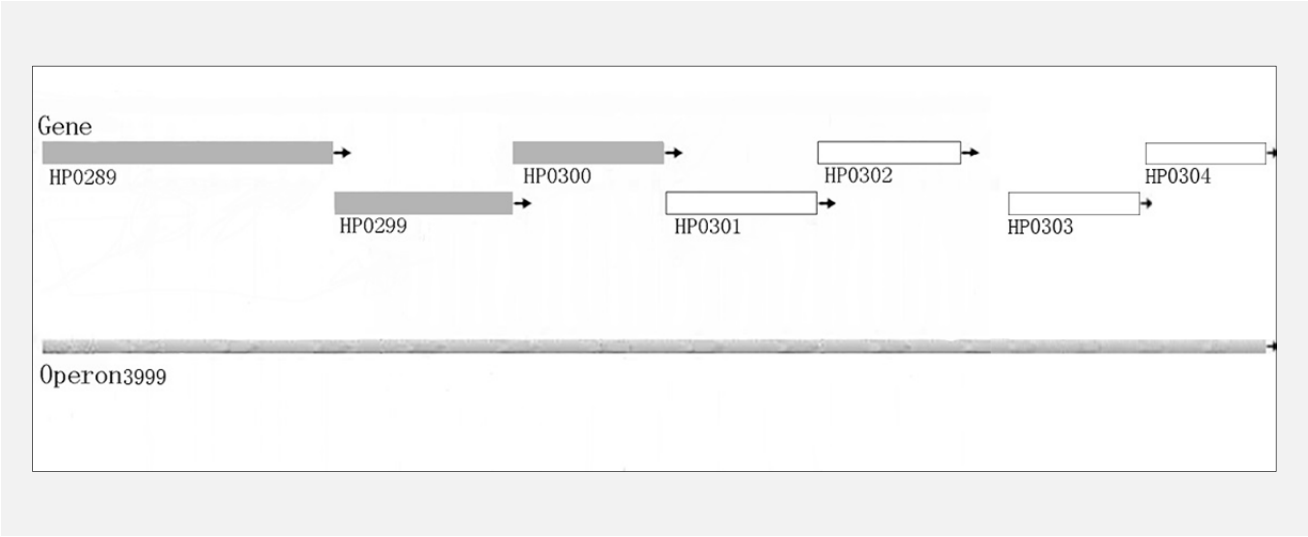

Figure 3. The *H. pylori* operon3999.

The upper part denotes the genes, and the lower part denotes the operon. Resistance genes were marked dark, while unknown genes are marked light.

superfamily of membrane proteins with diverse functions, with wide-spread expression in bacteria, archaea, and eukaryotes. The transporter has common global organization with three types of molecular components. It typically consists of two integral membrane proteins with six transmembrane segments per protein, two peripheral proteins that bind and hydrolyze ATP, and a periplasmic (or lipoprotein) substrate-binding protein in prokaryotes. However, the membrane spanning proteins and the ATP-binding proteins are fused to form a multidomain protein with a membrane-spanning domain (MSD) and a nucleotide-binding domain (NBD) in a typical eukaryotic ABC transporter [16,17]. All genes involved in this pathway are thought to be closely associated with the drug resistance of *H. pylori*. Therefore, we predicted that the remaining 17 genes of unknown function might also be classified as drug resistance genes.

The microcin C of peptide and nickel transporters pathway harbored four genes, among which HP0251, HP1251, and HP1252 were positively identified as drug resistance genes, and the role of HP0250 in drug resistance remained unknown. Similarly, five genes were included in the peptide and nickel transporters pathway where three genes were classified as drug resistance genes, and functions of HP0301 and HP0302 were unknown. Pathway hpy05120 is associated with epithelial cell signaling in *H. pylori* infection [18], and contains two major virulence factors of *H. pylori:* the vacuolating cytotoxin (VacA) and the cytotoxin-associated antigen (Cag) type IV secretion system (T4SS) as well as its translocated effector protein, CagA [18,19]. Gerhard et al. revealed a strong association of hpy05120 with pathogenic and membrane proteins of *H. pylori* [20]. One mechanism leading to antibiotic resistance of *H. pylori* is the alteration in cell membrane permeability; therefore, hpy05120 is hypothesized to be associated with drug resistance genes.

Hpy01120 is the pathway of microbial metabolism in diverse environments, which exists in all microbes. Since gene mutations are one way for *H. pylori* to become resistant to antibiotics, we predicted involvement of many drug resistance genes in this particular pathway.

The results of the present study showed that the percentage of drug resistance genes was higher than or equal to that of functionally unknown genes in the relevant pathways described above. For example, pathways hpy03020 carried 66.7% of the resistance genes (Table 3 and Figure 1). In pathway hpy03420, the genes are all resistance genes (Table 3). Many resistance genes (more than 50%) were found in both pathway hpy00020 and hpy00650, where unknown genes HP0192 and HP0193 were involved (Table 3). All genes involved in these pathways are thought to be closely associated with *H. pylori* resistance to antimicrobials. We also found that HP1541 [21], HP1470 [22], HP1022 [23], HP0615 [24], and HP0191 [24] have all been shown to be resistance genes through literature review. Therefore, we predicted that the unknown genes HP0776, HP0192, and HP0193 might also be classified as drug resistance genes.

Correlation analysis of H. pylori resistance gene and gene function

According to the mechanisms of antibiotic drug resistance, we divided the resistance genes into five types: ABC transporter-related resistance genes, membrane permeability-related resistance genes, penicillin binding

protein-related resistance genes, rdxA mutation-related resistance genes, and peptide transferase-related resistance genes.

In the present study, a total of 18 ABC transporter-related drug resistance genes were found to be involved in five relevant pathways. Among them, 15 genes participated in the hpy02010 pathway, whereas only three genes were distributed in the other four pathways associated with *H. pylori* pathogenicity. It is now believed that ABC transporters are a family of ubiquitous membrane-bound proteins that transport a variety of substrates [25], participating in the movement of most drugs and their metabolites across cell surface and cellular organelle membranes. Estrada-Tejedor et al. investigated the important roles of many ABC transporters in the host's defense mechanisms against foreign substances and cellular resistance to antibiotic drugs [26]. Gene mutations in ABC transporters trigger multidrug resistance in bacteria, which is supported by the results of Tsukamoto et al., who confirmed strong associations of ABC transporter gene expression with drug concentration and drug resistance in cells [27].

Bacterial biofilm (BBF), or bacterial film, is a special "capsule" formed by the irreversible attachment of bacteria to a solid surface and plays important roles in microbial adaptation to the natural environment. The bacteria make full use of BBF since it serves as protection from *antimicrobial agents* and host immune *defenses, leading* to a persistent and recurrent infection [28]. Kraupner et al. reported the significant association between BBF formation and drug resistance [29]. In this study, 63 drug resistance genes including hpy02020, hpy02040, and hpy5120 were involved in three pathways related to membrane protein permeability. Importantly, hpy5120 was found to not only be involved with membrane proteins but also with ABC transporter proteins. Previous studies have revealed that all of the genes in the hpy5120 pathway were located in the hpy_M00564 pathogenicity island, which is closely related to the overall pathogenicity of *H. pylori* [30,31]. Our previous study identified 39 pathogenic genes from 40 genes in the hpy5120 pathway [32]. In the present study, 14 of 40 genes in the hpy5120 pathway were identified as resistance genes (Table 1). Our previous and current studies demonstrated that the genes in the hpy5120 pathway include not only pathogenic genes but also resistance genes. Therefore, we hypothesized that the remaining 26 genes also function as novel resistance genes.

The pathogenic mechanisms of *H. pylori* are determined by colonization factors that are related to drug resistance. The flagella of *H. pylori* play an important role in the colonization of and adhesion to the gastrointestinal tract [33]. Kristich and Ordal reported associations of hpy02040 with flagella assembly and hpy02030 with bacterial chemotaxis, namely flagella movement [34]. Hpy02020 participates in a two-component signal transduction system (TCS) [35], which typically consists of a sensor histidine protein kinase (HPK) that receives external input signals and a response regulating (RR) protein that conveys the signal to cause a change in the bacterial cell physiology. Previous studies have demonstrated flagella functioning in variable stomach pH concentrations by regulating acid resistance genes and dynamic genes of *H. pylori* to colonize in gastric mucosa, which supports adaptation and survival in strong acid or weak acid environments for a long time [36,37]. Less than 30% of the resistance genes identified in the present study were part of the hpy2030, hpy02040, or hpy02020 pathways; therefore, we speculated that the other genes in these pathways were most likely associated with the pathogenicity and alternative drug resistance of *H. pylori* (Table 4).

Macrolides are the first-line of treatment for *H. pylori* [38]. However, the resistance rate of *H. pylori* to clarithromycin has increased to approximately 25% in China [39]. Clarithromycin prevents the synthesis of bacterial proteins by inhibiting the activity of polypeptide transferase [40]. Studies have demonstrated an association of mutations in the 23s rRNA polypeptide transferase domain of *H. pylori* with resistance to clarithromycin [41]. In the present study, we identified only seven resistance genes related to polypeptide transferase in 11 pathways. Among them, HP0154 was involved in nine pathways, followed by HP1110 and HP1111 in six pathways. As shown in Table 3, a correlation between pathways and resistance genes showed that most of the pathways in which HP1110 and HP1111 were present were associated with a resistance mechanism. Our results revealed that resistance genes related to polypeptide transferase in 11 pathways were involved in the regulation of multiple mechanisms of *H. pylori*. Therefore, it was hypothesized that the resistance genes of *H. pylori* may also act as pathogenic genes and essential genes simultaneously.

Penicillin binding proteins (PBPs) are a set of bacterial membrane proteins characterized by their affinity for and binding of penicillin [42]. In the present study, we discovered two resistance genes and two pathways (hpy01501: beta-lactam resistance, and hpy00550: peptidoglycan biosynthesis) that were associated with PBPs. Pathway hpy00550 included 14 genes, all of which were involved in the synthesis of cell wall peptides. There were no *H. pylori* genes found in the hpy01501 pathway. Chowdhury et al. reported an inhibition of antibiotics including penicillin to the biosynthesis of bacterial cell walls via binding to PBPs, causing bacterial cell death [43]. Bacterial morphology or sensitivity to antibiotics is affected by alternations in the number, type, or affinity of antibiotics to PBPs [44]. At present, bacterial resistance to antibiotics caused by changes in PBPs is still unclear. Among 14 genes in the hpy00550 pathway, only two (HP1565 and HP1556) were classified as resistance genes, and the remaining 12 genes were of unknown resistance gene functions.

H. pylori resistance to metronidazole is mainly caused by genetic mutations of rdxA and frxA nitrate reductase genes [45]. In the present study, only two rdxA related

resistance genes were identified, but they were not involved in the relevant pathway, indicating that the mechanism of resistance to metronidazole is unclear. As shown in Table 4, there were 58 genes of unknown function that were associated with 24 abundant and disorganized pathways. Further studies are needed to investigate the biological functions of these unknown genes in antimicrobial resistance.

Correlation analysis of H. pylori resistance genes and operons

An operon is a cluster of co-regulated genes with related or similar functions in prokaryotes. In the present study, we used statistical analysis to identify novel resistance genes of *H. pylori* in the operon where resistance genes were clustered. As shown in Table 5, the operon analysis of 148 resistance genes using the DOOR2 database revealed two operons with at least four drug resistance genes, four operons with three resistance genes, 15 operons with two genes, and 127 operons with one gene.

In the present study, all the genes in the four-gene operons (operon4062 and operon4167) were relevant and classified as resistance genes. Similarly, all of the genes in the three-gene operons (operon4208) were classified as resistance genes. The genes of an operon are usually functionally related, which can be useful to predict and classify biological functions of unknown genes of the same operon according to their relationships. For example, three genes of the five-gene operon (operon4073) were classified as resistance genes; the remaining two genes (HP0632, HP0633) were therefore predicted to be resistance genes. As shown in Table 6, three of seven genes in the seven-gene operon (operon3999) were classified as resistance genes. It can be speculated that the other four genes (HP0301, HP0302, HP0303, and HP0304) are most likely resistance genes. Two of three genes in both operon4039 and operon4158 are resistance genes, and it was therefore assumed that the other gene is also a resistance gene (Table 6). In the present study, we predicted that HP0475, HP1057, HP0632, and HP0633 are most likely pathogenic genes, whereas HP0301, HP0302, HP0303, and HP0304 might be resistance genes.

CONCLUSION

In the present study, a total of 148 drug resistance genes were identified with bibliometrics, and these genes were enriched in 46 pathways, identified using the KEGG database. The potential pathway association, functional classification, and operon association of these drug resistance genes were analyzed, from which 7 genes were speculated to be novel resistance genes of *H. pylori,* including HP0776, HP0192, HP0193, HP0475, HP1057, HP0632, and HP0633. These results provide the theoretical fundamentals for epidemiological prevention and the development of optimal treatment regimens for *H. pylori*-induced diseases. These data also strengthen our understanding of the molecular mechanisms of *H. pylori* resistance to antibiotics. Since resistance to commonly used antibiotics including clarithromycin and metronidazole seems to be a result of specific mutations in a small region of the responsible gene, "*omics*" technologies combined with advanced bioinformatics offer an attractive and effective approach for the discovery of *H. pylori* resistance genes.

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Declaration of Interest:

The authors declare that they have no competing interests.

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