

Supplementary Materials

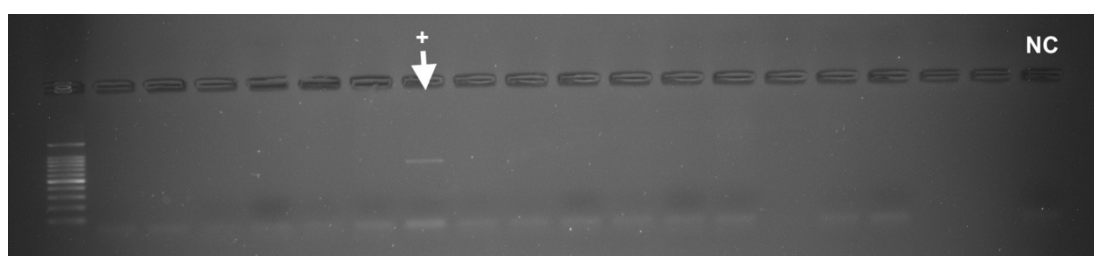


Figure S1. *frxA* PCR products electrophoresis gel; +: *frxA* positive sample; NC - Negative control

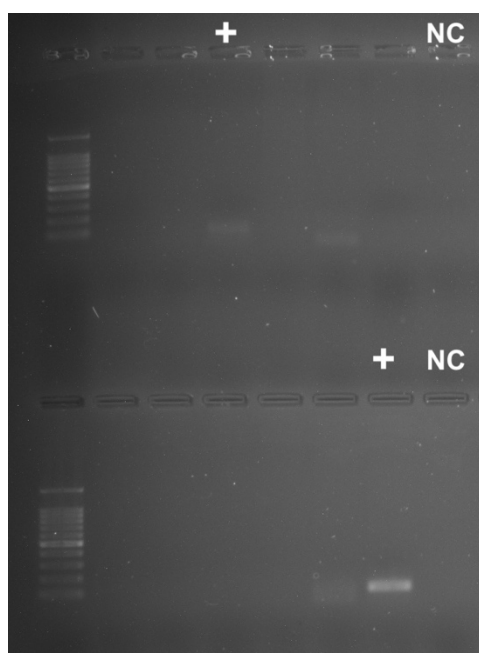


Figure S2. 16S *rRNA* PCR products electrophoresis gel; +: 16S *rRNA* positive samples confirmed by sequencing ; NC - Negative control

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBank Graphics Distance tree of results MSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Helicobacter pylori strain ALI572 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	1011	OP389222.1
Helicobacter pylori strain ALI514 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	980	OP389221.1
Helicobacter pylori strain ALI14 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	980	OP389218.1
Helicobacter pylori strain ALI12 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	980	OP389217.1
Helicobacter pylori strain ALI5 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	1503	OP389216.1
Helicobacter pylori strain SA514 16S ribosomal RNA gene, partial sequence	Helicobacter pylori	130	130	97%	2e-26	98.63%	1503	OP225843.1
Helicobacter pylori strain Hpfe0001 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1649959	CP094173.1
Helicobacter pylori strain Hpfe0002 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1615483	CP094172.1
Helicobacter pylori strain Hpfe0003 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1564612	CP094171.1
Helicobacter pylori strain Hpfe0004 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1560176	CP094170.1
Helicobacter pylori strain Hpfe0006 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1559683	CP094168.1
Helicobacter pylori strain Hpfe0007 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1562288	CP094167.1
Helicobacter pylori strain Hpfe0008 chromosome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1607782	CP094179.1
Helicobacter pylori strain Hpfe0011 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1621962	CP094165.1
Helicobacter pylori strain Hpfe0012 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1627796	CP094164.1
Helicobacter pylori strain Hpfe0013 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1613739	CP094163.1
Helicobacter pylori strain Hpfe0015 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1559327	CP094161.1
Helicobacter pylori strain Hpfe0016 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1564699	CP094160.1
Helicobacter pylori strain Hpfe0020 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1576980	CP094159.1
Helicobacter pylori strain Hpfe0021 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1615407	CP094157.1
Helicobacter pylori strain Hpfe005 chromosome, complete genome	Helicobacter pylori	130	260	97%	2e-26	98.63%	1595694	CP094156.1

Figure S3. Results of the basic alignment research tool for sequenced sample 1, 16s rRNA gene.

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBank Graphics Distance tree of results MSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Helicobacter pylori strain ALI572 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	1011	OP389222.1
Helicobacter pylori strain ALI514 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	980	OP389221.1
Helicobacter pylori strain ALI14 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	980	OP389218.1
Helicobacter pylori strain ALI12 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	980	OP389217.1
Helicobacter pylori strain ALI5 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	1503	OP389216.1
Helicobacter pylori strain SA514 16S ribosomal RNA gene, partial sequence	Helicobacter...	93.5	93.5	98%	1e-15	100.00%	1503	OP225843.1
Helicobacter pylori strain Hpfe0001 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1649959	CP094173.1
Helicobacter pylori strain Hpfe0002 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1615483	CP094172.1
Helicobacter pylori strain Hpfe0003 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1564612	CP094171.1
Helicobacter pylori strain Hpfe0004 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1560176	CP094170.1
Helicobacter pylori strain Hpfe0006 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1559683	CP094168.1
Helicobacter pylori strain Hpfe0007 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1562288	CP094167.1
Helicobacter pylori strain Hpfe0008 chromosome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1607782	CP094179.1
Helicobacter pylori strain Hpfe0011 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1621962	CP094165.1
Helicobacter pylori strain Hpfe0012 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1627796	CP094164.1
Helicobacter pylori strain Hpfe0013 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1613739	CP094163.1
Helicobacter pylori strain Hpfe0015 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1559327	CP094161.1
Helicobacter pylori strain Hpfe0016 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1564699	CP094160.1
Helicobacter pylori strain Hpfe0020 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1576980	CP094159.1
Helicobacter pylori strain Hpfe0021 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1615407	CP094157.1
Helicobacter pylori strain Hpfe005 chromosome, complete genome	Helicobacter...	93.5	186	98%	1e-15	100.00%	1595694	CP094155.1

Figure S4. Results of the basic alignment research tool for sequenced sample 2, 16s rRNA gene.

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Show 10 entries						
Bitscore	ARO tag	Name	Evalue	Identity	Species	Alignment
42	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.00000367872	100	Helicobacter pylori 26695	View
41	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000103761	94	Helicobacter pylori 26695	View
40	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000173553	94	Helicobacter pylori 26695	View

Figure S5. CARD analysis of PCR-positive samples for 16s rRNA gene, sample 1.

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<input type="text" value="Search"/>						
Show 10 entries						
Bitscore	ARO tag	Name	Evalue	Identity	Species	Alignment
41	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000100608	100	Helicobacter pylori 26695	View
40	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000238433	100	Helicobacter pylori 26695	View
39	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000327567	100	Helicobacter pylori 26695	View
39	ARO:3003510	Helicobacter pylori 16S rRNA mutation conferring resistance to tetracycline	0.0000450023	100	Helicobacter pylori 26695	View

Figure S6. CARD analysis of PCR-positive samples for 16s rRNA gene, sample 2.

blast.ncbi.nlm.nih.gov/Blast.cgi

Sequences producing significant alignments

Download Select columns Show 100

☒ select all 100 sequences selected

GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> Helicobacter pylori strain dRdM2addM2 chromosome, complete genome	Helicobacter pylori	1230	1230	92%	0.0	99.41%	1668735	CP026515.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 26695-dR chromosome, complete genome	Helicobacter pylori	1230	1230	92%	0.0	99.41%	1666471	CP026326.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 26695-dRdM1dM2 chromosome, complete genome	Helicobacter pylori	1230	1230	92%	0.0	99.41%	1666879	CP026323.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 26695-dRdM2 chromosome, complete genome	Helicobacter pylori	1230	1230	92%	0.0	99.41%	1666735	CP026324.1
<input checked="" type="checkbox"/> Helicobacter pylori strain dRdM1 chromosome, complete genome	Helicobacter pylori	1230	1230	92%	0.0	99.41%	1670779	CP026325.1
<input checked="" type="checkbox"/> Helicobacter pylori 26695-1CL DNA, complete genome	Helicobacter pylori 2...	1230	1230	92%	0.0	99.41%	1667239	AP013356.1
<input checked="" type="checkbox"/> Helicobacter pylori 26695-1CH DNA, complete genome	Helicobacter pylori 2...	1230	1230	92%	0.0	99.41%	1667302	AP013355.1
<input checked="" type="checkbox"/> Helicobacter pylori 26695-1 DNA, complete genome	Helicobacter pylori 2...	1230	1230	92%	0.0	99.41%	1667638	AP013354.1
<input checked="" type="checkbox"/> Helicobacter pylori R/f2, complete genome	Helicobacter pylori R...	1230	1230	92%	0.0	99.41%	1667890	CP003906.1
<input checked="" type="checkbox"/> Helicobacter pylori R/f1, complete genome	Helicobacter pylori R...	1230	1230	92%	0.0	99.41%	1667883	CP003905.1
<input checked="" type="checkbox"/> Helicobacter pylori 26695, complete genome	Helicobacter pylori 2...	1230	1230	92%	0.0	99.41%	1667892	CP003904.1
<input checked="" type="checkbox"/> Helicobacter pylori 26695, complete genome	Helicobacter pylori 2...	1230	1230	92%	0.0	99.41%	1667867	AE000511.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 26695-1MET, complete genome	Helicobacter pylori	1221	1221	92%	0.0	99.12%	1667303	CP010436.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 476-A2-EK2 chromosome, complete genome	Helicobacter pylori	1192	1192	92%	0.0	98.38%	1649543	CP032473.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 173-A-EK1 chromosome, complete genome	Helicobacter pylori	1190	1190	92%	0.0	98.38%	1690720	CP032903.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 24-A-EK1 chromosome, complete genome	Helicobacter pylori	1186	1186	92%	0.0	98.24%	1659500	CP032907.1
<input checked="" type="checkbox"/> Helicobacter pylori strain NCTC12823 genome assembly, chromosome:1	Helicobacter pylori	1186	1186	92%	0.0	98.24%	1632224	LR134519.1
<input checked="" type="checkbox"/> Helicobacter pylori strain Hp6-IRAN FrxA gene, complete cds	Helicobacter pylori	1186	1186	88%	0.0	99.54%	654	KT232265.1
<input checked="" type="checkbox"/> Helicobacter pylori strain LIM-006 chromosome, complete genome	Helicobacter pylori	1186	1186	92%	0.0	98.24%	1745296	CP051493.1
<input checked="" type="checkbox"/> Helicobacter pylori strain B147 chromosome, complete genome	Helicobacter pylori	1181	1181	91%	0.0	98.23%	1631093	CP024946.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 169-A-EK5 chromosome, complete genome	Helicobacter pylori	1181	1181	92%	0.0	98.09%	1610187	CP032904.1
<input checked="" type="checkbox"/> Helicobacter pylori strain 476-A-EK5 chromosome, complete genome	Helicobacter pylori	1181	1181	92%	0.0	98.09%	1634797	CP032900.1

Figure S7. Results of the basic alignment research tool for sequenced sample, *frxA*

CARD

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Bitscore	ARO tag	Name	Evalue	Identity	Species	Alignment
485	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	3.3928e-138	100	Helicobacter pylori 26695	View
461	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	9.57515e-131	100	Helicobacter pylori 26695	View
418	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	8.80777e-118	100	Helicobacter pylori 26695	View
318	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	2.33713e-115	100	Helicobacter pylori 26695	View
275	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	2.898e-129	100	Helicobacter pylori 26695	View
238	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	4.52394e-112	100	Helicobacter pylori 26695	View
201	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	2.898e-129	100	Helicobacter pylori 26695	View
108	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	4.52394e-112	97	Helicobacter pylori 26695	View
102	ARO:3007059	Helicobacter pylori frxA mutation conferring resistance to metronidazole	2.33713e-115	100	Helicobacter pylori 26695	View
92	ARO:3007059	Helicobacter pylori frxA mutation	4.52394e-	100	Helicobacter	View

Figure S8. CARD analysis of the *frxA* mutation gene positive sample sequence.

Results (all Loose hits shown)								
Search: <input type="text"/>								
RGI Criteria	ARO Term	SNP	Detection Criteria	AMR Gene Family	Drug Class	Resistance Mechanism	% Identity of Matching Region	% Length of Reference Sequence
Strict	Helicobacter pylori frxA mutation conferring resistance to metronidazole	Y62D	protein variant model	Antibiotic resistant Helicobacter pylori nitroreductase	nitroimidazole antibiotic	antibiotic target alteration	99.07	108.29
Previous 1 Next								

Figure S9. Resistance gene identifier analysis of the *frxA* mutation gene positive sample sequence

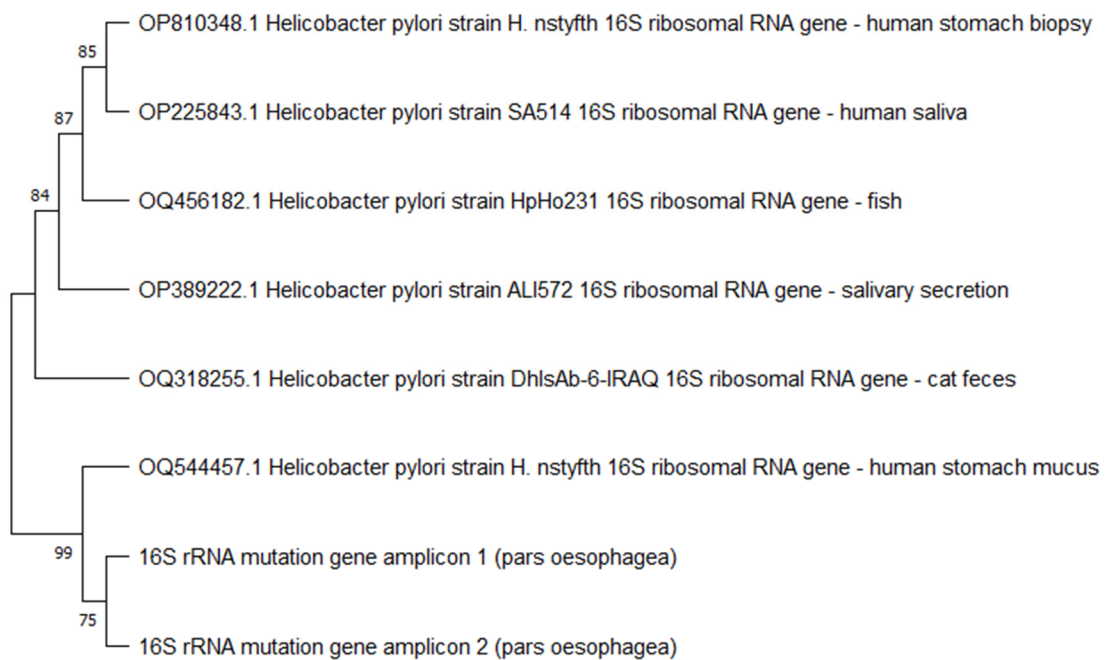


Figure S10. Phylogenetic tree based on comparison of 16S rRNA gene sequences.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The bootstrap consensus tree inferred from 1000 replicates [2] is taken to represent the evolutionary history of the taxa analyzed [2]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. This analysis involved 8 nucleotide sequences which were aligned using ClustalW. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1503 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [4].

Following the hyphen, the sample type from which the corresponding *H. pylori* strain was isolated is shown.

1. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **1987**, *4*, 406-425.
2. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783-791.
3. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* **2004**, *101*, 11030-11035.
4. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **2021**, <https://doi.org/10.1093/molbev/msab120>.



Figure S11. Phylogenetic tree based on comparison of *frxA* gene sequences.

The evolutionary history was inferred using the Neighbor-Joining method [1]. The bootstrap consensus tree inferred from 1000 replicates [2] is taken to represent the evolutionary history of the taxa analyzed [2]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. This analysis involved 10 nucleotide sequences which were aligned using ClustalW. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 735 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [4].

All reference strain sequences included, originated from *H. pylori* strains isolated from human gastric biopsy samples which showed metronidazole resistance with minimum inhibitory concentrations ranging from 16 to 256 mg/l. Following the hyphen, it is indicated what mutations were present in the reference strain sequences.

1. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **1987**, *4*, 406-425.
2. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783-791.
3. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* **2004**, *101*, 11030-11035.
4. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* **2021**, <https://doi.org/10.1093/molbev/msab120>.

Table S1. Primer sequences used for detection of *Helicobacter pylori*(-like organisms) and thermocycling conditions in previous research

Primer	Sequence	Target gene	Amplicon size	Thermal cycle conditions			<i>Helicobacter</i> strain used as positive control	Result of samples included in current study
				Nr. Cycles	Temp. (°C)	Time		
BFHpyl_F1	AAA gAg CgT ggT TTT CAT ggC g	ureAB	217 bp	45	94	30 sec	26695	Positive
BFHpyl_R1	ggg TTT TAC CgC CAC CgA ATT TAA				59	30 sec		
					72	1 min		
Hpy3F	TTATCGGTAAAGACACC AGAAA	glmM	144	45	94	30 sec	SS1	Negative
Hpy3R	ATCACAGCGCATGTCTTC				54	30 sec		
					72	30 sec		

From: Cortez Nunes, F.; Letra Mateus, T.; Taillieu, E.; Teixeira, S.; Carolino, N.; Rema, A.; De Bruyckere, S.; Gärtner, F.; Haesebrouck, F.; Amorim, I. Molecular detection of *Helicobacter* spp. and *Fusobacterium gastrois* in pigs and wild boars and its association with gastric histopathological alterations. *Vet Res* **2022**, 53, 78, doi:10.1186/s13567-022-01101-5.