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Prediction of Epitopes in the Proteome of *Helicobacter pylori*

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Abstract

Helicobacter pylori (H. pylori) is classified by the World Health Organization (WHO) as a group I carcinogen and is one of the most efficient human pathogens with over half of the world's population colonized by this gram-negative spiral bacterium. *H. pylori* can cause a chronic infection in the stomach during early childhood that persists throughout life due to diverse mechanisms of immune response evasion. *H. pylori* has several factors strongly associated with increased risk of disease such as toxins, adhesins, and chemoattractants, some of which are highly polymorphic, phase variable, and have different functions. Conventional treatments involve the use of antibiotics. However, treatment frequently fails due to the resistance *H. pylori* has progressively developed to antibiotics. This creates the need for different treatments made possible by identifying new therapeutic targets in the pathogen's genome.

The purpose of this study was an *in silico* prediction of T- and B- epitopes in *H. pylori* proteins. Twenty-two external membrane proteins from *H. pylori* Strain 26695 (accession number NC_000915) were identified using the web tool Vaxign (http://www.violinet.org/vaxign/). A total of one-hundred epitopes (60 class I epitopes and 40 class II epitopes) that could be used to develop novel non-antibiotics drugs for an *H. pylori* infection were predicted.

Keywords: *Helicobacter pylori*, epitopes, chronic infection in the stomach

1. Introducción

Helicobacter pylori (H. pylori) was first isolated by Warren and Marshall in 1982 (Ford & Axon, 2010), It is a slow-growing, Gram-negative, flagellated spiral-shaped microaerophilic organism (Jemilohun & Otegbayo, 2016).It has two to six flagella that provide mobility to withstand the rhythmic contractions in the stomach so they are able to penetrate the gastric mucosa (Spohn & Scarlato, 2001). It measures around 2.4 - 4.0 μm long and 0.5-1.0 μm wide (Refaeli et al., 2018).

H. pylori is an important pathogen in Public Health worldwide as one of the main causes of gastric diseases(Hooi et al., 2017). Several studies have shown that *H. pylori* is present in about 50% of the world population (Pilotto & Franceschi, 2014). In developing countries, the prevalence of infection can exceed 90% in adulthood(Hooi et al., 2017).

The host immune response induced by *H. pylori* is characterized by neutrophil recruitment followed by macrophages, mast cells, eosinophils, and T and B lymphocytes (Ayraud, Janvier, & Fauchère, 2002). Additionally,

there is an increase of IL-1, IL-8, and IL-6, and a reduction in the production of IL-17 (Kabir, 2011).

The current treatment of an *H. pylori* infection uses a combination of at least three compounds which include a proton pump inhibitor and two antibiotics. This treatment's decline in efficiency has been documented mainly due to its increased resistance to antibiotics (Fallone et al., 2016; Mégraud, 2012). Therefore, it is imperative to develop new therapeutic alternatives.

The rational design of a vaccine based on *In silico* prediction epitopes is a promising way to approach this problem (Moss et al., 2011). It has been demonstrated that surface proteins of some microorganisms have immunogenic epitopes, which could be used to design and develop novel, safe and efficient vaccines against pathogens (Ni, Wang, Liu, & Lu, 2010). However, the greatest requirement for the candidate epitopes is their ability to bind to molecules of Major Histocompatibility Complex (MHC) class I and / or class II. These molecules cause the epitopes to come to the cell surface to be subsequently recognition by the lymphocytes (Liao et al., 2009).

Vaxign is an online design system that predicts target proteins for vaccine design based on genome sequences using reverse vaccinology strategy (He, Xiang, & Mobley, 2010). The preestablished characteristics in Vaxign include the subcellular localization of the white protein and identifying epitopes that bind to MHC class I and class II (He et al., 2010; Xiang & He, 2009).

This study aimed to predict T- and B- epitopes from outer membrane proteins of *Helicobacter pylori* that can be used in later work to develop a vaccine against this microorganism or for alternative therapies such as passive immunization.

2. Methodology

2.1 Obtaining Genomic Sequences of H. pylori

The complete genome sequence of *H. pylori* was researched in RefSeq (Pruitt, Tatusova, & Maglott, 2005) and GenBank (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2006) databases..

2.2 Identification of Conserved Sequences

The OrthoMCL algorithm was used to find conserved proteins (Yu et al., 2010) and SPAAN software (Sachdeva, Kumar, Jain, & Ramachandran, 2005).The transmembrane helix topology analysis was carried out using optimized HMMTOP (Xiang & He, 2009) is freely available to non-commercial users at http://www.enzim.hu/hmmtop. Source code is also available upon request to academic users.

2.3 Identification of Conserved Sequences

The OrthoMCL algorithm was used to find conserved proteins (Chen, Mackey, Stoeckert, & Roos, 2006), OrthoMCL provides a scalable method for constructing orthologous groups across multiple eukaryotic taxa, using a Markov Cluster algorithm to group (putative) orthologs and paralogs. This method performs similarly to the INPARANOID algorithm when applied to two genomes, but can be extended to cluster orthologs from multiple species. A cut off of E-105 was used as the default value for all proteins that could cross-react with human but mouse and/or pig proteins were discarded.

2.4 Prediction of Epitopes

For *H. pylori* protein, the Vaxitop pipeline was used for the prediction of epitopes (http://www.violinet.org/vaxign/vaxitop), following the methodology described above by (Xiang & He, 2013). Calculations were done using a cut-off point of $p \leq 0.05$, which provides high sensibility, specificity and balance (He et al., 2010).

3. Results

3.1 H. pylori Genomes

A total of 683 *H. pylori* genomes were found, of which only 61 sequences corresponded to genomes strains of this microorganism, with an average of 1,571 and 1,454 protein genes (Table 1).

Table 1. Genomes of H. pylori strains

Strain	Assembly	Genes	Proteínas
Helicobacter pylori 26695	GCA_000008525.1	1555	1445
Helicobacter pylori J99	GCA_000008785.1	1607	1469
Helicobacter pylori Shi470	GCA_000020245.1	1584	1442
Helicobacter pylori B38	GCA_000091345.1	1545	1432
Helicobacter pylori 51	GCA_000011725.1	1545	1433
Helicobacter pylori 52	GCA_000023805.1	1522	1392
Helicobacter pylori 908	GCA_000148665.1	1501	1360
Helicobacter pylori SJM180	GCA_000148855.1	1579	1488
Helicobacter pylori Cuz20	GCA_000148895.1	1580	1487
Helicobacter pylori 35[a]	GCA_000178935.2	1530	1414
Helicobacter pylori India7	GCA_000185185.1	1602	1487
Helicobacter pylori F16	GCA_000270005.1	1531	1421
Helicobacter pylori F57	GCA_000270065.1	1560	1456
Helicobacter pylori 2017	GCA_000192315.1	1506	1382
Helicobacter pylori 2018	GCA_000192335.1	1516	1393
Helicobacter pylori 83	GCA_000213135.1	1562	1441
Helicobacter pylori Puno135	GCA_000224555.1	1585	1493
Helicobacter pylori Shi417	GCA_000277365.1	1586	1490
Helicobacter pylori Shi169	GCA_000277385.1	1559	1460
Helicobacter pylori Shi112	GCA_000277405.1	1607	1499
Helicobacter pylori PeCan18	GCA_000277425.1	1581	1480
Helicobacter pylori 26695	GCA_000307795.1	1632	1538
Helicobacter pylori Rif1	GCA_000307815.1	1630	1532
Helicobacter pylori Rif2	GCA_000307835.1	1630	1534
Helicobacter pylori OK113	GCA_000348865.1	1553	1418
Helicobacter pylori UM032	GCA_000392455.3	1549	1438
Helicobacter pylori UM299	GCA_000392475.3	1550	1439
Helicobacter pylori UM037	GCA_000392515.3	1634	1509
Helicobacter pylori UM066	GCA_000392535.3	1587	1474
Helicobacter pylori UM298	GCA_000439295.2	1551	1440
Helicobacter pylori SouthAfrica20	GCA_000590775.1	1552	1361
Helicobacter pylori 26695-1	GCA_000829095.1	1629	1539
Helicobacter pylori 26695-1CL	GCA_000829115.1	1628	1538
Helicobacter pylori 26695-1CH	GCA_000829135.1	1628	1538
Helicobacter pylori BM012A	GCA_000498315.1	1632	1495
Helicobacter pylori BM012S	GCA_000498335.1	1632	1495
Helicobacter pylori oki102	GCA_000600045.1	1566	1464
Helicobacter pylori oki112	GCA_000600085.1	1569	1456
Helicobacter pylori oki128	GCA_000600125.1	1496	1364
Helicobacter pylori oki154	GCA_000600145.1	1543	1412

Strain	Assembly	Genes	Proteínas
Helicobacter pylori oki422	GCA_000600165.1	1570	1441
Helicobacter pylori oki673	GCA_000600185.1	1534	1400
Helicobacter pylori oki828	GCA_000600205.1	1536	1399
Helicobacter pylori oki898	GCA_000600225.1	1563	1463
Helicobacter pylori J166	GCA_000685625.1	1569	1466
Helicobacter pylori	GCA_000685665.1	1551	1453
Helicobacter pylori	GCA_000685705.1	1626	1490
Helicobacter pylori	GCA_000685745.1	1551	1452
Helicobacter pylori NY40	GCA_000828955.1	1643	1476
Helicobacter pylori	GCA_000817025.1	1541	1413
Helicobacter pylori 26695-1	GCA_000826985.1	1630	1542
Helicobacter pylori	GCA_000827025.1	1631	1542
Helicobacter pylori J99	GCA_000982695.1	1594	1491
Helicobacter pylori	GCA_001433495.1	1617	1451
Helicobacter pylori	GCA_001653375.1	1584	1456
Helicobacter pylori	GCA_001653395.1	1573	1441
Helicobacter pylori	GCA_001653415.1	1590	1471
Helicobacter pylori	GCA_001653435.1	1462	1353
Helicobacter pylori	GCA_001653455.1	1498	1392
Helicobacter pylori	GCA_001653475.1	1491	1354

3.2 H. pylori Vaccine Candidate Protein Predition

H. pylori strain 26695 was arbitrarily selected for the prediction of protein candidates. For this strain, a total of 1,445 proteins have been described. In this study, 42 outer membrane proteins were identified, with a sticky probability ≥ 0.51. These proteins had no significant homology with pig, mouse or human proteins (Table 2). The outer membrane proteins with a greater likelihood adhesin (0.737) were: NP_207714.1 (a protein with fusion functions) and NP_207405.1 (a toxin-like protein).

Table 2. Selection of conserved outer membrane proteins of Helicobacter pylori, with probability of adherence ≥0.51

Protein Accession	Localization	Adhesin Probability
NP_206827.1	Outer Membrane	0.685
NP_207052.1	Outer Membrane	0.656
NP_207115.1	Outer Membrane	0.705
NP_207284.1	Outer Membrane	0.681
NP_207405.1	Outer Membrane	0.737
NP_207432.1	Outer Membrane	0.544
NP_207480.1	Outer Membrane	0.620
NP_207504.1	Outer Membrane	0.610
NP_207516.1	Outer Membrane	0.653
NP_207519.1	Outer Membrane	0.640
NP_207575.1	Outer Membrane	0.517

NP_207581.1	Outer Membrane	0.578
NP_207600.1	Outer Membrane	0.662
NP_207670.1	Outer Membrane	0.555
NP_207689.1	Outer Membrane	0.662
NP_207704.1	Outer Membrane	0.669
NP_207705.1	Outer Membrane	0.684
NP_207706.1	Outer Membrane	0.629
NP_207714.1	Outer Membrane	0.737
NP_208191.1	Outer Membrane	0.687
NP_208244.1	Outer Membrane	0.636
NP_208303.1	Outer Membrane	0.628

The outer membrane proteins with a greater likelihood adhesin (0.737) were: NP_207714.1 (a protein with fusion functions) and NP_207405.1 (a toxin-like protein).

Epitope Prediction of MHC Class I and II

Vaxitop (Xiang & He, 2013) was used to predict MHC class I and II binding epitopes. Likewise, other programs used to identify epitopes are listed in supplementary Table 1.

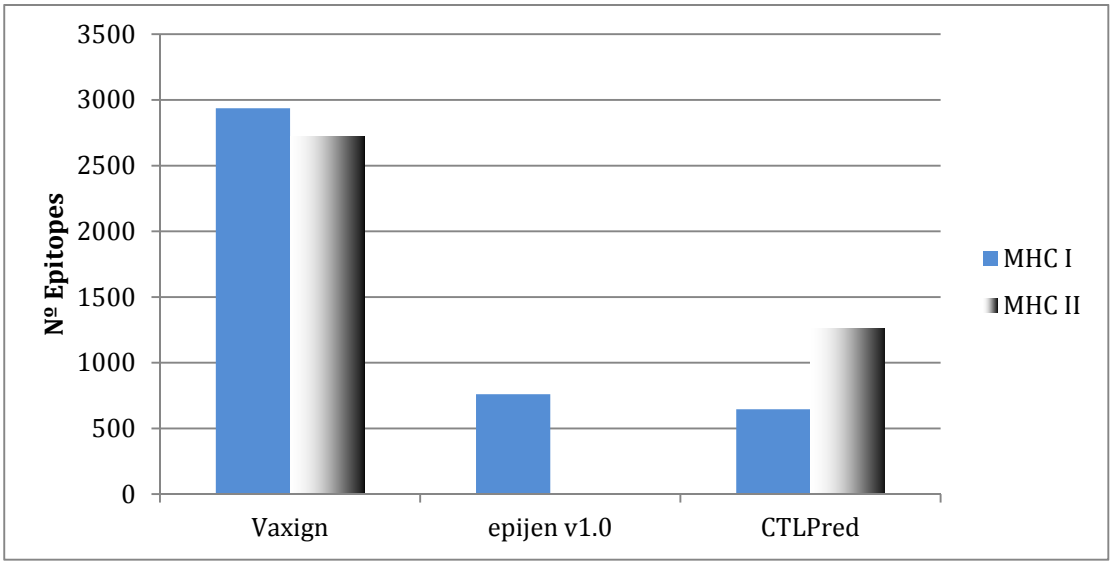

Figure 1. Total number of epitopes MHC I and MHC II predicted with different software

The greatest number of epitopes was obtained with Vaxign software (PSSM) with 2881 class I epitopes and 2634 class II epitopes. (Figure 1) The VacA protein (WP_000874574.1), an *H. pylory* outer membrane protein toxin, presented the highest number of epitopes, as follows: 60 epitopes for MHC class I (Table 3), and 40 epitopes is for MHC class II (Table 4).

Table 3. Predicted epitopes for MHC I in proteins of *Helicobacter pylori.*

Nº de acceso proteína	Epítope	alelo MHC	aminoacido Inicial	aminoacido final	P-valor
	TTLNEACPWL	HLA-A*01:01	264	273	0.0159
WP_000726314.1	GTSWLNSQYV	HLA-A*02:01	619	628	0.0244
	LYSVYLNYVF	HLA-A*24:02	700	709	6.50E-05
	LYSVYLNYVF	HLA-A*24:02	734	743	6.50E-05
WP_000713712.1	SWLNSEYVNL	HLA-A*24:02	655	664	0.00732
	DTLVNFKSRY	HLA-A*01:01	479	488	0.00163
	QYRGFSWKIL	HLA-A*24:02	384	393	0.00022
WP_001115603	KALMVADLKY	HLA-A*01:01	233	242	0.0173
	ITYDTNPNFN	HLA-A*01:01	267	276	0.0777
	RVKGLSIFYK	HLA-A*03:01	168	177	0.00044
WP_000709741.1	DYKRVVSVYL	HLA-A*24:02	286	295	0.0095
	LPYGFNTDLL	HLA-B*07:02	142	151	0.00792
	FIFDMMYTYK	HLA-A*03:01	645	654	0.00013
WP_000822057.1	PGLRYTFLNY	HLA-A*01:01	465	474	0.0247
	NVFGGVINVI	HLA-A*02:01	155	164	0.0184
	GYQNYFNDFI	HLA-A*24:02	508	517	0.0009
WP_001228436.1	QLTIENFLPY	HLA-A*01:01	95	104	0.0392
	FGDNLKTINL	HLA-A*01:01	333	342	0.0134
	KSDKAALGLY	HLA-A*01:01	79	88	0.00083
WP_001092132.1	YATGRFGNFY	HLA-A*01:01	332	341	0.018
	ATYRSNVANL	HLA-B*07:02	172	181	0.00582
	LYSVYLNYVF	HLA-A*24:02	597	606	6.50E-05
WP_010875534.1	RLYSVYLNYV	HLA-A*02:01	596	605	0.0002
	ITSTGPVTDY	HLA-A*01:01	203	212	0.00057
	FYSYGDKFHL	HLA-A*24:02	150	159	0.00018
WP_000911476.1	YVLYNSYLFY	HLA-A*01:01	142	151	0.00031
	VYRGFLWGIL	HLA-A*24:02	372	381	0.00063
	YMMDANAFTV	HLA-A*02:01	401	410	2.67E-05
WP_001108275.1	AYMQVDFTEL	HLA-A*24:02	67	76	0.00022
	FSADIKFEYY	HLA-A*01:01	452	461	0.00023
	FYFNYQRSYI	HLA-A*24:02	540	549	0.0002
WP_010875548.1	NTFKAYYQYY	HLA-A*01:01	275	284	0.00035
	QYNSYHPGTL	HLA-A*24:02	285	294	0.0016
	GLDYCGFDIY	HLA-A*01:01	677	686	0.00028
WP_000479960.1	DSLFEYGFNY	HLA-A*01:01	404	413	0.00613
	RPLRSNAIGL	HLA-B*07:02	257	266	0.0016
	FFDYNHAFIK	HLA-A*03:01	557	566	0.0174
WP_000753173.1	KQASIIITTL	HLA-A*02:01	260	269	0.0244
	QELGRNPFRK	HLA-A*03:01	509	518	0.0715

	FYTKIGYKQF	HLA-A*24:02	350	359	0.00069
WP_000592437.1	LSTIGSQTNY	HLA-A*03:01	149	158	0.0477
	QTYSTQAIQY	HLA-A*03:01	206	215	0.0536
	VYLTYMNSFL	HLA-A*24:02	197	206	0.00038
WP_000812546.1	NQLGNLIDLY	HLA-A*01:01	105	114	0.0159
	TYGVGTDVLY	HLA-A*01:01	410	419	0.0203
	MPRGNNTSYI	HLA-B*07:02	364	373	0.00061
WP_001248496.1	VTLAQVKVNL	HLA-A*01:01	62	71	0.0516
	MIMTTFPLYY	HLA-A*03:01	292	301	0.00064
	ALMSVSGQFV	HLA-A*02:01	1170	1179	0.00112
WP_000874574.1	GISGANGYEK	HLA-A*03:01	1214	1223	0.0193
	TTINLDDSVL	HLA-B*07:02	1097	1106	0.0241
	RYTYKDKFSF	HLA-A*24:02	605	614	0.00031
WP_000902542.1	AWCMTQHEGL	HLA-A*24:02	768	777	0.00732
	DHNVLTYFNY	HLA-A*01:01	564	573	0.00841
	YTNLSSQTNY	HLA-A*01:01	532	541	8.79E-05
WP_000915379.1	LPYNLNNIEL	HLA-B*07:02	135	144	0.00483
	LPASLFNDPQ	HLA-B*07:02	170	179	0.0212
	YYNHQNIFYY	HLA-A*24:02	191	200	0.00057
WP_000945747.1	LSRFVTNMYY	HLA-A*01:01	773	782	0.00221
	TTKGERTFEY	HLA-A*01:01	32	41	0.00358

Table 4. Epitopes for MHC class II predicted by Vaxign for *Helicobacter pylori*

Nº de acceso	**Epitopes**	**alelo MHC**	**aminoacido inicial**	**aminoacido final**	**Percentil (IEDB consensus)**
WP_000726314.1	IQDNYYIDSNIHSQV	HLA-DRB1*04:01	496	510	0.01
	QDNYYIDSNIHSQVQ	HLA-DRB1*04:01	497	511	0.01
WP_000713712.1	ENIADTLVNFKSRYS	HLA-DRB1*13:01	475	489	0.02
	NIADTLVNFKSRYSE	HLA-DRB1*13:01	476	490	0.02
WP_001115603	YAIFQRMYAPGINIT	HLA-DRB1*11:01	254	268	0.13
	FSNKYNIRMDLKLEY	HLA-DRB1*03:01	414	428	0.01
WP_000709741.1	LTPFNQVKSRTIFQL	HLA-DRB1*07:01	220	234	0.01
	NLTPFNQVKSRTIFQ	HLA-DRB1*07:01	219	233	0.01
WP_000822057.1	SFNTNYFVIFAKRYY	HLA-DRB1*13:01	556	570	0.01
	FNTNYFVIFAKRYYA	HLA-DRB1*13:01	557	571	0.01
WP_001228436.1	LINQNALPINYANLS	HLA-DRB1*13:01	437	451	1.52
	VATGLNYRYKHSKYS	HLA-DRB1*08:01	602	616	0.08
WP_001092132.1	QQYYEYFNNLARMIA	HLA-DRB1*11:01	194	208	0.06
	QYYEYFNNLARMIAL	HLA-DRB1*11:01	195	209	0.06
WP_010875534.1	QLQYRRLYSVYLNYV	HLA-DRB1*01:01	591	605	0.09
	TQLQYRRLYSVYLNY	HLA-DRB1*01:01	590	604	0.13

WP_000911476.1	GIKIHIDSNPKFRGL	HLA-DRB1*03:01	255	269	0.11
	EAPGIKIHIDSNPKF	HLA-DRB1*03:01	252	266	0.12
WP_001108275.1	VGYWGGLVGQKPWAS	HLA-DRB1*13:01	124	138	0.11
	WGGLVGQKPWASCGL	HLA-DRB1*13:01	127	141	0.11
WP_010875548.1	SVNASLQINNIFNMK	HLA-DRB1*13:01	749	763	0.07
	TSTDYFQIFNVMEGG	HLA-DRB1*13:01	562	576	0.45
WP_000479960.1	VFIIKLDYTIPKTGI	HLA-DRB1*03:01	652	666	0.11
	IIKLDYTIPKTGINL	HLA-DRB1*03:01	654	668	0.28
WP_000753173.1	ANFQFLFNMGVRMNL	HLA-DRB1*11:01	641	655	0.61
	NFQFLFNMGVRMNLA	HLA-DRB1*15:01	642	656	0.88
WP_000592437.1	SQYLYSLLGAYPTKL	HLA-DRB1*01:01	82	96	0.05
	PTIYNTYYKSAGTTV	HLA-DRB1*07:01	485	499	0.2
WP_000812546.1	SQKVRFLAPLSLALS	HLA-DRB1*11:01	19	33	0.15
	QSQKVRFLAPLSLAL	HLA-DRB1*11:01	18	32	0.19
WP_001248496.1	EKYFLTSSLSLLSFL	HLA-DRB1*07:01	4	18	0.01
	FIDIVTLAQVKVNLL	HLA-DRB1*11:01	58	72	0.34
WP_000874574.1	LLNFNGDTTLQNNAN	HLA-DRB1*03:01	999	1013	3.93
	QINTYTQQMSRLAKL	HLA-DRB1*01:01	2200	2214	4.16
WP_000902542.1	HRVTGSLQINNIFNM	HLA-DRB1*13:01	798	812	0.07
	TGDPSFIKSLGNNLL	HLA-DRB1*07:01	217	231	0.27
WP_000915379.1	NLTNLMLNMMAVFDS	HLA-DRB1*13:01	259	273	0.01
	VIYSYRVTNNLYVNL	HLA-DRB1*07:01	342	356	0.24
WP_000945747.1	FEYNNKMYIDRKELQ	HLA-DRB1*03:01	39	53	0.1
	MYIDRKELQQRQSNQ	HLA-DRB1*03:01	45	59	0.24

5. Conclusions

In the present study, bioinformatic analysis was made to the complete genome of *H. pylori* (Strain 26695 with Gen Bank access number of NC_000915). We identified 22 outer membrane proteins using the Vaxign web program. A total of 60 class I epitopes and 40 class II epitopes were predicted. These epitopes could be used in the improvement of active vaccines, for which we consider it important to combine diverse antigens of H. pylori, thus guiding the immune system to defend the host against this bacterium. Accordingly, this study was developed to predict antigenic determinants / epitopes of various outer membrane proteins of *H. pylori*. We propose probable epitopes of B and T cells that can trigger a desired immune response to these proteins. Additionally, they could be used in the design of therapeutic strategies without the use of antibiotics such as passive immunization, in which antibodies are administered to patients to help control infection by this microorganism, which we propose as the first line of action, before use of antibiotics.

Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

References

Ayraud, S., Janvier, B., & Fauchère, J.-L. (2002). Experimental colonization of mice by fresh clinical isolates of Helicobacter pylori is not influenced by the cagA status and the vacA genotype. *FEMS Immunology and Medical Microbiology*, *34*(3), 169-172. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12423767

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2006). GenBank. *Nucleic Acids Research*, *34*(Database issue), D16-20. https://doi.org/10.1093/nar/gkj157

Chen, F., Mackey, A. J., Stoeckert, C. J., & Roos, D. S. (2006). OrthoMCL-DB: querying a comprehensive

multi-species collection of ortholog groups. *Nucleic Acids Research*, *34*(Database issue), D363-8. https://doi.org/10.1093/nar/gkj123

Fallone, C. A., Chiba, N., van Zanten, S. V., Fischbach, L., Gisbert, J. P., Hunt, R. H., … Marshall, J. K. (2016). The Toronto Consensus for the Treatment of Helicobacter pylori Infection in Adults. *Gastroenterology*, *151*(1), 51-69.e14. https://doi.org/10.1053/j.gastro.2016.04.006

Ford, A. C., & Axon, A. T. R. (2010). Epidemiology of Helicobacter pylori infection and Public Health Implications. *Helicobacter*, *15*, 1-6. https://doi.org/10.1111/j.1523-5378.2010.00779.x

He, Y., Xiang, Z., & Mobley, H. L. T. (2010). Vaxign: The first web-based vaccine design program for reverse vaccinology and applications for vaccine development. *Journal of Biomedicine and Biotechnology*, *2010*. https://doi.org/10.1155/2010/297505

Hooi, J. K. Y., Lai, W. Y., Ng, W. K., Suen, M. M. Y., Underwood, F. E., Tanyingoh, D., … Ng, S. C. (2017). Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. *Gastroenterology*, *153*(2), 420-429. https://doi.org/10.1053/j.gastro.2017.04.022

Jemilohun, A. C., & Otegbayo, J. A. (2016). Helicobacter pylori infection: Past, present and future. *Pan African Medical Journal*. African Field Epidemiology Network. https://doi.org/10.11604/pamj.2016.23.216.8852

Kabir, S. (2011). The Role of Interleukin-17 in the Helicobacter pylori Induced Infection and Immunity. *Helicobacter*, *16*(1), 1-8. https://doi.org/10.1111/j.1523-5378.2010.00812.x

Liao, Y., Deng, J., Zhang, A., Zhou, M., Hu, Y., Chen, H., & Jin, M. (2009). Immunoproteomic analysis of outer membrane proteins and extracellular proteins of Actinobacillus pleuropneumoniae JL03 serotype 3. *BMC Microbiology*, *9*(1), 172. https://doi.org/10.1186/1471-2180-9-172

Mégraud, F. (2012). The challenge of Helicobacter pylori resistance to antibiotics: the comeback of bismuth-based quadruple therapy. *Therapeutic Advances in Gastroenterology*, *5*(2), 103-109. https://doi.org/10.1177/1756283X11432492

Moss, S. F., Moise, L., Lee, D. S., Kim, W., Zhang, S., Lee, J., … De Groot, A. S. (2011). HelicoVax: epitope-based therapeutic Helicobacter pylori vaccination in a mouse model. *Vaccine*, *29*(11), 2085–2091. https://doi.org/10.1016/j.vaccine.2010.12.130

Ni, X. D., Wang, N., Liu, Y. J., & Lu, C. P. (2010). Immunoproteomics of extracellular proteins of the Aeromonas hydrophila China vaccine strain J-1 reveal a highly immunoreactive outer membrane protein. *FEMS Immunology and Medical Microbiology*, *58*(3), 363–373. https://doi.org/10.1111/j.1574-695X.2009.00646.x

Pilotto, A., & Franceschi, M. (2014). Helicobacter pylori infection in older people. *World Journal of Gastroenterology*, *20*(21), 6364-6373. https://doi.org/10.3748/wjg.v20.i21.6364

Pruitt, K. D., Tatusova, T., & Maglott, D. R. (2005). NCBI Reference Sequence (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Research*, *33*(Database issue), D501-4. https://doi.org/10.1093/nar/gki025

Refaeli, R., Chodick, G., Haj, S., Goren, S., Shalev, V., & Muhsen, K. (2018). Relationships of H. pylori infection and its related gastroduodenal morbidity with metabolic syndrome: a large cross-sectional study. *Scientific Reports*, *8*(1), 4088. https://doi.org/10.1038/s41598-018-22198-9

Sachdeva, G., Kumar, K., Jain, P., & Ramachandran, S. (2005). SPAAN: a software program for prediction of adhesins and adhesin-like proteins using neural networks. *Bioinformatics*, *21*(4), 483-491. https://doi.org/10.1093/bioinformatics/bti028

Spohn, G., & Scarlato, V. (2001). *Motility, Chemotaxis, and Flagella*. *Helicobacter pylori: Physiology and Genetics*. ASM Press. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21290725

Xiang, Z., & He, Y. (2009). Vaxign: a web-based vaccine target design program for reverse vaccinology. *Procedia in Vaccinology*, *1*(1), 23-29. https://doi.org/10.1016/j.provac.2009.07.005

Xiang, Z., & He, Y. (2013). Genome-wide prediction of vaccine targets for human herpes simplex viruses using Vaxign reverse vaccinology. *BMC Bioinformatics*, *14 Suppl 4*(4), S2. https://doi.org/10.1186/1471-2105-14-S4-S2

Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., … Brinkman, F. S. L. (2010). PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics (Oxford, England)*, *26*(13), 1608-1615.

https://doi.org/10.1093/bioinformatics/btq249

Appendix

Table 1s. Comparison of four software for prediction of epitopes MHC I and MHC II classes of Helicobacter pylori

Accession #	sofware	predicion petidos		metodo de prediciòn
		MHC I	MHC II	
WP_000726314.1	Vaxign	90	86	PSSM
	epijen v1.0	40		AGMP
	CTLPred	28		ANNs y SVMs
	imtech		3	MCTT y ANNs
WP_000713712.1	Vaxign	92	131	PSSM
	epijen v1.0	42		AGMP
	CTLPred	29		ANNs y SVMs
	imtech		3	MCTT y ANNs
WP_001115603	Vaxign	94	112	PSSM
	epijen v1.0	28		AGMP
	CTLPred	29		ANNs y SVMs
	imtech		51	MCTT y ANNs
WP_000709741.1	Vaxign	66	48	PSSM
	epijen v1.0	18		AGMP
	CTLPred	17		ANNs y SVMs
	imtech		34	MCTT y ANNs
WP_000822057.1	Vaxign	186	154	PSSM
	epijen v1.0	51		AGMP
	CTLPred	36		ANNs y SVMs
	imtech		4	MCTT y ANNs
WP_001228436.1	Vaxign	151	134	PSSM
	epijen v1.0	28		AGMP
	CTLPred	32		ANNs y SVMs
	imtech		68	MCTT y ANNs
WP_001092132	Vaxign	114	99	PSSM
	epijen v1.0	28		AGMP
	CTLPred	23		ANNs y SVMs
	imtech		51	MCTT y ANNs
WP_010875534.1	Vaxign	122	109	PSSM
	epijen v1.0	27		AGMP
	CTLPred	32		ANNs y SVMs
	imtech		61	MCTT y ANNs

WP_000911476.1	Vaxign	112	110	PSSM
	epijen v1.0	26		AGMP
	CTLPred	26		ANNs y SVMs
	imtech		48	MCTT y ANNs
WP_001108275.1	Vaxign	118	96	PSSM
	epijen v1.0	27		AGMP
	CTLPred	21		ANNs y SVMs
	imtech		52	MCTT y ANNs
WP_010875548.1	Vaxign	174	155	PSSM
	epijen v1.0	27		AGMP
	CTLPred	36		ANNs y SVMs
	imtech		81	MCTT y ANNs
WP_000479960.1	Vaxign	184	132	PSSM
	epijen v1.0	27		AGMP
	CTLPred	30		ANNs y SVMs
	imtech		82	MCTT y ANNs
WP_000753173.1	Vaxign	142	126	PSSM
	epijen v1.0	27		AGMP
	CTLPred	16		ANNs y SVMs
	imtech		73	MCTT y ANNs
WP_000592437.1	Vaxign	102	88	PSSM
	epijen v1.0	27		AGMP
	CTLPred	27		ANNs y SVMs
	imtech	55		MCTT y ANNs
WP_000812546.1	Vaxign	112	113	PSSM
	epijen v1.0	27		AGMP
	CTLPred	26		ANNs y SVMs
	imtech		57	MCTT y ANNs
WP_001248496.1	Vaxign	127	110	PSSM
	epijen v1.0	29		AGMP
	CTLPred	24		ANNs y SVMs
	imtech		55	MCTT y ANNs
WP_000874574.1	Vaxign	514	506	PSSM
	epijen v1.0	130		AGMP
	CTLPred	96		ANNs y SVMs
	imtech		249	MCTT y ANNs
WP_000902542.1	Vaxign	189	143	PSSM
	epijen v1.0	46		AGMP
	CTLPred	36		ANNs y SVMs
	imtech		89	MCTT y ANNs
WP_000915379.1	Vaxign	167	122	PSSM

	epijen v1.0	59		AGMP
	CTLPred	46		ANNs y SVMs
	imtech		112	MCTT y ANNs
WP_000945747.1	Vaxign	193	148	PSSM
	epijen v1.0	47		AGMP
	CTLPred	36		ANNs y SVMs
	imtech		89	MCTT y ANNs