Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Online ISSN
Print ISSN
3007-3189
Volume 3, Issue 8 (2025)
http://amresearchreview.com/index.php/Journal/about

In-silico characterization of VacA virulence factor of *Helicobacter pylori*

Nadia Hussain
Department of Pharmaceutical Sciences, College of Pharmacy, Al Ain University, Al Ain Campus, 64141 Al Ain, United Arab Emirates. E-mail: nadia.hussain@aau.ac.ae
AAU Health and Biomedical Research Center, Al Ain University, Abu Dhabi Campus, P. O. Box 112612, Abu Dhabi, United Arab Emirates. E-mail: nadia.hussain@aau.ac.ae

Saboor Muarij Bunny
Research Associate, Pakistan Biosafety Clearing House-Pakistan Environmental Protection Agency. E-mail: saboorbunny73@gmail.com

Amal H. I. Al Haddad
Chief Operations Office, Sheikh Shakhbout Medical City (SSMC), PureHealth, Abu Dhabi, UAE. E-mail; ahhaddad@ssmc.ae

Wardah Shahid
Institute of Chemistry, The Islamia University of Bahawalpur, Pakistan. E-mail: wardahshahid@ymail.com

Shafia Arshad
Office of Research Innovation and Commercialization, The Govt. Sadiq College Women University Bahawalpur, Pakistan. E-mail: shafia.arshad@gscwu.edu.pk

Fatima Muccee (Corresponding Author)
School of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan. E-mail: fatima.sbb@pu.edu.pk

ABSTRACT

Helicobacter pylori is associated with incidence of gastric cancer due to several virulence factors. Its virulence can be reduced through inhibiting its toxins. Vacuolating cytotoxin (VacA) is one of its virulence factors. Current study focused at characterization of VacA. Its sequence was retrieved from Uniprot database and analyzed through SOPMA tool, ALPHAFOLD database, STRING tool and QuickGo. Analyses demonstrated that alpha helix, beta pleated sheets and random coil were composed of 17, 26.94 and 56.06% amino acids, respectively. The 3D configuration identified two major domains one significantly comprised of beta sheet and other of alpha helix. Protein interaction analysis revealed three protein partners with highest scores. i. e., ureB (0.733), hpaA (0.704) and dps (0.702). Gene ontology revealed its

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Online ISSN Print ISSN
3007-3189
Volume 3, Issue 8 (2025)
http://amresearchreview.com/index.php/Journal/about

function in toxicity. These findings might help in genetic alteration of VacA in order to inhibit its involvement in gastric cancer.

Keywords: *Helicobacter pylori*, vacuolating cytotoxin A, gene ontology, configuration, interaction

1. Introduction

Helicobacter pylori is a bacterium that infects stomach and intestinal lining and may cause gastritis, peptic ulcer and gastric cancer (Kusters et al. 2006, Malfertheiner et al. 2023). Pathogenesis of H. pylori depends on its virulence associated proteins that include colonization, immune evasion and disease induction factors. The colonization factors include adhesins, flagella, outer membrane proteins (OMPs), blood-group antigen binding adhesion (BabA) and sialic acid binding adhesin (SabA), *H. pylori* outer membrane protein (HopQ), outer inflammatory protein (OipA) and lipopolysaccharides (LPS) (Benktander et al. 2018, Pride and Blaser 2002, Sallas et al. 2019). Disease induction factors include vacuolating cytotoxin (VacA), Cag-pathogenicity island (CagPAI), outer-membrane vesicles (OMVs), high temperature requirement protein (HtrA) and *H. pylori* gamma glutamyl transpeptidase (ggt) (Kim et al. 2015, McGovern et al. 2001, Merdanovic et al. 2011, Ricci 2016, Schuelein et al. 2011). Immune evasion factors include urease, *H. pylori* neutrophil activating protein (HP-NAP) and superoxide dismutase (SOD) (Amedei et al. 2006, Baj et al. 2020, Sedarat and Taylor-Robinson 2024).

VacA encodes a toxin of 88 kDa which enters the host cells through binding with sphingomyelin localized in host cells (Papini et al. 2001). Once inside the cell, it gets accumulated in endosomes and is transferred to late endosome and then lysosome (Rassow 2011). Oligomerization of VacA occurs into hexamer during endosomal pathway. Oligomer causes depolarization of membrane which results in the formation of vacuole (Montecucco and De Bernard 2003b). The depolarization causes disturbance in calcium signaling pathways, T-cell inactivation, dysfunction of mitochondria and induction of autophagy (Luo et al. 2021, Montecucco and de Bernard 2003a, Rassow 2011).

Considering the association of VacA virulence factor with pathogenesis in human, we initiated current study targeting the characterization of VacA protein based on in-silico tools. This study might help us in exploring the molecular basis of this protein and predicting ways of blocking this toxin at molecular level.

2. Methodology

2.1 UNIPROT DATABASE

In current study, for analysis of properties of VacA protein, sequence was retrieved from Uniprot database (accessed on December 2024, available at https://uniprot.org). The protein comprised of 1288 amino acids with accession number Q9ZKW5 (Figure 1).

MEIQQTHRKINRPLVSLVLAGALISAIPQESHAAFFTTVIIPAIVGGIATGTA
VGTVSGLLSWGLKQAEEANKTPDKPDKVWRIQAGKGFNEFPNKEYDLY
KSLLSSKIDGGWDWGNAARHYWVKGGQWNKLEVDMKDAVGTYKLSGL
RNFTGGDLDVNMQKATLRLGQFNGNSFTSYKDSADRTTRVNFNAKNISID
NFVEINNRVGSGAGRKASSTVLTLQASEGITSSKNAEISLYDGATLNLASN
SVKLNGNVWMGRLQYVGAYLAPSYSTINTSKVQGEVDFNHLTVGDQNA

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Online ISSN Print ISSN
3007-3189
Volume 3, Issue 8 (2025)
http://amresearchreview.com/index.php/Journal/about

AQAGIIASNKTHIGTLDLWQSAGLNIIAPPEGGYKDKPNSTTSQSGTKNDK
KEISQNNNSNTEVINPPNNTQKTETEPTQVIDGPFAGGKDTVVNIFHLNTK
ADGTIKVGGFKASLTTNAAHLNIGKGGVNLSNQASGRTLLVENLTGNITV
DGPLRVNNQVGGYALAGSSANFEFKAGVDTKNGTATFNNDISLGRFVNL
KVDAHTANFKGIDTGNGGFNTLDFSGVTDKVNINKLITASTNVAVKNFNI
NELIVKTNGISVGEYTHFSEDIGSQSRINTVRLETGTRSIFSGGVKFKSGEKL
VINDFYYSPWNYFDARNVKNVEITRKFASSTPENPWGTSKLMFNNLTLGQ
NAVMDYSQFSNLTIQGDFINNQGTINYLVRGGKVATLNVGNAAAMMFN
NDIDSATGFYKPLIKINSAQDLIKNTEHVLLKAKIIGYGNVSTGTNGISNVN
LEEQFKERLALYNNNNRMDTCVVRNTDDIKACGMAIGNQSMVNNPDNY
KYLIGKAWRNIGISKTANGSKISVYYLGNSTPTENGGNTTNLPTNTTNNA
HSANYALVKNAPFAHSATPNLVAINQHDFGTIESVFELANRSKDIDTLYTH
SGAQGRDLLQTLLIDSHDAGYARQMIDNTSTGEITKQLNAATDALNNVAS
LEHKQSGLQTLSLSNAMILNSRLVNLSRKHTNHINSFAQRLQALKGQEFA
SLESAAEVLYQFAPKYEKPTNVWANAIGGASLNSGSNASLYGTSAGVDA
FLNGNVEAIVGGFGSYGYSSFSNQANSLNSGANNANFGVYSRFFANQHEF
DFEAQGALGSDQSSLNFKSTLLQDLNQSYNYLAYSATARASYGYDFAFFR
NALVLKPSVGVSYNHLGSTNFKSNSQSQVALKNGASSQHLFNANANVEA
RYYYGDTSYFYLHAGVLQEFAHFGSNDVASLNTFKINAARSPLSTYARA
MMGGELQLAKEVFLNLGVVYLHNLISNASHFASNLGMRYSF

Figure 1: Amino acid sequence of cytotoxin associated protein A (CagA) from *H. pylori* retrieved from Uniprot database

2.2 SOPMA TOOL

For prediction of 2D configuration, SOPMA Network Protein Sequence Analysis (NPS@) (accessed on December 2024, available at https://npsa.lyon.inserm.fr/cgi-bin/secpred_sopma.pl) was consulted.

2.3 ALPHAFOLD DATABASE

In order to analyze the 3D structure of VacA protein, ALPHAFOLD Protein Structure Database (accessed on December 2024, available at https://alphafold.ebi.ac.uk) was employed.

2.4 STRING TOOL

STRING tool (accessed on December 2024, available at https://string-db.org/cgi/input?sessionId=bR9wkraynabk&input_page_show_search=on) was consulted to analyze the interacting legends of VacA. Protein by sequence option was selected to perform analysis and amino acids sequence was given as input. *H. pylori* was selected as the organism. Protein interacting partners were predicted alongwith their scores.

2.5 QUICKGO

For gene ontology annotations, web based tool, QUICKGO (accessed in December 2024, available at https://www.ebi.ac.uk/QuickGO/api/index.html) was employed.

3. Results

3.1 Analysis of 2D configuration

Three aspects of 2D structure. i. e., alpha helix, extended strand and random coil

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 8 (2025)
Online ISSN
Print ISSN
3007-3189
http://amresearchreview.com/index.php/Journal/about

were predicted. The 219 amino acids were found as part of alpha helix which constituted 17% of total amino acids. Extended strand and random coil were composed of 347 amino acids (26.94%) and 722 amino acids (56.06%), respectively (Figure 2).

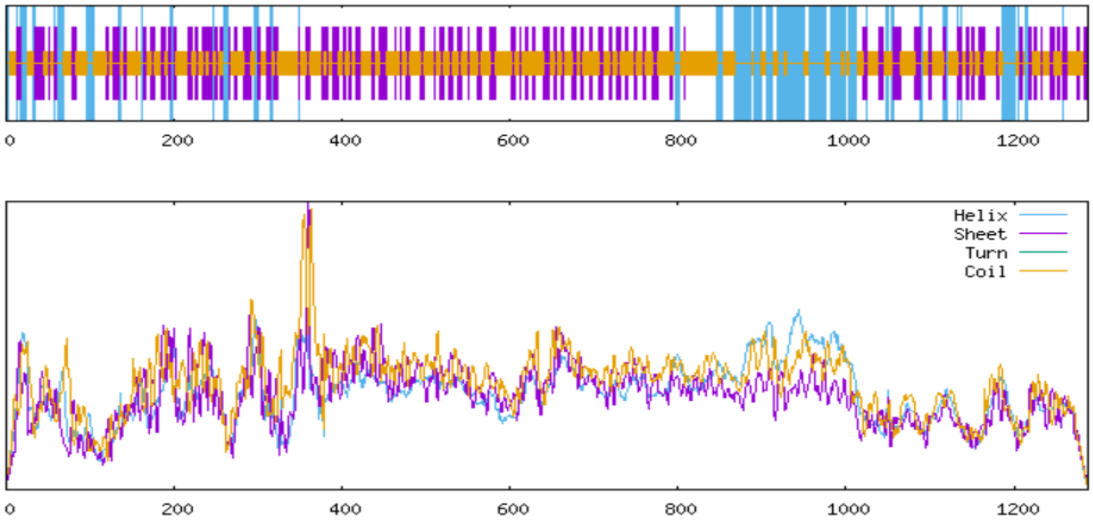
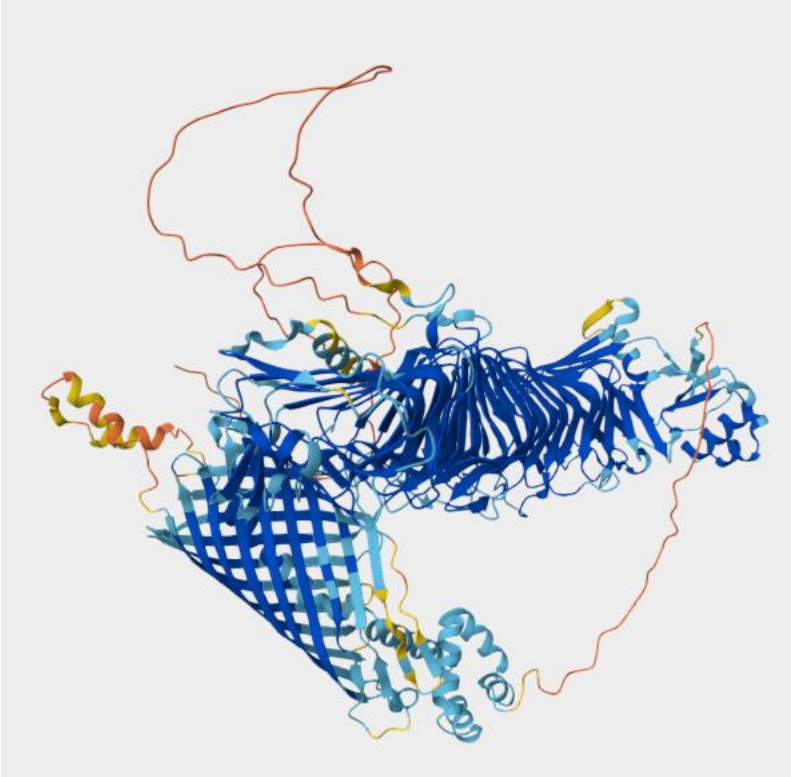


Figure 2: Prediction of 2D configuration of VacA protein based on SOPMA tool

3.2 Analysis of 3D configuration

The 3D structure analysis revealed both the alpha helix and beta pleated sheet components in equal proportions. Structure showed highly complex folding and coiling (Figure 3).



Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Online ISSN
Print ISSN
3007-3189
Volume 3, Issue 8 (2025)
http://amresearchreview.com/index.php/Journal/about

Figure 3: Prediction of 3D configuration of VacA virulence factor based on ALPHAFOLD database
Dark blue: very high confidence (pLDDT > 90), light blue: confident (90 > pLDDT > 70), yellow: low (70 > pLDDT >50), orange: very low (pLDDT < 50)

3.3 Analysis of protein-protein interactions

Total ten interacting protein partners were predicted for VacA virulence factor (Figure 3). Scores of urea amidohydrolase (ureB), flagellar sheath adhesin (hpaA), neutrophil activating protein (dps), Cag pathogenicity island protein 26 (CagA), hypothetical protein (HP_0902), Cag pathogenicity island protein 23 (CagE), cysteinyl-tRNA synthetase (cysS), urease protein (glmM), chaperone and heat shock protein (groL) and gamma glutamyltranspeptidase (HP_1118) were found as 0.733, 0.704, 0.702, 0.625, 0.610, 0.587, 0.556, 0.542, 0.525 and 0.512, respectively (Figure 4).

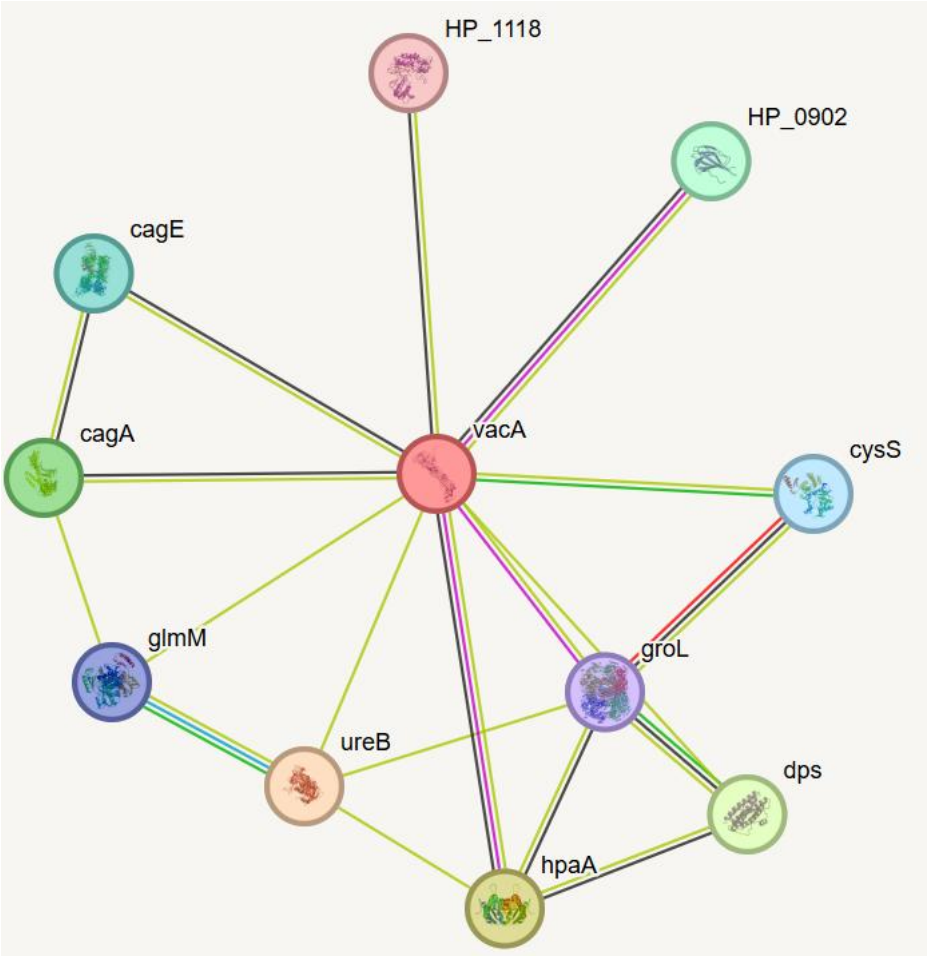


Figure 3: STRING tool based prediction of protein-protein interactions of VacA virulence factor from *H. pylori*
VacA: vacuolating cytotoxin autotransporter, ureB: urea amidohydrolase, glmM: urease protein C, hpaA: flagellar sheath adhesin hpaA, dps: neutrophil activating protein, groL: chaperone and heat shock protein, cycA: Cag pathogenicity island protein, cagE: Cag pathogenicity island protein 23, cysS: cysteinyl-tRNA synthetase,

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Online ISSN
Print ISSN
3007-3189
Volume 3, Issue 8 (2025)
http://amresearchreview.com/index.php/Journal/about

HP_1118: gamma glutamyltranspeptidase, HP_ 0902: hypothetical protein

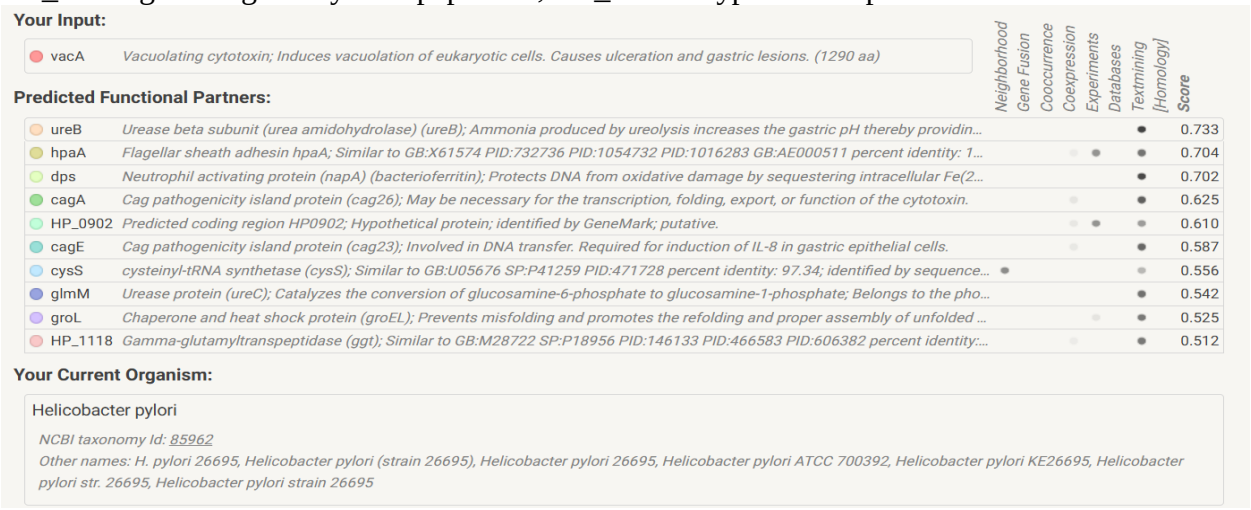


Figure 4: Protein interacting partners of VacA virulence factor of *H. pylori* and their scores, predicted through STRING tool analysis

3.4 Analysis of gene ontology

Gene ontology analysis predicted following functions of VacA. Toxin activity (GO:0090729), modulation of process of another organism (GO:0035821), extracellular region (GO:0005576), cell outer membrane (GO:0009279), cell surface (GO:0009986), membrane (GO:0016020), outer membrane (GO:0019867) and periplasmic space (GO:0042597).

4. Discussion

Several genotypes of VacA virulence factor has been documented in literature as associated with gastric cancer. E. g., vacAs1/il/ml, m1, il, c1 and i region polymorphisms (Bakhti et al. 2016, Jang et al. 2010). This association highlights it as a crucial factor that can be blocked to prevent incidence of gastric cancer.

The 2D configuration of VacA predicted in current study is consistent with previous findings (Gangwer et al. 2007). The 3D structure analysis revealed two domains in VacA configuration. This finding is in accordance with earlier literature which documents two domains. i. e., p33 and p55, for this virulence factor (Chambers et al. 2013). . Only little information is available in earlier published work regarding p33 but detailed x-ray crystallography based structure has been predicted for p55 (Gangwer et al. 2007).

Identification of urea amidohydrolase, neutrophil activating protein, CagA, CagE and ggt as interacting protein partners of VacA is consistent with previous work (Day et al. 2000, Fu 2014, Ling et al. 2015, Nejati et al. 2018, Olivera-Severo et al. 2006). However, no study has documented cysS, groEL and glmM direct physical interaction with VacA, hence our study is inconsistent with literature in this regard (De Reuse et al. 1997, Junaid et al. 2016, 翟康乐 et al. 2023).

Biological function of VacA found via ontology analysis demonstrated its association with cell outer membrane and periplasmic space which is consistent with literature (Czajkowsky et al. 1999, Iwamoto et al. 1999, Tombola et al. 1999a, Tombola et al. 1999b). Gene ontology analysis revealed the function of VacA as toxin which is consistent with earlier literature (Boquet and Ricci 2012, Chauhan et al. 2019, Cover and Blanke 2005, Foegeding et al. 2016).

This detailed insight into structure and molecular interactions of VacA may be

considered to find out the possible ways of interfering its mechanism of pathogenesis.

Statements and Declarations:

Informed consent: N/A

Ethical approval: N/A

Competing interests: *The authors have no relevant financial or non-financial interests to disclose.*

Funding: *The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.*

Author contributions: N.H. write up of original draft, S.M.B. formal analysis, A.H.I.A.H. formal analysis, W.S. data curation, S.A. data visualization, F.M. supervision and perceived the idea

Acknowledgements: The authors would like to extend their gratitude to School of Biochemistry and Biotechnology (SBB), University of the Punjab, Lahore.

Submission declaration and verification: The manuscript has not been submitted anywhere else and is not under consideration by any other journal.

Data availability statement: The sequence of VacA documented in current study is available at https://uniprot.org

References

Amedei A, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G. 2006. The neutrophil-activating protein of Helicobacter pylori promotes Th1 immune responses. The Journal of clinical investigation 116:1092-1101.

Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, Maciejewski R. 2020. Helicobacter pylori virulence factors—mechanisms of bacterial pathogenicity in the gastric microenvironment. Cells 10:27.

Bakhti Z, Latifi-Navid S, Mohammadi S, Zahri S, Bakhti F, Feizi F, Siavoshi F. 2016. Relevance of Helicobacter pylori vacA 3′-end Region Polymorphism to Gastric Cancer. . Helicobacter 21:305-316.

Benktander J, Barone A, Johansson MM, Teneberg S. 2018. Helicobacter pylori SabA binding gangliosides of human stomach. Virulence 9:738-751.

Boquet P, Ricci V. 2012. Intoxication strategy of Helicobacter pylori VacA toxin. Trends in microbiology 20:165-174.

Chambers MG, Pyburn TM, González-Rivera C, Collier SE, Eli I, Yip CK, Takizawa Y, Lacy DB, Cover TL, Ohi MD. 2013. Structural analysis of the oligomeric states of Helicobacter pylori VacA toxin. Journal of molecular biology 425:524-535.

Chauhan N, Tay ACY, Marshall BJ, Jain U. 2019. Helicobacter pylori VacA, a distinct toxin exerts diverse functionalities in numerous cells: An overview. Helicobacter 24:e12544.

Cover TL, Blanke SR. 2005. Helicobacter pylori VacA, a paradigm for toxin multifunctionality. Nature Reviews Microbiology 3:320-332.

Czajkowsky DM, Iwamoto H, Cover TL, Shao Z. 1999. The vacuolating toxin from Helicobacter pylori forms hexameric pores in lipid bilayers at low pH. Proceedings of the National Academy of Sciences 96:2001-2006.

Day AS, Jones NL, Lynett JT, Jennings HA, Fallone CA, Beech R, Sherman PM. 2000. cagE Is a Virulence Factor Associated with Helicobacter pylori—Induced Duodenal Ulceration in Children. The Journal of infectious diseases 181:1370-1375.

De Reuse H, Labigne A, Mengin-Lecreulx D. 1997. The Helicobacter pylori ureC gene codes for a phosphoglucosamine mutase. Journal of bacteriology 179:3488-3493.

Foegeding NJ, Caston RR, McClain MS, Ohi MD, Cover TL. 2016. An overview of Helicobacter pylori VacA toxin biology. Toxins 8:173.

Fu H-W. 2014. Helicobacter pylori neutrophil-activating protein: from molecular pathogenesis to clinical applications. World journal of gastroenterology: WJG 20:5294.

Gangwer KA, Mushrush DJ, Stauff DL, Spiller B, McClain MS, Cover TL, Lacy DB. 2007. Crystal structure of the Helicobacter pylori vacuolating toxin p55 domain. Proceedings of the National Academy of Sciences 104:16293-16298.

Iwamoto H, Czajkowsky DM, Cover TL, Szabo G, Shao Z. 1999. VacA from Helicobacter pylori: a hexameric chloride channel. FEBS letters 450:101-104.

Jang S, Jones KR, Olsen CH, Joo YM, Yoo Y-J, Chung I-S, Cha J-H, Merrell DS. 2010. Epidemiological link between gastric disease and polymorphisms in VacA and CagA. Journal of clinical microbiology 48:559-567.

Junaid M, Linn AK, Javadi MB, Al-Gubare S, Ali N, Katzenmeier G. 2016. Vacuolating cytotoxin A (VacA)–A multi-talented pore-forming toxin from Helicobacter pylori. Toxicon 118:27-35.

Kim JH, Lee J, Park J, Gho YS. 2015. Gram-negative and Gram-positive bacterial extracellular vesicles. Pages 97-104. Seminars in cell & developmental biology: Elsevier.

Kusters JG, Van Vliet AH, Kuipers EJ. 2006. Pathogenesis of Helicobacter pylori infection. Clinical microbiology reviews 19:449-490.

Ling SSM, Khoo LHB, Hwang L-A, Yeoh KG, Ho B. 2015. Instrumental role of Helicobacter pylori γ-glutamyl transpeptidase in VacA-dependent vacuolation in gastric epithelial cells. PloS one 10:e0131460.

Luo J, Bai L, Tao J, Wen Y, Li M, Zhu Y, Luo S, Pu G, Ma L. 2021. Autophagy induced by H. pylori VacA regulated the survival mechanism of the SGC7901 human gastric cancer cell line. Genes & Genomics 43:1223-1230.

Malfertheiner P, Camargo MC, El-Omar E, Liou J-M, Peek R, Schulz C, Smith SI, Suerbaum S. 2023. Helicobacter pylori infection. Nature reviews Disease primers 9:19.

McGovern K, Blanchard T, Gutierrez J, Czinn S, Krakowka S, Youngman P. 2001. γ-Glutamyltransferase is a Helicobacter pylori virulence factor but is not essential for colonization. Infection and immunity 69:4168-4173.

Merdanovic M, Clausen T, Kaiser M, Huber R, Ehrmann M. 2011. Protein quality control in the bacterial periplasm. Annual review of microbiology 65:149-168.

Montecucco C, de Bernard M. 2003a. Immunosuppressive and proinflammatory activities of the VacA toxin of Helicobacter pylori. The Journal of experimental medicine 198:1767-1771.

---. 2003b. Molecular and cellular mechanisms of action of the vacuolating cytotoxin (VacA) and neutrophil-activating protein (HP-NAP) virulence factors of Helicobacter pylori. Microbes and infection 5:715-721.

Nejati S, Karkhah A, Darvish H, Validi M, Ebrahimpour S, Nouri HR. 2018. Influence of Helicobacter pylori virulence factors CagA and VacA on pathogenesis of gastrointestinal disorders. Microbial pathogenesis 117:43-48.

Olivera-Severo D, Wassermann GE, Carlini C. 2006. Ureases display biological effects independent of enzymatic activity: is there a connection to diseases

caused by urease-producing bacteria? Brazilian journal of medical and biological research 39:851-861.

Papini E, Zoratti M, Cover TL. 2001. In search of the Helicobacter pylori VacA mechanism of action. Toxicon 39:1757-1767.

Pride DT, Blaser MJ. 2002. Concerted evolution between duplicated genetic elements in Helicobacter pylori. Journal of molecular biology 316:629-642.

Rassow J. 2011. Helicobacter pylori vacuolating toxin A and apoptosis. Cell communication and signaling 9:26.

Ricci V. 2016. Relationship between VacA toxin and host cell autophagy in Helicobacter pylori infection of the human stomach: a few answers, many questions. Toxins 8:203.

Sallas ML, Dos Santos MP, Orcini WA, David ÉB, Peruquetti RL, Payão SLM, Rasmussen LT. 2019. Status (on/off) of oipA gene: their associations with gastritis and gastric cancer and geographic origins. Archives of microbiology 201:93-97.

Schuelein R, Everingham P, Kwok T. 2011. Integrin-mediated type IV secretion by Helicobacter: what makes it tick? Trends in microbiology 19:211-216.

Sedarat Z, Taylor-Robinson AW. 2024. Helicobacter pylori outer membrane proteins and virulence factors: potential targets for novel therapies and vaccines. Pathogens 13:392.

Tombola F, Carlesso C, Szabò I, De Bernard M, Reyrat JM, Telford JL, Rappuoli R, Montecucco C, Papini E, Zoratti M. 1999a. Helicobacter pylori vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. Biophysical journal 76:1401-1409.

Tombola F, Oregna F, Brutsche S, Szabò I, Del Giudice G, Rappuoli R, Montecucco C, Papini E, Zoratti M. 1999b. Inhibition of the vacuolating and anion channel activities of the VacA toxin of Helicobacter pylori. FEBS letters 460:221-225.

翟康乐, 宫雅楠, 肖迪, 孙路, 何利华, 房梦飏, 尤元海, 王磊, 闫笑梅, 张建中. 2023. Interacting proteins of Helicobacter pylori GroEL protein. Acta Microbiologica Sinica 63:1970-1981.