REVIEW

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Overview of *Helicobacter pylori* Infection in Indonesia: What Distinguishes It from Countries with High Gastric Cancer Incidence?

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Helicobacter pylori infects more than half the human population. However, the prevalence in Indonesia is low, as is the prevalence of gastric cancer. Hence, it could be instructive to compare these prevalence rates and their determining factors with those of countries that have high gastric cancer incidence. Ethnicity and genetic characteristics of *H. pylori* are important determinants of the *H. pylori* infection rate in Indonesia. The infection rate is higher in Bataknese, Papuans and Buginese than in Javanese, the predominant ethnic group. Ethnicity is also an important determinant of the genetic characteristics of *H. pylori*. Analysis of CagA in the EPIYA segment showed that the predominant genotypes in Papuans, Bataknese and Buginese are ABB-, ABD- and ABC-type CagA, respectively. Meanwhile, in the countries with high gastric cancer incidence, almost all strains had East Asian type CagA. An antibiotic susceptibility evaluation showed that the standard triple therapy can still be used with caution in several cities. There is a very high rate of resistance to second-line regimens such as levofloxacin and metronidazole. Recent studies have shown that furazolidone, rifabutin and sitafloxacin are potential alternative treatments for antibiotic-resistant *H. pylori* infection in Indonesia. Rather than focusing on early detection and eradication as in countries with high gastric cancer prevalence, countries with low gastric cancer prevalence should focus on screening the several groups that have a high risk of gastric cancer. **(Gut Liver, Published online July 6, 2020)**

Key Words: *Helicobacter pylori*; Indonesia; Prevalence; Virulence, Gastric cancer risk

INTRODUCTION

Helicobacter pylori is the most successful human pathogen and is associated with dyspepsia. It is also a common and curable cause of peptic ulcers and gastric cancer.[1-3] The infection prevalence was high is many areas such as in Africa and East Asia which reported as 79.1% and 56.1% respectively.[4] Despite their high prevalence, the gastric cancer incidence was varied. East Asian region has high age-standardized rates (ASR) of 22.4 and its countries Korea, Mongolia, and Japan were known for the highest gastric cancer incidence in the world with the ASR of 39.6, 33.1, 27.5, respectively.[5] However, the African region has very low ASR; 4.2 per 100,000 people. On contrary, there was also region that have both low prevalence of *H. pylori* infection and low incidence of gastric cancer; such as a populous archipelago country, Indonesia. The investigation on this low prevalence either infection and gastric cancer incidence is also necessary as a comparison to find the risk factor and *H. pylori* properties that may contributes to infection and gastric cancer pathogenesis.

Indonesia is the fourth most populous country in the world, with 267,842,296 people belonging to 300 ethnic groups in a total area of about 1,900,000 km^2. Based on GLOBOCAN 2018, Indonesia was categorized as a low gastric cancer risk country with ASR of 1.22/100,000 (gco.iarc.fr).[6] Our neighbor country, Malaysia was also reported to have low prevalence of *H. pylori* infection and low gastric cancer incidence (ASR of 5.2). Study

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in Malaysia reported that ethnicity play role in the *H. pylori* infection and gastric cancer and Malay ethnic is reported as low risk for gastric cancer compared to Chinese and Indian. Malay ethnic is also one of a large ethnic group in Indonesia. Indonesian's Malayan descent have low gastric mucosal severity,[7,8] similar to that among Malayans in Malaysia and Singapore.[9,10] Moreover, Indonesia consists of more ethnics that may provide more insight on the role of ethnicity and other factors.

H. pylori can damage epithelial cells and induce an inflammatory response, resulting in severe histopathology, suggesting that *H. pylori* should be eradicated even in populations with a low prevalence of *H. pylori*.[11] Detecting the presence of *H. pylori* is critically important, and validation of the tests for *H. pylori* is required, especially in areas with a low prevalence of *H. pylori*.[12] In Indonesia, urine tests have been validated with sensitivity of 83.3% and specificity of 94.7%, and serological tests have been validated with sensitivity of 86.7% and specificity of 96.8%.[13] Validation of the noninvasive urea breath test and stool antigen test is still required. Gastrointestinal endoscopy has limited availability and is predominantly utilized on Java Island, and it is not fully covered by health insurance.[14] Invasive detection of *H. pylori* infection in Indonesia is still an unfavourable choice.

In this review, we provided the current status of *H. pylori* in low risk gastric cancer country about what factor differentiating to those with high incidence of gastric cancer, including prevalence and its risk factors, *H. pylori* associated diseases, virulence factors and the antibiotic resistance, with some proposed recommendations.

PREVALENCE AND RISK FACTORS OF *H. pylori* INFECTION

In general, the prevalence of *H. pylori* infection is more than 90% in peptic ulcer patients who are not using nonsteroidal anti-inflammatory drugs[15] and 20% to 40% among patients with functional dyspepsia according to various diagnostic methods.[16] In six studies of dyspeptic patients in Indonesia, the prevalence of *H. pylori* infection varied from 5.7% to 68%.[17-19] The highest prevalence was found in Jakarta using the rapid urease test, culture and histology.[20] The results of the urea breath test varied but usually found a low prevalence of *H. pylori* infection, from 0% to 11.2%.[18,19,21] We found several reasons for these inconsistent results, including the accuracy of each *H. pylori* test, differences between location of the biopsy, differences between pathologists and variation in the evaluation criteria.[22] Furthermore, most of these studies were conducted in the region where Javanese was the predominant ethnicity.

To clarify the reasons for these inconsistent results, our study in Surabaya, Java Island applied five different methods to detect *H. pylori* infection: culture, histology, immunohistochemistry, rapid urease test and urine antibody test and found that the prevalence of *H. pylori* infection was low (11.5%) based on at least one positive result among those five tests.[22] We then expanded the study to include 849 patients from the five largest islands representing at least 13 ethnic groups. The prevalence was still considerably low with only 10.4% (Table 1).[23,24] In contrast with neighboring countries such as Thailand and the Philippines, with prevalence rates of 76.1% and 60%, respectively,[25,26] we found a low prevalence of 22.1%.[27] Another survey in North Sulawesi province using urine tests in 251 adult patients of predominantly Minahasanese and Mongondownese ethnicity detected *H. pylori* in only 14.3% of subjects. A prevalence of *H. pylori* infection of only 3.8% was reported in a population of children in North Sulawesi, Indonesia.[28]

The prevalence of *H. pylori* infection in Indonesia was generally low, in concordant with Malaysia and Singapore but in contrast with several other areas in Southeast Asia such as Vietnam or Thailand. Thus, epidemiology of *H. pylori* infection can be varied among regions.[29] This low prevalence of *H. pylori* may be correlated with the low gastric cancer incidence in Indonesia. East Asian country such as Korea and Japan reported a high ASR of gastric cancer and also showed a high *H. pylori* infection prevalence; 54.0% and 51.4 % respectively (Table 2),[4,5,8,23,27,30-37] even in some cases there were some probability to be infected with non-*H. pylori* organism.[38] These phenomena may support the role of *H. pylori* in the gastric cancer pathogenesis.

A collaborative study among South Asian countries reported that host genetic susceptibility, genetic diversity of *H. pylori* and environmental factors were associated with *H. pylori* infection.[39-41] Several habits may significantly increase *H. pylori* prevalence, such as eating food with fingers, rarely washing hands before eating, eating cucumbers more than once a week and drinking alcohol.[42] Other risk factors, such as salary, source of water, type of latrine, history of medicine intake, religion, and smoking were associated with the prevalence of *H. pylori* infection in Indonesia.[27] The most important factors related to the risk of *H. pylori* infection are differences in ethnicity and geographical areas in Indonesia. For example, we showed that, after adjustment for age and sex, the risk of *H. pylori* infection in people of Papuan, Batak, and Buginese ethnicity was 30.57-, 28.39- and 23.23-fold, respectively, higher than that in Javanese. Moreover, the prevalence of *H. pylori* infection among young people of these ethnic groups was higher than that among Javanese of the same age group.[27]

H. pylori-ASSOCIATED DISEASE

1. Dyspepsia and gastritis

Dyspepsia is a major associated disease of *H. pylori* infection. Dyspepsia is estimated to be present in 25% of Indonesian population.[43] Several factors cause dyspepsia, including *H. pylori* infection, stress, metabolic disease, medication and functional dyspepsia. Dyspepsia has been shown to decrease the quality of life in Indonesia. Additionally, several factors, such as female sex, older age and more severe dyspepsia symptoms, can

Table 1. Summary of *Helicobacter pylori* Infection Prevalence, Virulence Factor and Antibiotic Resistance in Indonesia

Characteristic	Bali	Java	Kalimantan	Papua	Sumatera	Sulawesi	Timor	Total	Reference
H. pylori prevalence	7 (11.5)	17 (4.0)	6 (6.7)	9 (42.9)	26 (19.8)	13 (14.9)	14 (40.0)	88 (10.4)	23
Subjects (number)	61	424	90	21	131	87	35	849	
Virulence factor									23
cagA positive	6 (100)	11 (78.6)	5 (100)	7 (100)	18 (100)	13 (100)	13 (92.9)	73 (94.8)	
cagA type									
East Asian-type	3 (50.0)	10 (71.4)	2 (40.0)	1 (14.3)	18 (100)	9 (69.2)	6 (42.9)	49 (67.1)	
Western-type	3 (50.0)	1 (7.1)	3 (60.0)	0	0	4 (30.8)	7 (50.0)	18 (24.7)	
ABB-type	0	0	0	6 (85.7)	0	0	0	6 (8.2)	
vacA s1/m1	6 (100.0)	7 (50.0)	5 (100)	7 (100)	9 (50.0)	6 (46.2)	12 (85.7)	52 (67.5)	
oipA “on”	6 (100.0)	12 (85.7)	4 (80.0)	6 (85.7)	18 (100)	12 (92.3)	14 (100)	72 (93.5)	
dupA negative	6 (100.0)	11 (78.6)	3 (60.0)	7 (100)	17 (94.4)	13 (100)	8 (57.1)	65 (84.4)	
Strain (number)	6	14	5	7	18	13	14	77	
Antibiotic resistant rate									23
Clarothromycin	1 (16.7)	3 (21.4)	0	1 (14.3)	1 (7.7)	1 (5.6)	0	7 (9.1)	
Amoxicillin	0	0	0	1 (14.3)	1 (7.7)	1 (5.6)	1 (7.1)	4 (5.2)	
Metronidazole	2 (33.3)	7 (50.0)	1 (20.0)	3 (42.9)	4 (30.8)	16 (88.9)	3 (21.4)	36 (46.7)	
Levofloxacin	1 (16.6)	7 (50.0)	1 (20.0)	2 (28.6)	2 (15.4)	8 (44.4)	3 (21.4)	24 (31.2)	
Tetracycline	0	2 (14.3)	0	0	0	0	0	2 (2.6)	
Strain (number)	6	14	5	7	18	13	14	77	
Furazolidone	0	0	0	0	0	0	0	0	24
Sitafloxacin	0	0	0	0	0	0	0	0	
Garenoxacin	0	2 (15.4)	0	0	0	3 (16.7)	0	5 (6.5)	
Rifaximin	3 (50.0)	4 (30.7)	3 (60.0)	2 (28.5)	6 (46.1)	3 (16.7)	6 (42.8)	27 (35.5)	
Rifabutin	0	0	0	0	0	0	0	0	
Strain (number)	6	13	5	7	18	13	14	76	

Data are presented as number (%).

Table 2. Comparison of *Helicobacter pylori* Profiles of Indonesia and Japan

Variable	Indonesia, %	Japan, %	Korea, %	References
Prevalences of *H. pylori* infection	10.1	51.7	54.0	4, 8
Disease in *H. pylori* positive				
Duodenal ulcer	2.3	22.7	18.4	27, 30, 31
Gastric ulcer	11.4	16.4	23.9	27, 30, 31
Gastric cancer	0.9	13.1	13.4	5
Virulence type				
CagA positive	97.7	100	94.1	27, 31, 32
East Asian type	60.5	97.7	96.2	27, 32, 33
ABB type	18.6	-	-	27
VacA s1m1	70.4	96.5	77.6	27, 32, 34
DupA	6.8	21.0	48.0	27, 35
Antibiotic resistance				
Amoxicillin	5.2	13.0	9.5	23, 34, 36
Clarithromycin	9.1	48.0	17.8	23, 34, 36
Metronidazole	46.7	49.0	29.5	23, 34, 36
Levofloxacin	31.2	15.0	37.0	23, 34, 37

worsen the quality of life of individuals with dyspepsia.[44] Most dyspepsia cases are due to inflammation of the gastric mucosa, which can be observed by endoscopic or histological examination. Observation of 247 dyspeptic individuals in the three cities found that a lower pepsinogen I/II ratio was correlated with a higher inflammation score in the antrum and corpus.[7] In addition, an inverse correlation was observed between pepsinogen levels and *H. pylori* infection status.[7] An Indonesian study of 1,139 endoscopic patients found that *H. pylori* infection was the main factor in acute and chronic gastric mucosal inflammation, regardless of ethnicity and geographical location, increasing the odds of acute and chronic gastric mucosal inflammation by 185 and 300, respectively.[8] These findings suggest an important role of *H. pylori* infection in the progression of gastric inflammation, even in areas with a low prevalence of *H. pylori* infection.

2. Peptic ulcer

Although dyspepsia is one of the most common complaints of patients who visit healthcare providers, the prevalence of peptic ulcer in Indonesia is low.[14] Our nationwide survey of 17 cities in Indonesia found that 77 of 1,139 dyspeptic patients who underwent endoscopic examination had a sign of ulceration. This finding indicates a low prevalence of peptic ulcer in Indonesia compared with that in neighboring countries, such as 50.9% in Malaysia,[45] 47.6% in Thailand,[46] and 5.6% to 14.7% in Vietnam.[47,48]

Peptic ulcer disease (PUD) consists of the duodenal ulcer and gastric cancer. The development of duodenal ulcer and gastric cancer is associated with different cascade mechanisms,[49] suggesting that the mechanism of the diseases may be more complex than we previously expected. Gastric ulcer has the higher risk to become gastric cancer while the duodenal ulcer was less likely.[50] The first cohort study in Japan by Uemura *et al*.[51] mentioned that the gastric ulcer was likely found in the patients with the *H. pylori* positive. In that study, 23.8% of the *H. pylori* positive patients had gastric ulcer and 16.4% in the other study in Japanese population.[30,51] While in Korea, 23.9% of *H. pylori* infected patient has gastric ulcer. Concordant with the result in Indonesia, 11.3% of the *H. pylori* positive patients were also had gastric ulcer (Table 2). Even though the total number of gastric ulcer patients in Indonesia may be lower than Japan due to the national low infection prevalence, we still need to be cautious on treating the patients in some specific ethnics that showed higher infection prevalence.

The development of ulceration in the stomach and duodenum involves a complex inflammatory pathway that induces degeneration of the gastric mucosa.[52,53] A study showed that *H. pylori* has a virulence factor that has a strong association with duodenal ulcer but a protective effect against gastric cancer, known as the duodenal ulcer promoting (*dupA*) gene.[35] In fact, there was no intact long-type *dupA*, the real virulence marker of *dupA* for severe outcomes,[54] in the Indonesian strain. In our study, short-type *dupA* was predominant, and all peptic ulcer patients were *dupA*-negative.[55] On contrary, the rate of dupA positive in the area with higher gastric cancer risk such as Korea and Japan, showed higher prevalence; 48% and 21% respectively (Table 2). Further research on the development of ulceration in the gastric and duodenal mucosa needs to be performed, especially in areas of low *H. pylori* infection, such as Indonesia.

3. Gastric cancer

H. pylori infection can cause inflammatory symptoms of gastritis, but only 10% to 20% of cases develop into gastric ulcers, and only 1% to 2% are at risk for developing non-cardiac gastric cancer.[51] Gastric ulcer, duodenal ulcer, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma are diseases of the gastrointestinal tract known to be caused by *H. pylori* infection.[56,57] A meta-analysis reported that *H. pylori* infection was strongly associated with gastric cancer, with an odds ratio (OR) of 3.00 and a 95% confidence interval of 2.42 to 3.72.[58] Antral predominant gastritis and corpus predominant gastritis can lead to the two different pathogenesis of disease. In antral-predominant gastritis, there is an increase in gastric acid production, resulting in a high flow of acid to the duodenum and metaplasia of the duodenum. The location of this tissue change is called the gastric mucosa island, which facilitates other *H. pylori* infections. *H. pylori* infection and high acid exposure are destructive, leading to duodenal ulcers. In predominantly corpus infections, there is a disruption of acid production by parietal cells, the majority of which are in the corpus. As a result, stomach acid production decreases and the stomach become hypochlorhydric. Reduced acidity causes an increase in *H. pylori* infection, both in the corpus and in the antrum, increasing the risk of developing gastric ulcers and gastric cancer.[59]

H. pylori infection is associated with severe atrophic gastritis. Atrophic gastritis increases gastric pH and induces bacterial overgrowth that alters the local metabolism. In atrophic gastritis, gastric gland structure is replaced by connective tissue. This is called non-metaplastic atrophy, whereas replacement by an inappropriate glandular structure is called metaplastic atrophy.[60] Gastric cancer is initially marked by severe atrophic gastritis, infiltration of lymphocytes/plasma cells and neutrophils in the corpus and intestinal metaplasia in the antrum and/or corpus.[8] Corpus-predominant gastritis is common in countries with a high risk of gastric cancer, whereas antrum-predominant gastritis is more common in low-risk countries.[24,61-63] Monitoring atrophic gastritis could be a method of determining the incidence of gastric cancer.

Studies of gastric mucosal status and gastric cancer risk analysis in Indonesian populations showed that antrum-predominant gastritis is predominant in patients with chronic gastritis.[8] Corpus-predominant gastritis is associated with a high incidence of gastric cancer in China (ASR of 22.73/100,000). Antrum-predominant gastritis can develop into duodenal ulcer,

but it is less likely to develop into gastric cancer. This supports the finding that Indonesia is a country with a low risk of gastric cancer. The prevalence of gastric ulcer is lower in Indonesia than in other Asian countries, such as India and Malaysia.[27]

A study in Indonesia[8] showed that almost half of individuals with dyspeptic symptoms had histological abnormalities, including acute, chronic with atrophic gastritis, as well as intestinal metaplasia. The association between the prevalence of chronic active gastritis and the prevalence of atrophic gastritis supports the consensus that chronic active gastritis can progress to atrophic gastritis, which can further develop into cancer.[8] In cases in which atrophic gastritis is absent, gastric cancer can develop from the increased activity of inflammation related to *H. pylori* infection.[64] Some changes in biological functions, including increased oxidative stress along with genetic and epigenetic alterations, are capable of causing *H. pylori*-induced gastric cancer. The pathway involves degradation of the proinflammatory matrix and angiogenic pathway along with the involvement of peptidyl-prolyl cis. *H. pylori* trans-isomerase (HP0175), which affects the gastric Th17 response and also provides a link between *H. pylori* infection and gastric cancer.[65,66] In addition, our multivariate regression analysis showed that Timor ethnicity (OR, 8.531), age (OR, 1.107), and *H. pylori* infection (OR, 22.643) are independent risk factors for atrophic gastritis. Hence, people of Timor ethnicity had the highest gastric cancer risk score.[8] These data show that even though Indonesia is regarded as a country with a low risk of gastric cancer, severe gastric mucosal conditions were observed in several ethnic groups.[7] This finding suggests that policymakers in Indonesia should pay attention to ethnic groups with a high risk of gastric cancer, especially for gastric cancer screening and eradication of *H. pylori*.

VIRULENCE OF INDONESIAN *H. pylori*

H. pylori is regarded as the most diverse and successfully colonizing pathogenic bacterium worldwide.[67] These attributes are due to a high genetic diversity in its genome, which is a result of evolutionary pressures that produce genetic adaptations during human gastric colonization.[68] Horizontal gene transfer, mutation, migration and genetic deviation are mechanisms associated with bacterial genetic diversity. The definition of a virulence factor refers to bacterial genes that may be responsible for inducing and developing a disease with a spectrum of severity.[50] There are several well-known virulence factors of *H. pylori*, such as vacuolating cytotoxin A (*vacA*), cytotoxin-associated gene A protein (*cagA*) and outer inflammatory protein A (*oipA*). Adherence factors, such as blood group antigen-binding adhesin (*babA*) and sialic acid-binding adhesin (*sabA*), are also regarded as *H. pylori* virulence factors. The presence of intact Cag pathogenicity island (*cag* PAI) distinguishes the most virulent *H. pylori* strain, which is associated with gastric inflammation and cancer.[69] We discuss here the *H. pylori* virulence factors identified in Indonesia.

1. Cag pathogenicity island

In 1996, a huge protein complex was discovered that encoded a secretion system that was later confirmed as a type IV secretion system (T4SS).[69] This T4SS is a 40 kB DNA consisting of a complete virB-like protein complex that forms a syringe-like pilus structure on the surface of *H. pylori* and has a main function of translocation of an oncogenic protein, CagA.[70] Among the Cag PAI genes, the most extensively studied is *cagA*. The *cagA* gene is located in the 3' terminal of the Cag PAI. It is believed to be the main cause of the inflammation of gastric epithelial cells after infection by *H. pylori*. *H. pylori* can be differentiated into *H. pylori* that produces CagA (CagA-positive) or that does not produce CagA (CagA-negative). The *cagA* gene has the highest diversity among Cag PAI genes.[71] This high diversity is due to the Glu-Pro-Ile-Tyr-Ala (EPIYA) amino acid motif and its surrounding amino acids, which are known as the EPIYA segment. The EPIYA-A and EPIYA-B segments are called the first repetitions, and the EPIYA-C and EPIYA-D segments are called the second repetitions. Based on the different amino acid motifs of the second repetitions, CagA can be distinguished as the Western-type, which is determined as CagA ABC or ABCC, and the East Asian-type, which is determined as CagA ABD. The difference in these amino acid motifs affects the binding affinity of CagA to the Src homology-2 domain-containing phosphatase 2 (SHP2). The East Asian-type CagA with the EPIYA-D motif had a 100-fold greater binding affinity than the Western-type CagA with the EPIYA-C motif.[50] Stronger binding to SHP2 increases cellular Ras-extracellular receptor kinase activation, which has the clinical consequence of increasing the risks of peptic ulcer and/or gastric cancer.[72]

In Indonesia, East Asian-type CagA is predominant, similar to neighboring countries such as Malaysia and Vietnam, with rates of 56% and 96%, respectively. However, in Cambodia and Thailand, Western-type CagA is predominant, with rates of 59% and 54%, respectively. Studies of *H. pylori* strains in the five largest islands in Indonesia found that 60.5% were East Asian-type CagA, 20.9% were Western-type CagA and 18.6% were ABB-type CagA. Interestingly, Indonesia had other interesting types of CagA that were isolated from *H. pylori* strains Jayapura had an ABB-type CagA, which is very rare and has only been found outside of Indonesia in strains isolated from Aboriginals in Australia.[55,73] When the result from Indonesia was compared with the result from the high gastric cancer incidence such as Japan, this lower proportion of East Asian CagA may explain why the gastric cancer risk was generally low in Indonesia. It is reported that East Asian CagA is predominant in Japan and South Korea with 97.7% and 96.2%, respectively.[32,33] Therefore, the detection of East Asian CagA may become a biomarker for developing gastric cancer.[74] However, in Mongolia which was reported as the second highest ASR of gastric cancer (ASR of 33.1/100,000

population), those individuals mostly infected by non-East Asia *H. pylori*.[75,76] These data suggest, the development of gastric cancer could not be explained by the CagA type alone.

In addition to the CagA EPIYA segment differences, there is another important determinant factor known as the pre-EPIYA region. This region, which is located about 300 base pairs (bp) upstream of the first EPIYA motif, has also been investigated as a virulence factor.[77] Geographical area analysis showed specific pre-EPIYA types. The pre-EPIYA region can contribute to the incidence of gastric cancer. Most strains from East Asia have 39 bp deletion-type pre-EPIYA, whereas in Western countries most strains have no deletion of pre-EPIYA.[55] This contrasts with the findings in Indonesia, where most of the East Asian-type *cagA* strains are not the 39-bp deletion-type pre-EPIYA that are commonly found but 6 bp deletion-type pre-EPIYA (48.8%), followed by 18 bp deletion (25.6%), no deletion (18.6%) and 39 bp deletion (7%).[55] The finding is also different from that in other Asian countries in Vietnam, which is dominated by strains with 18 bp deletion-type pre-EPIYA (75%).[55] In fact, the risk of gastric cancer is higher in Vietnam than in Indonesia. Therefore, a new genetic genomic diversity marker for *H. pylori* strains in Indonesia could use a 6 bp deletion-type pre-EPIYA.

2. Integrating conjugative elements of *H. pylori*

In addition to the Cag PAI, there is another interesting PAI that is also the result of horizontal gene transfer, known as Integrating Conjugative Elements of *H. pylori* (ICE*Hptfs*) (Fig. 1). Because it carries T4SS, it is reported that ICE*Hptfs*4b have higher inflammatory action than PAIs carrying incomplete T4SS or no T4SS.[78,79] Its activity is reported to be enhanced in acidic and adherent conditions.[80]

The worldwide distribution of ICE*Hptfs* is extensive, with more than 80% of *H. pylori* carrying ICE*Hptfs*.[81,82] However, recent observations found that only about half of Indonesian *H. pylori* carried ICE*Hptfs*, consisting of ICE*Hptfs3-tfs4a* (42.8%) and ICE*Hptfs3* (16.3%), and its variability among all Indonesian *H. pylori* was determined by the ethnic group of the infected host. Virulence analysis of ICE*Hptfs* and *cag* PAI showed that inflammation was more severe in patients infected with intact *cag* PAI-ICE*Hptfs*-positive strains than in those infected with non-intact *cag* PAI-ICE*Hptfs*-negative strains.[79] This finding suggests that ICE*Hptfs* is associated with the more virulent strains of *H. pylori*.

3. Other *H. pylori* virulence factors

VacA is a virulence factor that has been comprehensively studied in *H. pylori*. Its vacuolating activity varies according to the *vacA* sequence structure of the signal region (s1 and s2) and the middle region (m1 and m2). The *vacA* s1 type is divided into s1a, s1b, and s1c and the *vacA* m1 type is divided into m1a, m1b, and m1c.[50] These sequence variations result in differences in secreted proteins and expression levels that are associated with disease severity.[83] Studies in Latin America, West Asia, and Africa reported that the risk of peptic ulcer or gastric cancer was higher in patients infected with the s1 m1 *vacA* type than in those infected with the s2 m2 *vacA* type.[11] The high toxicity of s1 m1 strains was supported by an *in vitro* study showing that s1 m1 strains consistently induced cell vacuolation.[73] Observations of variability in *vacA* in Indonesia showed that s1/m1 was the predominant type, followed by s1/m2 and s2/m2, suggesting that the *H. pylori* circulating in Indonesia was the virulent strain.[55]

The outer membrane protein OipA is another virulence factor that induces interleukin 8 (IL-8) and aids the adhesion of *H. pylori*. Slipped-strand mispairing based on the number of CT dinucleotide repeats in the 5′ region of the *oipA* gene regulates the functional status (on or off) of OipA.[84] *H. pylori* colonization density, IL-8 levels and neutrophil infiltration were higher in the human stomach infected with an *oipA* "on" strain,[85] even though the OipA receptor has not been identified.

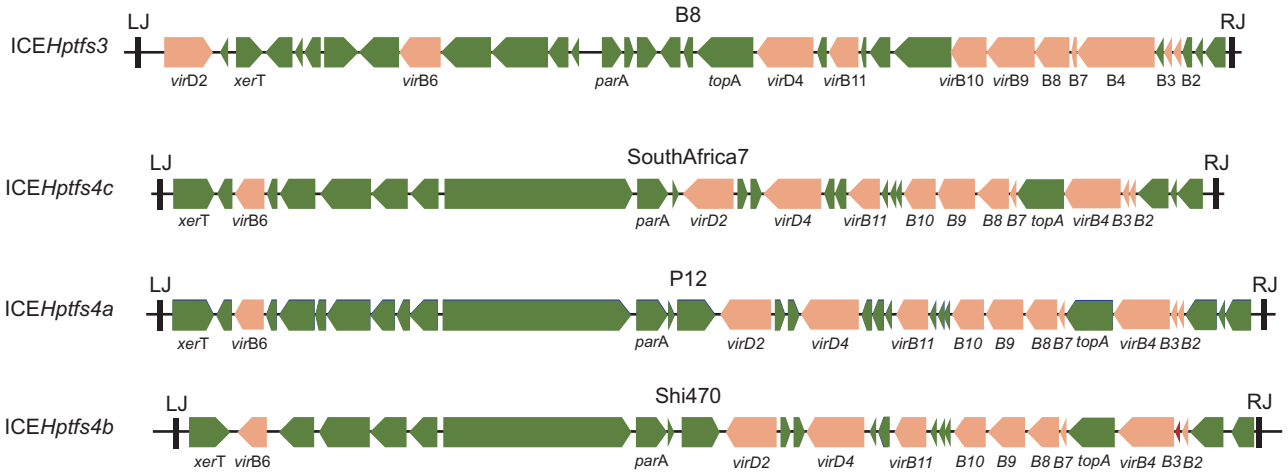

Fig. 1. Schematic figure of several Integrating Conjugative Elements of *H. pylori* (ICE*Hptfs*) types. The ICE*Hptfs* has two distinct varieties, ICE*Hptfs4* and ICE*Hptfs3*. Its genetic variability is mainly due to the different genetic characteristics of *virB8, virB9* and *virD4*.

The previous study reported strong associations of *oipA* "on" status with *vacA* s1/m1, *cagA*-positive and *babA*2-positive genotypes.[86] Furthermore, patients infected with strains with *oipA* "on" status are at higher risk for duodenal ulcer, regardless of other virulence factors.[87] Higher risks for PUD (OR, 3.97) and gastric cancer (OR, 2.43) with infection by the *oipA* "on" strain were also reported in the previous meta-analysis.[88] Thus, analysis of the presence or absence of the *oipA* gene without regard to functional on/off status may be unable to predict the risk of PUD or gastric cancer.[88] However, a recent study found that the *oipA* "on" type was observed in more than 80% of Indonesian *H. pylori*.[23] Based on these findings, Indonesian *H. pylori* can be considered as virulence type 1; however, the prevalence of gastric cancers in Indonesia was very low. This observation showed that the pathogenesis of gastric cancer, especially in Indonesia, is more complex than we predicted previously.

ANTIBIOTIC RESISTANCE

Because of the ability of *H. pylori* to remain in an asymptomatic host, *H. pylori* infection is a serious infectious disease. *H. pylori* can also cause gastritis and other complications, such as gastric ulcer and gastric cancer. Thus, according to the Kyoto global consensus conference on *H. pylori* gastritis in 2014, *H. pylori* infection needs to be eradicated.[89] Recent guidelines have proposed indications to start the treatment and regimens used for treatment in the Asia-Pacific and Association of Southeast Asian Nations (ASEAN) regions.[90,91] With regard to regimens used for *H. pylori* infection, standard triple therapy with proton pump inhibitors, clarithromycin and amoxicillin is the most popular in areas with clarithromycin resistance rates lower than 15%. There are several regimens that available for first-line therapy and salvage therapy according to Asia-Pacific consensus.[92] However, besides all of the possible combinations of drugs, the best option is to choose the best antimicrobial agents for each patient, according to susceptibility tests.[93]

In the ASEAN region, more attention should be paid to *H. pylori* resistance to metronidazole and clarithromycin. Metronidazole-resistant *H. pylori* remains a major antibiotic-resistant pathogen in the region. The prevalence of metronidazole resistance varies among ASEAN countries. Cambodia has the highest prevalence (73%), followed by Vietnam (70%), Singapore (48%), Myanmar (36.5%), Thailand (36%), and the Philippines (30%). Clarithromycin resistance is now at a high level, and the use of clarithromycin-based triple therapy is no longer recommended,[94] although Southeast Asia is the region in Asia with the lowest clarithromycin resistance rates among Asian countries.[95] The prevalence of clarithromycin resistance varies among ASEAN countries, being high in Cambodia (43%), Vietnam (30%), moderate to high in Singapore (17%) and low in Malaysia (6.8%), the Philippines (2%), and Myanmar (0%).[94] In comparison with the East Asian counties, such as Japan, Clarithromycin resistance in Indonesia was also considered to be low. However, metronidazole resistance was almost similar.[36]

Studies of the antibiotic susceptibility of *H. pylori* in Indonesia showed that Indonesian strains had high prevalence of metronidazole and levofloxacin resistance and low prevalence of clarithromycin, amoxicillin and tetracycline resistance.[23] A study from Indonesia in 2016 of the antibiotic susceptibility of *H. pylori* in Indonesia found that strains from Java and Bali Islands had clarithromycin resistance rates greater than 15% (21.4% and 16.7%, respectively), which is the recommended resistance limit for eradication therapy without conducting susceptibility tests. Java Island had a high rate of tetracycline resistance of 14.3%. Java and Sumatra Islands had the highest rates of levofloxacin resistance, with 50% and 44.4%, respectively. The highest rate of metronidazole resistance occurred in Sumatra (88.9%), followed by Java (50.0%), Papua (42.9%), Bali (33.3%), Sulawesi (30.8%), Timor (21.4%), and Kalimantan (20.0%). All these rates are taken from study reports based on geographical location in Indonesia. Research on the prevalence of antibiotic resistance based on ethnic groups showed that strains from Ambonese (50.0%), Chinese (20.0%), and Balinese (16.6%) had high prevalence of clarithromycin resistance that exceeded the recommendations of the Maastricht Guidelines (>15% to 20%).[90] Dayak was the only ethnic group in which *H. pylori* was sensitive to all antibiotics (clarithromycin, amoxicillin, metronidazole, levofloxacin and tetracycline). In areas with a high prevalence of clarithromycin resistance (>15%), standard triple therapy should not be used as first-line treatment and quadruple therapy would be a better choice. At present, *H. pylori* eradication with standard triple therapy was reported <70% eradication rate which is ineffective in several countries worldwide including ASEAN countries.[23,94] Therefore, *H. pylori* antibiotic therapy should be administered carefully in some regions of Indonesia. Furthermore, the low prevalence of amoxicillin resistance in Indonesia suggests that the use of amoxicillin as a first-line therapy needs to be considered.

The mechanism of antibiotic resistance by *H. pylori* may guide the doctor to achieve the treatment target and initiate the development of new drugs. Clarithromycin is commonly used in first-line regimens for *H. pylori* treatment with bacteriostatic mechanisms. The bacteria become resistant due to a decrease in the affinity of the ribosomes for the drug, by mutations in various points in domain V of the ribosomal RNA (rRNA) 23R (Table 3).[92,95] We found an interesting point mutation, A2143G, in Indonesian clarithromycin-resistant strains. When we performed next-generation sequencing, a novel mutated sequence in *hp1314* (*rpl22*), including 19 bp deletions at position 535, was found.[23]

There are at least four mechanisms of resistance to metronidazole that summarized in Table 3.[23,92,95,96] Levofloxacin is a broad-spectrum fluoroquinolone drug that eradicates *H. pylori*.[92,95] Indonesian strains have amino acid substitutions at Asp-

Table 3. Mechanisms of Antibiotic Action and Resistance for Clarithromycin, Metronidazole and Levofloxacin[23,92]

Antibiotics	Mechanism of drug action	Mechanism of resistant
Clarithromycin	Binds to 23S rRNA (part of 50S subunit of the bacterial ribosome) so it can inhibit the protein translation	Mutation in the V segmen of 23s rRNA gene especially in locus 2142G, A2142C and A2143G and the most rarely found are 2144T, T2717C and C2694A. Alteration in translation initiation of IF2. L22 ribosomal proteins gene mutation. Increasing expression of efflux pump.
Metronidazole	Degrade bacterial DNA	Reduction in the uptake of the antibiotic and/or an increase in the efflux of the antibiotic through the bacterial wall. Mutation in *fdxB* gene encoding ferredoxin-like protein. Mutations of the *rdxA* and *frxA* genes.
Levofloxacin	Inhibit DNA gyrase so that DNA synthesis is disrupted	Mutations in the *gyr*A DNA, subunits A or B, which caused the D91 position to be changed to G, N, A or Y, N87K and A88V. Mutation in the *gyrB* gene such as D481E or R484K.

rRNA, ribosomal RNA.

91 and Asn-87 that are associated with the highest minimum inhibitory concentration values observed. In addition, amino acid substitutions at Arg-484 and Ser-479 in the *gyrB* subunit were observed.

The guidelines for managing *H. pylori* infection are still being developed based on variations in geographical area.[97] According to the Bangkok consensus report recommendation, each country should develop its own appropriate first-line regimen based on its antibiotic resistance pattern. The second-line regimen should consist of a drug that has never been used in the country and to which *H. pylori* is susceptible in most of the population.[90] Vonoprazan has recently been approved in Japan for eradication of *H. pylori* strains that are resistant to clarithromycin with a mechanism of blocking potassium competitive acid P-CAB.[98] It might be another alternative due to the high prevalence of clarithromycin resistance in some areas in Indonesia. Other alternative regimens, such as furazolidone, rifabutin and sitafloxacin, have been suggested in Indonesia where there is an increase in levofloxacin resistance in the absence of bismuth treatment.[24] Antibiotic susceptibility tests, such as the E-Test or molecular methods, can be used before starting appropriate treatment in cases of more than two treatment failures.[90,99] However, the use of antibiotic susceptibility tests is difficult in Indonesia due to the lack of facilities and high costs. Estimating the prevalence of antibiotic resistance in local situations from local surveillance and/or local clinical experience can help provide appropriate care in eradicating *H. pylori* in each case. In addition, a detailed evaluation of the patient's previous use of antibiotics is important to reduce the resistance of *H. pylori* to antibiotics in Indonesia.

OTHER MICROBIOTA THAN *H. pylori*

Microbiota is a term for the micro-organisms living in a particular location. The location may be a macro-environment such as a national park or a microenvironment such as soil, seawater or even human skin or stomach. The diversity of microbiota may explain several matters, such as how environmental conditions affect what kind of bacteria can live in that particular environment, how bacteria evolve with changes in environmental conditions and how changing the environment may change the bacterial composition.[100] The microbiota of the gastric environment in relation to the pathogenesis of diseases is an interesting topic to discuss. It was believed that the stomach was a somewhat sterile environment due to its high acidity and the low level of cultured bacteria from inside the stomach. However, recent advanced technology has shown that the stomach has a large and diverse bacterial community with a density ranging from 10 to 1,000 colony-forming units.[101] Not only *H. pylori*, gastric microbiota may play roles in the gastric epithelial homeostasis and carcinogenesis.[102]

Utilizing a clone of small subunit 16S rDNA, the results from 23 gastric biopsies revealed a diverse bacterial community of 128 phylotypes, with the majority of bacteria belonging to the Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria phyla.[103] These data provide evidence of a great diversity of gastric microbiota due to a large degree of intersubject variability. In addition, diet and environmental change have a strong relationship with changes of the gastric microbiota.[104] However, even though the microbiota can be changed rapidly by changing the diet, the human gastric microbiota has a consistent pattern of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria as the predominant phyla and *Streptococcus* as the predominant genus.[104-106]

In addition to altering its composition due to changes in dietary habits and environmental changes, changing the gastric microbiota may also affect the gastric condition. A study analyzing the gastric microbial community in different stages of

the Correa cascade showed alterations of the gastric microbial composition. Along the Correa cascade, the microbial diversity and abundance of *H. pylori* were lower in chronic gastritis patients than in gastric cancer patients.[107] There was an overrepresentation of bacterial genera that included commensal bacteria from the intestine and an increase in nitrosating potential, suggesting a role of microbiota and *H. pylori* in carcinogenesis due to the genotoxic capability. Another comparison study of the microbiota in gastritis and gastric cancer showed a significant microbial dysbiosis in individuals with intestinal metaplasia and gastric cancer, with significant enrichment of 21 and depletion of 10 bacterial taxa in gastric cancer compared with gastritis.[108] In addition, five bacterial species (*Peptostreptococcus stomatis, Streptococcus anginosus, Parvimonas micra, Slackia exigua, and Dialister pneumosintes*) were enriched in patients with gastric cancer.[109] In *H. pylori*-infected patients, the proportion of Bacteroidetes decreased while the proportions of Firmicutes and Proteobacteria increased compared with *H. pylori*-negative patients.[110] The previous metagenomic analysis showed that T4SS genes were frequently observed in patients with intestinal metaplasia. This study not only analyzed the 16S rRNA sequence from gastric biopsies but also sequenced the whole genome of the microbiome. It was concluded that abundant T4SS might induce more severe gastritis and carcinogenesis.[111] These data suggest that gastroduodenal diseases may have a strong association with other bacteria in addition to *H. pylori*. This hypothesis also led us to the intriguing phenomenon in Indonesia of a low prevalence of *H. pylori* but a very high prevalence of dyspepsia. Our preliminary analysis of the gastric microbiota of *H. pylori*-negative patients with gastric inflammation produced several candidate bacteria that were associated with gastritis superficialis and also with precancerous lesions, including atrophic gastritis and intestinal metaplasia (unpublished data). Discussion of the development of gastroduodenal diseases, especially of the causative agents, should not consider only *H. pylori* as the pathogen. Alteration of the gastric commensal bacteria may also change the gastric environment and lead to disease.

CONCLUSIONS

Low gastric cancer incidence countries are mostly supported by low *H. pylori* infection, even the virulence is similar. It is necessary to support research on the latest developments in *H. pylori* infection. In contrast with high gastric cancer prevalence countries focusing on early detection and eradication, low gastric cancer countries should pay attention for screening to the several groups with high risk of gastric cancer.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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