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IN VITRO STUDY AND MOLECULAR ANALYSIS OF *babA* AND *sabA* ADHESIN GENES OF CLINICAL AND ENVIRONMENTAL *H. pylori* ISOLATES AND THEIR ROLE IN *H. pylori* COLONIZATION AND PATHOGENESIS

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Abstract

Helicobacter pylori (*H. pylori*) colonization in the gastric mucosa is the initial stage of infection to occur and ultimately causing gastric diseases such as chronic gastritis, peptic ulcer, and gastric cancer. *H. pylori* produce several virulence factors, including *babA* and *sabA,* that are essential for colonization of the stomach. This study aims to determine the expression of *babA* and *sabA* of different *H. pylori* isolates *in vitro* and its role in *H. pylori* colonization and pathogenesis. *H. pylori S3 (cagA$^-$), S5 (cagA$^+$), C7 (cagA$^-$)* and *C8* (*cagA$^+$*) strains from humans and cockroaches were co-cultured into AGS cells for 6h. Bacterial adherence was determined using Giemsa staining and dilution plating. The expression of *babA* and *sabA* was conducted using quantitative real-time PCR (qRT-PCR). Fold differences in the gene expression were determined using the $2^{-\Delta\Delta Ct}$ method. Bacteria adherence rate was higher in infected AGS cells with *H. pylori S5* (*cagA$^+$*) and *C8* (*cagA$^+$*). The expression of the *babA* gene was significantly upregulated in *H. pylori cagA$^+$*. However, *babA* gene expression was downregulated in *H. pylori cagA$^-$* strains after interaction with the AGS cells. In contrast, the expression of the *sabA* gene was upregulated in all *H. pylori* strains. Higher expression of the *babA* and *sabA* gene in *H. pylori cagA$^+$* increased the bacteria adherence rate in which permit the *H. pylori* to invade the epithelial cells. Hence, this study postulates that *babA* and *sabA* gene plays a vital role in *H. pylori* colonization. The adhesion of *H. pylori* to the gastric epithelium is mediated by the adhesin and allows the release of *cagA* into the host cells that facilitates colonization, induces mucosal inflammation and enable persistent infection that can lead to severe clinical outcomes.

INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative, spiral-shaped, and microaerophilic bacterium[1, 2]. This bacterium is urease, catalase, and oxidase-positive and possesses 3 to 5 polar flagella for motility[3, 4]. Almost half of the world population is infected with *H. pylori* [5],[6]. Long-term carriage of *H. pylori* significantly increases the risk of developing site-specific diseases if left untreated. Several gastrointestinal diseases, such as gastritis, peptic ulcer, duodenal ulcer, and gastric adenocarcinoma have been proven to be highly associated with *H. pylori* infection[7, 8].

The diverse clinical presentation of *H. pylori* infection is a result of the interaction between bacterial virulence, host genetic and environmental factors. The virulence factors of *H. pylori* can be categorized to be related to three major pathogenic processes; colonization, immune escape and disease induction [3, 9]. Major virulence factors in *H. pylori* strains is cytotoxin-associated gene A (*cagA*) [8, 10]. *cagA* is a well-recognized oncoprotein and highly immunogenic

protein in which translocated into host cells by the type IV cag secretion system after bacterial attachment [9, 11]. This gene usually induces bacteria adhesion and invasion of the gastric epithelial cells. Moreover, the *cagA* gene has been shown to be involved in persuading inflammation, ulceration and carcinogenesis [12, 13].

Besides that, among the other virulence factors that are also hypothesized to mediate host-bacterial interactions and maintain colonization of the *H. pylori* in the stomach are blood group antigen-binding adhesion (*babA*) and sialic acid-binding adhesion (*sabA*). *H. pylori babA* and *sabA* are bacterial cell-surface proteins that enable bacterial adherence to the gastric epithelial cells [2, 7, 14]. Besides that, it is also reported to be involved in numerous processes during early and chronic phases of infection [7, 9].

However, the different expression levels of the *babA* and *sabA* gene may cause a certain degree of *H. pylori* infection and thus affect the severity of the clinical outcomes. Therefore, this study aims to determine the expression of *babA* and *sabA* of different *H. pylori* isolates *in vitro* and its role in *H. pylori* colonization and pathogenesis.

MATERIALS AND METHODS

Bacterial Strains

H. pylori strains used in this study were *H. pylori S3 (cagA$^-$), S5 (cagA$^+$), C7 (cagA$^-$)* and *C8 (cagA$^+$)* strains. For comparison, a reference strain of *H. pylori* ATCC43504 was used in this study. *H. pylori S3 (cagA$^-$)* and *S5 (cagA$^+$)* strains were isolated from gastric biopsies of patients with *H. pylori* infection at Pusat Perubatan UiTM Sungai Buloh (PPUiTM). The gastric biopsies were transferred in tube containing brain heart infusion broth (BHI) and transported to the laboratory in ice (4°C). All procedures were approved by the UiTM Research Ethics Committee (REC/62/16). The gastric biopsies were homogenized and inoculated onto blood agar supplemented with Dent Supplement and incubated in microaerophilic conditions at 37°C for five to seven days, at high humidity.

Meanwhile, *H. pylori C7 (cagA$^-$)* and *C8 (cagA$^+$)* strains were isolated from cockroaches collected from eateries in Sungai Buloh. The cockroaches were manually hand caught and place in a sterile universal container before sending to the laboratory. The cockroaches were first frozen at 0°C for 5 to 10 minutes. After immobilization by freezing, the cockroaches were transferred into 15 mL tube. A total of 2 mL of sterile normal saline was added to the tube before thorough shaking for 2 minutes. Aliquots (0.01 mL) of the suspensions were then inoculated onto blood agar supplemented with Dent Supplement and incubated in microaerophilic conditions at 37°C for five to seven days, at high humidity.

Bacterial Strains Growth Condition

All strains were streaked and grown on blood agar supplemented with Dent Supplement containing 10mg/L vancomycin, 5mg/L trimethoprim lactate, 5mg/L cefsulodin and 5mg/L amphotericin B (Oxoid, England) and incubated in microaerophilic conditions at 37ºC for five to seven days, at high humidity.

The bacterial colonies were sub-cultured on Brain Heart Infusion broth (Oxoid, England) supplemented with glucose (Sigma, USA) and fetal bovine serum (Gibco, USA) and grown until mid-log phase (OD600nm = 0.5 or $1x10^8$ CFU/mL). The broth culture was incubated in microaerophilic conditions at 37ºC for two days, at high humidity.

Cell Line Growth Conditions

The human gastric cancer cell line AGS (ATCC CRL-1739) was purchased from American Type Culture Collection (ATCC, USA). AGS cells were routinely maintained in 25 cm^2 tissue culture flasks (SPL, Korea) and grown in complete growth media. Complete growth media consists of Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 2% penicillin-streptomycin (Gibco, USA) [15]. The AGS human stomach epithelial cell line was grown to confluent at 37°C in humidified 5% CO_2. The cells were harvested by trypsinization and centrifuged at 1500 x g speed for 5 min. The cell pellet was resuspended in complete media growth, and cell counting was carried out with a hemocytometer using trypan blue (Gibco, USA) [15, 16].

Morphological Observation by Giemsa Staining

Giemsa staining was done to detect the adherence *H. pylori* to the AGS cells. Giemsa staining method was adapted from a previous study with some modifications [15, 17]. Briefly, AGS cells were seeded in 6 well plates at $2x10^5$ cells per well (2 mL/well) and incubated at 37°C in the presence of 5% CO_2 for 18 to 24 hours. Then, the cells were infected with 2 mL of bacterial suspension in DMEM supplemented with 10% FBS for 6 hours. The infected cells were washed with phosphate-buffered saline, and the cells were fixed with 100% methanol for 5 min. The fixed cells were stained with 5% Giemsa for 30 min and washed three times with PBS. The adherence of *H. pylori* to the host cells was observed using an inverted microscope (Olympus 1x81, Japan) at 40x magnification. All experiments were done in triplicates.

Infection of AGS Cell Line with *H. pylori* strains

This method was adapted from a previous study with some modifications [15]. The bacterial pellet from overnight cultured in brain heart infusion broth was suspended in

DMEM (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and the suspension was adjusted to the concentration of $1x10^8$ CFU/mL using Densichek Plus (Biomerieux, USA). The AGS cell line was grown in complete media growth in a 25 cm^2 tissue culture flask at 37°C in the presence of 5% CO_2 until 90% confluency (~$4x10^6$ cells). Next, the AGS cells were washed with PBS, and the cells were infected with 2 mL of the bacterial suspension in DMEM supplemented with 10% FBS for 6 hours at a multiplicity of infection (MOI) of 50:1 (bacteria: cells). Then, the AGS cell was washed three times with PBS to remove non-adherent bacteria. The infected AGS cells were detached using cell dissociation reagent (Gibco, USA). For adherence assay, dilution plating was performed to measure the adherence rate of *H. pylori* to the AGS cells. The final number of adherent bacteria was determined by serial dilution of the infected AGS cell suspension in PBS followed by standard spread plate technique on blood agar supplemented with Dent supplement. All experiments were done in triplicates, and results were averaged.

RNA Extraction and cDNA Synthesis of Bacterial RNA from Infected Cell

RNA from the *H. pylori*-infected AGS cells and *H. pylori* culture were extracted using GeneJET Genomic RNA Purification Kit (*Thermo Fisher Scientific Inc, USA*) according to the manufacturer's protocol. Bacterial RNA without host cells was used as control. The RNA extracts were then quantitated using SpectraMax® QuickDrop™ UV-Vis spectrophotometer (Molecular Devices, USA) to measure the concentration and quality of the RNA. RNA extracts were stored at -20°C until required or immediately proceed to cDNA synthesis for qRT-PCR analysis. The RNA integrity and visualization were done on a 1.2% (w/v) agarose gel in 1xTBE (Tris-borate-EDTA).

The cDNA synthesis of RNA extracts was performed using Maxima First Strand cDNA Synthesis Kit (*Thermo Fisher Scientific Inc, USA*) following the manufacturer's instructions. The same amount of total RNA (1000ng/20µL) was reverse transcribed. Control reactions were performed under the same conditions without the reverse transcriptase enzyme (no-RT control) to determine the possibility of genomic DNA contamination. The product of cDNA synthesis was stored at -20°C until required or immediately used for qRT-PCR analysis.

Gene Expression Quantification Using a qRT-PCR assay

The *babA* and *sabA* gene expression were determined by quantitative real-time (qRT-PCR). Final reaction volume of 10 µL contained Maxima Sybr Green qPCR Master Mix 2x (*Thermo Fisher Scientific Inc, USA*), 1 µL bacterial cDNA and 0.5 µM of each primer (Sigma Aldrich Pte Ltd, Singapore). Primers used in this study were designed using the BLAST program at the National Center for Biotechnology Information (NCBI) website. The primers sequences used in this study were listed in Table 1. 16s rRNA was used as the normalizing gene. The qRT-PCR assay was performed on a CFX96™ Real-Time System (BIORAD, USA) with the following two-step cycling protocol: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 second and 60°C for 1 minute. qRT-PCR products were analyzed by melting curves of 65°C to 95°C with an increasing temperature of 0.5°C for 5 seconds for unspecific product or primer dimer formation. Standard curves of the genes were performed with 10-fold serial dilutions of the cDNA product to determine primers and amplification efficiency. The efficiency of qRT-PCR should be between 90-110% ($-3.6 \geq$ slope ≥ -3.3). For assay reproducibility, the qRT-PCR was performed in triplicates from three independent experiments.

Table 1. List of target and normalizing genes used in real-time PCR

Gene	Primer sequences	Reference
babA-F	GGAAGCGAAAGTTTGAGTGG	[18]
babA-R	GAGAGGCTTAGCGGGACTTT	
sabA-F	GAGCGTTGCTTACGGTTGAG	
sabA-R	CCCAACAAAACGCTACCACT	
16SrRNA-F	TCGGATTGTAGGCTGCAACTC	[19]
16SrRNA-R	CCGCAACATGGCTGATTTG	

Data Analysis

Data were obtained as threshold cycle (C_t) values using Biorad CFX Manager 3.1 Relative expression ratio of the gene was determined using the $2^{-\Delta\Delta Ct}$ method [20].

The relative expression ratio of the target gene was determined in a sample versus control and expressed in comparison to a reference gene. Statistical analysis was performed using IBM SPSS Statistics 22 and GraphPad Prism 8. Mann-Whitney U test was used for differences between two groups. A *p*-value <0.05 was considered to be statistically significant [15].

RESULTS

The AGS cells that were infected by *H. pylori* isolates were observed under an inverted microscope. Adherence of bacteria strains was seen on the surface of the infected AGS cells (Figure 1). Cytoplasm and nucleolus of AGS cells stained bluish violet while the nuclei stained purple with Giemsa staining.

The results of bacterial adherence to AGS cells were expressed as colony-forming unit per millilitre (CFU/mL) of adhered bacteria. The bacteria spread over a blood agar plate supplemented with Dent supplement by using serial

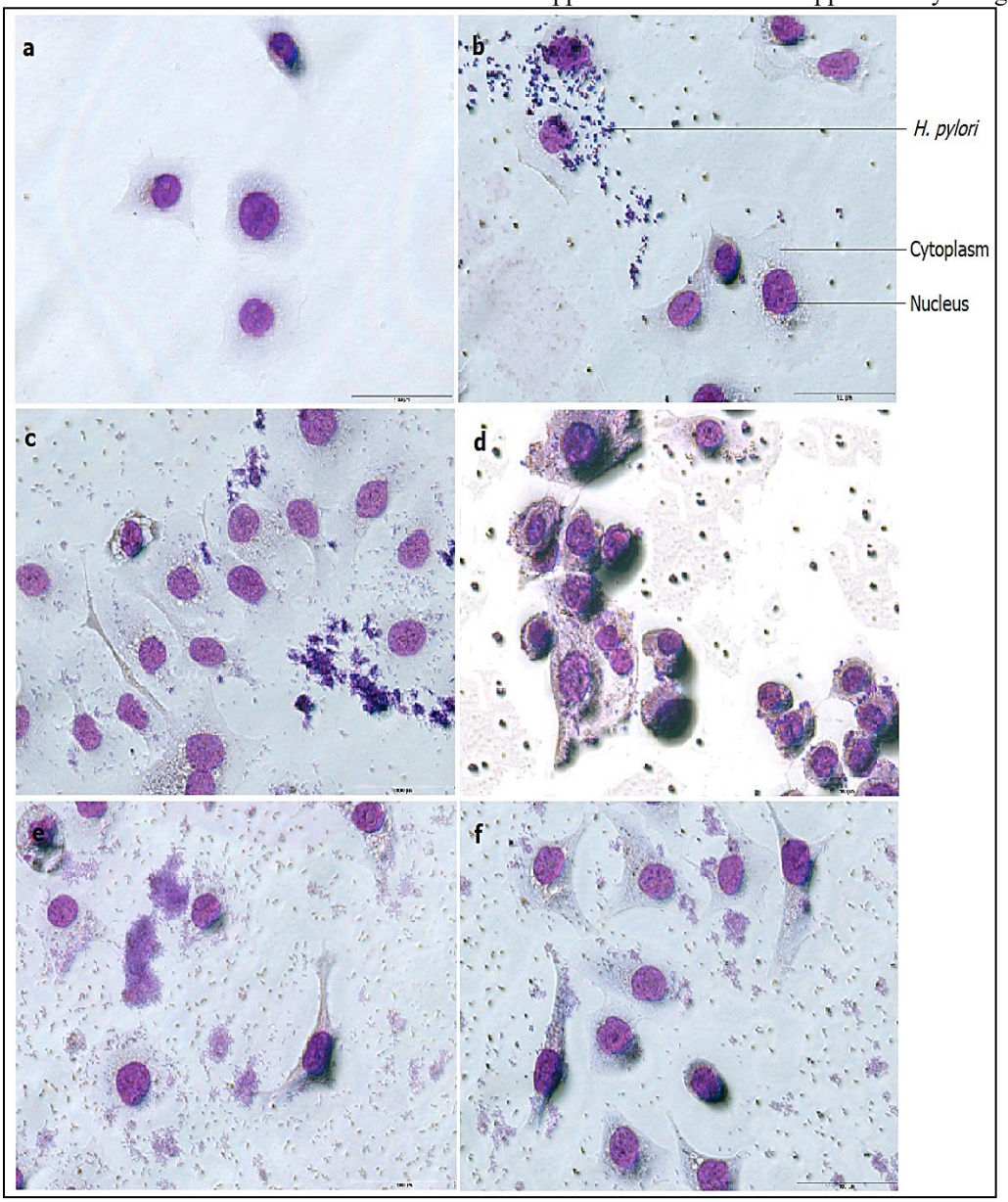

Figure 1. Giemsa staining of bacteria from *H. pylori* strains adhered to AGS cell was observed under an inverted microscope at 40x magnification; (a): Uninfected Cell; (b): *H. pylori* ATCC43504; (c): *H. pylori S3 (cagA-)*; (d): *H. pylori S5 (cagA+)*; (e): *H. pylori C7 (cagA-)*; (f): *H. pylori C8 (cagA+);* The data is representative of three independent experiments.

dilutions, and the numbers of bacteria colonies that grow on each plate are counted. The plate that has numbers of colonies between 30 and 300 are counted. The number of adhered bacteria was determined by multiplying the count on the plate with the dilution factor. A one-way between-group analysis of variance (ANOVA) was conducted to explore the number of bacteria adherences to the host cells. There was a statistically significant difference at the *p-value* < 0.05 level among different isolates when compared to the uninfected cell as a control group.

The adherence rate of different *H. pylori* strains were in the range of 23% to 75% (Figure 2). As for *H. pylori* isolate from gastric biopsies, *H. pylori S5* (*cagA*$^+$) shows the highest adherence rate (75%) with a value of 2.25 x 10^8 CFU/mL; meanwhile, *H. pylori S3* (*cagA*-) shows the lowest adherence rate (26%) with a value of 7.70 x 10^7 CFU/mL. Whereas *H. pylori* isolate from cockroaches, *H. pylori C8* (*cagA*$^+$) showed the highest adherence rate (67%) with a value of 2.01 x 10^8 CFU/mL; meanwhile, *H. pylori* C7 (*cagA*$^-$) showed the lowest adherence rate (23%) with a value of 6.80 x 10^7 CFU/mL. Bacteria adherence rate of the positive control group, *H. pylori ATCC43504* was 95% with a value of 2.86 x 10^8 CFU/mL.

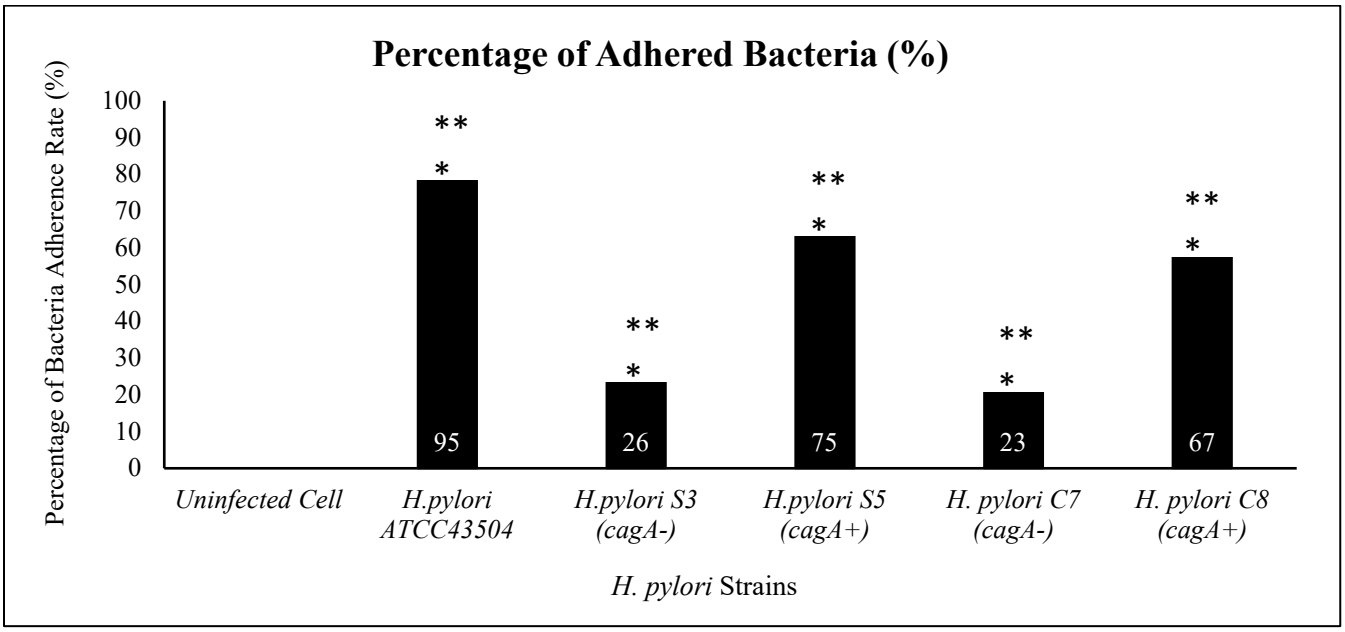

Figure 2. Graph of percentage bacteria adherence rate, *H. pylori* isolates to AGS cells (%); * indicates significant when *p-value* < 0.05 compared to uninfected cell; ** indicates significant when *p-value* < 0.05 compared to *H. pylori* ATCC43504

The integrity of RNA was checked by gel electrophoresis showed that the bacterial RNA (23S and 16S) and host cell RNA (23S and 18S) which was harvested after the infection into AGS cells. The expression of adhesin gene *babA* and *sabA* were detected by real-time RT-PCR after different isolates of *H. pylori*-infected into the AGS cells. Expression data for each gene were normalized against 16S rRNA as a housekeeping gene. There was a statistical difference in the expression level of the *babA* gene in *H. pylori S5*(*cagA*$^+$) and *H. pylori C8* (*cagA*$^+$) strains after infected into the AGS cells compared to bacterial culture without host cell as a control **(Figure 3)**. The expression *babA* gene was significantly upregulated in *H. pylori S5*(*cagA*$^+$) and *H. pylori C8* (*cagA*$^+$) strains after infected into the AGS cells, with more than two-fold changes (*p*-value<0.05). The gene expression of *babA* in the *H. pylori S5*(*cagA*$^+$) and *H. pylori C8* (*cagA*$^+$) strains infected cells were at 11.97 and 5.92 folds higher than control, respectively. In contrast, *H. pylori S3* (*cagA*$^-$) and *H. pylori C7* (*cagA*$^-$) showed down-regulation of the *babA* gene at 1.68, and 4.23 folds lower than the control.

Meanwhile, the findings were obtained for the relative quantification of *sabA* gene expression were slightly different from the *babA* gene expression after interaction with the AGS cells. All *H. pylori* isolates (*cagA*$^{+/-}$) showed upregulation of *sabA* gene after infected into the AGS cells compared to control, with more than two-fold changes **(Figure 4)**. However, only *H. pylori S5*(*cagA*$^+$) and *H. pylori C8* (*cagA*$^+$) strains showed significantly different in the expression of the *sabA* gene after infected into the AGS cells, with *p*-value<0.05. The gene expression of *sabA* in the *H. pylori S5* (*cagA*$^+$) and *H. pylori C8* (*cagA*$^+$) strains infected cells were at 15.45 and 8.40 folds higher than control, respectively. Meanwhile, there were no significant changes in the gene expression of *sabA* of *H. pylori S3 (cagA-) and H. pylori C7 (cagA-)* strains after infected into the AGS cells. The gene expression of *sabA* in *H. pylori S3 (cagA*$^-$*)* and *H.*

pylori C7 (*cagA*$^{-}$) strains infected AGS cells were 2.15 and 1.22 folds, respectively.

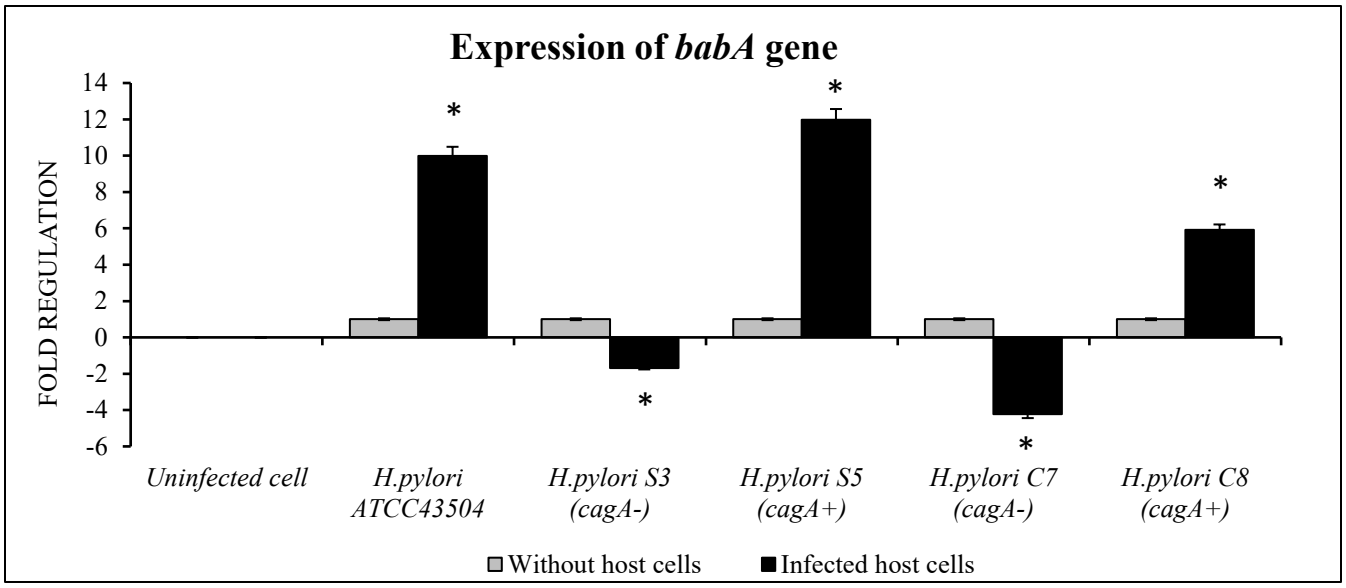

Figure 3. The expression of *babA* gene shows differential gene expression among the *H. pylori* stains after interaction with AGS cells. Relative normalized expression was determined using the $2^{-\Delta\Delta Ct}$ method. Data are means ± SEM of each reaction. Changes are considered significant when *p*-value<0.05 indicated by *.

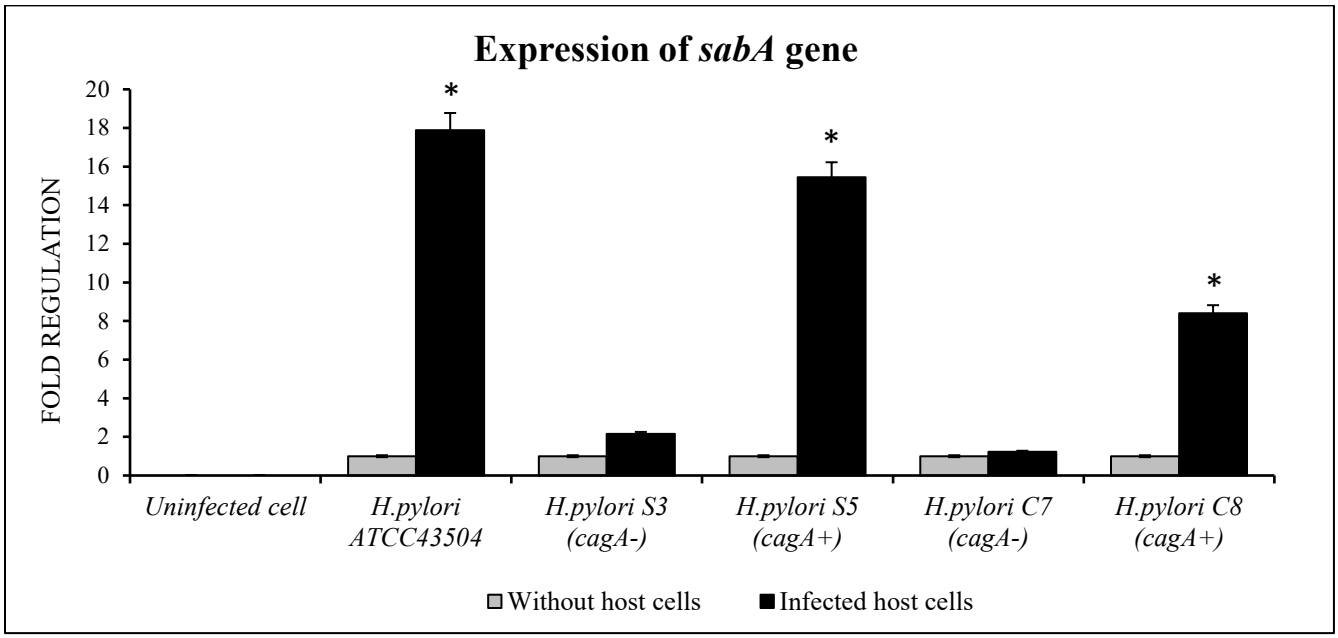

Figure 4. The expression of *sabA* gene shows differential gene expression among the *H. pylori* stains after interaction with AGS cells. Relative normalized expression was determined using the $2^{-\Delta\Delta Ct}$ method. Data are means ± SEM of each reaction. Changes are considered significant when *p*-value<0.05 indicated by *.

DISCUSSION

Adherence of *H. pylori* to the gastric mucosal surface constitutes a vital step in the establishment of profitable interactions with the host gastric epithelial cells, contributing decisively to the high prevalence and chronicity of *H. pylori* infection [21]. Furthermore, the evolution of the bacterial virulence factors, host, and environmental factors facilitates the persistence of *H. pylori* infection and intricate the severity of various clinical outcomes [7, 14]. Among the *H. pylori* virulence factors that are responsible for initiating the bacterial colonization are urease, flagella, chemotaxis system, and adhesins. Besides that, the virulence factors are also hypothetically involved in the immune escape that help *H. pylori* evade from host immune clearance and allow its persistence in the human stomach [9, 22].

Primarily, the urease activity and flagella-mediated motility of *H. pylori* enable the bacteria to survive in a harsh acidic environment and movement toward the mucus layer of the epithelium. Following that, *H. pylori* secrete adhesins which are *babA* and *sabA*, that interact with the receptors on the host epithelium cells [23, 24].

In this study, the expression level of the *babA* gene in *H. pylori cagA+* (*H. pylori S5* and *C8*) are higher compared to *H. pylori cagA-* strains. The expression *babA* gene in *H. pylori cagA+* was significantly upregulated in with more than two-fold changes (p-value<0.05). Based on these findings, it is suggested that the *babA* adhesin mediates high-affinity binding of *H. pylori* to the ABO blood group antigen-glycosylated gastric mucosa and ultimately enables tight mucosal bacterial adherence to the gastric epithelial cells [25]. Moreover, differential expression of the *babA* gene might possess different domains for bacterial binding to the host ABO/Leb binding sites in the gastric mucosa [26].

The findings were obtained for the relative quantification of *sabA* gene expression were slightly different from the expression of the *babA* gene after interaction with AGS cells. The expression of *sabA* gene was significantly upregulated in the *H. pylori S5*(*cagA*$^{+}$) and *H. pylori C8* (*cagA*$^{+}$) infected AGS cells. Meanwhile, there were no significant changes in the gene expression of *sabA* gene in the infected AGS cells with *H. pylori S3 (cagA*$^{-}$) and *H. pylori C7* (*cagA*$^{-}$) strains. Therefore, *H. pylori cagA*$^{+}$ strains showed significantly different in the expression of the *sabA* gene with p-value<0.05 after infected into the AGS cells. The regulation of the *sabA* gene probably due to the carbohydrate structure sialyl-Lewis antigen expressed on the gastric epithelium [27]. Lewis antigens are common in infected and inflamed gastric mucosa. Thus, alteration in the glycosylation patterns in the gastric mucosa with upregulation of inflammation-associated sLex antigens interacting with *sabA* enhances *H. pylori* colonization and enhance persistent infection and chronic inflammation [28, 29]. Moreover, higher *sabA* gene expression can also rapidly respond to changing conditions in the stomach or in different regions of the stomach that permit *H. pylori* to adapt to varying microenvironments or host immune responses to ensure long-term colonization and infection [29]. Additionally, *sabA* positive was previously reported to be associated with higher gastric cancer risk. However, the pathophysiological importance of the *sabA* is remaining uncertain [30].

The findings from the *babA* and *sabA* gene expression are consistent with the bacteria adherence assay and morphological observation by Giemsa staining. A higher number of the adhered bacteria onto the epithelial cells could be observed microscopically. It indicates that a higher bacterial load of the *H. pylori cagA+* strains invade the epithelial cells after interaction with the AGS cells. Cytotoxin-associated gene A (*cagA*) is an oncoprotein that is highly immunogenic. It is usually translocated into the host cells after bacterial attachment by the type IV cag secretion system [9, 11].

Once attached, bacterial effector molecules, *cagA* and *vacA* are secreted by the *H. pylori* and modulate the gastric epithelial cell behaviour leading to loss of cell polarity, the release of nutrients and chemokines [31]. It is then lead to the damage of host tissue and intracellular replication [32]. Besides that, *cagA* could also alter the intracellular signal transduction pathways that facilitate the malignant transformation of gastric epithelial cells [9, 22].

The persistence of *H. pylori* infection has also been linked with the activity of *babA* and *sabA* adhesin. The adhesins could adapt to changing environmental conditions during long-term colonization due to strong bacteria adherence onto the gastric epithelial cells [25]. Moreover, higher expression of the *babA* and *sabA* adhesins permits the bacterium to rapidly adapt to the changes of glycosylation of the host gastric mucosa that occurs during infection and enable to evade from the inflammatory response [21].

Bacterial adherence mediated by *babA* adhesin also enhances the ability of the T4SS system to contact the host cells, thus strengthen the inflammatory response. Besides that, when the *babA* gene binding to Leb, it contributes to gene mutations through the formation of double-stranded DNA breaks in host cell lines [33, 34]. Similarly, the *sabA* adhesin can facilitate colonization by binding to the sialyl-Lewis antigens and mediate binding of *H. pylori* to sialylated structures of neutrophils. Hence, it is also suggested that *babA* and *sabA* might be involved in carcinogenesis as an abundance of sialyl-Lewis antigens is commonly enhanced in inflamed or cancerous gastric tissues [33, 35].

Therefore, higher expression of *babA* and *sabA* gene in *H. pylori cagA+* strains postulates that these strains are more virulent, potent, invasive and cause a higher degree of *H. pylori* infection. It is supported by the findings of the previous study stating *H. pylori babA*-positive status is closely associated with severe gastric injury, high *H. pylori* density and poor clinical outcome [22, 36]. Furthermore, it is also demonstrated that *babA* and *sabA* gene plays a vital role in *H. pylori* colonization that enables persistent infection and eventually could lead to severe clinical outcomes. It is also suggested that active form of *babA* and when in

conjunction with *cagA* and *vacA s1* alleles are associated with a higher risk of severe duodenal ulcer and gastric cancer [30, 37].

Additionally, bacteria adherence of the *H. pylori* isolated from humans and cockroaches upon infection into the AGS cells in this study demonstrated a comparable expression of *babA* and *sabA* gene. These findings showed that the *H. pylori* strain isolated from the cockroaches can attach and adhere to the human gastric epithelial cell. Hence, cockroaches may potentially act as vectors or the sources of *H. pylori* infection in humans. Nevertheless, further study of gene expression analysis of host immune response should be conducted to unravel the association of the *H. pylori* isolates from human and cockroaches and establish the link between higher expression of *babA* and *sabA* gene with severe clinical outcomes.

CONCLUSION

Higher expression of the *babA* and *sabA* gene in *H. pylori* $cagA^+$ strains increased the bacteria adherence rate in which permit the *H. pylori* to invade and proliferate in the gastric epithelial cells. It thus enhances the delivery of other virulence factors such as *cagA* and *vacA* that are involved in inducing the inflammatory response. Hence, this study postulates that the *babA* and *sabA* gene plays a vital role in *H. pylori* colonization that help in bacterial adherence and enable the persistent infection in which eventually could lead to severe clinical outcomes. However, structural and biophysical characterization of the *H. pylori babA* and *sabA* should be explored to provide insights into *H. pylori* adherence mechanisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

ETHICAL APPROVAL

Acquisition of the gastric biopsies used in this study was approved by UiTM Ethics Approval (REC/62/16).

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