

Original Article

Duodenal ulcer promoting gene (DupA), plasticity region genes and sigma factors in *H. pylori* strains from Nigeria

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Abstract

Introduction: *Helicobacter pylori* is a principal cause of gastric cancer. The aim of this study was to determine the prevalence and contribution of duodenal ulcer promoting gene A (*dupA*), the plasticity region genes and sigma factors in relation to their pathological expression of *H. pylori* infections in the Nigerian population.

Methodology: Polymerase Chain Reaction was used to analyze a total of forty-nine *H. pylori* strains isolated from patients attending various endoscopic units in tertiary hospitals in Nigeria for complete *dupA* (G27 variant), *jhp0917*, *jhp0918*, other plasticity region genes *jhp 914/917*, *jhp0914*, *jhp0940* and sigma factors.

Results: PCR results indicated that the prevalence of complete *dupA* (G27 variants), *jhp0917*, *jhp0918* and other plasticity region genes *jhp0914*, *jhp0914/0917* and *jhp0940* in the *H. pylori* strains were 4%, 53%, 88%, 73%, 12% and 0% respectively. The prevalence values of the sigma factors were 96%, 92%, 80% for *rpoN*, *fliA* and *rpoD* respectively. However, the endoscopic findings showed that erosion, normal mucosal, ulcer, hyperaemic stomach, mucosal atrophy and oedematous stomach in the patients where the *H. pylori* strains were isolated were 40.8%, 32.7%, 10.2%, 8.2%, 2.0% and 6.1% respectively. There was significant association between *jhp0917*, *jhp0914/917* and G27 variant and the endoscopic findings, while other plasticity genes showed no association with the endoscopic findings.

Conclusion: These results suggest that the presence of *jhp0917*, *jhp0914/917* and G27 variant could be used as marker to predict the pathological effect of severity in Nigeria patients with *H. pylori* infection.

Key words: *Helicobacter pylori*; sigma factors; plasticity region genes; *DupA*; transcription, pathology.

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Introduction

Helicobacter pylori is a spiral-shaped, microaerophilic, gram-negative, highly motile bacterium found exclusively in the mucous layer of the human stomach [1,2]. It is implicated in peptic ulcer disease, gastric adenocarcinoma; gastric mucosa associated lymphoid tissue (MALT) lymphoma, and stomach cancer [3]. Apart from the *cag* pathogenicity island that contains a type IV secretion system and the carcinogen *cagA* of *Helicobacter pylori*, the plasticity

region genes have been implicated in the pathogenicity of the bacterium.

The *cag* pathogenicity island and the plasticity region contain strain-specific genes, half of which are located in the plasticity regions in J99 [4-5]. Currently, studies show that some specific genes or combination of genes in the plasticity region may play significant roles in the pathogenesis of *H. pylori*-associated gastro-duodenal diseases [5-8]. All *Helicobacter pylori* strains have plasticity zones of different sizes in a defined

location on the chromosomes [8]. P12 genome features three different plasticity zones two of which are integrated as genomic islands into restriction-modification system pseudo-genes. One of the plasticity zones is said to harbour a complete set of genes encoding a novel type IV secretion system that seems to be involved in horizontal transfer of DNA between *H. pylori* strains [4,9].

The duodenal ulcer promoting gene A (*dupA*) and *jhp0940*, *0945*, *0947*, *0949* which are located in a plasticity region of the *H. pylori* genome has been reported to be a marker that contributes to the increased risk of duodenal ulcer and reduced risk for gastric atrophy and cancer. These have been reported to be prevalent in isolates from patients with gastritis, duodenal ulcer and gastric cancer [10-13].

H. pylori strain J99 has been reported to harbour the genes *jhp0914* to *jhp0961* in a plasticity region and that the open reading frames ORFs in the plasticity region display diversity [14]. The *jhp0917* and *0918* are tagged duodenal ulcer promoting gene (*dupA*) and encode homologues of *virB ATPase*, which is thought to be involved in DNA uptake, transfer and protein transfer [13,15-16]. Many genes have also been reported in *H. pylori* that are associated with virulence including *cagA*, *vacA*, urease, flagellin and *dupA*, but there is little knowledge about the genetic and regulatory function in *H. pylori* virulence [16-18].

Based on sequence similarity searches, *Helicobacter pylori* genome encodes three sigma factors which are sigma 70 (σ^{70}) which is the factor encoded by the gene HP0088 (*rpoD*), sigma 28 (σ^{28}) encoded by the gene HP1032 (*fliA*) and sigma 54 (σ^{54})

encoded by the gene HP0714 (*rpoN*) [19-20]. *Helicobacter pylori* σ^{70} (*rpoD*) is the primary (housekeeping) sigma factor, which is essential for general transcription in exponentially growing isolates [21]. σ^{70} is specific to the recognition of *cagA* promoter. In *H. pylori* mutant *rpoD* gene could not be isolated and this suggested an absolute requirement of σ^{70} for the viability of the bacteria [21]. σ^{28} and σ^{54} are two alternative sigma factors dedicated mostly to control expression of flagella components [22]. In *H. pylori* σ^{28} and σ^{54} allows RNA polymerase to recognize genes involved in flagella biosynthesis which are essential for motility and colonization of the human gastric mucosa. Most genes involved in flagella biosynthesis require σ^{54} for transcription [23-24]. σ^{54} in *H. pylori* is also important in gene regulation for survival in nutrient deficient environment, slows down proliferation process by negatively regulating genes involved in energy metabolism and biosynthesis and enhanced stress resistant ability by positively regulating genes involved in protein synthesis and redox reaction [25].

The genomic plasticity region genes, *dupA* and the alternate sigma factors of *H. pylori* isolates from Nigeria have not been investigated. This current study was designed to evaluate their roles in disease manifestation in *H. pylori* infections in Nigeria.

Methodology

Study Subjects

Four hundred and eighteen (418) subjects comprising one hundred and seventy-six (176) males and two hundred and forty-two (242) females aged between 12 and 86 years were recruited for the study

Table 1. Primers used in the detection of *dupA*, plasticity region genes and sigma factors.

Primers	Sequences(5'-3')	Amplicon length	References
<i>jhp914</i> (WS539F) 2329	ATGTCCAGTAAGGAATTTGTC	3000bp (J99)	Fischer et al. [29]
<i>jhp917</i> (WS606R) 2460	AGCAATAAAAACGCTTAAAAGTCTC		
<i>jhp917</i> (<i>dupA</i> 113F) UB106	GACGATTGAGCGATGGGAATAT	971bp (J99)	Richard et al. [42]
<i>jhp917</i> (<i>dupA</i> 1083R) UB107	CTGAGAAGCCTTATTATCTTGTGG		
<i>jhp918</i> (<i>dupA</i> 1202F)UB108	TAAAATCACAAAGGGGAAAAGATC	509bp (J99)	Richard et al. [42]
<i>jhp918</i> (<i>dupA</i> 918R)UB 109	AAGCTGAAGCGTTTGTAACG		
<i>jhp 940</i> (2318F)	GCCAGAGATTTCAATGATGTC	970bp (J99)	Rizwan et al. [34]
<i>jhp 940</i> (2319R)	AAAAATTTAGGCTCAAAGTCTG		
<i>jhp914</i> (2328F)	ATGTCCAGTAAGGAATTTGTC	500bp (J99)	Fischer et al. [29]
<i>jhp914</i> (2329F)	CTACAATATAGCTCTCAAAG		
<i>dupAG27</i> variant(<i>dupA</i> 2856F)677	GTATTCCTAGCCAATATCTTTAG	600bp (G27)	Fischer et al. [29]
<i>dupAG27</i> variant(<i>dupA</i> 2857R)678	AAAAATTTAGGCTCAAAGTCTG		
σ^{70} (<i>rpoD</i>) F	AAACGCTTGAATACAACTGC	290bp	Sun et al. [36]
σ^{70} (<i>rpoD</i>) R	GATCGGAAATCAACTCCCTC		
σ^{54} (<i>rpoN</i>) F	GCATTTCTTTAGCATCGCCTTAG	200bp	Sun et al. [36]
σ^{54} (<i>rpoN</i>) R	GAGCAGTTGGCGGTATTTGGT		
σ^{28} (<i>fliA</i>) F	GGGGCGATGTTTAGATTATTG	100bp	Sun et al. [36]
σ^{28} (<i>fliA</i>) R	TTCCCATGCTCATTAAGGTGT		

*Plasticity region genes (*jhp917*, *jhp918*, *jhp914/917*, *jhp914*), complete *dupA*– (G27), sigma 70 – *rpoD*, sigma 54 – *rpoN*, Sigma 28 - (*fliA*), forward primers – (F), reverse primers – (R), base pair – (bp), duodenal ulcer promoting gene A- (*dupA*), J99 – *H. pylori* African strain.

after informed consent was obtained. These subjects were outpatients that attended the endoscopic unit of the following teaching hospitals, Lagos State University Teaching Hospital (LASUTH), Lagos University Teaching Hospital (LUTH), University of Benin Teaching Hospital (UBTH) and Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) in Nigeria. Subjects who were currently on antibiotics and proton pump inhibitors were excluded from the study.

Collection and Processing of Samples

Biopsies were obtained from the corpus and antrum of the patient’s stomach and transported to the laboratory in Portagerm pylori (Biomerieux, Marcy l’Etoile, France) within two hours. The biopsies were cultured on GC agar plates (Difco) containing dent antibiotic selective supplement (Oxoid, Basingstoke, United Kingdom) at PH 6.8 -7.0, vitamin mix (1%) and horse serum (9%). (Serum plates) were incubated in a microaerophilic condition (85% N₂, 10% CO₂, 5% O₂) at 37 °C for minimum of 3 days after which the serum plates were examined for possible growth of *Helicobacter pylori* strains. Rapid urease test was used for biochemical confirmation of the isolates [15]. Biopsies were also sent for histological examination for cancer, ulcer, erosion, dysplasia, metaplasia, atrophy and inflammation.

DNA Extraction and PCR Analysis

DNA extraction from forty-nine cultured isolates was carried out using QIAGEN DNA kit and polymerase chain reaction (PCR) fragments were amplified with a PAN-Script DNA polymerase (PAN Biotech, Aidenbach, Germany) for the *dupA* and plasticity region genes and FIREPol[®] DNA polymerase (Solis BioDyne, Tartu, Estonia) for the sigma factor with different primer pairs (Table 1). The PCR cycling conditions for the *dupA* and the plasticity region genes were as follows: 95 °C for 5 min, then 35 cycles at 95 °C for 30 s, 54 °C for 30 seconds and extension at 72 °C for 1 to 5 minutes depending on the gene size. The cycling conditions of PCR for the sigma factors

were as follows: 95 °C for 5 minutes, then 30 cycles at 95 °C for 30 seconds, 50°C for 30 seconds and extension at 72 °C for 1 minute. Amplicon were separated using Agarose gel electrophoresis and viewed under UV light [15].

Ethical Approval

Ethical approval was obtained from IRB NIMR (registration number IORG0002656).

Statistical Analysis

SPSS version 21 was used to analyze the association between the sigma factors, *dupA* gene and the plasticity region genes. The association between *dupA* gene, the plasticity region genes and the endoscopic findings were determined using fisher’s exact test, multivariate linear regression analysis was used to check the effect or degree of influence of dependent variable *rpoD*, *rpoN*, *fliA* (sigma factors) on the plasticity region genes. Similarly, binary logistic regression was used to determine association of dependent variables (*rpoD*, *rpoN*, *fliA*) and independent variables (*jhp917*, *jhp918*, *jhp914/917*, *jhp914*, *G27*) which were included in the model with the significant level set at P < 0.05.

Results

Histological findings revealed that none of the forty-nine patients’ biopsies had cancer, ulcer, erosion, dysplasia, metaplasia and atrophy. Examination for inflammatory chronicity and activities showed moderate chronicity in twenty-two biopsies, low chronicity in fourteen, severe chronicity in six, autolysis in four and no inflammation in two. There were no inflammatory activities in twenty-nine of the biopsies, low activity in thirteen, moderate in three while four of the biopsies were observed to be autolysed.

Comparative analysis of the *dupA*, plasticity region genes and endoscopic findings showed that the *jhp0918* gene was harboured by 88% (43/49) of the isolates, the *jhp0918* was more common in strains from patients

Table 2. Fisher’s Exact Test on *dupA* and plasticity region genes association with endoscopic findings.

<i>dupA</i> and plasticity region genes	p-values	Conclusion
<i>jhp917</i>	0.000	Significant
<i>jhp914/917</i>	0.042	Significant
<i>jhp917/918</i>	0.009	Significant
<i>jhp918</i>	0.411	Not significant
<i>jhp914</i>	0.234	Not significant
<i>jhp940</i>	Gene is absent	incomparable

*Accepted p-values was set at < 0.05, Duodenal ulcer promoting gene A- (*dupA*), Plasticity region genes (*jhp917*, *jhp918*, *jhp914/917*, *jhp914*), Complete *dupA*- (*G27*)*jhp917/918*.

Table 3. Percentage distribution of the sigma factors in Nigerian isolates.

Sigma factors	Frequency of positive isolates	Frequency of negative isolates
σ^{54} (<i>rpoN</i>)	95.9% (47)	4.1% (2)
σ^{28} (<i>fliA</i>)	91.8% (45)	8.2% (4)
σ^{70} (<i>rpoD</i>)	79.6% (39)	20.4% (10)

*Sigma 70 - (*rpoD*), sigma 54 - (*rpoN*), sigma 28 - (*fliA*).

with erosion 42% (18/43) than those with normal mucosa 35% (15/43). Subjects with mucosal atrophy, oedematous and hyperaemic stomach were 14% (6/43). Similarly, subjects with ulcer were 9% (4/43).

The *jhp0914* gene was detected in 36 (73%) isolates out of which 14 (39%) were from subjects with erosion, 10 (28%) of the isolates were from subjects who had normal mucosa. Eight (22%) of the isolates were from subjects with mucosal atrophy, oedematous and hyperaemic stomach while four (11%) of the isolates were from subjects who presented with ulcer.

Twenty-six (53%) of the isolates harboured *jhp0917* gene, out of which nine (35%) were from subjects who had normal mucosa, eight (31%) from mucosal atrophy, oedematous and hyperaemic stomach, five (19%) from ulcer while four (15%) were from subjects with stomach erosion.

Six (12%) of the isolates harboured the *jhp0914/0917* genes at the truncated part of the plasticity region. Of the six isolates, four (67%) were from subjects who had normal mucosa, two (33%) from mucosal atrophy, oedematous and hyperaemic stomach.

The *jhp917/918*, also called complete (G27 variant) was present in only two (4%) of the isolates, these two isolates were obtained from subjects who presented with gastric ulcer.

The *jhp0940* gene was not detected in any of the isolates.

Using fisher’s exact test to find out the significant relationship between *dupA*, plasticity region genes and gastro-duodenal diseases, the *jhp0917* gene showed significant association with endoscopic findings, with $p < 0.001$. Gene *jhp 914/917* showed significant association with endoscopic findings with $p = 0.042$ while *jhp917/918* which was possessed by only two

isolates with significant association of $p = 0.009$. The *jhp0918* and *0914* were not associated with the endoscopic findings with $p = 0.411$ and 0.234 respectively while *jhp0940* was absent in all the isolates (Table 2).

The results of the alternate sigma factors indicated that 95.9% (47/49) of the isolates characterized possessed σ^{54} (*rpoN*) while 91.8% (45/49) possessed σ^{28} (*fliA*). Similarly, 79.6% (39/49) of the isolates possessed σ^{70} (*rpoD*). Thus σ^{54} (*rpoN*) was the highest in frequency amongst the sigma factors evaluated while σ^{70} (*rpoD*) was the lowest. Overall, 73.5% (36/49) of all the strains possessed the three sigma factors, 18.4% (9/49) possessed both σ^{54} (*rpoN*) and σ^{28} (*fliA*) but not σ^{70} (*rpoD*), two (4.1%) also possessed both σ^{70} (*rpoD*) and σ^{54} (*rpoN*) but not σ^{28} (*fliA*), two (4.1%) possessed only σ^{70} (*rpoD*) (Table 3).

There was significant association between σ^{70} (*rpoD*) and the plasticity region genes *jhp 917* and *jhp 914* using logistic regression with $p = 0.014$ and 0.046 respectively (Table 4). The level of influence of σ^{70} (*rpoD*) on the *dupA* and plasticity region genes was 27%. The σ^{54} (*rpoN*) and σ^{28} (*fliA*) does not have association with *dupA* and the plasticity region genes and the level of influence was 17% and 23% respectively (Table 5).

Discussion

The relationship between the presence of some of the genomic plasticity region genes, *dupA* and clinical outcome is apparent in this study. From the results gene *jhp0917* was significant for gastro-duodenal diseases such as erosion, ulcer, mucosal atrophy and hyperaemia with $p < 0.001$. This is in line with previous findings which reported *jhp0917* to be the first disease-specific

Table 4. Logistic regression analysis of sigma factors (σ^{70} (*rpoD*), σ^{54} (*rpoN*), σ^{28} (*fliA*)) significance on *dupA* and plasticity region genes.

<i>dupA</i> , plasticity region genes	Sigma factors		
	σ^{70} (<i>rpoD</i>) p-values	σ^{54} (<i>rpoN</i>) p-values	σ^{28} (<i>fliA</i>) p-values
<i>jhp917</i>	0.014	0.997	0.997
<i>jhp918</i>	0.767	0.997	0.997
<i>jhp914/917</i>	0.999	1.000	1.000
<i>jhp 914</i>	0.046	0.997	0.997
<i>jhp917/918</i>	0.117	1.000	1.000
<i>jhp 940</i>	Gene is absent	Gene is absent	Gene is absent

*Accepted p-values was set at < 0.05 , Duodenal ulcer promoting gene A- (*dupA*), Plasticity region genes (*jhp917*, *jhp918*, *jhp914/917*, *jhp914*), Complete *dupA*- (G27), Sigma 70 - (*rpoD*), Sigma 54 - (*rpoN*), Sigma 28 - (*fliA*).

Table 5. Linear Regression Analysis of Sigma Factors (σ^{70} (*rpoD*), σ^{54} (*rpoN*), σ^{28} (*fliA*) Influence level on *dupA* and plasticity region genes.

Sigma factors	R squared values	Level of Influence on <i>dupA</i> and plasticity region genes.
σ^{70} (<i>rpoD</i>)	0.267	27%
σ^{28} (<i>fliA</i>)	0.231	23%
σ^{54} (<i>rpoN</i>)	0.176	18%

* Duodenal ulcer promoting gene A- (*dupA*), plasticity region genes (*jhp917*, *jhp918*, *jhp914/917*, *jhp914*), complete *dupA*- (G27) sigma 70 - (*rpoD*), sigma 54 - (*rpoN*), sigma 28 - (*fliA*).

virulence marker in *H. pylori* [10]. The *jhp0917* was also reported to play a role in IL-8 production, activation of transcription factors responsible for IL-8 promoter activity and increased survival at low pH. Both *jhp0917* and *0918* genes have been designated as duodenal ulcer promoting (*dupA*) gene [26-27].

Similarly, the *jhp0918* gene was the most prevalent among the plasticity region genes examined and erosion was observed to be the highest recorded pathological outcome in subjects harbouring the strains with the *jhp0918* gene, followed by mucosal atrophy, oedematous, hyperaemic stomach and ulcers, while none had cancer. In a study involving strains from Brazilian children and adults, the prevalence of *dupA* gene was extremely high (92%; 445/482) irrespective of the nature of gastro-duodenal diseases such as gastritis, duodenal ulcer and gastric cancers [28-29].

The prevalence of *jhp0917* and *jhp0918* was observed to be 53% and 73.5% respectively in the isolates studied. Findings around the world indicated that their prevalence vary from 6% to 92% [12,26,30].

The truncated plasticity region gene *jhp0914/0917* was found in only 12% (6/49) of the isolates studied with statistical association with gastro-duodenal diseases with $p = 0.042$, studied isolates showed that 88% (43/49) of these isolates had deletion of this gene. Previous study has reported that most *Helicobacter pylori* strains from Western Africa have this deletion in a part of the plasticity region [31].

The *jhp917/918* (G27 variance) also known as complete *dupA* was present in only two (4%) of the isolates and the patient from which it was obtained had ulcer. Takahashi *et al.* [32] reported that the *jhp917/918* is significantly associated with gastric ulcer and gastric cancer than gastritis [32].

With respect to *jhp0914*, the observed prevalence of 73% (36/49) indicated their wide spread presence in Nigeria strains, which is in agreement with previous report that the gene is specifically present in the hp Africa1 population and it was noted to be a general signature of hp Africa1 strains [31,33]. Evidence has shown that *jhp0914* in J99 is a good marker for gastro-duodenal diseases. This was corroborated with observed clinical outcome in the present of erosion 39%

(14/36), mucosal atrophy, oedematous and hyperaemic stomach 22% (8/36) and ulcer 11% (4/36) [5].

None of the isolates studied possessed the *jhp0940* gene. In a previous study, the prevalence of this gene was found to vary from 1.5% in Brazil to 100% in South African isolates and it was reported that they may be positively associated with increased risk of gastric cancer and decreased risk for duodenal ulcer in some populations [34]. Previous studies have also reported the gene to encode host interaction factor, contributing to *H. pylori* virulence and evolution of the infection [14,35-37].

Majority of *H. pylori* studied had sigma factors. This is in agreement with studies conducted in Italy [21]. The σ^{54} (*rpoN*) is known to controls several bacterial regulatory processes involved in energy metabolism, biosynthesis, oxidative stress and virulence [38]. σ^{28} (*fliA*) has been reported to regulates the expression of outer membrane proteins, lipopolysaccharide synthesis, DNA restriction and *cagA* expression as well as control of LPXC gene which is involved in the early steps of lipid A synthesis of *H. pylori* [39-41]. Similarly, previous report indicated that σ^{54} (*rpoN*) and σ^{28} (*fliA*) control the expression of flagella components [22,42]. From a previous study, it was reported that σ^{70} (*rpoD*) is highly expressed and stimulated in the presence of chloramphenicol or tetracycline [42,43]. The high frequency of sigma factors in Nigerian isolates (σ^{54} (*rpoN*) (95.9%); σ^{28} (*fliA*) (79.6%); σ^{70} (*rpoD*) (79.6%)) is indicative of how essential this sigma factor is in *H. pylori*. Previous study indicated that sigma factors provide efficient mechanisms for simultaneous regulation of virulent gene expression in an unfavourable environment [43]. This is the first report on the prevalence of sigma factors in Nigerian isolate.

Conclusion

The present work revealed that the presence of *jhp0917* genes and *jhp914/917* were significantly associated with gastro-duodenal diseases and that because of the high prevalence of *jhp0917* and *0918* they are better biomarkers than the *jhp0914/0917*, *0940* and the *jhp917/918* (G27 variance) for the detection of

the presence of plasticity region genes in the Nigerian *H. pylori* strains. The *jhp0918*, *0914* and *0940* genes were however not associated with gastro-duodenal diseases. The high prevalence of sigma factors in the isolates may be contributory to the expression of these virulent factors.

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Authors' contributions

Manuscript preparation and Clinical studies-JTF, Manuscript Editing and Clinical studies- AIA, SSI, NH, PP, Clinical studies – FMA, OC, UR, AI, LO, ND, AO, AI, BM, NF.

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