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Development of a Synergistic Novel Therapy against Antimicrobial Resistance Strains of *Helicobacter pylori*

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Abstract

Increasing Antimicrobial Resistance (AMR) compromises the treatment of *Helicobacter pylori* infection globally. This study aimed to use probiotics together with an antibiotic regimen and a proton pump inhibitor to eradicate *H. pylori*. Stools from positive *Helicobacter pylori* Stool Antigen (HpSAg), totaling 114 (47.7%) were cultivated on Columbia-based Dent agar (Oxoid) at different times. D Berg's commercially sourced brand of *Lactobacillus rhamnosus/casei* probiotic was used. Following biochemical and molecular identification. A per-clinical-factorial-experimental design was employed for the study. Animals, aged 40 weeks old and above were prepared and grouped into six subsets labeled T1 - T6, each defining a particular experimental variable with n = 20. The *H. pylori* isolates 1×10^8 CFU/ml in normal saline were intraperitoneally administered to the test animals (T2 - T6) over 21 days. Different preparations of probiotics and antibiotics-probiotic therapies were given to the relevant subsets and the outcomes were monitored daily. Fisher's exact software was used to calculate the error value at 5% ($p < 0.05$). Sensitive antibiotics alone gave an 80% (P value = 0.058) success rate while antibiotic-probiotic treatment, gave 100% (P value = 0.001). This study demonstrated that the success of *H. pylori* treatment lies in a good Antimicrobial Sensitivity Test-

ing (AST) platform and that only a probiotic-antibiotic regimen can guarantee total eradication (100%) of *H. pylori*.

Keywords

Helicobacter pylori, Probiotics, *Lactobacillus rhamnosus* Biovar Casei, HpSAg, Columbia-Dent Agar

1. Introduction

Fundamental principle behind the successful establishment of any infection or disease lie in the functional capacity of the microbiome and the consequent dysbiosis or altered ratio (usually reduced) of the beneficial/useful microorganisms to the pathogens. Recently, researchers have been exploring this new ground to investigate the mechanisms of the host-microbe relationship to fully elucidate and provide a simple explanation of the roles of probiotics in diseased and healthy conditions [1]-[3]. The human gut *microbiome* is a complex and dynamic ecosystem of microorganisms residing in the gastrointestinal tract [4] [5]. It comprises trillions of bacteria, viruses, fungi, and other microbes. The gut *microbiome* plays a crucial role in maintaining health and disease developmental processes [6] [7]. Recent advancements in sequencing technologies and bioinformatics have revolutionized our understanding of this microbial ecosystem [8], shedding light on their composition, functions, and interactions with the host. Each individual's *microbiome* is unique and influenced by genetics, diet, and environmental factors.

Helicobacter pylori colonizes the gut of more than 40% of the global population [1]. Most of the commonly used antibiotics in the eradication treatment are becoming inefficient as a result of the pathogen's increasing resistance due to quorum sensing, and horizontal gene transfer among other factors [2] [3]. The gut microbiome plays a crucial role in the human internal environment [4]-[6]. They evolve with the host [7] [8] and perform essential physiological functions for the host, which include preventing infection from various pathogens, promoting the maturation of the immune system, participating in the regulation of the immune response, aiding digestion to enhance nutritional absorption and metabolism, as well as promoting anti-cancer functions. They are otherwise referred to as commensal or probiotics.

This study encompasses multiple disciplines, including Microbiology, Gastroenterology, antibiotic resistance, and therapeutic innovation, with a focus on improving H. pylori eradication strategies through the combined use of probiotics and antibiotics.

Aim of the Study

This study was undertaken to explore the efficacy of probiotics-supplemented therapy in *Helicobacter pylori* eradication treatment plans, which could bring

about an improved strategy for effective eradication of *H. pylori* from the human gut.

2. Literature Review

2.1. Epidemiology and Demographic Factors

Helicobacter pylori is a Gram-negative, spiral-shaped, microaerophilic bacterium that colonizes the human gastric mucosa, playing a central role in the development of gastritis, peptic ulcer disease, and gastric cancer, as well as gastric Mucosa-Associated Lymphoid Tissue (MALT) lymphoma [1]. It is one of the most prevalent chronic bacterial infections globally, with changing demographic distribution influenced by public health interventions and socioeconomic conditions [2]. The pathogen evolves genetically diverse but specific virulence factors such as but not limited to *cagA* and *vacA* that enable the bacteria to facilitate chronic colonization of the gut epithelium, achieve immune evasion, and consequently cause tissue damage [3]. Studies have observed that *H. pylori* exists as a syndemic co-pathogen. This means that it more often exists alongside viruses, parasites, and protozoa, occasioned by shared risk factors such as poor sanitation and low socioeconomic status. The World Health Organization characterized and classified *H. pylori* as a biological carcinogen, capable of initiating gastric carcinogenesis through inflammation, DNA damage, and modulation of signaling pathways like NF-κB and STAT3 [4]. Generally, eradication of *H. pylori* is central to reducing gastric disease burden. However, this has become a great challenge due to increasing antibiotic resistance exhibited by the pathogen.

Despite a general global decline in prevalence due to improvements in sanitation and antibiotic access, *H. pylori* infection remains highly endemic in many developing countries, with clear demographic disparities based on age, socioeconomic status, and geographic location. Epidemiological studies from 2020 to 2024 consistently underscore that *H. pylori* infection is primarily acquired during early childhood and persists into adulthood if left untreated [5] [6]. However, the dynamics of *H. pylori* transmission and persistence are complex. While it is commonly believed that infection is primarily acquired in childhood, some studies suggest a continuous risk of acquisition throughout adulthood, with an estimated annual incidence of 0.5% to 2%. This challenges the notion that *H. pylori* infection is predominantly a childhood-acquired condition and highlights the need for further longitudinal studies to understand transmission dynamics fully.

Studies show specific distributions of *H. pylori* incidence among various age groups. Among schoolchildren, Zhang *et al.* [7] found the incidence to be 18.4% in China. The strains possess high resistance to clarithromycin, raising concerns about future eradication challenges [7]. However, Al Atrash *et al.* [8] observed high infection rates among children in the United States, highlighting treatment failures and regional resistance patterns. Studies in Nigeria by Afolabi *et al.* [9] and Odetunde *et al.* [10] reported pediatric prevalence rates of 35.3% and 42%, respectively. These incidents are often linked to poor sanitation, shared bedding,

and contaminated water sources. The age most affected is between 14 and 19 years. This contrasts starkly with findings from developed countries. For instance, McDowell *et al.* [11] observed a prevalence of just 7.1% among children in the USA. In Europe, studies in Germany and the UK report similar figures, with pediatric prevalence typically below 10% and declining further due to routine pediatric care and antibiotic stewardship programs [12].

Prevalence among adult populations has been consistently high compared to pediatric distribution across the globe. In the US and Europe, for instance, CDC report (2021) estimates adult prevalence in the US at around 30%, with higher rates among older adults. European data also show regional differences: while countries like Sweden and the Netherlands report adult prevalence rates below 20%, Southern European nations like Portugal and Italy show slightly higher rates (30% - 40%), often tied to earlier birth cohorts before public health improvements took effect [13]. Adult populations in West Africa continue to carry the highest burden. Jajere [14] reported adult prevalence rates in northern Nigeria between 70.5% and 87.5%, particularly among women and economically disadvantaged groups. This is accounted for by socio-economic disposition, hygiene status, persistent childhood infection, and limited access to diagnostic and eradication treatments.

2.2. Virulence Factors and Genetic Determinants

The pathogenicity of *H. pylori* is primarily mediated by virulence factors such as *cagA*, *vacA*, and *babA*. Kobayashi *et al.* [15], Hu *et al.* [16], and Jajere [14] highlighted the role of *cagA*-positive strains, showing a strong association with chronic gastritis and atrophic changes. CagA, once translocated into gastric epithelial cells via a type IV secretion system, disrupts cellular junctions and promotes oncogenic pathways. *CagA* synergistically interacts with host epithelial signaling pathways to upregulate pro-inflammatory cytokines like IL-8, thereby sustaining chronic inflammation and oxidative stress which are key precursors to malignant transformation of affected tissues [16].

Similarly, the functional consequences of *vacA* allelic variations in clinical isolates demonstrated that high-toxicity *vacA* genotypes (s1/m1) correlated with increased epithelial apoptosis and more severe mucosal inflammation [17] [18]. *CagA* and *vacA* prevalence varied significantly by region, with East Asian strains exhibiting higher virulence than those from Africa or Europe. However, even within less virulent strains, factors like *babA*2 were consistently associated with enhanced mucosal adherence and persistent colonization [19]. West African isolates from patients with serious gastritis showed predominantly *vacA* s1/m1 and *cagA* genes [20].

Successful colonization of the gastric mucosa by *Helicobacter pylori* is critically dependent on its ability to adhere tightly to the host epithelium. This process is mediated by a family of Outer Membrane Proteins (OMPs), particularly BabA (blood group antigen-binding adhesin) and SabA (sialic acid-binding adhesin),

which bind to Lewis b and sialylated antigens, respectively. On the other hand, HopQ and AlpA contribute to both adherence and immune modulation. This highlights the fact that OMP subsets facilitate firm bacterial attachment via redundant and adaptive adhesin systems, which allows for persistence in case of individual adhesins' disruption either by genetic disposition or by host immunity [21] [22].

However, the correlation between these virulence factors and disease severity is not consistent across all populations. Some studies have not found a significant association between the presence of *cagA*, *vacA*, or *babA*2 genes and the development of peptic ulcers or gastric cancer in certain cohorts. This suggests that host genetic factors, environmental influences, and bacterial strain diversity contribute to disease outcomes, indicating that virulence factors alone may not be definitive predictors of disease progression [23] [24].

2.3. Host-Pathogen Interaction and Immune Evasion

A hallmark of *Helicobacter pylori*'s pathogenicity lies in its ability to evade host immune surveillance while simultaneously triggering a chronic inflammatory response that contributes to tissue injury. One major mechanism involves the modulation of cytokine signaling pathways. *H. pylori* infection induces the secretion of interleukin-8 (IL-8), a potent neutrophil chemoattractant, and other pro-inflammatory cytokines, through *cagA*-mediated activation of the NF-κB and AP-1 transcription factors. This persistent recruitment of immune cells exacerbates oxidative damage and mucosal inflammation which when unresolved leads to carcinogenesis [25] [26]. According to studies by Serrano *et al.* (2021), *H. pylori* also alters dendritic cell function, skewing T-cell responses toward a regulatory phenotype that permits bacterial persistence. Their findings revealed reduced expression of MHC class II and co-stimulatory molecules in infected dendritic cells, effectively blunting adaptive immune responses [27].

Further, the bacterium's ability to down-regulate Toll-Like Receptor (TLR) signaling, especially TLR2 and TLR4, through the action of *vacA* dampens immunity [28]. This allows *H. pylori* to avoid early innate immune detection, facilitating long-term colonization and increasing the risk for chronic gastritis and neoplastic transformation. Dampening of the immune system by *H. pylori* can also occur through the induction of regulatory T cells (Tregs). This immune modulation is linked to an increased risk of neoplastic transformation due to unresolved inflammation [19] [29]. These studies illustrate that *H. pylori* does not merely trigger inflammation, but strategically manipulates immune pathways to ensure its survival. By maintaining a delicate balance between immune activation and evasion, the bacterium establishes chronic infection, laying the groundwork for peptic ulcers and gastric malignancies. Nevertheless, the extent to which *H. pylori* manipulates the immune system varies among individuals. Factors such as host genetic polymorphisms, variations in bacterial strains, and environmental influences contribute to this variability [30]-[33]. For example, certain cytokine gene polymor-

phisms in the host can influence the severity of the inflammatory response to *H. pylori* infection. This underscores the complexity of host-pathogen interactions and the need for personalized approaches in managing *H. pylori*-associated diseases [34]-[36].

2.4. Progression to Disease States

The progression of *Helicobacter pylori* infection to serious gastrointestinal diseases—such as chronic gastritis, peptic ulcer disease, and gastric cancer—is influenced by a multi-factorial interplay of bacterial virulence, host genetics, and environmental factors. Long-term colonization with virulent *H. pylori* (cagA) strains significantly increases the risk of atrophic gastritis and intestinal metaplasia, both of which are precursors to gastric carcinoma [37]. While *cagA*-positive strains are associated with a higher risk of gastric cancer, not all individuals infected with such strains develop malignancies. This indicates that additional factors, such as dietary habits, smoking, and genetic predispositions, play significant roles in disease progression.

CagA-positive strains with active *vacA* alleles lead to more rapid progression from non-atrophic gastritis to peptic ulcer and gastric dysplasia [38]. A cohort study in Southeast Asia linked these virulence markers with higher histological activity scores and increased epithelial turnover [38]. In sub-Saharan Africa, Adedokun *et al.* identified a high prevalence of chronic gastritis among infected adults, often presenting with severe dyspepsia. While gastric cancer incidence remains relatively low in the region, the burden of ulcer disease is disproportionately high, possibly due to late diagnosis and limited access to eradication therapy [39].

Persistent infection with *H. pylori* has been shown to result in altered expression of gastric epithelial genes involved in cell cycle regulation and apoptosis. These genetic changes not only promote mucosal damage but also create a mutagenic micro-environment conducive to neoplastic transformation [40]. A meta-analysis across Europe and Asia shows that individuals infected with strains expressing multiple virulence factors (e.g., *cagA*+, *vacA* s1m1, and *babA*+) had significantly higher odds of developing gastric adenocarcinoma [41]. This supports and promotes the phenomenon known as the virulotype model, where disease risk correlates with the combination of pathogenic traits.

2.5. Bacterial Isolation

The isolation of gastric Helicobacter species is difficult, and it has low sensitivity. Growth typically requires incubation on selective media such as Columbia agar base under microaerophilic conditions for 5 to 7 days. Isolation is advantageous in research settings because it allows subsequent identification of the organism using conventional biochemical testing, whole-cell protein profiling, and DNA analysis. It also permits antimicrobial susceptibility testing, which has recently been recommended before treating humans for *H. pylori* infection due to the increasing prevalence of antimicrobial resistance [42].

2.6. Available Treatment Options Against *H. pylori*

Treatment regimens for *H. pylori* eradication are based on the combination of a strong acid suppressant and antibiotics. First-line therapy is selected according to locoregional or individual *H. pylori* antibiotic resistance patterns [43] [44]. The increasing resistance of *H. pylori* to commonly used antibiotics poses significant challenges to eradication efforts. First-line therapies often fail due to resistance to clarithromycin and other antibiotics, necessitating alternative treatment regimens [43]. Second-line therapy needs to consider the first-line regimen and antibiotic resistance status. Confirmation of treatment success should be done no earlier than 4 weeks after the end of therapy [45].

2.7. Probiotics

Probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts [46] [47]. A wide range of probiotic strains has been studied for their potential roles in combating pathogens such as, but not limited to, diarrhea-inducing bacteria [47]. Among the strains widely studied include: *Lactobacillus rhamnosus* GG, *L. reuteri*, *Bifidobacterium bifidum*, and *Saccharomyces boulardii* as the most promising strains [48]. These are naturally distributed throughout the gastrointestinal tract, with *Lactobacillus* and *Bifidobacterium* species predominating in the small intestine and colon, respectively [49]. Those inhabiting the gut are highly adapted to the high concentration of bile acids that characterize the anatomical site [47]. While *S. boulardii* can transiently colonize the stomach and upper small bowel, exerting localized anti-inflammatory effects, *L. johnsonii* is native to the human stomach and competes with *H. pylori* for niche dominance [50] [51]. Probiotics have been proposed as adjuncts to antibiotic therapy, aiming to enhance eradication rates and reduce side effects. Some studies report that certain probiotic strains, such as *Lactobacillus* and *Bifidobacterium*, can improve treatment outcomes and mitigate antibiotic-associated side effects [52]-[57]. However, other studies have found no significant benefit, and concerns remain regarding the optimal strains, dosages, and treatment durations. Additionally, the long-term safety of probiotic supplementation, especially in immunocompromised individuals, requires further investigation [58]-[61]. According to Tan *et al.* [61], there is great variability in probiotic strains, dosages, and treatment duration, which can lead to inconsistency in results regarding the benefits of probiotics. The study emphasized the need for further research on the long-term safety of probiotics, especially in immunocompromised individuals.

The anti-pathogen mechanisms of probiotics include competitive exclusion, antimicrobial production, and immune modulation [62]-[66]. Furthermore, Gao *et al.* [67] showed that probiotics upregulate IL-10 and TGF-β, both anti-inflammatory cytokines, while downregulating pro-inflammatory factors IL-8 and TNF-α. In a similar study, it was proposed that probiotics enhance dendritic cell tolerance and increase regulatory T cell populations, thereby reducing immune-medi-

ated mucosal injury. Particularly, it was observed that *Lactobacillus* strains suppress *H. pylori* urease activity, which is crucial for the bacterium's survival in acidic environments [68]-[70].

3. Materials and Methods

3.1. Study Area

This study was undertaken at Chukwuemeka Odumegwu Ojukwu University, Cheznik Specialist Laboratory Nigeria.

3.2. The Sampling Population

The sampling population was drawn from both Nnamdi Azikiwe Teaching Hospital (NAUTH), Nnewi, and Cheznik Specialist Laboratory, Awada Obosi with the voluntary consent of the participants following ethical approval. Follow-up analyses were undertaken at Cheznik Medical Laboratory, Awada Obosi in Onitsha metropolis and molecular diagnostics were conducted at Inqaba Biotech, Ibadan.

3.3. Study Design

The pre-clinical-factorial-experimental design, as reviewed by Johnson *et al.* [71] and Baker *et al.* [72], was adopted. A total of one hundred and twenty (120) Wistar rats were divided into six groups (n = 20 per group). All groups were fed standard animal feed and controlled for different experimental variables.

3.4. Sample Analysis

3.4.1. Culture Media Cultivation

Isolation of *Helicobacter pylori* from freshly voided human stool samples positive for stool antigen (HpSAg) tests (**Figure 5**) was performed. Columbia agar base with defibrinated sheep blood agar (Oxoid, Thermo Fisher Scientific, United Kingdom) was used in conjunction with Dent selective antibiotics to effectively cultivate *H. pylori* [73].

3.4.2. Media Preparation

The media were prepared following the manufacturer's instructions (**Table S1a** and **Table S1b**). An equivalent of 39 g/L of powdered media was dissolved in distilled water and gently mixed. The media were autoclaved at 121˚C for 15 minutes. Dent supplements comprising Vancomycin (5.0 mg), Trimethoprim (2.5 mg), Cefsulodin (2.5 mg), and Amphotericin B (2.5 mg) per 500 ml of Columbia agar base were added to the sterilized media upon cooling to 50˚C. Sheep blood (35 ml per 500 ml) was also added aseptically before pouring into sterile Petri dishes.

3.4.3. Gram Staining

A differential staining technique developed by Hans Christian Gram in 1884 and updated by Osman Erkmen [74] in 2021 was used. This involved primary staining with gentian violet, mordanting with Lugol iodine, decolorizing with 95% alcohol, and counterstaining with Safranin to differentiate between Gram-positive and

Gram-negative microorganisms.

3.4.4. Biochemical Testing

Biochemical tests were performed to confirm characteristic physiological products or metabolites of *H. pylori* based on morphology. The tests included stool antigen test, urease test, and oxidase test.

1) Stool Antigen Detection

Stool Antigen Testing (SAT) was conducted using the Immunochromatographic Assay (ICA) method as described by Bordin *et al.* [79]. A small stool sample was dissolved in the manufacturer-provided buffer (Diaspot brand) and mixed thoroughly. After 3 minutes, three drops of the mixture were applied to the sample well of the immunochromatography cassette. Test validity was confirmed by the appearance of a precipitation line in the control region. A positive result was indicated by the presence of two lines—one in the control region and one in the test region (**Figure 5**).

2) Urease Test

The urease test was conducted following the method described by Graham and Miftahussurur [75]. Overnight broth cultures of *H. pylori* were transferred into a medium containing urea and phenol red as a pH indicator. The cultures were incubated for 12 - 48 hours. A color change of the medium along with gas bubbles in the Durham tube confirmed the presence of *H. pylori*.

3.4.5. Antimicrobial Susceptibility Assay

Antimicrobial susceptibility testing was performed using the disc diffusion method as described by Tenover [76], the Clinical and Laboratory Standards Institute (CLSI) [77], and Okorie-Kanu *et al.* [78]. The McFarland standard was employed to standardize bacterial inoculum density and evaluate the efficacy of selected antimicrobial agents against *Helicobacter pylori*.

3.4.6. 16S rRNA Sequencing (PCR) Method

Polymerase Chain Reaction (PCR) amplification was performed using a high-throughput next-generation sequencing platform. The amplified 16S rRNA gene fragments were analyzed by aligning sequences against reference databases using the Basic Local Alignment Search Tool (BLAST) algorithm on the National Center for Biotechnology Information (NCBI) platform. The NCBI, a division of the United States National Library of Medicine (NLM) under the National Institutes of Health (NIH), was established in 1988 to provide access to biomedical and genomic information.

3.5. Preparation and Induction of Laboratory Animals

A total of 120 Wistar rats were randomly divided into six groups (T1 - T6) and subjected to different experimental protocols. All animals were fasted for 12 hours prior to feeding and treatment administration.

- **T1 (Negative Control):** Not induced with *H. pylori*.
- **T2 (Positive Control):** Induced with *H. pylori* at 1×10^8 CFU/ml in saline

via intraperitoneal injection; no treatment administered.

- **T3:** Induced as in T2; treated with standard antibiotic therapy consisting of Amoxicillin (7 µg/g t.d.s), Levofloxacin (3 µg/g b.i.d), Clarithromycin (7 µg/g b.i.d), and a proton pump inhibitor (PPI) (0.3 µg/g b.i.d) for at least 10 days.
- **T4:** Induced as in T2; treated with probiotic *Lactobacillus* alone at 6 µg/g daily for a minimum of 10 days.
- **T5:** Induced as in T2; treated with a combination of 3 µg/g probiotic *Lactobacillus* plus the standard antibiotic regimen for at least 10 days.
- **T6:** Same as T5 but receiving a higher probiotic dose of 6 µg/g body weight daily.

H. pylori (1×10^9 CFU/ml) was administered every two days in all induced groups to maintain infection [80].

Hint: 3 µg/g equiv = 200 mg, 7 µg/g equiv = 400 mg, 0.3 µg/g equiv = 20 mg.

3.6. Safety Assessment on Test Animals

Safety evaluation was conducted at various stages to monitor potential adverse effects of probiotics-supplemented therapy. Animals were randomly selected and humanely sacrificed using mild chloroform sedation. Renal and liver function tests were performed to assess biochemical markers indicative of organ function and systemic toxicity.

3.7. Statistical Analysis

Data were analyzed using SPSS v25.0. Proportions were compared using Fisher's exact test, and results were expressed as mean ± SD A p-value < 0.05 was considered statistically significant.

4. Results

Figure 1. Colonial appearance of *H. pylori* on dent.

The isolates grown on Columbia-based Dent agar exhibited typical *H. pylori* colony morphology. Colonies appeared translucent to milky (**Figure 1**), Gram-negative, spiral-shaped, and tested positive for catalase, oxidase, and urease (**Figure 2**). On the other hand, the MRS confirmed *Lactobacillus rhamnosus* (*casei*) ap-

peared with whitish discrete colonies (Figure 3) and showed gram positive reaction (Figure 4).

Figure 2. Morphological appearance of selective Columbia-base agar *Lactobacillus rhamnosus* (*casei*) on *MRS* agar.

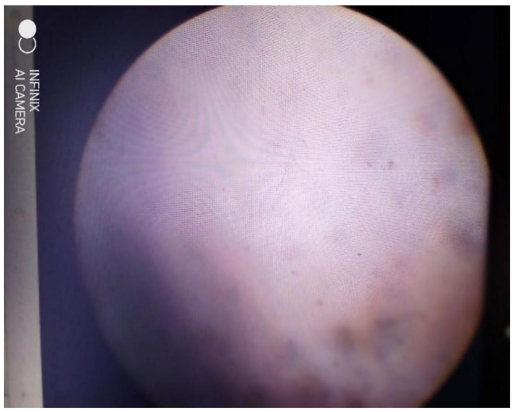

Figure 3. Gram negative staining *Helicobacter pylori* using X40 objective.

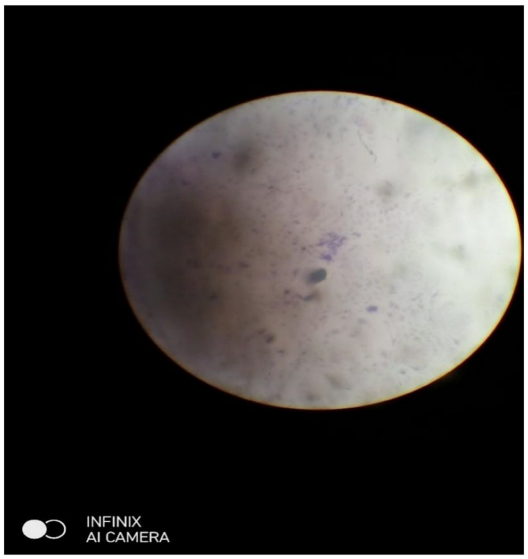

Figure 4. Gram positive staining *Lactobacillus rhamnosus* using X40 objective.

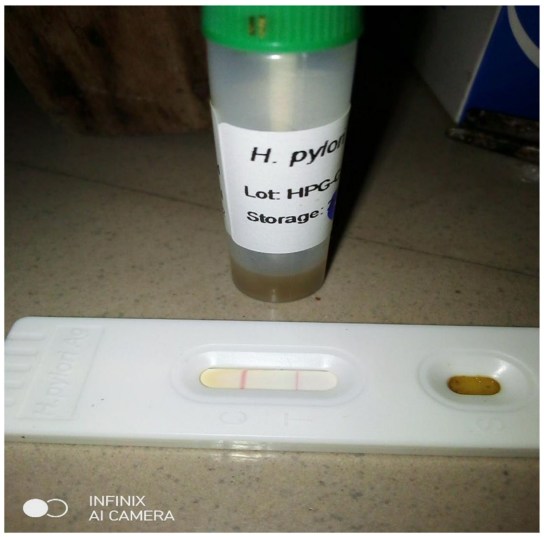

Figure 5. *Helicobacter pylori* stool antigen test (HpSAg) showing positive test.

Table 1. Morphological appearance of the isolates.

Isolates	Morph Catalase	Gram Staining	Oxidase	Urease
H1	Translucent/Milky Pinkish smooth	Negative, pleomorphic Spiral, coccoid and bacilli	+	+
H2	Large mucoidal Greenish pigment foul smell	Negative, bacilli	+	+
P1	Opaque color large colony	Variable staining budding and pseudo-hyphae	NA	NA
P2	Large colony Wet and smooth	G + vebacilli	-	-

Key: H1, H2, P1, P2 = unknown isolates, NA= not applicable, + = positive.

Table 2. Antimicrobial sensitivity result.

Antibiotics	Std Conc	Std zone of inhibition		Test zone of inhibition
	1.5 × 108 cfu	(mm)	(mm)	
		I	S	N = ave of 3 readings
Levofloxacin	1.5 × 108	14 - 16	>17	23.0 (n) + 1.3
Amoxicillin	1.5 × 108	17 - 19	>20	20.5 (n) + 1.9
Cefotaxin	1.5 × 108	18 - 20	>21	17 (n) + 1.2
Metronidzole	1.5 × 108	14 - 17	>18	12.5 (n) + 2.8
Ciprofloxacin	1.5 × 108	16 - 20	>21	22 (n) + 2.2
Clarithromycin	1.5 × 108	14 - 17	>18	17 (n) + 0.8
Lactobacillus probiotics	1.5 × 108	unknown	unknown	13 (n) + 1.4

Keys: cfu = colony forming units, Conc = Concentration or density of inoculum, Std = Standard, I = Intermediate sensitivity, S = Significant sensitivity.

Antibiotic sensitivity patterns are shown in Table 1 and Table 2. Levofloxacin and Amoxicillin demonstrated the highest inhibition zones (>20 mm), indicating strong

efficacy against *H. pylori*. Metronidazole exhibited weak inhibition (<13 mm), consistent with known resistance patterns [1]. Probiotic inhibition zones were modest (~13 mm), supporting their role as adjuvants rather than standalone treatments.

As presented in Table 4, rats treated with *Lactobacillus rhamnosus* alone (T4) demonstrated significant weight recovery compared to positive controls (T2) (P = 0.049). This indicates partial mitigation of disease burden, though probiotics alone did not fully eradicate the infection.

Table 3 shows the summary of the weekly average weight analysis of the experimental animals. The least average weight loss was recorded by animals in T2 which were induded to infection without any treatment interventions. T1 group which were without any infectious or treatment intervention showed highest average weight gain followed by the recuperating animals in T5 dosed on 400 mg eqv inclusive treatment regimen.

Table 3. Averages weights of experimental animals across groups weeks W2 - W6 and W7 - W16).

Group	W0 (Week 1)	Wx (Weeks 2 - 6)	Wf (Weeks 7 - 16)
Normal Control (T1)	127.91	135.31	158.64
Positive Control (T2)	114.50	101.15	70.90
Standard Drug (T3)	126.61	134.82	86.04
Std. Drug + 200 mg eqv Probiotics (T4)	110.30	141.23	97.26
Std. Drug + 400 mg eqv Probiotics (T5)	118.25	114.12	115.76

(*Values rounded to* 2 *decimal places*).

Table 4. Effects of the probiotic monotherapy (W_6 - W_{16}).

Groups	Animal Weights per week (g)			
	W_6	W_7	W_{12}	W_{16}
Normal control	166.70 ± 25.18	172.40 ± 25.80	169.60 ± 26.17	182.25 ± 24.96
Positive control	109.08 ± 16.39*	112.31 ± 16.98*	114.23 ± 18.33*	111.23 ± 18.49*
Probiotic only	102.11 ± 23.43*	105.06 ± 23.57*	128.33 ± 18.42*	147.22 ± 16.70*
Lvalue	51.945	54.309	36.524	61.432
p-value	<0.056	<0.054	<0.051	<0.049

Table 5. Effects of the probiotic-supplemented therapy with 200 mg/kg equiv.

Group (n = 20)	Average Weight of the Animals Per Week (g)			
	W_6	W_7	W_{12}	W_{16}
Negative control	166.70 ± 25.18	172.40 ± 25.80	169.60 ± 26.17	182.25 ± 24.96
Positive control	109.08 ± 16.39*	112.31 ± 16.98*	114.23 ± 18.33*	111.23 ± 18.49*
standard drug mg probiotic	118.33 ± 23.06*	123.53 ± 23.09*	136.07 ± 20.38*	148.33 ± 17.48*
f-value	40.968	41.187	32.496	59.592
p-value	0.029	0.019	0.029	0.011

Table 6. Effects of the probiotic-supplemented therapy with 400 mg probiotics (W6 - W16).

Group (n = 20)	Average Weight of the Animals Per Week (g)			
	W_6	W_7	W_{12}	W_{16}
Normal control	166.70 ± 25.18	172.40 ± 25.80	169.60 ± 26.17	182.25 ± 24.96
Positive control	109.08 ± 16.39*	112.31 ± 16.98*	114.23 ± 18.33*	111.23 ± 18.49*
Standard + 200 mg probiotics	117.17 ± 24.42*	121.50 ± 24.49*	133.58 ± 24.43*	147.92 ± 24.70*
f-value	38.951	40.458	29.287	48.055
p-value	0.028	0.025	0.020	0.019

The effects of combining probiotics with antibiotics are shown in Table 4 and Table 6. Key findings showed that both 200 mg equiv and 400 mg equiv probiotics-supplemented regimens significantly improved eradication rates compared to antibiotics alone (p = 0.058). No statistically significant difference was observed between the two probiotic doses (p = 0.021). Given the comparable efficacy, the 200 mg/kg equiv dose is preferable due to its better safety profile.

Table 7 and Table 8 summarize liver and kidney enzyme levels. Probiotic-supplemented groups maintained stable ALT, AST, BUN, and creatinine levels throughout the experiment. No signs of systemic toxicity were observed, confirming the safety of probiotic therapy.

Table 7. Blood urea nitrogen and creatinine results.

Group	T0		Tm		Tf	
	Crea	BUN	Crea	BUN	Crea	BUN
	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL
T2	8.1 + 1.8	41.2 + 3.1	8.3 + 1.5	43.0 + 2.3	8.4 + 2.1*	42.0 + 4.2
T2	8.1 + 1.8	41.2 + 3.1	20.3 + 1.5	70.0 + 20	18.4 + 2.1*	79.0 + 40
T3	7.9 + 1.9	42.5 + 2.6	11.5 + 1.4*	50.0 + 10*	11.7 + 2.2*	59.0 + 5.0
T4	8.3 + 2.2	42.1 + 3.2	12.1 +1.7*	55.0 + 13	12.5+ 1.3*	60.0 + 9.0
sT5	8.2 + 1.6	41.7 + 3.8	11.7 + 1.6*	57.0 + 10	10.2 + 1.8*	59. 0 + 3.2
T6	8.2 + 2.0	42.7 + 2.1	12.2 + 1.2*	54.8 + 12	12.1 + 1.4*	60.0 + 2.2
f-value	0.0630	0.405	64.315	3.097	30.784	2.217
p-value	0.001	0.001	0.017	0.025	0.013	0.022

Table 8. Liver function assessment test.

Group	T0		Tm		Tf	
	Crea	BUN	Crea	Crea	BUN	Crea
	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL
T1	17.2 + 3.0	57.3 + 5.0	17.9 + 2.8	58.4 + 5.0	17.5 + 3.0	58.6 + 5.5
T2	19.0 + 2.5	56.2 + 7.0	39.4 + 10.2*	91.4 + 3.9*	8.0 +7.5*	100.5 + 5.2*
T3	17.5 + 2.9	56.5 + 9.0	31.1 + 8.5*	95.0 + 6.1*	20.3 + 3.3*	44.8 + 10.5*

Continued

T4	18.2 + 1.9	55.3 + 11.0	288.3 + 11.7*	92.4 + 7.5*	24.0+ 2.9*	48.0 + 11.0
T5	17.1 + 2.2	59.4 + 4.8	44.8 + 6.4*	89.6 + 10.2*	28.0 + 2.4*	41.0 + 8.9*
	18.1 + 2.0	57.5 + 5.8	36.4 + 9.6*	93.0 + 7.1*	25.3 + 4.7*	43.0 + 5.5*
f-value	0.869	0.356	36.054	10.501	20.327	0.617
p-value	0.001	0.001	0.015	0.021	0.001	0.012

5. Discussions

This study investigated the therapeutic potential of combining probiotics with antibiotics for the eradication of *H. pylori* [80]. Results show that probiotics enhanced eradication rates, improved weight recovery, and reduced potential adverse effects associated with antibiotic Monotherapy.

Probiotic supplementation increased treatment efficacy and reduced treatment-associated side effects. This supports integrating probiotics into standard therapy for refractory *H. pylori* infections, particularly in regions with high antibiotic resistance [81]-[83].

Similarly, according to Homan and Orel [84], probiotics could reduce the adverse effects of antibiotics, improve treatment tolerability, and potentially enhance eradication success (**Table 5** and **Table 6**). They emphasized the adjuvant role of probiotics in traditional antibiotic-based therapies rather than as standalone treatments, providing valuable insights into optimizing eradication protocols for *H. pylori*. This study established the therapeutic potential of probiotics at various dosages (200 mg equiv and 400 mg equiv) in synergy with sensitive antibiotics in the holistic eradication of *H. pylori* infection. No significant difference was observed in dosage administration of the probiotics at 200 mg equiv ($p = 0.011$) and 400 mg equiv respectively ($p = 0.019$). This follows that administering a higher dose of probiotics in excess of 400 mg equiv formula could only have more effect on the overall weight (**Table S2**) than 200 mg equiv and not possibly on the therapeutic index.

Table 5 and **Table 6** revealed so much about the synergistic efficiency of antibiotic-probiotic therapy. It showed that Probiotics improve treatment outcomes through several mechanisms including Competitive exclusion in which probiotics compete with *H. pylori* for adhesion sites on the gastric mucosa, limiting colonization. Urease inhibition, in which *Lactobacillus* strains suppress *H. pylori* urease activity, impairing its acid survival mechanism. And finally Immune modulation in which probiotics increase anti-inflammatory cytokines (IL-10, TGF-β) while down-regulating pro-inflammatory mediators (IL-8, TNF-α), enhancing gastric mucosal healing. These agree with the separate findings of other authors such as Chen and Takahashi [85]-[88].

However, some researchers [29] [90] support the standalone use of probiotics in managing specific infections, although the effectiveness varies depending on the condition and probiotic strain used. Rokka and Nami independently found out that probiotics or combination of both could promote recovery of the gut mi-

crobiota after disruptions caused by infectious agents or allergens. The studies also linked increased susceptibility to infections to an imbalanced gut microbiome that often leads to chronic complications if not managed appropriately. Therefore, any intervention that can help modulate immune system could also take care of other common pathogens.

Safety concerns (Table 7 and Table 8) show that probiotic usage either as prophylaxis against gut imbalance or as synergistic therapy against pathogens poses no serious health challenge. Liver and kidney enzyme functions were found to be within the acceptable normal range [90]-[91]. Death recorded during the experiment was below the proposed rate (Table 3). Test probiotic-antibiotic combined therapy holds a lot of hope and promises in eradication of the bacterium thereby improving the treatment outcome.

6. Conclusion

This work has contributed a solution to the global challenge of antimicrobial resistance using a microbial (probiotic) antibiotic synergistic strategy. The work has demonstrated the safety of the new therapy through animal studies and the potential of probiotics not just to eradicate *H. pylori* in pylori infection but indeed in other cases of infectious diseases. This option offers a better alternative that can somewhat guarantee more effective, consistent, and holistic *H. pylori* eradication therapy.

Limitations

While this study provides promising insights, it was performed on animal models. Clinical trials are required to validate dosage optimization, evaluate long-term safety, and establish standardized probiotic formulations.

Recommendation

A long-term study needs to be carried out to investigate the standalone potential of probiotics as a therapeutic agent, perhaps using strain (s) specific models. Also, long-term studies need to be carried out to ascertain impact of probiotics in viral infections, as an evidence-based therapeutic index has so far been established in bacterial infections.

Data Availability

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author/s.

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Conflicts of Interest

The authors declare no conflict of interest whatsoever in the form of financial involvement, or responsibility to any sponsor or agency. All the authors agreed to send this manuscript to Advances in Infecious Diseases (AID) for publication.

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Supplementary Documents

A selective supplement for the isolation of *H. pylori* from clinical specimens is Columbia Blood Agar Base Code: Cm0331.

Table S1a. *Helicobacter pylori* SELECTIVE Medium.

Formula	gm/liter
Special peptone	23.0
Starch	1.0
Sodium chloride	5.0
Agar	10.0
pH 7.3 ± 0.2	

Directions

Add 39 g to 1 litre of distilled water. Boil to dissolve and sterilize by autoclaving at 121˚C for 15 minutes.

Table S1b. *Helicobacter pylori* selective supplement (Dent). Code: Sr0147.

Vial contents (each vial is sufficient for 500 ml of medium)	per vial	per litre
Vancomycin	5.0 mg	10.0 mg
Trimethoprim	2.5 mg	5.0 mg
Cefsulodin	2.5 mg	5.0 mg
Amphotericin B	2.5 mg	5.0 mg

Quality control

Positive controls:	Expected results
Helicobacter pylori ATCC® 43526	Good growth; colourless colonies.
Negative control:	
Candida albicans ATCC® 10231	Inhibited or no growth

*This organism is available as a Culti-Loop®.

Table S2. Constituent of commercially produced pelleted grower's feed per 25 kg.

Ingredient	Ingredient
Cereals/grain,	Vegetable Protein
Premix (Vitamins/Minerals)	Essential Amino Acids
Salt	Antioxidant
Anti-toxins	Prebiotic
Enzymes	
The above are contained in the specific	Composition below:
Crude protein 15% (Min)	Calcium 1.0% (Min)

Continued

Fat 7% (Max)	Available Phosphorus 0.35% (Min)
Crude Fibre 10% (max)	
Metabolizable 2550 Kcal/Kg (Min)	
Energy	

Feed composed by Grand Cereals, a subsidiary of UAC of Nig plc is a Vital feed brand. Source: Ezeumeh *et al.* (2022).

Consent Declaration Draft

We, the undersigned, hereby agree to participate in the research project titled Effect of probiotics-supplemented Antibiotic Therapy in Eradication of *Helicobacter pylori*, conducted by the research team led by Prof. C.N. Umeaku. We understand that our involvement is voluntary, and we have been fully informed about the purpose, procedures, and any potential risks or benefits of the study. We acknowledge that our contributions, data, and personal information will be handled confidentially and used solely for this research. We have been allowed to ask questions, and all our concerns have been addressed to our satisfaction. We understand that we may withdraw from the study at any point without penalty or loss of benefits.

By signing this document, we consent to participate and agree to abide by the research protocols as explained.

Signature:

Name:

Date:

Top of Form

Bottom of Form

Sample S1. Research participation consent note.

Our Reference: COOU/EC/2021/06/012

Date: 23/06/2021

To:

Whom it may concern

Subject: Ethical Approval for Research Involving Human Participants

The Ethical Committee of Chukwuemeka Odumegwu Ojukwu University has reviewed your research proposal titled Effect of probiotic-supplemented antibiotic therapy in eradication of *Helicobacter pylori*, which involves human participants. After careful consideration of the ethical aspects of your study, we are pleased to inform you that ethical approval has been granted to proceed with your research. This approval is contingent upon your adherence to the ethical guidelines set forth by the university, ensuring the confidentiality and protection of all participants. You are also required to inform the committee of any significant changes to the study protocol.

We wish you success in your research and expect that it will contribute meaningfully to knowledge advancement.

Sincerely,

Chairman, Ethical Committee

Chukwuemeka Odumegwu Ojukwu University

Sample S2. Ethical approval letter excer.