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EFFECT OF COMMERCIAL PROBIOTICS AND ANTIBIOTICS ON THE GROWTH OF *CAMPYLOBACTER* ISOLATED FROM CHICKEN MEAT IN HO CHI MINH CITY MARKETS

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SUMMARY

This study explores the antibiotic susceptibility of *Campylobacter*, a prominent foodborne pathogen, isolated in Ho Chi Minh city markets and the efficacy of commercial probiotics in inhibiting these bacteria for enhancing food safety and treating *Campylobacter* infections. Bacteria were isolated from chicken meat in modified coal deoxycholate cefoperazone agar (mCCD), followed by characterization as per standard procedures. Ten isolates with Gram negative, catalase positive and oxidase positive characteristics were collected. Antibiotic susceptibility is ascertained through the determination of the inhibited zone and minimum inhibitory concentration (MIC) of five distinct antibiotics against *Campylobacter* on Muller Hinton agar plates, culminating in a comprehensive assessment after a 24-hour incubation duration. The antibiotic susceptibility results underscore substantial diversity in *Campylobacter* isolates among meat samples, thereby accentuating discernible distinctions among the various antibiotic products. The research also evaluated the suitability of 5 commercial probiotic products (re-named as A, B, C, D, and E for fair assessment) by examining their impact on the growth of *Campylobacter* colonies. The antimicrobial effect of probiotics against *Campylobacter* is assessed using the agar well diffusion assay and co-culture method. We obtained consistent results from two methods, indicating no variation in *Campylobacter* species among meat samples but significant variations among probiotic products. The outcomes of this research provide valuable insights into the antimicrobial potential of each probiotic and antibiotic on *Campylobacter*, informing recommendations for food hygiene practices and underscoring the role of both probiotics and antibiotics in combating *Campylobacter* infections.

Keywords: antibiotic susceptibility, co-culture inhibitory method, minimum inhibitory concentration (MIC), probiotics

INTRODUCTION

Campylobacter is one of the leading foodborne pathogens worldwide. According to the Centers for Disease Control and Prevention, about 1.5 million Americans are infected with *Campylobacter* infections each year. The European union Food Safety report in 2019 showed that *Campylobacter* was the third most frequently causative agent in food-borne outbreak at EU level with about 220.000 cases (European_CDC, 2021). According to statistics from the Food Safety Department (Ministry of Health), each year, Viet Nam has about 7.000 - 10.000 people infected and 100 - 200 deaths from the epidemic. *Campylobacter* is found in a variety of food sources, mainly poultry, unpasteurized milk, or unprocessed meat foods, and then infects humans through the gastrointestinal tract (Humphrey *et al.,* 2007).

Campylobacter infection has become one of the most serious infectious diseases in recent years (Kaakoush *et al.,* 2015). *C. jejuni* and *C. coli* are recognized as human gastrointestinal pathogens. The "expanding" *Campylobacter* species including *C. concisus. C. upsaliensis.* and *C. ureolyticus* could be other pathogens that lead to gastrointestinal and extra gastrointestinal illnesses (Man, 2011). The *Campylobacter* infection shares many of the same symptoms as the so-called stomach flu (which is not the same as influenza, a respiratory illness). Possibly, the infected people have diarrhea (sometimes with blood), fever, headache, nausea and vomiting, and stomach cramps.

Campylobacter bacteria are helical, rod-shaped, or curved Gram-negative species with a single polar flagellum, a bipolar flagellum, or no flagellum absolutely (Kaakoush *et al.,* 2015). By ingesting contaminated food, drinking contaminated water, or occupational contact with diseased animals, people can get campylobacteriosis. Consuming raw poultry, meat, or eggs, as well as cross-contaminating foods by using the same cutting board or cutlery for both raw meat and raw vegetables without cleaning them first, are frequent ways to get sick. It only takes a single drop of juice from raw meat or poultry to have enough *Campylobacter* to infect a human. Additionally, consuming raw (unpasteurized) milk containing *Campylobacter* can cause infection in humans and animals. Some individuals get an infection after coming in contact with the sick dog's or cat's waste (poop). Although many chicken flocks have *Campylobacter* infections, the individual birds are unaffected. Bacteria can spread from an infected bird's intestines to its flesh after slaughter. *Campylobacter* was isolated on specific antibiotic-containing media, under microaerobic conditions, and incubated at 37-42^{o}C to inhibit the growth of some heat-resistant *Campylobacter* species.

Although most *Campylobacter* infections recover within a few days, the disease can be life-threatening in people with weakened immune systems, especially children, the elderly, and patients with the disease HIV/AIDS. Treatment of *Campylobacter* with broad-spectrum antibiotics supplemented with consuming probiotics is currently common therapy. In parallel, the use of beneficial microorganisms is also being studied extensively (Balta *et al.,* 2022). The current research promisingly shows the potential of lactic acid bacteria in inhibiting the growth of *Campylobacter*. The study of Dec and colleagues on many species of *Lactobacilli* indicates that this probiotic could effectively inhibit *Campylobacter*

under co-culture conditions, but the culture of *Lactobacilli* had no inhibitory activity. This result also suggests that many strains of lactic acid bacteria have the potential for use in probiotic supplementation for poultry and humans (Dec *et al.,* 2018).

Current probiotic products commonly contain *Lactobacillus*, *Bifidobacterium,* and/or *Saccharomyces.* This preparation is to increase beneficial microbes in the intestinal tract, reducing the growth of harmful bacteria or chemicals in the digestive system that cause symptoms like diarrhea, indigestion, etc. Probiotic products play a role in balancing the number of intestinal bacteria by competing with and inhibiting harmful bacteria (Ansari *et al.,* 2023). *Bacillus subtilis* bacteria produce enzymes such as proteases that help digest meat proteins. In addition, antimicrobial peptides from *B. subtilis* are instrumental in the treatment of bacterial infections with their rapid killing activity against a wide range of pathogens (Su *et al.,* 2020). However, different ratios of probiotics in product preparations may have different effects on *Campylobacter* inhibition. Therefore, study the activity of probiotic preparations on *Campylobacter* could help to reveal the potential of these microbial combinations on inhibition.

This investigation studies the antibiotic susceptibility of *Campylobacter* isolated from the Ho Chi Minh city markets and the effect of probiotics on inhibiting these bacteria. Employing a range of commonly used antibiotics, including cephalexin, nalidixic acid, streptomycin, erythromycin, and tetracycline, the response of *Campylobacter* would be remarked and correlated to the inhibitory potential of probiotics. This data adds further understanding of *Campylobacter* and some suggestions for use.

MATERIALS AND METHODS

Experimental design

Chicken meat samples were randomly collected from some open markets in Ho Chi Minh city and brought to the laboratory for *Campylobacter* isolation following standard procedures. Bacterial isolates were then tested with different antibiotics in susceptibility assays. Probiotics products were collected from pharmacy and cultured in Muller Hilton medium to check the colony forming unit. The growth of *Campylobacter* isolates was examined in co-culturing with probiotic and with probiotic culture broth alone. Data of bacterial growth was collected and analyzed. The experimental design was sketched in Figure 1.

Samples collection

According to standard guidelines for collecting and handling with fresh meat samples in Viet Nam (QCVN-01-04:2009/BNNPTNT, 2009), six poultry meat samples from three Ho Chi Minh City markets: Phuoc Long market (two samples), Thu Duc market (two samples), and Ba Chieu market (two samples) were collected using the carcass wash method. Samples, taken deep within the pectoral muscle, were placed in a sterile bag with 100 ml of diluted peptone brine. Gentle squeezing for 2-3 minutes ensured thorough rinsing. To preserve *Campylobacter* sensitivity, samples were stored at 3°C ± 2°C and promptly analyzed, avoiding freezing.

Probiotics material

Five popular commercial probiotic

products from Viet Nam were collected from pharmacy based on their popularity and composition. To ensure the privacy, the probiotic products were rebranded and named as A, B, C, D and E, allowing a fair assessment of their effect on *Campylobacter* growth. Table 1 presents the compositions and the quantity of each product used in the inhibition study. The net weight used of the product powder for the experiments was calculated equivalent to 10^7 CFU of each probiotic product, then culture this probiotic powder in 10 ml of LB medium.

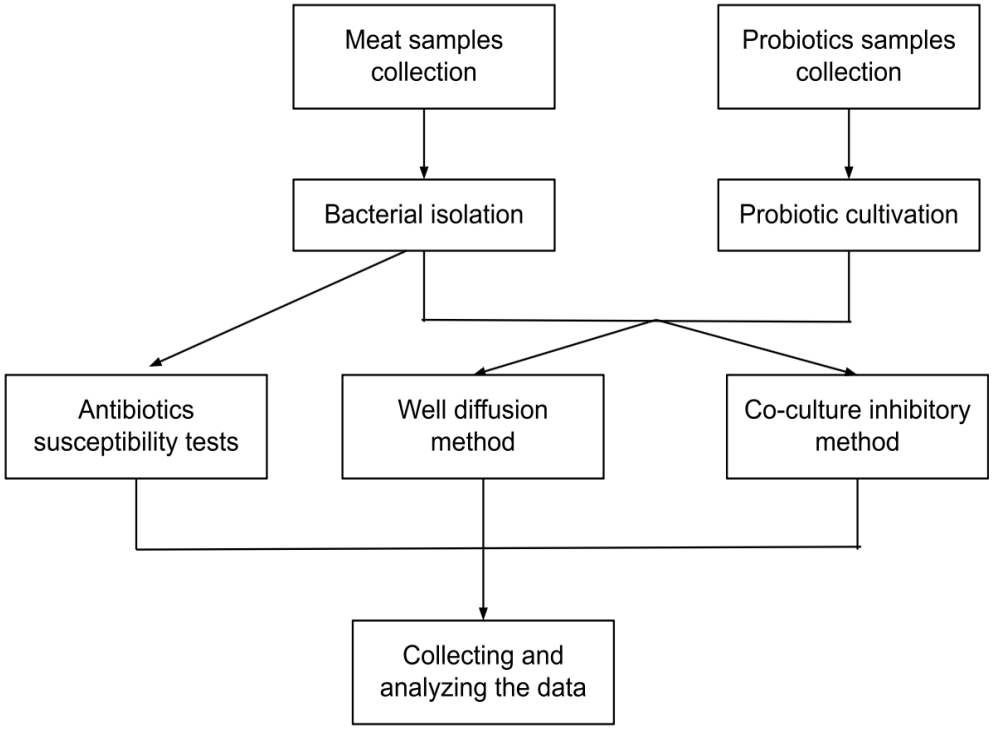


Figure 1. The experimental design of the study.

Campylobacter isolation

Meat broth was cultured on modified coal deoxycholate cefoperazone agar (mCCD) for *Campylobacter* isolation (Dudzic *et al.,* 2016). The mCCD components are dissolved in distilled water, pH adjusted to 7.4 ± 0.2, and sterilized. Cefoperazone and amphotericin B were added, and the cooled medium was poured into sterile Petri dishes. A 10 µl sample was inoculated onto the mCCD agar plate using a sterile culture rod. After incubation at 37°C in a microaerobic environment for 24 hours, *Campylobacter* colonies were collected for purification through repeated streaking. Further tests or subculturing were performed for identification and characterization following the standard procedures.

Gram staining

Gram staining involves initially applying crystal violet dye to a bacterial

smear, staining all bacteria purple. Iodine serves as a mordant, fixing the dye in the cell wall. A decolorizing agent, usually ethanol or acetone, is then used, removing the dye from gram-negative bacteria but not from gram-positive bacteria. Gram-negative bacteria are subsequently counterstained with basic safranin, giving them a pink color. *Campylobacter*, being Gram-negative, appears pink to red with a distinctive tilde shape (Tripathi & Sapra, 2023).

Table 1. The net weight used of five probiotics products. The names of all probiotic products were marked as A, B, C, D and E for research purposes.

Probiotics products	Net weight in the packages	Probiotics containing	Net weight used
Product A	1 g	10^8 CFU *Lactobacillus acidophilus*	0.1 g
Product B	1 g	10^8 CFU *Bacillus clausii*	0.1 g
Product C	4 g	10^8 CFU *Lactobacillus*. 10^8 CFU *Bacillus subtilis* 10^8 CFU *Bacillus clausii*. 10^8 CFU *Saccharomyces boulardii*	0.1 g
Product D	500 mg	10^7 CFU *Lactobacillus acidophilus*. 10^7 CFU *Lactobacillus sporogenes*. 10^7 CFU *Lactobacillus kefir*	0.167 g
Product E	3 g	10^8 CFU *Lactobacillus acidophilus*. 10^8 CFU *Bifidobacterium longum*. 10^8 CFU *Streptococcus faecalis*	0.1 g

Catalase testing

The catalase test identifies the presence of catalase enzymes in bacterial cells by introducing hydrogen peroxide to a bacterial culture. A positive result, indicated by bubbling or effervescence within seconds, confirms catalase activity. This test is frequently employed in microbiology laboratories to distinguish between catalase-positive and -negative bacteria. *Campylobacter*, a positive reaction is observed with the production of air bubbles during the catalase test (Dudzic *et al.,* 2016).

Oxidase reaction

The oxidase test detects cytochrome *c* oxidase in bacterial cells by applying an oxidase reagent to a bacterial culture. A positive result, indicated by a rapid dark blue or purple color change, confirms the enzyme's presence. This test is valuable for distinguishing between oxidase-positive and -negative bacteria. *Campylobacter* shows a positive reaction, marked by a distinct purple color, aiding in bacterial identification (Dudzic et al., 2016).

Inhibition of *Campylobacter* in solid medium by probiotics

Campylobacter colonies were spread on Muller Hinton agar plates then antimicrobial activity was assessed using the agar well diffusion assay. Seven wells (9 mm diameter) were created, with five for 5 probiotic broths, one for positive control (ampicillin 30 µg), and one for negative control (LB broth). Probiotic culture broth from 10^7 CFU in 10 ml LB medium was collected, filtered, and added (80 µl) to the wells. After 24 hours of incubation at 37°C, growth inhibition zone diameters were measured. This process was triplicated.

Co-culture inhibitory effect of probiotic

Co-culturing involved combining 10^7 CFU of *Campylobacter* with 10^7 CFU of probiotic in 10 ml Muller Hinton liquid medium and incubated at 37°C for 24 hours. Post-incubation, 100 µl of liquid from both experimental and control flasks were spread onto mCCD agar to quantify *Campylobacter* colony-forming units. The control with only *Campylobacter* was included. This process was triplicated.

Antibiotic susceptibility

Antibiotic susceptibility was first evaluated with cephalexin (Cp) (30 µg), nalidixic acid (Ng) (30 µg), streptomycin (Sm) (10 µg), erythromycin (Er) (15 µg), and tetracycline (Te) (30 µg). Bacterial layers on Mueller Hinton agar were exposed to antibiotic disks, and incubated for 24 hours at 37°C, inhibition zones were then measured. Minimum Inhibitory Concentration (MIC) was examined using various antibiotic disk concentrations on bacterial layers. MIC was determined at the lowest concentration exhibiting inhibitory effects after another 24-hour incubation. This approach offers both qualitative and quantitative insights into antibiotic efficacy (Siddiqui *et al.,* 2015).

Statistical analysis

Data presented as mean ± SD of diameter of inhibition zone on agar plates, and of number of colonies after co-culturing probiotics and *Campylobacter.* Data were analyzed statistically using ANOVA: Two-factor with replication test, and $p \leq 0.05$ indicates significant difference between treatments.

RESULTS

Isolation of *Campylobacter* colonies

Ten *Campylobacter* colonies, labeled as S1 to S10, were isolated and subjected to analysis. The selective growth of *Campylobacter* was obtained by culturing the colonies on mCCD agar supplemented with antibiotics, a specialized media designed for *Campylobacter* isolation. Colonies appeared bright pale on the dark background of medium (Figure 2).

Characterization of *Campylobacter*

A total of 10 colonies (S1 to S10) were further analyzed in biochemical tests to confirm their morphology. *Campylobacter* was selectively obtained from specialized media (mCCD agar). Gram staining showed that they were Gram negative. In addition, positive catalase reaction and positive oxidase reaction indicate the biochemical characteristics of the species. The results presented in Table 2 unequivocally confirm the presence of *Campylobacter* colonies, validating their identification.

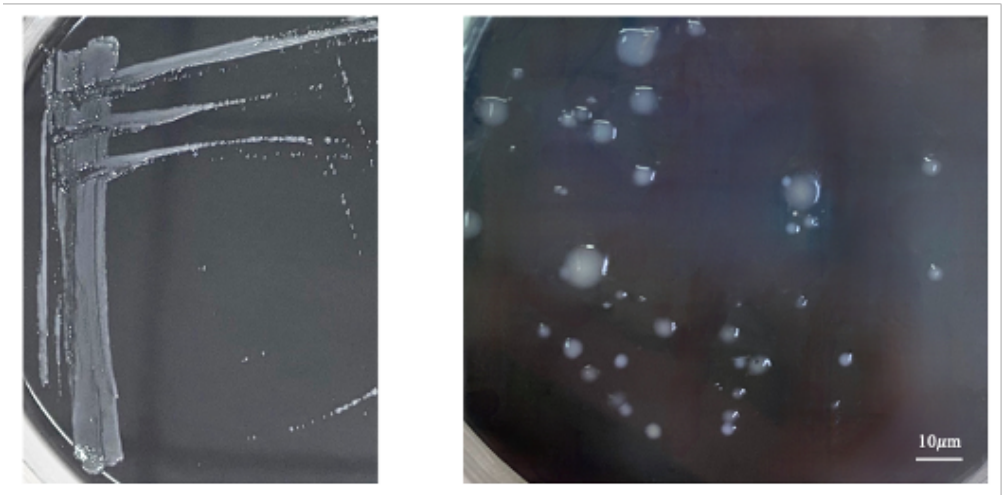
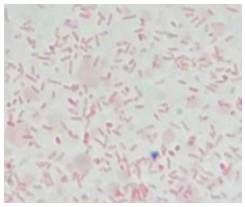
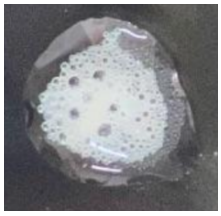
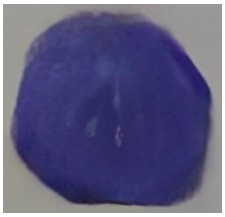


Figure 2. *Campylobacter* colonies were isolated on the mCCD agar

Table 2. The result of biochemical reactions of *Campylobacter* testing.

Types of test	mCCD growing	Gram stain	Catalase reaction	Oxidase reaction
Result				
	Positive	Gram negative	Positive	Positive

Inhibition of *Campylobacter* in solid medium by probiotics

Each probiotic from Table 1 was cultured in 10 ml of LB liquid medium for 24 hours. Culture was filtered to collect all broth, and 80 µl of this broth was used for each well diffusion test. Ten isolates were incubated with the same sample of each probiotic broth and inhibition zones were recorded (Figure 3). Values of inhibition zones were statistically analyzed between isolates and between probiotics. Among the meat samples and isolates, no significant difference was obtained among colonies tested ($p > 0.05$) indicating that each probiotic has the same effect of inhibition on all *Campylobacter* isolates. However, analysis on effect of five probiotic products (A, B, C, D, and E) on each isolate yielded a p-value<0.001, proving that 5 probiotic products differ significantly in inhibiting the growth of *Campylobacter* (Table 3). Variations in their characteristics and composition might explain their effectiveness. Among them, A and E have the lowest and highest impact on *Campylobacter* inhibition, respectively.

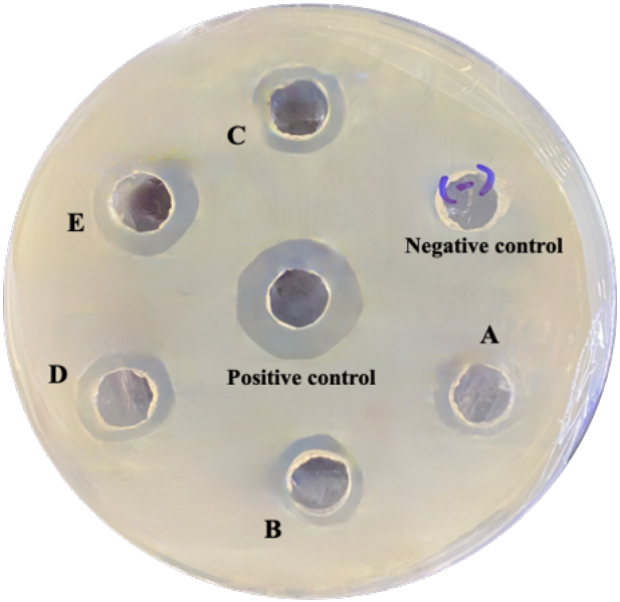


Figure 3. Inhibition of *Campylobacter* by probiotics on Muller Hinton agar. *Campylobacter* and broth of probiotics product A (A); product B (B); product C (C); product D (D); product E (E); Positive control contained ampicillin 30 µg; Negative control contained LB medium.

Table 3. The inhibition of *Campylobacter* from well diffusion plates by five probiotics products. Data obtained as mean of inhibition zone diameter in millimeters of replicates for each sample (Average + SD).

	Product A	**Product B**	**Product C**	**Product D**	**Product E**	**The control**
S1	12.0 ± 1.0	14.0 ± 1.0	15.7 ± 0.6	15.0 ± 1.0	17.0 ± 1.0	20.3 ± 0.3
S2	13.7 ± 1.5	14.3 ± 0.6	14.7 ± 1.5	16.0 ± 1.0	17.3 ± 1.5	21.7 ± 1.5
S3	12.7 ± 0.6	15.3 ± 1.2	15.7 ± 0.6	15.7 ± 0.6	16.3 ± 1.5	21.0 ± 1.0
S4	10.3 ± 1.0	13.7 ± 0.6	15.0 ± 1.0	16.3 ± 0.6	16.3 ± 1.5	20.7 ± 0.6
S5	11.3 ± 1.2	15.3 ± 1.2	14.3 ± 0.6	15.0 ± 1.0	17.3 ± 1.5	21.0 ± 1.0
S6	14.0 ± 1.0	13.3 ± 1.5	15.7 ± 0.6	14.7 ± 0.6	18.3 ± 1.2	22.0 ± 1.0
S7	11.0 ± 1.0	14.0 ± 1.7	15.3 ± 1.2	14.7 ± 1.2	15.7 ± 1.2	21.0 ± 1.0
S8	12.7 ± 1.2	15.3 ± 1.5	15.3 ± 0.6	15.7 ± 0.6	19.0 ± 1.0	22.0 ± 1.0
S9	12.0 ± 1.0	14.7 ± 0.6	15.0 ± 1.0	15.0 ± 1.0	17.8 ± 0.6	21.0 ± 1.0
S10	12.3 ± 2.1	15.0 ± 1.0	15.3 ± 0.6	15.0 ± 1.0	17.7 ± 1.5	20.3 ± 0.6

Values in the same column are not significant different ($p>0.05$); Values in the same raw are significantly different among five probiotics samples ($p<0.001$).

Co-culture inhibitory effect of probiotic

In order to evaluate the effect of probiotics against *Campylobacter* in liquid culture, we carried out co-culturing of this bacteria with each of products A, B, C, D, and E. The same amount equivalent to 10^7 bacterial cells of *Campylobacter* was mixed with 10^7 CFU of probiotic calculated from data in product label. The mixture was then cultured in Mueller-Hinton liquid medium for 24 hours followed with spreading the same amount of co-culture on mCCD agar plate. Positive control contains the same amount of *Campylobacter* without co-culturing with probiotic. Number of *Campylobacter* colonies on the plates was then recorded and statistically analyzed. The results are presented in Table 4.

Table 4. The number of *Campylobacter* colonies recovered in MH plates after co-culture with each of five probiotics products (Average + SD). Control contained only *Campylobacter* in the culture.

Isolates\ Probiotics	A	B	C	D	E	The control
S1	43 ± 3.0	51 ± 2.0	44 ± 1.0	48 ± 1.0	32 ± 2.0	94 ± 4.0
S2	44 ± 2.0	55 ± 2.0	50 ± 7.0	47 ± 3.0	31 ± 3.0	
S3	45 ± 2.0	53 ± 7.0	43 ± 9.0	43 ± 9.0	31 ± 1.0	
S4	45 ± 4.0	49 ± 1.0	43 ± 1.0	47 ± 2.0	35 ± 2.0	
S5	44 ± 9.0	50 ± 2.0	43 ± 1.0	49 ± 13	33 ± 2.0	
S6	42 ± 4.0	51 ± 4.0	44 ± 4.0	48 ± 7.0	32 ± 7.0	
S7	42 ± 7.0	52 ± 2.0	42 ± 3.0	46 ± 4.0	35 ± 2.0	
S8	43 ± 3.0	51 ± 4.0	43 ± 12	45 ± 2.0	35 ± 7.0	
S9	43 ± 3.0	51 ± 9.0	44 ± 2.0	51 ± 9.0	31 ± 7.0	
S10	43 ± 2.0	48 ± 12	45 ± 3.0	46 ± 7.0	33 ± 2.0	

Values in the same column are not significant different ($p>0.42$); significant difference among five probiotics samples ($p<0.05$).

Number of *Campylobacter* colonies were recorded to see if co-culturing with probiotics reduces the number of colonies forming units of the bacteria. More colonies formed indicated the weak inhibition effect of probiotic used. The statistical analysis was performed among isolates (S1 to S10) and among probiotic products (A to E). No difference found when treating *Campylobacter* isolates with the same probiotics among samples S1-S10 ($p>0.42$). However, among five probiotic products (A, B, C, D, and E), there is a statistical difference ($p<0.05$). Number of colonies recovered from incubating with product B is higher significantly than other products

while the least number is found in product E. These results indicated that product E effectively inhibited *Campylobacter* growth, revealing the promising antibacterial properties against *Campylobacter* infections.

Antibiotic susceptibility

Each *Campylobacter* isolate was examined with commonly available antibiotics. *Campylobacter* was first spreaded on agar plate of MH medium, then antibiotic disk containing each of cephalexin (30 µg Cp), nalidixic acid (30 µg Ng), streptomycin (10 µg Sm), erythromycin (15 µg Er) and tetracycline (30 µg Te) was placed onto a bacterial layer, followed with an incubation period for 24 hours at 37°C. The inhibition zone from each test was measured and triplicated (Figure 4), and all results were analyzed together in statistical analysis (Table 5). Among *Campylobacter* isolates when treated with antibiotics, a significant difference was obtained (p<0.001). This suggests that the different meat samples used in the study exhibit variations in some aspect or attribute, which could be related to factors such as origin, quality, or processing methods. Notably, each antibiotic exhibits a unique value for the extent of inhibition zones, providing valuable insights into their respective efficacy against the bacteria.

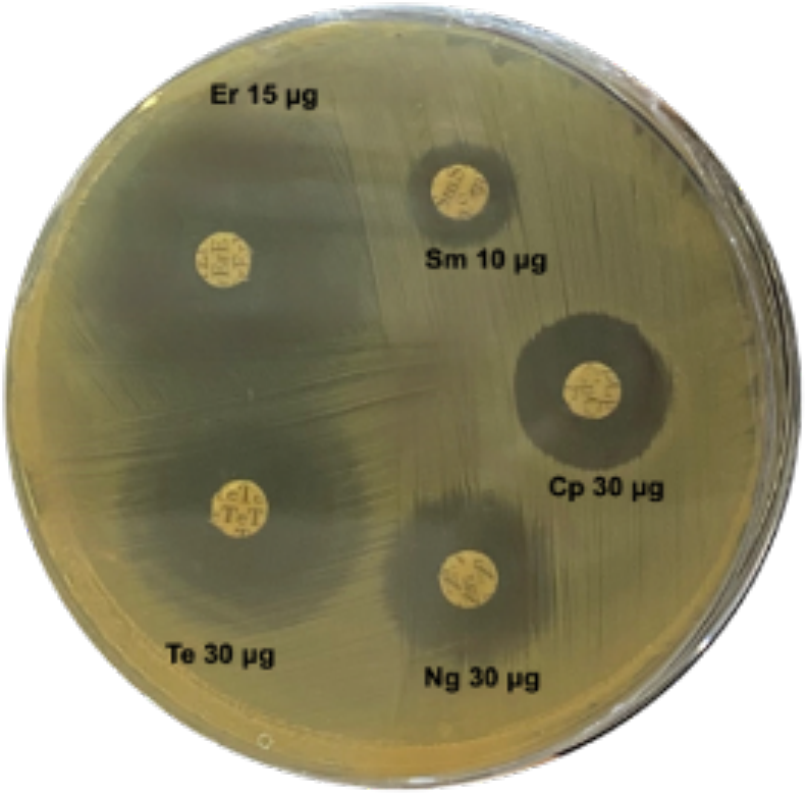


Figure 4. The inhibition zones created when incubating five antibiotics with *Campylobacter*. Cephalexin (Cp); Nalidixic acid (Ng); Streptomycin (Sm); Erythromycin (Er); Tetracycline (Te).

Table 5. The inhibition of *Campylobacter* by five antibiotics. Data obtained as mean of inhibition zone diameter in millimeters of replicates for each sample. (Average + SD).

	Cp 30 µg	Ng 30 µg	Sm 10 µg	Er 15 µg	Te 30 µg
S1	19.5 ± 0.5	20.5 ± 0.5	12.0 ± 1.0	22.5 ± 2.5	22.0 ± 1.0
S2	21.0 ± 1.0	22.0 ± 1.0	13.5 ± 1.5	24.0 ± 1.0	24.0 ± 1.0
S3	17.0 ± 1.0	18.5 ± 1.5	11.5 ± 1.5	22.5 ± 2.5	22.0 ± 2.0
S4	21.5 ± 1.5	21.5 ± 1.5	12.0 ± 1.0	26.0 ± 1.0	24.0 ± 1.0
S5	22.0 ± 3.0	19.0 ± 1.0	12.5 ± 1.5	25.0 ± 2.0	25.0 ± 1.0
S6	25.0 ± 1.0	20.0 ± 1.0	14.5 ± 0.5	27.5 ± 2.5	26.5 ± 1.5
S7	25.5 ± 0.5	24.5 ± 0.5	16.0 ± 1.0	29.5 ± 0.5	29.0 ± 1.0
S8	23.0 ± 2.0	24.5 ± 0.5	16.0 ± 2.0	30.0 ± 1.0	25.5 ± 1.5
S9	18.5 ± 0.5	21.5 ± 1.5	12.0 ± 1.0	29.0 ± 1.0	25.5 ± 1.5
S10	19.5 ± 0.5	23.5 ± 1.5	12.5 ± 0.5	**30.5 ± 1.5**	26.5 ± 2.5

Significant difference obtained among the isolation samples in the same column ($p<0.001$) and among five antibiotics samples in the same raw ($p<0.001$).

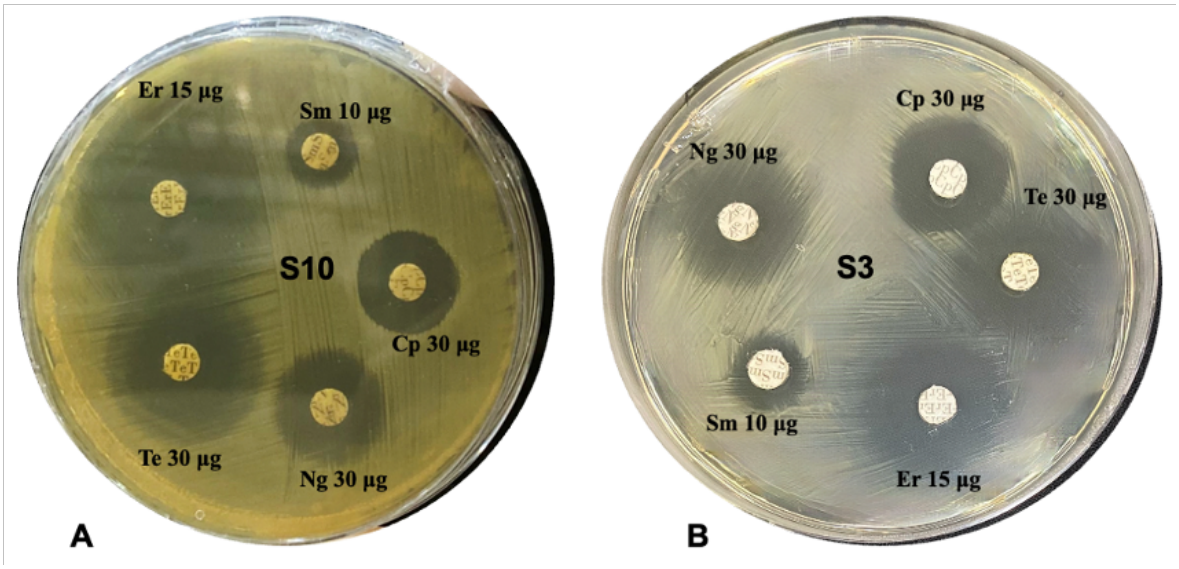


Figure 5. The inhibition zones created when incubating 5 antibiotics with sample S10 (A) and S3 (B).

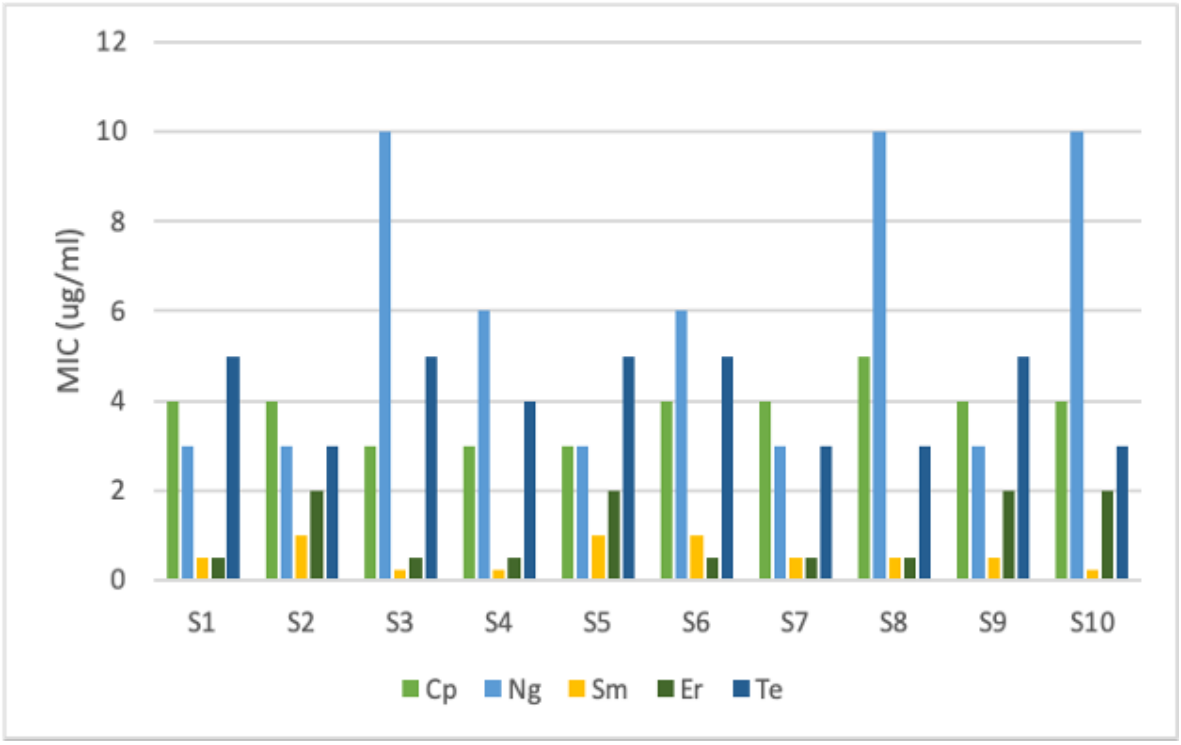


Figure 6. The MIC of five antibiotics against ten samples of *Campylobacter.*

Table 6. The value of the MIC of 5 antibiotics against 10 samples of *Campylobacter* (µg/ml).

	Cp	Ng	Sm	Er	Te
S1	4.0	3.0	0.5	0.5	5.0
S2	4.0	3.0	1.0	2.0	3.0
S3	3.0	10.0	0.25	0.5	5.0
S4	3.0	6.0	0.25	0.5	4.0
S5	3.0	3.0	1.0	2.0	5.0
S6	4.0	6.0	1.0	0.5	5.0
S7	4.0	3.0	0.5	0.5	3.0
S8	5.0	10.0	0.5	0.5	3.0
S9	4.0	3.0	0.5	2.0	5.0
S10	4.0	10.0	0.25	2.0	3.0

Further examination of each antibiotic on *Campylobacter* inhibition was carried out. Acquiring the random antibiotic amount from study of Siddiqui (Siddiqui et al., 2015), we tested on each isolate (Figure 4). For example, sample S10 was treated with 15 µg erythromycin, displaying the most substantial inhibition zone (30.5 ± 1.5 mm) among isolates suggesting that erythromycin, at this specific concentration, exhibited a robust anti-*Campylobacter* effect. In contrast to the significant inhibition zone observed in sample S10 with erythromycin, sample S3 treated with 10 µg

streptomycin displayed the smallest inhibition zone with only 11.5 ± 1.5 millimeters in diameter (Figure 5).

To examine the effect of antibiotics on *Campylobacter* isolates, we carried out the Minimum Inhibitory Concentrations (MIC) test. We prepared ranges of antibiotic concentrations and tested on each bacterial isolate. Notably, nalidixic acid at a MIC of 3 µg/ml was found to effectively inhibit *Campylobacter* in half of the tested samples. Cephalexin with an MIC of 4 µg/ml exhibited in 60% of samples. Minimal inhibition concentrations of each antibiotic were summarized in Figure 6 and Table 6. These findings provide essential information of antibiotic concentrations required for effectively controlling *Campylobacter* growth, promisingly suggest probiotics in the treatment of *Campylobacter* infections.

DISCUSSION

Using the well diffusion assays and co-culture inhibitory method consistently demonstrated the antibacterial potential of various probiotic products. However substantial variations were observed among the tested probiotics, suggesting diverse inhibitory activities against *Campylobacter*. These results underscore the potential for targeted probiotic selection to combat specific *Campylobacter* infections. Five probiotic products differ in species composition and nutritional additives in which E showed the highest potential of inhibition in liquid broth and co-culturing assays. Product E shared the same *Lactobacillus* as other products and supplemented with *Bifidobacterium longum* with equal amount. This combination is shown potential for *Campylobacter* inhibition.

When testing with antibiotics of broad coverage, difference is obtained among colonies. Even being treated well with probiotics, the difference in antibiotic susceptibility was obtained, indicating that the isolates might possess particular biological characteristics. The result also indicates that the probiotic amount used in this research is sufficient in inhibiting *Campylobacter* strains.

Combining probiotics and antibiotics may be considered for optimal outcomes, addressing both pathogenic bacteria and microbiota health. While antibiotics rapidly resolve infections, their use can disrupt the gut microbiota leading to side effects and antibiotic resistance (MacDougall & Polk, 2005). Probiotics comprising live microorganisms will promote a healthy gut balance which are generally safe and have preventive benefits (Sanders et al., 2016). They can be well-tolerated and reduce antibiotic-associated issues. However, their effectiveness may vary, especially in severe infections, and standardization challenges exist (Hill et al., 2014). Choosing between them depends on infection severity, individual health, and treatment goals. Further research is essential to elucidate the relationships between specific probiotic species, concentrations, and nutritional components, providing insights for targeted probiotic interventions and strategies to enhance food safety.

CONCLUSION

Ten isolates of *Campylobacter* from chicken meat samples have been studied and the inhibitory effects of probiotics and antibiotics were evaluated. Each probiotic had similar impacts on all isolates, however, there were some noticeable differences

across the formulations. Product E with *Lactobacillus* and *Bifidobacterium* was the most effective one. All *Campylobacter* isolates were susceptible to tested antibiotics including cephalexin, tetracycline, erythromycin, streptomycin, and nalidixic acid at indicated amounts, in which MIC for each antibiotic was observed. The results highlight some characteristics of *Campylobacter* isolated from chicken meat and potential of lactic acid bacteria for *Campylobacter* inhibition. The study suggests further investigation on broader isolation and formulation probiotics to get effective inhibition of this bacterial infection.

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