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In-vitro antibacterial activity of commercially available probiotics on food-borne pathogens along with their synergistic effects with synthetic drugs

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ABSTRACT

Background: Probiotics are put forward as food to ensure the maintenance of the equilibrium of the intestinal flora. Prolonged usage of probiotics in food ingredients for human as well as in animal feed has not exposed any side effects yet. Present study attempted to justify the effects of some commercially available probiotics (Good-gut, Lubenna, Probio and Protein restro) and commonly used antibiotics (Streptomycin, Gentamycin, Ampicillin, Methicillin, Azithromycin, Erythromycin, Ceftrizone, Imepenem, Ciprofloxacin and Tetracycline) on the bacteria which were previously isolated from food samples.

Methods: The anti-bacterial potential of the probiotics was aimed to be checked through the agar well diffusion method and the antibiogram of the synthetic drugs was determined by disc-diffusion method (Kirby Bauer technique). The minimum inhibitory concentration (MIC) of the probiotics were examined through broth micro dilution assay.

Results: Almost all the probiotic samples exhibited antibacterial activity against the tested bacteria within the range of 10 mm–30 mm except *Bacillus* spp. and *Salmonella* spp. The lowest MIC values 3 mg/ml was determined with Luvena for *Pseudomonas* spp. and *Shigella* spp. while the maximum MIC 20 mg/ml was recorded for Good gut and Probio against *Salmonella* spp. and *E. coli*. Meanwhile, majority of the tested pathogens were detected to be resistant against more than one antibiotic as MDR strains except gentamycin, streptomycin and azithromycin. During the combination method, the zone diameter increased remarkably with a clear indication of synergistic effects compared to their individual activity.

Conclusion: This study substantiated that the deployment of a combination of two antibacterial medications in order to combat the multi-drug resistant bacteria would rather be efficacious than the application of either antimicrobial agent alone.

1. Introduction

Throughout the world, every year a large percentage of death is still caused by the bacterial and the fungal infections by reason of the up-surge in multidrug resistant bacteria as well as the acute inefficacy of available antibiotics against the increasing microbial infections [1–4].

Although, antibiotics play a crucial role in the treatment of various diseases, there are adverse side effects associated with them, such as, allergic reactions, gastrointestinal effects (e.g., nausea, loss of appetite, bloating, vomiting, abdominal pain, diarrhoea) resulting from the disturbance of gut flora and so on [5,6]. The patients receiving antibiotics for the treatment of periodontal diseases are exposed to numerous adversities of antibiotics which entail allergies, nephritis, haematological problems, gastrointestinal problems, disturbance in nervous system, low level of electrolytes etc [7]. Antibiotics-associated adverse drug events (ADEs) commonly experienced by inpatients on antibiotics

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are gastrointestinal, renal hematologic abnormalities and so forth [8].

With the inevitability of side effects, antibiotics should be prescribed for the short-term weighing the probability of benefit greater than the risk of detriment [7]. The overconsumption of antibiotics and the irrational and inappropriate use of synthetic drugs, sometimes without the recommendation of the registered physicians, appear to be the major reasons of the uncontrollably growing bacterial resistance and the drastic decline in antibiotic aptitude [9–11]. The extended use of antibiotics may instigate greater antibiotic resistance not only at the individual patient level but also at the community, country, and regional levels [8].

Hence, to formulate new drugs beside the available antibiotics, the natural components from plant origin should come into focus in terms of undermining the potential antibiotic resistant bacteria [11,12]. Several researchers have published their article regarding the practices of using the combination drug therapy against different microbial agent [13,14]. The bioactive compound from natural sources may act as an adjuvant of commercially available drugs and induces their activity against the pathogenic microflora [1]. In human body, beneficial bacteria are plentiful with the potential in their possession to safeguard the body from harmful bacteria. Such types of bacteria are recognized as probiotics [15,16]. Probiotic is a live microbial supplement, which should have some special attribute such as lack of virulent properties, acid tolerant ability, adherence ability towards the cell and highly dominating against human pathogens [17]. In accordance with the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), the most common probiotic microorganisms are Lactic Acid Bacteria, *Bifidobacterium*, *Propionibacterium* and as a fungi *Saccharomyces boulardii* is very effective [15,18–20].

Several earlier studies emphasized that the antimicrobial activity of probiotics against many pathogenic bacteria including the MDR strain of *S. aureus, E. faecalis*, *K. pneumonia, P. aeruginosa, Escherichia coli, S. typhii* and other *Salmonella* spp [21,22]. was profoundly noteworthy. In recent time, scientists have proved that the synergistic interaction of probiotics and antibiotics can possibly reduce the widespread bacterial diseases effectively with the addition of a notable number of health benefits of the host [23,24].

Furthermore, the oral consumption of probiotic microorganisms produces a defensive effect on the gut flora. Following a plethora of research and studies, it has been evinced that probiotics have multitudinous beneficial effects on the host body during the diseases caused by gut micro flora but its medical application is yet to be known [18,25,26]. Through the deployment of probiotics, the hosts could be protected from various intestinal disorders, resulting in a considerable increase in the number of beneficial bacteria in gut [27,28]. Despite the numerous benefits of probiotics, some cautions are essential while taking the right doses of probiotics based on the individual's age and physical complications in order to prevent the risks associated with probiotic use [29].

Meanwhile, beside the use of probiotics in medical and clinical purpose, its demand has increased in industrial sectors as well [17]. Having utilized the beneficial microorganisms, a huge number of commercially available enzymes, tablets and some fermented food are manufactured which are now very popular as probiotic products in the market [18,30,31]. In our study, we attempted to determine the specific effects of commercially available probiotics against different pathogenic microbiota and most importantly to evaluate their ability to enhance the efficiency of common antibiotics which have already been reported as ineffective/less-effective against bacteria.

2. Materials and methods

2.1. Collection of different probiotics and antibiotics disc

Five (5) types of commercially available probiotics such as Good-gut, Luvena, Probio, Protein restro and Acteria were purchased from the different drug house in Dhaka city. Among the 5 probiotics, protein restro was manufactured by the SabahCare Pharmaceutical, Malaysia and rest of the samples were manufactured by the renowned pharmaceuticals in Bangladesh as cell-free metabolites (Supplement 1). Ten (10) types of antibiotics disc (Streptomycin 10 μg, Gentamycin 10 μg, Ampicillin 10 μg, Methicillin 30 μg, Azithromycin 15 μg, Erythromycin 15 μg, Ceftrizone 30 μg, Imepenem 30 μg, Cipofloxacin 5 μg, Tetracycline 30 μg) were used in this study (Hi Media Laboratories Pvt. Limited, Mumbai 400086, India). The time frame of the study was from September 2019 to November 2019. Dates of manufacturing and expiry were checked prior to evaluate the antimicrobial activity of both samples [9].

2.2. Sources of pathogenic bacteria

The most common food-borne pathogens (*Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Bacillus* spp.) were collected from the Institution of Food Science and Technology, Bangladesh Council of Scientific & Industrial Research (BCSIR) in Dhaka City. The bacterial culture plates were transported into the research laboratory (Microbiology research Lab, Stamford University Bangladesh) in thermal stabilizing box [9].

2.3. Determination of anti-bacterial activity of the probiotics

To examine the efficacy of probiotics against different tested bacteria: this study was introduced agar well diffusion methods on Muller Hinton Agar [32,33]. According to the suggested method by Clinical and Standared Laboratory Institute; a loopfull culture of the tested bacteria (*Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Bacillus* spp.) was inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England) to make the bacterial suspension and the bacterial lawn was prepared onto the surface of the MHA media. Then wells (8 mm) were made on the inoculated MHA media and Granule of probiotics was added into each of the wells. Three replications were used for each of the probiotics. After incubation at 37 °C for 24 h, the presence of clear zone around the sample solution (if any) was analyzed for the existence of the antibacterial activity of the samples tested [34].

2.4. Determination of minimal inhibitory concentration (MIC) of probiotics through broth micro dilution techniques

To confirm the result of agar well diffusion method, we implemented the broth micro dilution assay to determine the lowest concentration of each of the probiotics capable of trimming down the extent of viability of the test bacteria as (MIC) [33]. 5 ml of stock solutions were prepared for each of probiotics by using the double distilled water (DDW). According to the suggested method by Clinical and Standard Laboratory Institute: two-fold serial broth dilution methods were used to determine the MIC (Clinical and Laboratory Standards Institute 2006). An aliquot of 100 μL of the overnight (~12 h) culture of each of the test bacteria was inoculated into the appropriately labeled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England) at the turbidity adjusted with 0.5 McFarland standard and the different volumes of probiotic samples (2 μL, 4 μL, 8 μL, 16 μL, 32 μL, 64 μL, 128 μL, 256 μL, 512 μL and 1024 μL) were introduced. Three replications were used for each of the probiotics. All the tubes were incubated at 37 °C for 24 h. The MIC value was determined by recording the lowest concentration (mg/mL) of each sample that could inhibit the multiplication of the tested bacteria, as judged visually by a lack of turbidity in the tube comparable to the McFarland standard.

2.5. Detection of the efficacy of antibiotics against the bacteria

According to the modified Kirby–Bauer method, all the pathogenic

bacteria were tested to determine their antibiotic susceptibility pattern against 10 antibacterial drugs (including first, second, and third generation drugs) by disc diffusion assay on Mueller–Hinton Agar (Difco, Detroit, MI) [34]. A single colony of each bacterium was inoculated into 2 mL of Mueller–Hinton broth and incubated at 37 °C for 4 h. The turbidity of the culture was then adjusted to a McFarland standard of 0.5. Sterile cotton swabs were dipped in the suspensions and distributed evenly across the surface of Muller–Hinton agar. On the surface of the MHA media, all the antibiotic disc (Streptomycin 10 μg, Gentamycin 10 μg, Ampicillin 10 μg, Methicillin 30 μg, Azithromycin 15 μg, Erythromycin 15 μg, Ceftrizone 30 μg, Imepenem 30 μg, Cipofloxacin 5 μg and Tetracycline 30 μg) were placed at appropriate spatial distance of 5 mm [9,32,35]. Three replications were used for each of the antibiotics. The plates were then inverted and incubated at 37 °C for 24 h. After incubation, the zone of inhibition was measured and the response of bacteria against the drugs (resistant or sensitive) was determined through comparing the data with standard chart [32,34,35].

2.6. Combination of probiotics and antibiotics

Previously, the efficacy of probiotics and antibiotics were separately looked out. But during combination method, the effectiveness of both probiotics and antibiotics were measured together. Firstly, suspension of pathogenic strains (*Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. and *Bacillus* spp.) were lawn onto Muller-Hinton agar plates. Then 20 μL of each 5 commercial probiotics were incorporated to ten antibiotics disc and placed on to the MHA agar. Then, the plates were incubated at 37 °C for 24 h. Afterward the inhibitory zone diameters were measured and recorded [36].

2.6.1. Statistical analysis

During the study period, all data were statistically analyzed by determining p-value and the significant level was considered as <0.05. The GraphPad Prism (San Diego, CA) was customized to calculate the one-way analysis of variance (one-way ANOVA).

3. Result

3.1. Effects of different commercial probiotics samples against the food borne bacteria

In this study all the probiotics samples were found to be effective against all the tested bacteria except *Bacillus* spp. and *Salmonella* spp. (Fig. 1). In case of Good-gut, the zone diameter was estimated within the range of 10 mm–15mm against *E. coli*, *Klebsiella* spp., *Staphylococcus* spp., *Shigella* spp. and *Vibrio* spp. while 19 mm was recorded against *Pseudomonas* spp. The zone of inhibition range was 18 mm–30mm by Luvena against all the tested bacteria except *Pseudomonas* spp., which was 13 mm. In terms of Probio, the zone diameter was noticed within the range of 10 mm–18mm whereas the highest zone diameter was recorded for Protein restro 29 mm against *Shigella* spp. and the lowest diameter was recorded 9 mm against *Staphylococcus* spp. Acteria produced zone diameter within the range of 18 mm–27mm against the tested bacteria (Fig.1).

3.2. Determination of minimal inhibitory concentration (MIC) of probiotics against the food borne pathogens

The minimal inhibitory concentration (MIC) or broth micro-dilution assay was used in addition to the agar well diffusion method to identify the lowest concentration of each of the experimental probiotic samples capable of reducing the test bacteria's viability [33].

The antibacterial activity of the probiotics was also evaluated by determining the value of the Minimal Inhibitory Concentration (MIC), which is the lowest concentration of probiotics required to prevent

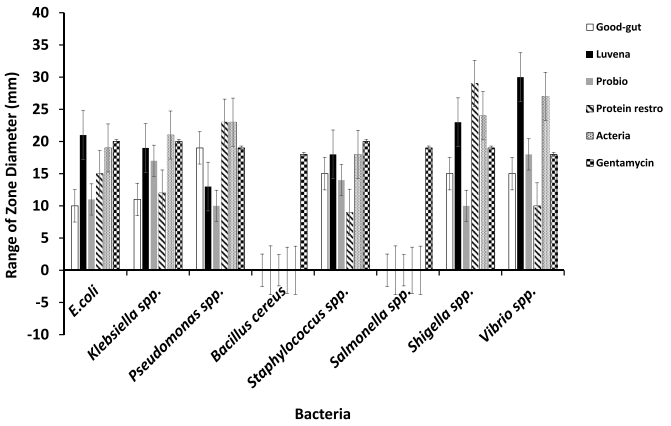

Fig. 1. Antibacterial activity of different probiotics against food borne pathogens: the figure indicates the bacterial growth retardation ability of the commercially available probiotics, a total of 5 probiotics were used, the white bar presenting the effectivity of good-gut, the black bar showing the activity of luvena, the ash bar displaying the activity of probio, the zebra cross bar is showing the activity of protein restro and the ash dot bar is indicating the efficacy of acteria. According to the graph, all the probiotics showed the satisfactory range of antibacterial activity in contrast of the positive control gentamycin which indicated by big black dot pattern bar. All the experiments were performed three times.

observable bacteria growth. The MIC values for the probiotics were found to be between 3 and 20 mg/ml (Table 1). The lowest MIC values 3 mg/ml was determined with Luvena against *Pseudomonas* spp. and *Shigella* spp. while the maximum MIC 20 mg/ml was recorded for Good gut and Probio against *Salmonella* spp. and *E. coli* respectively. *Bacillus* spp. and *Salmonella* spp. were also inhibited by action of the probiotics those were not killed by agar well diffusion method.

3.3. Efficacy of the synthetic drug on the growth of food borne pathogens

The regular introduction of multiple synthetic drugs into the market has been brought about by a considerable number of pharmaceutical companies in light of the enormous potential of those drugs to deplete the likelihood of bacterial multiplication [9,37,38]. With declining antibacterial activity, antibiotics are losing their resistance to bacteria on account of our lack of proper knowledge in medication and the frequent genetic diversity in microorganisms [39]. Eventually, scientists are rather concerned about the discovery of alternate methods for synthesizing more effective medications and seeking novel components in terms of the formulation of new drugs [12]. Based on this ground, present study executed this experiment on very common antibiotics to determine their antibacterial activity against the bacteria isolated form different food items. The zone diameter was not so satisfactory against all the bacteria. The highest zone measured 20 ± 1.63 mm and lowest zone diameter was recorded as 0 ±0 mm. All pathogenic strains (except *Staphylococcus*) were resistant to methicillin. *Vibrio* spp. showed resistance to five antibiotics (ampicillin, erythromycin, imipenem, ceftriaxone, and tetracycline). Meanwhile, erythromycin produced 19 ± 2.16 mm and 19.66 ± 2.05 mm of zone diameter against only *Bacillus* spp. and *Staphylococccus* spp. respectively (Table 2). However, Ampicillin was unable to inhibit the growth of *E.coli, Klebsiella* spp. and *Pseudomonas* spp. while tetracycline could not kill *Salmonella* spp.

3.4. Combined effects of antibiotics and probiotics against food borne pathogens

Antibiotics were unable to kill or suppress pathogenic isolates in several circumstances. In most of the cases the range of zone diameter indicated that the pathogenic bacteria were resistant except

Table 1
Minimum Inhibitory Concentration (MIC) of the samples.

Probiotics	Organisms							
	E.coli	*Klebsiella* Spp	*Pseudomonas* spp	*Bacillus* spp	*Staphylococcus* spp	*Salmonella* spp	*Shigella* spp	*Vibrio* spp
Good gut	18 mg/mL	19 mg/mL	14 mg/mL	20 mg/mL	18 mg/mL	20 mg/mL	16 mg/mL	13 mg/mL
Luvena	12 mg/mL	13 mg/mL	3 mg/mL	16 mg/mL	17 mg/mL	19 mg/mL	3 mg/mL	7 mg/mL
Probio	20 mg/mL	18 mg/mL	13 mg/mL	18 mg/mL	18 mg/mL	19 mg/mL	16 mg/mL	16 mg/mL
Protein restro	17 mg/mL	14 mg/mL	10 mg/mL	18 mg/mL	10 mg/mL	18 mg/mL	10 mg/mL	10 mg/mL
Acteria	14 mg/mL	11 mg/mL	11 mg/mL	16 mg/mL	7 mg/mL	17 mg mL	4 mg/mL	6 mg/mL

The experiments were conducted three times independently, and the results were found to be reproducible.

Table 2
Antibiotic Susceptibilities of food-borne pathogens.

	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* Spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	20 ± 1.63	20 ± 1.63	20 ± 1.63	18 ± 1.63	20 ± 1.63	20 ± 1.63	18 ± 1.63	13 ± 0.81
Gentamycin	20 ± 1.63	20 ± 1.63	19 ± 0.82	18 ± 1.63	19.66 ± 2.05	20 ± 1.63	19 ± 0.81	18.66 ± 0.94
Ampicillin	0 ± 0	0 ± 0	0 ± 0	11 ± 1.41	11.66 ± 1.24	12.33 ± 2.05	12 ± 2.16	0 ± 0
Methicillin	0 ± 0	0 ± 0	0 ± 0	0 ± 0	18 ± 1.63	0 ± 0	0 ± 0	0 ± 0
Azithromycin	20 ± 1.63	20 ± 1.63	17 ± 0.82	19 ± 0.82	20 ± 1.63	20 ± 1.63	19 ± 0.82	16.66 ± 0.94
Erythromycin	0 ± 0	0 ± 0	0 ± 0	19 ± 2.16	19.66 ± 2.05	0 ± 0	0 ± 0	0 ± 0
Ceftrizone	19.66 ± 2.05	20 ± 1.63	19.66 ± 1.69	14.66 ± 1.24	19.33 ± 0.47	19 ± 0.82	18 ± 0.82	0 ± 0
Imepenem	20 ± 1.63	15 ± 2.16	19 ± 1.63	13.33 ± 1.88	18 ± 1.63	10 ± 0.81	16 ± 1.41	0 ± 0
Ciprofloxacin	19 ± 0.82	16 ± 1.41	15.66 ± 0.94	14 ± 0.82	15 ± 0.81	10.33 ± 1.24	14 ± 2.16	13 ± 0.81
tetracycline	15.66 ± 1.69	19.33 ± 2.05	12.66 ± 1.24	11.66 ± 0.47	12 ± 0.81	0 ± 0	8.67 ± 1.24	0 ± 0

The experiments were conducted three times independently. Mean ± SD values have been shown here.

streptomycin, gentamycin, and azithromycin (Table 2). Five of the commercially available probiotics and 10 of the commonly prescribed antibiotics were individually implemented on the bacteria tested in this study. In case of probiotics, the zone diameter varied between 10 mm and 30mm while the antibiotics produced zone diameter within the range of 0 mm–20mm (Fig. 1 and Table 2). To observe the combined effects of probiotics and antibiotics, we implemented a mixture of probiotics and antibiotics against all pathogenic isolates. The zone diameter of all combination was found to be enlarged than the individual effects. The combination of good gut with each of the antibiotics formed zone diameter within the range of 15 ± 1.63 mm-33 ± 0.81 mm (Table 3). The zone diameter varied between 13 ± 0.81 mm-34 ± 2.98 mm in case of the combination of luvena with the antibiotics (Table 4). The mixture of probio with antibiotics showed zone diameter 13 ± 0.81 mm-30 ± 1.41 mm (Table 5) while the zone of inhibition was estimated 10 ± 0.81 mm-32 ± 1.63 mm for protein restro (Table 6). The combination of acteria with antibiotics exhibited the zone diameter 10 ± 1.63 mm-32.66 ± 2.62 mm (Table 7). Some of the antibiotics were totally unable to kill the bacteria but after combination the zone diameter was recorded in more higher range than the single effects. Combination of synthetic drugs and probiotics can kill or inhibit the growth better than the single drugs can do and produce a bigger zone [10,40,41].

4. Discussion

The infectious diseases caused by bacteria are contributing to the death toll around the world, especially in poverty-stricken countries, with the situation further aggravated by the participation of human fungal infections as hidden killers [1–3]. The morbidity and mortality rates among the world population are alarmingly on the rise as consequences of the widespread bacterial and fungal infections due to the emergence of the multidrug resistance pathogens and the declining efficacy of the available antibiotics against them [1]. Hence, seeking alternative ways through the utilization of the antimicrobial agents from natural sources for the improvement of the potency and the effectiveness of the currently available antibiotics along with the eradication of the detrimental effects associated with them should attract urgent concern at this juncture [1]. A number of published studies showed that the combination drug therapy could successfully be used as antimicrobial agents [13,14]. Furthermore, some research demonstrated that the far-reaching beneficial effects on hosts could be achieved by the altercation of micro-biota and in this case, probiotics have become the centre of attention because of their profound health benefits and especially, their antimicrobial and antagonistic activity against the antibiotic resistant pathogens [15,17,21,22,42–44]. In this study, some

Table 3
Synergistic effect of Good gut and antibiotics on the tested microorganisms.

Good gut with	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	30.33 ± 1.24	29 ± 0.81	32 ± 0.81	30.66 ± 1.24	27.66 ± 1.24	30 ± 1.41	28 ± 1.63	26.66 ± 0.94
Gentamycin	33 ± 0.81	30.33 ± 1.24	31 ± 0.81	29.66 ± 2.49	33 ± 0.81	30 ± 1.63	29 ± 2.16	27 ± 0.81
Ampicillin	17 ± 1.63	14.5 ± 0.5	20.33 ± 1.24	16 ± 2.16	21 ± 3.26	17.66 ± 2.49	19 ± 2.16	16.66 ± 2.37
Methicillin	17 ± 0.81	15 ± 1.63	20.66 ± 1.69	18 ± 2.16	25.66 ± 1.25	17.66 ± 2.49	15 ± 1.63	15 ± 1.63
Azithromycin	20.33 ± 1.24	15 ± 1.63	17 ± 0.81	31 ± 1.41	26.66 ± 2.04	28 ± 3.55	26 ± 2.94	19 ± 1.63
Erythromycin	21 ± 0.81	17 ± 1.24	20.33 ± 1.25	25.66 ± 2.94	21 ± 1.63	25.33 ± 2.05	18 ± 2.16	15 ± 1.63
Ceftrizone	23.33 ± 1.24	28.33 ± 1.69	28 ± 1.63	24.33 ± 1.69	24 ± 1.63	29 ± 2.16	27 ± 0.81	17.66 ± 1.69
Imepenem	22.66 ± 2.05	19 ± 1.63	26 ± 2.94	21.66 ± 1.24	23 ± 0.81	19 ± 1.63	26.33 ± 1.24	19 ± 2.16
Ciprofloxacin	23 ± 2.16	26 ± 0.81	27 ± 0.81	27 ± 0.81	24 ± 1.63	18 ± 2.44	19 ± 1.63	22 ± 0.81
Tetracycline	20 ± 1.63	21 ± 0.81	18 ± 1.63	19.66 ± 3.85	23 ± 1.63	18 ± 1.63	17 ± 0.81	17.66 ± 0.47

The experiments were conducted three times independently. Mean ± SD values have been shown here.

Table 4
Synergistic effect of Luvena and antibiotics on the tested microorganisms.

Luvena with	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	29 ± 0.81	31.66 ± 1.69	28.33 ± 1.24	27 ± 1.24	27 ± 3.55	25 ± 0.94	25.33 ± 1.39	31 ± 1.41
Gentamycin	26.66 ± 0.94	25 ± 0.81	28 ± 0.81	24 ± 2.16	33 ± 2.44	23.66 ± 1.69	26.66 ± 1.24	33.66 ± 1.69
Ampicillin	21.33 ± 2.05	20 ± 0.81	11.66 ± 1.24	20 ± 0.81	21 ± 0.81	15 ± 1.63	24 ± 0.81	13 ± 0.81
Methicillin	23.66 ± 2.05	19.33 ± 1.39	13 ± 0.81	30 ± 1.63	20 ± 1.63	16 ± 1.69	23.66 ± 1.69	33.33 ± 2.94
Azithromycin	29.66 ± 1.24	28 ± 1.63	27.66 ± 1.24	25.66 ± 1.66	28 ± 1.63	24 ± 1.63	28.33 ± 1.64	34 ± 2.98
Erythromycin	22.66 ± 1.23	19 ± 0.81	13 ± 0.81	18 ± 2.44	22 ± 0.81	16 ± 0.81	23.66 ± 1.24	30 ± 1.63
Ceftrizone	24.33 ± 1.24	22 ± 0.81	29 ± 1.63	15.33 ± 1.24	19.66 ± 0.47	16.33 ± 3.09	25 ± 0.81	27 ± 2.16
Imepenem	27 ± 0.81	28.33 ± 1.64	26.66 ± 1.24	14.33 ± 2.62	21 ± 0.81	15 ± 1.63	24 ± 1.63	29.33 ± 0.94
Ciprofloxacin	24 ± 1.63	26 ± 0.81	23 ± 0.81	26 ± 0.81	24 ± 1.69	15.33 ± 1.69	26 ± 1.63	33 ± 2.44
Tetracycline	25 ± 0.94	29 ± 1.63	20 ± 0.81	24 ± 2.16	20 ± 0.81	19.66 ± 0.66	24 ± 1.63	30 ± 0.81

The experiments were conducted three times independently, Mean ± SD values have been shown here.

Table 5
Synergistic effect of Probio and antibiotics on the tested microorganisms.

Probio with	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	27 ± 2.16	24 ± 0.94	28 ± 0.81	29 ± 1.63	27.33 ± 1.64	30 ± 1.41	29 ± 1.94	29 ± 1.41
Gentamycin	27 ± 3.55	20 ± 1.63	24 ± 1.69	29.33 ± 0.94	31 ± 2.16	23.66 ± 2.05	28.33 ± 1.64	25 ± 0.81
Ampicillin	14.33 ± 2.62	16 ± 0.81	15.33 ± 1.69	16 ± 0.81	17 ± 1.63	16.33 ± 3.09	13 ± 0.81	18 ± 0.81
Methicillin	15.66 ± 1.66	28 ± 1.63	15.66 ± 1.66	17 ± 0.81	23 ± 0.81	19 ± 1.69	15.66 ± 1.63	18.33 ± 1.69
Azithromycin	30 ± 1.41	28.66 ± 1.69	19 ± 0.81	29 ± 0.94	28 ± 2.16	28.33 ± 1.64	29 ± 1.63	30 ± 1.41
Erythromycin	16 ± 1.69	30 ± 1.41	15.66 ± 1.69	18.66 ± 1.69	19.33 ± 1.39	18 ± 1.69	14 ± 2.16	19.66 ± 0.66
Ceftrizone	29 ± 2.44	28 ± 0.81	28 ± 0.81	15 ± 1.63	29 ± 0.81	19 ± 1.63	20.33 ± 1.25	17.66 ± 1.63
Imepenem	27 ± 0.81	18.66 ± 1.69	29 ± 1.69	16.33 ± 3.09	28.33 ± 1.64	19.66 ± 1.69	19 ± 0.81	18.33 ± 1.39
Ciprofloxacin	28 ± 1.63	29 ± 0.81	27.33 ± 1.64	28.33 ± 1.64	24 ± 2.16	16 ± 2.16	16 ± 1.63	26 ± 0.81
Tetracycline	18 ± 0.81	21.66 ± 1.69	15.33 ± 1.69	14 ± 2.16	17.66 ± 1.66	11.66 ± 0.47	13.33 ± 1.88	18 ± 1.69

The experiments were conducted three times independently, Mean ± SD values have been shown here.

Table 6
Synergistic effect of Protein restro and antibiotics on the tested microorganisms.

Protein restro with	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	26.33 ± 2.05	21.66 ± 1.24	25.66 ± 2.49	24 ± 0.81	26 ± 0.81	21.66 ± 1.24	32 ± 1.63	17 ± 0.81
Gentamycin	27.66 ± 1.69	22 ± 0.81	25.66 ± 1.69	24 ± 2.44	31 ± 1.69	23 ± 0.81	29.66 ± 2.05	21 ± 1.63
Ampicillin	15 ± 1.63	14.66 ± 1.69	25 ± 1.63	12 ± 1.63	12.33 ± 1.69	10 ± 0.81	30 ± 0.81	13 ± 2.16
Methicillin	15 ± 0.81	17 ± 1.63	23 ± 0.81	11 ± 0.81	21 ± 0.81	11 ± 1.41	29 ± 1.69	11 ± 0.81
Azithromycin	31.33 ± 1.24	24 ± 0.81	26.33 ± 2.05	29 ± 0.81	28 ± 1.41	24 ± 0.81	31.33 ± 1.24	19 ± 1.63
Erythromycin	15.66 ± 1.66	13 ± 0.81	20.66 ± 1.69	18.66 ± 1.63	18.66 ± 1.69	12 ± 1.63	30.33 ± 1.24	12 ± 1.63
Ceftrizone	23.66 ± 0.94	21.33 ± 1.63	24 ± 2.44	15 ± 1.63	20.66 ± 1.24	19 ± 0.81	31.33 ± 1.24	10.66 ± 1.69
Imepenem	24 ± 1.63	16 ± 1.66	25.66 ± 1.69	13 ± 2.16	19 ± 0.81	19 ± 1.63	30 ± 1.63	10 ± 0.81
Ciprofloxacin	22.33 ± 1.60	19 ± 0.81	24 ± 1.63	22 ± 0.81	23 ± 0.81	10.66 ± 1.69	31.33 ± 1.69	12 ± 2.16
Tetracycline	19 ± 0.81	20.33 ± 1.66	25 ± 1.63	12 ± 1.63	13 ± 0.81	13 ± 2.44	30 ± 0.81	12 ± 1.63

The experiments were conducted three times independently, Mean ± SD values have been shown here.

Table 7
Synergistic effect of Acteria and antibiotic on the tested microorganisms.

Acteria with	Zone of inhibition(mm) against tested organisms							
	E.coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Salmonella* spp.	*Shigella* spp.	*Vibrio* spp.
Streptomycin	22.66 ± 1.69	26 ± 0.81	29.66 ± 1.24	23.66 ± 1.24	28 ± 1.69	18.33 ± 1.69	32.66 ± 2.62	31.66 ± 1.69
Gentamycin	25 ± 1.63	25.66 ± 1.69	24 ± 1.63	27 ± 0.81	32 ± 1.63	23 ± 0.81	27.66 ± 2.06	28 ± 0.81
Ampicillin	19 ± 1.63	21 ± 0.81	20.66 ± 1.69	10 ± 1.63	20.33 ± 1.24	10 ± 1.63	26 ± 0.81	29.33 ± 2.05
Methicillin	19 ± 0.81	21 ± 1.63	25 ± 1.63	13 ± 2.44	22 ± 1.63	14.33 ± 2.35	24 ± 0.81	25.33 ± 1.69
Azithromycin	31.66 ± 1.69	25.66 ± 1.69	30 ± 0.81	29 ± 1.63	29 ± 1.69	23.66 ± 1.24	31 ± 1.63	32.66 ± 2.62
Erythromycin	20.66 ± 1.64	20.33 ± 1.69	23.33 ± 1.24	18 ± 1.69	20 ± 1.63	13 ± 0.81	24 ± 1.63	25.33 ± 1,24
Ceftrizone	23.66 ± 1.24	23 ± 0.81	26 ± 1.63	17 ± 1.63	22 ± 0.81	19.66 ± 1.69	25 ± 0.81	27 ± 0.81
Imepenem	24 ± 1.63	21.66 ± 0.47	24 ± 1.63	13 ± 0.81	21 ± 0.81	19 ± 1.63	25 ± 1.69	27 ± 1.69
Ciprofloxacin	22 ± 0.81	22.66 ± 0.94	27.66 ± 2.06	22 ± 1.63	23.66 ± 1.24	10 ± 1.63	26 ± 1.63	28 ± 1.63
Tetracycline	20.66 ± 1.24	22.66 ± 1.69	24 ± 0.81	12.33 ± 1.69	20.66 ± 1.69	12.33 ± 1.69	25.33 ± 1.63	27 ± 1.63

The experiments were conducted three times independently, Mean ± SD values have been shown here.

commercially available probiotics were screened for the determination of their antimicrobial activity on different food-borne pathogens along with their synergistic effects in combination with synthetic drugs. The probiotics displayed antimicrobial activity against the pathogens used in this experiment. The effectiveness of the synthetic drugs was also observed but the synergistic effects of probiotics and antibiotics were more prominent than their individual effects on the pathogens. However, both *Bacillus* spp. and *Salmonella* spp. showed resistance to all the probiotics and among the antibiotics, Methicillin failed to affect the growth of *Bacillus* spp. while *Salmonella* spp. was resistant to Methicillin, Erythromycin and Tetracycline. Through this research, it is evident that these are the pathogens with the potential to withstand synergistic effects of probiotics and antibiotics, hence, they are more likely to undergo further mutations in order to give rise to more severe infections. So, the antimicrobial agents such as probiotics should be incorporated into more advanced research combined with other drugs for the invention of novel therapy against the multi-drug resistant bacteria. However, it has also been reported by the researcher that the administration of probiotics is not always advantageous, particularly for the newborns and immunocompromised patients with clinical issues such as malignancies, leaky gut, diabetes, and post-organ transplant convalescence [45]. Some probiotic strains could exploit the people's weakened immune systems and turn into opportunistic infections, causing life-threatening pneumonia, endocarditis, and sepsis [45]. In fine, this primary research findings would be impactful for the highly populated and poor economical countries like Bangladesh where the amount of physician is not enough for the current citizen, or the people may not afford to manage the cost of physician's consultancy. Not only that but also, many of the country could not yet establish proper law and order in order to retail the drugs without the prescription by the registered physician. The current findings would help the people to know the alternative way of antibiotic therapy.

5. Conclusion

The current exploration established that the probiotic samples have potent antimicrobial activity against food borne pathogenic culture. Besides, it examined the efficacy of the synthetic drugs against the bacteria and the synergistic effects of probiotics and antibiotics against the same pathogens. In this study, both *Bacillus* spp. and *Salmonella* spp. exhibited resistance to all the probiotics. Meanwhile, with the exception of methicillin, all other synthetic drugs were able to kill the pathogens. Though the range of their zone diameter was less than the recommended zone diameter for effective drugs. Moreover, methicillin was unable to inhibit the growth of other bacteria also. Erythromycin could only inhibit the *Bacillus* spp. and *Staphylococcus* spp., while the rest of the bacteria were found to be resistant. Both pathogens appear to possess the ability to subdue combined drugs, thereby having the substantial potential to give rise to more complex mechanisms through drastic changes in their genes by mutations or the direct influence of the environmental factors. By and large, the likelihood of microorganisms developing self-protection mechanisms should serve as a stark warning for all of us with the available drugs losing their efficacy to eradicate the pathogens at this juncture. Only the invention of new drugs through further research could save lives from the pathogenic killings. In this case, the antagonistic relation of probiotics with the pathogenic microbes is a testament to their antimicrobial activity which could be successfully targeted for the treatment and the prevention of disease with less side effects. This finding would be suggestive for the physician to prescribe probiotics as a therapeutic means instead of using synthetic drugs to reduce the drug resistant bacteria as well as the formation of superbugs. Extensive further study is required to optimize the combined dose of lower effective antibiotics with probiotics to formulates the new combination of antimicrobial therapeutics in future. As the future strategy, our research group have already started to work on others probiotics to evaluate their potency not only against the food borne pathogens but also the pathogens isolated from the clinical sources.

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CRediT authorship contribution statement

Mrityunjoy Acharjee: Conceptualization, Methodology, Software, Supervision, Writing – review & editing. **Fhamida Hasan:** Data curation, Writing – original draft. **Tamanna Islam:** Data curation, Writing – original draft. **Ifra Tun Nur:** Visualization, Investigation, Writing – review & editing. **Nila Begum:** Data curation, Writing – original draft. **Chayanika Mazumder:** Data curation, Writing – original draft. **Mahabuba Akter Lubna:** Data curation, Writing – original draft. **Nagma Zerin:** Writing – review & editing. **Asif Shahriar:** Software, Validation. **Md Rayhan Mahmud:** Writing – review & editing.

Declaration of competing interest

Authors have no conflict of interest.

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Appendix A. Supplementary data

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