Original Article

Antibacterial Effects of Compound Bifilact on *E.coli* and *Campylobacter jejuni*

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ABSTRACT

Background and Objectives: Probiotics are live microorganisms that function through various mechanisms and affect the alteration of the commensal microbiota against pathogens. Nowadays, given the problems associated with antibiotics use, probiotic strains offer a novel and appropriate alternative for the treatment of diseases such as diarrhea. The aim of this study was to investigate the antibacterial synergism of *Lactobacillus spp*., *Bifidobacterium spp*. and *Escherichia coli* strain Nissle 1917 (ECN) on the clinical sample of diarrheagenic *E.coli* and *Campylobacter jejuni.*

Methods: A paper disk-diffusion technique was used to evaluate the antibacterial activity. Sterile 6 mm paper disks were saturated with probiotic suspensions made by settling probiotic medications into distilled water. Three kinds of disk were prepared. One disk was prepared for *Lactobacillus spp*. and *Bifidobacterium spp*., another for ECN, and the third was made by combined probiotics. Clinical samples of diarrheagenic *E.coli* and *Campylobacter jejuni* were cultivated on Muller Hinton agars, and disks were placed on the inoculated Muller Hinton agars. All plates were incubated under microaerophilic and appropriate conditions.

Results: The zone of inhibition (ZOI) of the bacterial growth was measured. All pathogenic microorganisms showed sensitivity to the probiotic disks. The combined disks had better effects against pathogens compared with single disks.

Conclusion: A considerable synergistic effect was observed in the results of combined probiotics; therefore, combined strains can be more efficient against intestinal pathogens in comparison with single probiotics.

Keywords: Probiotic, *Lactobacillus*, Bifidobacterium, *Escherichia coli* Nissle, Diarrhea, *Campylobacter jejun.i.*

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INTRODUCTION

Probiotics are live microorganisms typically of human origins. They help prevent and treat certain diseases such as diarrhea. Probiotics are generally preferred for their low cost and favorable antimicrobial properties. These bacteria are able to control pathogenic organisms and their pathogenicity. Lactic-acid-producing bacteria are among the promising probiotics in the medical and industrial community. With the establishment of the gastrointestinal tract, these probiotics occupy the positions of the pathogenic bacteria and prevent the binding of pathogens to the receptors. Probiotics further generate antimicrobial and bactericidal factors such as lantibiotics and cyclic antimicrobial peptides. They act through different mechanisms, affecting the commensal microbiota and potentially improving and maintaining gut microecology against pathogens (1, 2). Probiotics are the most efficient microbiota against pathogens because they consume nutrients required by pathogens [3], The substances which have lower the pH [4], produce bacteriocins (5), create an anaerobic environment by scavenging oxygen (6), inhibit epithelial and mucosal adherence (7), and stimulate immunity [8]. Nowadays, antibiotic resistance, antibiotic-associated diarrhea (AAD), and its detrimental effects on normal microbial flora and allergic reactions to antibiotics have necessitated researchers finding a better substitute for antibiotics and restricting antibiotic consumption to certain conditions. Probiotic utilization, with its multiple benefits, is an effective remedy for this problem (7,8). Moreover, the examined prophylactic role of probiotics has shown that many strains of probiotics have a great potential for prophylaxis and treatment of a range of gastrointestinal disorders (9). *Lactobacillus* and *Bifidobacterium spp*. are the most common probiotics. Furthermore, a human commensal biotype of *E. coli* is *E.coli* strain Nissle 1917 (ECN) utilized in some European countries as a therapeutic and preventative food or drug in the treatment of diarrhea [1, 3]. Latest statistics have revealed an extensive rise in the incidence of diarrheagenic *E.coli* (DEC) and Campylobacteriosis in both developed and developing countries (10, 11). DEC strains have been isolated in more than half of the children with diarrhea and these pathogenic microorganisms are among the main causes of diarrhea, particularly in developing countries (12). *Campylobacter jejuni* infection is widely reported in industrialized countries, and it is bound to challenge global health in the future (10). About 85% of children suffering from diarrhea had a Campylobacter positive stool sample (14). The objective of this study was to investigate the antibacterial synergism of *Lactobacillus* and *Bifidobacterium spp*. and *E.coli* Nissle 1917 on pathogenic *E.coli* and *Campylobacter jejuni*.

MATERIALS AND METHODS

Lactobacillus species (Lactobacillus *casei, Lactobacillus rhamnosus, Lactobacillus acidophilus and Lactobacillus bulgaricus*), *Bifidobacterium species* (*Bifidobacterium breve and Bifidobacterium longum*) and *Escherichia coli* strain Nissle 1917 (ECN) were employed as probiotics in the present research . *E.coli* Nissle 1917 was isolated from Mutaflor (a probiotic medication), and *Lactobacillus spp* and *Bifidobacterium spp*. were provided by Fami-Lact capsules. Each capsule contained specific bacteria ideal for use in this test. The suitable concentration of probiotics was determined through settling capsules into distilled water and diluting them to a 1×10^6CFU/ml concentration. To prepare 0.5 McFarland (1.5×10^8 CFU/ml) stocks, a suspension obtaining the fresh and pure culture of the pathogenic microorganisms (clinical sample of *Campylobacter jejuni* and diarrheagenic *E.coli*) was used. Pathogenic *E.coli* and *Campylobacter jejuni* were cultured on Muller Hinton agars with a 1×10^6CFU/ml final concentration.

Agar diffusion method has been widely employed to assay antimicrobial activity. Disk-diffusion method is generally used for preliminary screening of potential antimicrobial efficacy against pathogens (15, 16). In the method, sterile 6 mm paper disks were saturated with 30 µl of probiotic suspension. Three kinds of disks were made: one for *Lactobacillus* and *Bifidobacterium spp*., another for *Escherichia coli* strain Nissle 1917 (ECN), and the third was prepared through equally mixed probiotics (15µl of *Lactobacillus* and *Bifidobacterium spp*. and 15µl of ECN suspension). Disks were placed on the inoculated Muller Hinton agar surface at a distance of 3 cm (according to the CLSI

2017). The plates were incubated with a microaerophilic gas pack inside an anaerobiosis jar for 24 h at 37 °C. The inhibitory zone diameter of growth was reported according to the CLSI 2019.

RESULTS

The zone of inhibition (ZOI) of bacterial growth was measured. For the tested probiotics, all pathogenic microorganisms showed sensitivity to the single probiotic disks.

The diameter of the growth inhibition zones is reported in Table 1. Results of disk diffusion by probiotics showed that probiotics had appropriate effects against the growth inhibition of *E.coli* and *C. jejuni*. The combined disks had synergistic effects in the test, inhibiting these microorganisms more than single probiotics.

Table1- Results of disk diffusion probiotics

Probiotic disk	*E.coli* (ZOI)	*Campylobacter jejuni* (ZOI)
Lactobacillus and *Bifidibacterium*	10 mm	20 mm
Escherichia coli Nissle 1917	30 mm	20 mm
Combined probiotics	40 mm	35 mm

DISCUSSION

Probiotic bacteria offer a novel alternative for the treatment of diarrhea and eradication of bacteria involved in the pathogenesis of diarrhea. A paper disk-diffusion test was performed in order to evaluate the synergistic effect of *Lactobacillus spp, Bifidobacterium spp*. and *Escherichia coli* strain Nissle 1917 (ECN) on diarrheagenic *E.coli* and *Campylobacter jejuni*. Via this technique, combined probiotics presented a better performance in both cases. The production of organic acids by *Lactobacillus spp*. showed a potent inhibitory effect against gram-negative bacteria owing to the permeabilizing capacity of the bacterial outer membrane, hence considered as a main antimicrobial compound (17). Furthermore, *Lactobacillus* strains showed a pH-dependent inhibitory activity against *Campylobacter jejuni* (18). The growth of *Campylobacter* strains below pH 4.9 was restricted, and low pH killed this microorganism [19]. Intestinal colonization by pathogens is reduced by the inhibition of their ability to adhere to epithelial cells as an important prerequisite for their colonization. *Escherichia coli* Nissle 1917 (ECN) was able to adhere to human intestinal epithelial cells as a strong adherent and suppress the colonization of other pathogens (7). ECN formed a biofilm of non-pathogenic microorganisms preventing pathogenic bacteria from adhering to the cell surface; this potent colonizer was able to effectively colonize the intestine of newborns within a few days as a beneficial microflora (20). The synergistic effect of probiotics has been investigated in some studies. Combination of *Lactobacillus* and *Bifidobacterium spp*. resulted in a wider antimicrobial effect, superior induction of IL-10, and silenced pro-inflammatory cytokines (21). According to the obtained results, probiotic strains had a significant synergistic effect against pathogens, and their combination represented a better therapeutic effect. Nowadays, owing to the preventative and therapeutic effects of probiotics, investigating the antagonistic and synergistic effects of probiotic strains contributes to discovering a more useful mixture in dealing with different diseases (22, 23).

The results of this study showed that probiotics in the laboratory were able to inhibit *E. coli* and *Campylobacter jejuni* intestinal pathogens. It has also been proven that the simultaneous administration of several probiotics has better antimicrobial effect. Therefore, it is suggested that for immunocompromised patients and those who use broad-spectrum antibiotics or even healthy people, probiotics arecapable of periodically reconstructing the digestive probiotics. Therefore, they are recommended in the first two weeks of each season for the prevention of the infection and inoculation of pathogens in the body.

CONCLUSION

Today, the application of general probiotic microorganisms and bactericines and their replacement with antibiotic compounds have been considered. Because bacteria are not resistant to these antimicrobial peptides, bacteriosins are considered as a substitute for antibiotics. Therefore, given the urgent need of the current world to find effective methods for controlling bacterial pathogens, it is important to study bacteriosins or probiotics as alternative antibiotic candidates.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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