

Influence of the probiotic *Escherichia coli* strain Nissle 1917 on the growth of different pathogenic bacteria isolated from patients with diarrhea

Anahita Dezfulian¹, Ali Motavelly zadeh Ardekani², Mohammad Mehdi Aslani³, Hosein Dabiri¹, Mohammed Reza Zali¹

¹ Research Institute for Gastroenterology and Liver Disease, Shahid Beheshti University, M.C., Tehran, Iran

² Department of Reproductive Endocrinology, Reproductive Biotechnology Research Center, Avicenna Research Institute, Shahid Beheshti University, M.C., Tehran, Iran

³ Department of Microbiology, Pasture Institute of Iran, Tehran, Iran

ABSTRACT

Aim: The aim of this study was to investigate the in vitro inhibitory effect of probiotic *E.coli* Nissle 1917 (EcN) strain against pathogenic bacteria isolated from patients with diarrhea.

Background: Probiotics are viable microorganisms that are shown to have beneficial effects on human health. EcN is a typical example of probiotics; however, there are few reports of it being administered for treatment of diarrhea.

Patients and methods: The inhibitory effect of EcN was assessed against bacteria associated with diarrhea, including 30 diarrheagenic *E.coli* strains, 10 *Salmonella* spp, 10 *Clostridium difficile* and 10 *Campylobacter* spp, using spot method inoculation. The microcin sensitive strain (*E.coli* K12 H 5316) was used as control.

Results: In vitro growth inhibition was recorded in none of cultured bacterial samples.

Conclusion: The inhibitory activity of EcN on different bacteria probably relies on different in vivo complementary mechanisms.

Keywords: Nissle 1917, Pathogenic Bacteria, Diarrhea, Probiotic.

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INTRODUCTION

Bacterial diarrhea is one of the most common causes of morbidity and mortality of infants and young children in developing countries. The annual death rate in Asia, Africa and America is estimated to be around 4.6–6 million (1). In the past decades, diarrhea was one of the major causes of infant death in Iran. Enteropathogenic bacteria

such as pathogenic strains of *E.coli*, *Salmonella*, and *Shigella* were the most common causative bacterial agents of water and food borne intestinal infections (2). Nevertheless, considerable effort has been made for prevention or minimization of these diarrheic infections.

Antibiotic therapy is the primary choice in bacterial diarrhea at present. There is, however a risk of bacterial resistance (3). Therefore, development of alternative methods seems to be necessary. Probiotic products seem to be promising in this respect (4). Probiotics are viable

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Reprint or Correspondence: Anahita Dezfulian, MD.
Research Institute for Gastroenterology and Liver Diseases,
Shahid Beheshti University, M.C., Tehran, Iran
E-mail: Dezfulian_an@yahoo.com

microorganisms usually belonging to the resident microflora. These bacteria are non-pathogenic and contribute to the health and well being of the host. They may also represent effective tools to control or prevent the infections (5). Indeed, various genera of probiotic bacteria have shown that they can interfere with the growth of a number of pathogens (6, 7).

The efficacy of probiotic bacteria in diarrhea has been investigated in clinical trials (8,9). Probiotic *E.coli* strains interfere with bacterial invasion or stimulate the immune system (10). The non-pathogenic *E.coli* Nissle 1917 (EcN) was originally isolated during World War I from a soldier who withstood a severe outbreak of diarrhea. With antagonistic activity against enteric pathogens, this strain has been proposed for treatment of acute diarrhea in infants and toddlers. Moreover, it has been reported that the administration of EcN to newborn infants prevents the colonization of the intestine by microbial pathogens (11).

In vitro studies have shown that EcN strain is able to compete with certain *E.coli* strains and other enterobacteria. EcN produces bactericidal products known as microcins (12). It is active against microorganisms associated with diarrhea. We designed this in vitro study to examine whether this probiotic strain could inhibit the growth of pathogenic bacterial species, isolated from patients with diarrhea.

PATIENTS and METHODS

Bacterial Strains: Sixty strains of different bacteria species were isolated from patients with diarrhea and were included in this study. The organisms used as indicator were 30 diarrheagenic *E.coli* strains (10 Enteropathogenic *E.coli* (EPEC), 10 Enterotoxigenic *E.coli* (ETEC), 10 Shigatoxin producing *E.coli* (STEC)), 10 *Salmonella* spp, 10 *Campylobacter* spp and 10 *Clostridium difficile* strains. These bacteria were identified according

to morphologic and biochemical features. The EcN was obtained from Ardeypharm GmbH (Mutaflor®, Herdecke, Germany).

Spot method inoculation for antagonism testing: For testing inhibitory effect, different species were cultured on appropriate media. Aerobic bacteria were cultivated in Brain Heart Infusion Broth (BHI) (Merck, Germany) at 37°C for 24 hours. *Clostridium difficile* was cultured on the *Clostridium difficile* Medium (Himedia, India) enriched with 7% sheep blood and enrichment supplement. The plates were incubated for 48 hours at 37°C in anaerobic conditions. The *Campylobacter* strains were subcultured on *Brucella* blood–agar (Merck, Germany) enriched with 7% Sheep blood and enrichment supplement. The plates were incubated in a microaerophilic atmosphere for 48 hours at 37°C. EcN was propagated in a special broth (M 63, minimal medium supplemented with glucose (12) for 24 hours at 37°C).

For testing antagonism, one loop of the aerobic bacterial broth culture and anaerobic bacterial culture were transferred to 5 ml of normal saline separately. These suspensions were diluted with normal saline up to half density according to McFarland's score. Of these suspensions, 0.1 ml aliquots were plated onto surface of suitable solid medium, Luria Bertani (LB) agar medium for aerobic bacterial species and *brucella* blood agar and *Clostridium difficile* medium, for *Campylobacter* and *Clostridium difficile* respectively (13,14).

Plates were spread to obtain a uniform bacterial lawn. After drying the surface of the agar medium at 37°C for 30 min, a loopful drop of the broth culture of Nissle strain in the M63 Media was spotted on the surface of each plate. After incubation at 37°C in the appropriate atmosphere for 24 to 72 hours, the plates were examined for inhibition zones (15).

Microcin production assay of *E.coli* Nissle 1917-
Control Experiment: In order to test the ability of

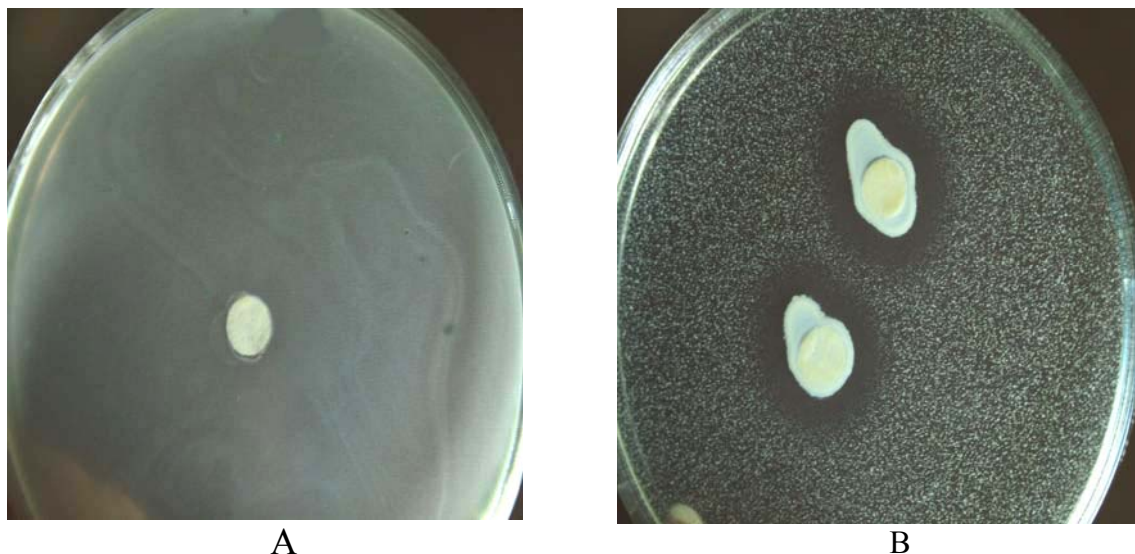


Figure 1. The effect of microcin-producing bacteria on pathogenic enteric bacteria. *E. coli* Nissle did not inhibit growth of *Salmonella* (A) although it secreted enough microcin to inhibit growth of microcin sensitive *E. coli* (B)

EcN to produce microcins, plates containing 6 mg of soft agar and 100 μ l of overlying microcin-sensitive *E. coli* strain K12-H5316 were used. Blanc Discs of 5 mm diameter were placed on solidified agar and a 10 μ l drop of *EcN* suspension was inoculated onto each disc. Eventually, plates were incubated at 37°C overnight and zones of growth inhibition were determined the following day (16).

RESULTS

Inhibitory effect of *EcN* was tested against the pathogenic bacteria isolated from patients with diarrhea. *E. coli* strain Nissle 1917 did not inhibit the growth of different pathogenic strains of *E. coli* (EPEC, ETEC, STEC). The inhibitory activity of *E. coli* strain Nissle 1917 against *Clostridium difficile* and *Campylobacter* spp was tested also; *EcN* did not cause marked inhibition of these species either. Furthermore, *EcN* did not inhibit growth of *Salmonella* spp (Figure 1A).

Microcin production by *EcN* was observed on LB agar. Zones of growth inhibition of *E. coli* K12 H 5316 were observed to be 13 mm (Figure 1B)

DISCUSSION

One well-known mode of action of *EcN* is its antagonistic activity against intestinal pathogens. This activity might be due to the production of specific antimicrobial substances, such as microcins (17, 18). However, the microcin negative isogenic mutant of *EcN* has been shown to be as effective as the parent strain in interfering with pathogenic bacteria. In fact, because of their narrow spectrum of bacteriocin activity, microcins are unlikely to be responsible for the inhibitory effect of *E. coli* Nissle 1917 (19).

Effective adherence of *EcN* to intestinal epithelial cells may block necessary receptors for internalization of invasive bacteria. The strong adhesion to intestinal epithelial cells enables the *E. coli* Nissle 1917 to form an *in vivo* biofilm of non-pathogenic bacteria that may prevent pathogenic microorganisms from accessing the

cell surface (11,20). However, the stabilizing effect of EcN on intestinal microbiology, and its preventive effect on colonization of enteric pathogens cannot be achieved in vitro.

According to some other studies, E.coli Nissle 1917 and other probiotic strains may exert their beneficial effects through stimulation of the synthesis of endogenous epithelial antimicrobial peptides such as human Beta Defensin-2 (21,22,23,24).

The growth and metabolic activity of E.coli may also cause changes in the pH or chemical composition of the colonic lumen that would be unfavorable to the pathogenic bacteria (11, 25). This might especially be true, but it cannot be important in the test medium after the growth of EcN, because these changes are not enough to inhibit growth of different bacteria. In vivo evaluation of antagonistic effect of EcN and description of the mechanisms involved enable a more complete understanding of the inhibitory activity of this strain.

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