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Original Article

Antibacterial Effect of Non-industrial Yogurt on Salmonella and Shigella

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Abstract

Background and Aim: Yogurt is one of the most consumed foods with different probiotic effects. The aim of this study was to evaluate antimicrobial effects of non-industrial yogurts on two common enteropathogens – *Salmonella* and *Shigella*.

Methods: In this experimental study, we evaluated the antimicrobial effects of 30 various non-industrial yogurt samples on 11 *Shigella sonnei*, 7 *Shigella flexneri*, 2 *Shigella boydii*, and 15 *Salmonella enteritidis* isolates. All bacterial isolates were locally isolated from patients suffering from diarrhea. Using the well diffusion method, we evaluated the antimicrobial effect of whey protein from yogurt before and after 24 hours incubation at 42°C. The data was analyzed using independent *t* test and chi-square in SPSS (version 11.5). *P* value less than 0.05 was considered significant.

Results: None of the yogurts had antimicrobial effect on *Shigella* and *Salmonella* isolates before 24 hours incubation at 42°C. After incubation, however, 9 (30%) and 12 (40%) samples out of the 30 yogurt samples showed antimicrobial effects on 25%-50% and less than 25% of isolates, respectively. Six yogurt samples (20%) did not have any antimicrobial effects. Only 1 yogurt sample exhibited antimicrobial effect on more than 50% of the tested isolates.

Conclusion: Our results suggested that some samples of non-industrial yogurts have an antibacterial effect on *Shigella* and *Salmonella*. However, neither of them had an influence on more than 51.5% of isolates. Therefore, reliance on yogurt as a standalone treatment is not recommended.

Keywords: Yogurt, *Salmonella*, *Shigella*, Gastroenteritis, Diarrhea.

Introduction

In industrialized countries, 30% of people suffer from food-borne diseases on a yearly basis. In 2000, at least 2 million people died from diarrheal diseases worldwide (1). In the last two decades, global attempts have been intensified for controlling acute watery diarrhea, and significant successes have been achieved in this regard (2). Nevertheless, the disease in developing countries has remained as a main cause of morbidity and mortality among children (3). *Shigella* and *Salmonella* are among the most important bacterial causes of diarrhea (4) – a disease that puts a big burden on families and healthcare system. *Shigella flexneri* is a facultative intracellular pathogen that is responsible for human bacillary dysentery or shigellosis, a disease characterized by acute rectocolitis. Shigellosis is a major cause of childhood morbidity and mortality leading to approximately 1 000 000 deaths globally each year (5).

Salmonella species are gram-negative, motile, anaerobic facultative, intracellular bacilli. Infection with different serotypes of *Salmonella enteric* species cause diseases ranging from self-limiting diarrhea and localized gastrointestinal inflammation to typhoid fever, a systemic infection with high lethality rates (6). Hence, extensive studies have been performed worldwide to find an inexpensive and effective treatment for controlling the diarrhea (3).

The beneficial effects of microbiota on the immune system have suggested the use of some non-pathogenic bacteria known as probiotics for treating diarrhea (6). Probiotics are foods containing live bacteria that exert beneficial effects on human health through the digestive system. They have a health benefit on the host when administered in adequate doses (7,8). Several studies have demonstrated that a wide variety of dairy products' *Lactobacillus* influence the immune system (9) and serum cholesterol (8); prevent mutation (8), genital infections (10), and gastrointestinal infections (7,8), and treat constipation. *Lactobacillus acidophilus* is a well-known probiotic strain commonly used in the dairy industry. It is added to conventional yogurt or is alternatively used as the main starter culture for the production of yogurt or fermented milks (11).

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A large share of research studying the effect of *Lactobacillus* on the prevention or treatment of diarrhea is classified into two groups (12). The first group investigates the beneficial effects of yogurt containing various *Lactobacillus* strains on the treatment or prevention of gastrointestinal infections in humans or animal models (13). This group of studies have demonstrated that yogurt mainly reduces the duration and severity of diarrhea rather than its prevention (14). The second group includes studies with in vitro investigation of antibacterial effects of isolated *Lactobacillus* from various dairy products on reference strain of enteric pathogens (15). These studies have shown that various species of *Lactobacillus* have different inhibitory effects on enteric pathogens; however, none of them have examined the antibacterial effects of yogurt. Moreover, they have usually used only single strain (mostly the standard strains) of enteric pathogens for susceptibility test.

In many developing countries including Iran, non-industrial produced yogurts are sold on retail market. These yogurts are prepared without industrial starter, using the remainder of previously used yogurt. These yogurts are prepared using a variety of starters and processed in different incubation temperatures and time durations. However, the antimicrobial activity of these food products has remained unknown. In the present study, we evaluated the antimicrobial effects of non-industrial yogurts from Iranian retail sale on locally isolated *Salmonella* and *Shigella* isolates. This study emphasizes that since various trains are used in non-industrial production of yogurt in some regions, it is necessary to choose the best strains with greatest probiotic impact in order for study purposes.

Methods

In this experimental study, we examined the antimicrobial effect of thirty various non-industrial yogurts on 20 *Shigella* and 15 *Salmonella*, isolated from the patients suffering from acute diarrhea.

Microbial Strains

We collected a variety of microbial isolated from diarrhea patients in different clinical laboratories of Birjand, Iran, including 20 *Shigella* and 15 *Salmonella*. While all the *Salmonella* isolates were *Salmonella enteritidis*, the *Shigella* isolates consisted of *S. sonnei* (n = 11), *S. flexneri* (n = 7) and *S. boydii* (n = 2).

Yogurt Samples

Thirty various yogurt samples were purchased from markets in different areas of Birjand such that each yogurt sample was from a unique individual producer. The reason for using whey was to determine the amount of dry material per unit of yogurt water volume in order to compare the various yogurt products and also to produce water yogurt with double concentration. Generally, yogurt water contains all features of the yogurt product.

Each sample was divided into two equal parts. In order to extract whey, one part of the sample was centrifuged (3000 g, 10 minutes) with a centrifuge (Zentrifungen 1011, Tletich, Germany). The supernatant containing the extracted whey was sterilized through filtration using a 0.22 µm syringe filter (GVS, USA). Another part of the sample was incubated (Memmert, Germany) for 24 hours at 42°C and then was used to extract the whey as mentioned above. The extracted whey was lyophilized and kept at -20°C for further evaluations (16).

Specifications of yogurt samples including the yogurt solid mass, dry weight of extracted whey, and pH of whey pre- and post-incubation were measured.

To test antibacterial activity, the whey solution was prepared by dissolving the lyophilized whey in a sterile solution of phosphate buffer with pH = 7 and various concentrations (0.5, 1 and 2 times of normal concentration) under aseptic conditions.

Sensitivity Test

Using the well diffusion method, we evaluated the inhibitory effects of whey with various concentrations on *Shigella* and *Salmonella* isolates (17). In summary, the Mueller-Hinton Agar plates were prepared as the manufacturer had advised. The inocula were prepared by suspending ten young colonies of test isolates grown on blood agar plates in 0.85% saline, and their turbidity was adjusted equivalent to 0.5 McFarland standards. The 90 mm Mueller-Hinton Agar plates were inoculated with the inocula, and then 4 wells (6 mm diameter) were punched in each plate. Three wells on each plate were filled with 100 µl of different concentrations of whey solution and the fourth was filled with sterile phosphate buffer as the control sample (pH = 7). The plates were incubated for 24 hours at 37°C, and the diameter of inhibition zone around each well was recorded.

Statistical Analysis

We used SPSS version 11.5 for data analysis. We used paired *t* test to compare the mean of pH and dry weight of whey pre- and post-incubation of yogurt at 42°C, as well as Pearson test to evaluate the correlation between various specifications of yogurt samples. We also used *t* test (independent samples) in order to evaluate the relationship between antibacterial effects of whey and various specifications of yogurt samples. The chi-square test was used to evaluate the relationship between different genus of bacteria (*Shigella* versus *Salmonella*) and inhibitory effect of whey. *P* value less than 0.05 was considered significant.

Results

The results of this study show that there is a significant difference between pH of whey pre- and post-incubation at 42°C ($P < 0.001$); however, there was no significant difference ($P = 0.739$) in dry weight of whey pre- and post-incubation (Table 1). Additional incubation time is for equalizing the incubation conditions for all yogurts since different manufacturers may use different time durations for incubating milk into yogurt. Furthermore, no significant relationship was found between the different characteristics of yogurt samples. We found that the whey extracted from yogurt samples which isolated pre-incubation at 42°C did not have any antibacterial effect, in any tested concentration, on *Salmonella* and *Shigella* species.

However, the whey extracted from some yogurt samples at the double concentration showed inhibitory effects on some of the *Salmonella* and *Shigella* species post-incubation (as shown in Figure 1). According to the results, 8 (26.7%) yogurt samples did not show any antimicrobial effect on any *Salmonella* and *Shigella* isolates, while 12 (40%) samples showed antimicrobial effect on less than 25% of the isolates. Moreover, 9 (30%) samples showed antimicrobial effect on 25%-50% of isolates. However, 1 sample demonstrated antimicrobial effect on 51.5% of isolate samples (Figure 1).

Except for 2 samples, which showed different effects on *Salmonella* and *Shigella* genus (sample no. 22 [$P = 0.003$] and sample no. 24 [$P = 0.017$]), there was no significant difference in the inhibitory effect of most of the yogurts on two bacterial genus (*Shigella* versus *Salmonella*). In general, there was no significant differences in sensitivity to whey samples between *Salmonella* and *Shigella* genus ($P = 0.255$).

We evaluated the effects of pH and dry weight of whey pre- and post-incubation at 42°C on their overall antibacterial activity as well as their inhibitory effect on *Shigella* and *Salmonella* isolates. Our studies indicated that the dry weight of whey after incubation at 42°C has a significant effect on the overall inhibitory activity of *Shigella* and *Salmonella* isolates ($P < 0.001$) as well as an inhibitory effect on *Salmonella* ($P < 0.001$) and *Shigella* ($P < 0.001$) individually. Other characteristics including solid mass of yogurt and pH of whey pre- and post-incubation at 42°C did not have any significant effect on the inhibitory activity of yogurts.

Discussion

In this study, we evaluated the inhibitory effect of non-industrial yogurts on two most common enteric pathogens: *Salmonella* and *Shigella*. The aim of this study was to evaluate if these yogurts have any antimicrobial property for *Salmonella* and *Shigella*, and if so, whether there is

Table 1. Characteristics of Yogurt Specimens Before and After Incubation at 42°C

	Minimum	Maximum	Mean	Standard Deviation	*P* Value (*T* Test)
Dry weight of yogurt	65.00[a]	164.00[a]	100.59[a]	23.47	-
Dry weight of whey before incubation	21.00[a]	70.00[a]	41.73[a]	11.72	0.739
Dry weight of whey after incubation	20.00[a]	60.00[a]	40.65[a]	11.40	0.739
pH of whey before incubation	3.70	4.24	3.98	0.14	<0.001
pH of whey after incubation	3.42	4.20	3.69	0.19	<0.001

[a] mg/ml.

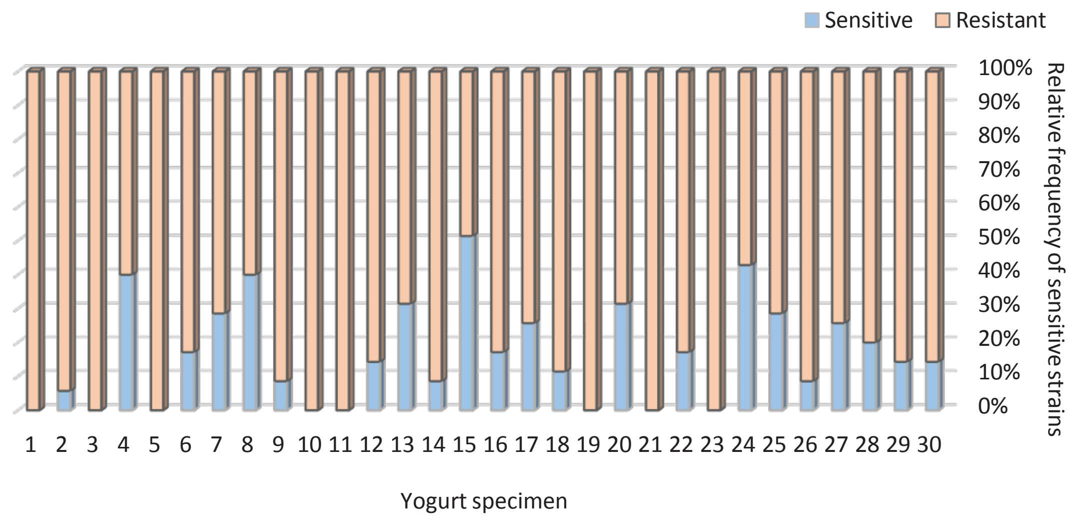

Figure 1. Relative Frequency of Sensitive Isolates of *Shigella* and *Salmonella* to the Whey Extracted From Yogurt Samples After Incubation for 24 Hours at 42°C in 2X Concentration.

any difference between yogurts in terms of this property. Finally, we aimed to see whether there is any correlation between the incubation time, other characteristics of yogurts, and their antimicrobial effect.

We observed a large difference between yogurt samples for their antibacterial effect on *Salmonella* and *Shigella*. Eight out of 30 yogurt samples did not show any antimicrobial effect on any isolated *Salmonella* and *Shigella*; 12 samples showed antimicrobial effects on less than 25% of isolated samples, 9 samples on 25%-50% of isolates, and finally only 1 sample showed antimicrobial effect on 51.5% of isolates. Although there is no exact similar study to the current investigation, our findings are consistent with other comparable studies in which antimicrobial effect of various strains of isolated *Lactobacillus* from dairy products have been tested on reference strains of enteropatogens (18,19). The difference in antibacterial effect of yogurts could be due to various factors such as differences in optimum growth temperature of *Lactobacillus*, final pH of yogurt, incubation period, the ability of *Lactobacillus* in producing different organic acids, production of antimicrobial substances (bacteriocin) by *Lactobacillus*, and finally simultaneous presence of other bacteria in yogurt (19,20).

Our results indicated that incubation period has a significant effect on antimicrobial effect of yogurt. Several studies have shown that incubation period, temperature, and pH of yogurt affects the production of bacteriocin by *Lactobacillus* (21-24). In this study, we observed a significant decrease in the pH after incubation of the samples at 42°C ($P<0.001$).

Our studies indicated that dry weight of whey after incubation at 42°C has a significant effect on the overall inhibitory activity of *Shigella* and *Salmonella* isolates ($P<0.001$) as well as an inhibitory effect on *Salmonella* ($P<0.001$) and *Shigella* ($P<0.001$) individually.

Conclusion

When incubated for a longer period, some non-industrial yogurts exert an antibacterial effect on some *Shigella* and *Salmonella* isolates. However, as none of them is effective on more than 51.5% of isolates, reliance on yogurt as a single treatment is not recommended.

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