An *in vitro* study on the antibacterial effect of kefir against some food-borne pathogens

**Kefirin gıda kaynaklı patojenlere karşı antimikrobiyal etkisi üzerine bir in-vitro çalışma**

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**SUMMARY**

Kefir which is acidifying and alcoholic fermented milk is accepted as a good example of a probiotic mixture of bacteria and yeast. In this study; we planned to investigate the antimicrobial effect of 24 and 48 hours fermented kefir against *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 11778), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 7644) and *Escherichia coli* (ATCC 8739) by using disk diffusion method. The best antimicrobial effect of 24 and 48 hours kefir was seen against *Staphylococcus aureus* with the diameter zones of 21.4 and 21.1 mm respectively at the end of fermentation period. Similar results were obtained from 24 and 48 hours fermented kefir on the 1st, 4th and 7th days of storage at +4 ºC. In all cases, although the antimicrobial activity decreased or did not change during the storage; it increased only against *Salmonella enteritidis*.

**Keywords:** Kefir; antimicrobial activity, disk diffusion method, food-borne pathogens, probiotic

**ÖZET**


**Anahtar kelimeler:** Kefir; disk difüzyon metodu, gıda kaynaklı patojenler, probiotik

**INTRODUCTION**

Probiotic foods contain live bacteria and have some beneficial effects against pathogenic microorganisms and one of the mechanisms of antimicrobial activity is the inhibitory effect of acidifying microorganisms in probiotic foods (1). The term “probiotic” dates back to 1965 when it referred to any substance or organism that contributes to intestinal microbial balance (2). Kefir which is an acidifying and mildly alcoholic fermented milk originated from the Caucasian mountains, is accepted as a good example of a probiotic mixture of bacteria and yeast (3). It is described as a symbiotic association between lactic and acetic bacteria and yeast and claimed to act against the pathogenic genera *Salmonella*, *Helicobacter*, *Shigella*, *Staphylococcus* and *E. coli* (4). With the emergence of antibiotic-resistant bacteria, it is reasonable to explore new sources of natural compounds with antibacterial compounds. This property makes the kefir use of al-
ternative treatment for these pathogenic infections (5). The function of the microorganisms constituting the kefir may include production of lactic acid, antibiotics and bactericides, which inhibit the growth of undesirable and pathogenic microorganisms (6).

Kefir drink contains lactic acid, mainly the L(+) form, of about 0.8-0.9%, as well as formic, succinic and propionic acids, CO₂, ethyl alcohol, different aldehydes (propionic, acetic) and trace amounts of isoamyl alcohol and acetone (7). This probiotic dairy drink is widely used in various parts of the world for the treatment of tuberculosis, cancer and gastrointestinal disorders that caused by pathogenic microorganisms (8). Koroleva (9) indicated that kefir is used in hospitals and sanatoria for a variety of illnesses, including metabolic disorders.

However scientific interest in kefir is growing due to its health benefits, a limited number of in vitro experimental studies were performed in order to understand the antimicrobial mechanism of kefir's microbial flora (10). It has been reported that kefir causes a bacteriostatic effect against E. coli possibly due to competition for nutrients between kefir microbiota and the test strain and/or due to substances that could appear at early stages in the fermentation of milk (1).

Alm (11) and Korneva et al. (12) assessed the antibacterial activity of kefir against Salmonella, Shigella and Staphylococcus species. Cevikbas et al. (13) studied the antibacterial and antifungal activities of kefir and kefir grain on Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Bacillus subtilis and some Candida spp. It is reported that Lactococcus lactis DPC3147 a strain isolated from an Irish kefir grain produces a bacteriocin with a broad spectrum of inhibition (14). Rodrigues et al. (15) investigated the antimicrobial and healing activity of kefir and kefiran extraction.

In this study, it is aimed to investigate the antimicrobial effect of 24 and 48 hours fermented kefir against Staphylococcus aureus (ATCC 29213), Bacillus cereus (ATCC 11778), Salmonella enteritidis (ATCC 13076), Listeria monocytogenes (ATCC 7644) and Escherichia coli (ATCC 8739) in vitro conditions. In order to evaluate the antimicrobial effect of kefir, comparison made with ampicillin and gentamycin. pH changes of the kefir were also observed during the storage period.

**MATERIAL AND METHODS**

**Kefir Production.** Commercially bottled, homogenized UHT milk was used to produce kefir. 0.01 U freeze dried, mild aromatic kefir culture PROBAT KC3 (Danisco, Denmark) was used as starter culture to ferment 1 L of milk. It was reported that, using defined cultures to produce kefir is in progress toward standardizing the kefir production (16). Because of that we preferred to use standard, freeze dried culture instead of using traditional kefir grain. The composition of starter culture was Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis, Leuconostoc mesenteroides subsp. Cremoris, Lactobacillus kefyr, Kluyveromyces marxianus var. marxianus, and Saccharomyces unisporus. Fermentation was done in two groups at 24-26 °C for 24 and 48 hours. At the end of fermentation time, kefir was cooled to approximately to +4 °C and stored at this temperature for 7 days. At the end of fermentation and during storage, antibacterial activity was tested in the 1st, 4th and 7th days of storage. The traditional starter culture of kefir takes the form of grains of variable sizes which resembles cauliflower flowers in shape and these grains contain a wide and varying microflora (17). Because of that commercially prepared and freeze-dried kefir culture was preferred in order to optimize the fermentation and initial flora of kefir.
Preparations of bacterial solutions. *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 11778), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 7644) and *Escherichia coli* (ATCC 8739) were used as test microorganisms which are the most common food borne pathogenic bacteria. They were activated in nutrient broth by fermentation at 35°C for 24 hours. A loop full of the bacteria that activated and enriched in nutrient broth were transferred to sterile saline water and emulsified to a turbidity of McFarland 0.5 density. The final bacterial cell concentration approximated to 10^{8}/ml with spectrophotometric method.

Testing antimicrobial activity. Antibiotic activity of kefir was evaluated using the disk diffusion method as described by the National Committee for Clinically Laboratory Standards (18). Ampicillin and gentamycin were used to compare the antimicrobial activity and 10μg/ml of antibiotics was pipetted on to 5 mm diameter paper disk. 24 and 48 hours fermented kefir were pipetted at the amount of 0.1 ml and 1.2 mg/ml as described by Rodrigues et al. (15). The paper disks with antibiotics and experimental kefir were applied to the agar surface previously inoculated with 0.1 ml organism suspension. These plates were incubated at 37°C for 24 hours and the inhibition zones were measured at the end of fermentation period. Experiments were performed in triplicates and mean values were used.

pH Measuring. pH of 24 and 48 hours fermented kefir were measured by Hanna HI 9321 Microprocessor pH meter. pH was measured within the same day of antimicrobial activity testing by taking about 35-40 ml of kefir to a separate glass under aseptic conditions.

RESULTS AND DISCUSSION

The antimicrobial activity was first tested at the end of fermentation period, before storage at +4 °C. No significant difference was obtained between antimicrobial zone diameters of 24 and 48 hours fermented kefir. The best antimicrobial effect of 24 and 48 hours kefir was seen against *Staphylococcus aureus* with the diameter zones of 21.4 and 21.1 mm respectively. Similar results were reported by other researchers. Cevikbas et al. (13) reported the greatest activity of kefir against gram-positive coccus, *Staphylococcus aureus* and gram-positive bacillus. Rodrigues et al. (15) obtained the best antimicrobial effect against *S. aureus* and *Pseudomonas aeruginosa* with the diameter zones of 30.0 and 30.2 mm respectively. Also according to this study kefir causes good inhibition zones against *S. pyogenes* and *S. salivarius* with the diameter zones of 27.2 mm and 24.9 mm respectively. Another study that has investigated the antimicrobial activity was performed by Zacconi et al. (19). According to their results; kefir has antimicrobial effect against wide variety of gram-positive and gram-negative bacteria.

In our study, gentamycin and ampicillin were used to compare the antimicrobial effect and caused similar inhibition diameter zones with the zones caused by kefir. Table 1 shows the diameters of antimicrobial inhibition zones of 0.1 ml of 24 and 48 hours fermented kefir at the end of fermentation and before storage, compared with gentamycin and ampicillin. The results represent the mean zone diameters of triplicate trials in mm.

**Table 1.** Inhibition zones (diameter in mm) of antimicrobial activity of 24 and 48 hours fermented kefir after fermentation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampicillin</th>
<th>Gentamycin</th>
<th>Kefir (24 h)</th>
<th>Kefir (48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>25.3</td>
<td>22.6</td>
<td>21.4</td>
<td>21.1</td>
</tr>
<tr>
<td>E. coli</td>
<td>20.2</td>
<td>20.8</td>
<td>19.5</td>
<td>18.6</td>
</tr>
<tr>
<td>B. cereus</td>
<td>21.8</td>
<td>21.2</td>
<td>20.6</td>
<td>20.2</td>
</tr>
<tr>
<td>S. enteretidis</td>
<td>21.3</td>
<td>19.8</td>
<td>18.4</td>
<td>18.9</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>20.0</td>
<td>21.5</td>
<td>18.3</td>
<td>18.2</td>
</tr>
</tbody>
</table>
According to our study it was seen that; no significant change in antibacterial activity was occurred in the 1st, 4th and 7th days of storage for 24 hours fermented kefir. The best antibacterial activity was seen against Staphylococcus aureus with unimportant changes during the storage days. Table 2 represents the diameters of antimicrobial inhibition zones of 24 hours fermented kefir during the storage at + 4 °C.

Table 2. Inhibition zones (diameters in mm) of antimicrobial activity of 24 hours fermented kefir during the storage at + 4 °C.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampicillin (day 1)</th>
<th>Ampicillin (day 4)</th>
<th>Kefir (day 1)</th>
<th>Kefir (day 4)</th>
<th>Kefir (day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>25.1</td>
<td>22.2</td>
<td>21.2</td>
<td>21.0</td>
<td>21.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>20.4</td>
<td>21.4</td>
<td>19.5</td>
<td>19.4</td>
<td>19.4</td>
</tr>
<tr>
<td>B. cereus</td>
<td>21.0</td>
<td>21.7</td>
<td>20.5</td>
<td>20.2</td>
<td>20.1</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>21.7</td>
<td>20.2</td>
<td>18.8</td>
<td>20.9</td>
<td>21.2</td>
</tr>
<tr>
<td>L. monocytogene</td>
<td>20.4</td>
<td>20.9</td>
<td>18.0</td>
<td>18.0</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Similar results were obtained from the tests on 48 hours fermented kefir. The results of 48 hours fermented kefir's antimicrobial activity testing during the storage at + 4 °C were given in Table 3.

Inhibition zones of kefir were similar sizes as those found using other probiotics. Matijasik and Rogelsj (20) showed Lactobacillus K7 strain produced inhibition zones of 19 and 22 mm against Clostridium tyrobutyricum and C. perfringens, respectively.

Table 3. Inhibition zones (diameters in mm) of antimicrobial activity of 48 hours fermented kefir during the storage at + 4 °C.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Ampicillin (day 1)</th>
<th>Ampicillin (day 4)</th>
<th>Kefir (day 1)</th>
<th>Kefir (day 4)</th>
<th>Kefir (day 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>25.5</td>
<td>22.2</td>
<td>21.0</td>
<td>20.8</td>
<td>20.8</td>
</tr>
<tr>
<td>E. coli</td>
<td>20.0</td>
<td>21.1</td>
<td>18.4</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>B. cereus</td>
<td>22.1</td>
<td>21.0</td>
<td>20.0</td>
<td>19.8</td>
<td>19.7</td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>20.4</td>
<td>19.9</td>
<td>19.0</td>
<td>21.6</td>
<td>22.0</td>
</tr>
<tr>
<td>L. monocytogene</td>
<td>19.8</td>
<td>21.2</td>
<td>18.2</td>
<td>18.1</td>
<td>17.9</td>
</tr>
</tbody>
</table>

At the end of fermentation period pH of 24 and 48 hours fermented kefir were 5.52 and 4.89 respectively. During the storage of kefir, pH decreased slightly till the 7th day of storage. Atasver et al. (21) observed the pH changes of kefir during 15 days of storage. According to the results of their study; pH was measured as 4.55 at the first day; 4.36 at the 7th day. These results are parallel to the ones that we obtained. In Table 4, pH changes of 24 and 48 hours fermented kefir during the storage at + 4 °C were given.

In conclusion; this study demonstrates that kefir possesses antibacterial effect against Staphylococcus aureus (ATCC 29213), Bacillus cereus (ATCC 11778), Salmonella enteritidis (ATCC 13076), Listeria monocytogenes (ATCC 7644) and Escherichia coli (ATCC 8739) and the present studies mentioned above confirm our conclusion.

It has to be underlined that the inhibitoriest effect was seen against Staphylococcus aureus. On the other hand, it was seen that the
antibacterial effect decreased or did not change during the storage at + 4 °C except of *Salmonella enteretidis*. The data presented in this study suggests that kefir may be a good antimicrobial agent in food technology for food safety. More researches have
to be performed related to this subject, in order to put kefir’s antimicrobial activity in practice for food technology.

REFERENCES