Screening Kulfi for Staphylococcal Enterotoxins with the Thermonuclease Test

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(Received for publication May 31, 1979)

ABSTRACT

Forty kulfi samples collected from local push-cart vendors and restaurants were screened for thermonuclease and staphylococcal enterotoxins. Viable staphylococcal counts were also determined in these samples. Thermonuclease was detected in four kulfi samples. The incidence was, however, more (15%) in samples from push-cart vendors as compared to those from restaurants (5%). Enterotoxins were also detected in thermonuclease-positive samples. The incidence of enterotoxins A and B was 10% as compared to those from restaurants (5%). Enterotoxins were detected in thermonuclease-positive samples. The incidence of enterotoxins A and B was 10% as compared to those from restaurants (5%). Enterotoxins were also detected in thermonuclease-positive samples. The incidence of enterotoxins A and B was 10% as compared to those from restaurants (5%). Enterotoxins were also detected in thermonuclease-positive samples. The incidence of enterotoxins A and B was 10% as compared to those from restaurants (5%). Enterotoxins were also detected in thermonuclease-positive samples. The incidence of enterotoxins A and B was 10% as compared to those from restaurants (5%).

Kulfi is a frozen indigenous milk product popular in many parts of North India (21) and Pakistan (10). This product is prepared from cow, buffalo or mixed milk which is concentrated to approximately half of its volume in a large open pan (karahi), kept over a fire hearth (chula). The concentrated milk is cooled for a while at ambient temperature. Sugar at the rate of 15-20% is then added to milk and stirred for thorough mixing. Spices and nuts (cardamom, almonds, pistachio, cashew) or fruit juices (mango) are added to the sweetened milk and mixed. The mixture is then filled in conical containers and frozen in an earthen pot containing an ice-salt mixture. The frozen product is consumed either as such or along with semya (prepared from wheat flour, drawn in the form of long threads and immersed in water before use). Kulfi is marketed either by halwais (sweetmeat makers) in restaurants or from push carts by roadside vendors.

The hygienic quality of kulfi is yet to be known to many people. In a recent investigation, Rao (16) has isolated various types of microorganisms including staphylococci from this product. Staphylococcus aureus has gained importance in recent years in view of its ability to grow and produce enterotoxins in foods, including milk and milk products (7,9,19), leading to food poisoning outbreaks. A large number of coagulase producing staphylococci have been reported to produce thermostable DNase (thermonuclease) and between thermonuclease and enterotoxin production has been very well established in dairy products (6,19). Cords and Tatini, Park et al. and Batish et al. (3,6,15) have recommended thermonuclease test as a rapid and reliable method for the detection of staphylococcal enterotoxin in dairy products.

In the present investigation, an attempt has been made to assess the quality of market kulfi samples by determining staphylococcal population as well as thermonuclease.

MATERIALS AND METHODS

A total of 40 samples of kulfi were collected according to Standards Methods (1) from the Karnal market during summer (March to June, 1978). These included 20 from push-cart vendors and 20 from restaurants. Samples obtained from push-cart vendors had a candy-like appearance containing a wooden stick and the product was frozen in a conical iron container. Kulfi sold in restaurants was available in either metal or plastic containers. The containers, sealing material (rubber bands) and the earthen pot are shown in Fig. 1.

Kulfi samples were examined for staphylococcal counts on Staphylococcus medium 110 (5). Thermonuclease was extracted (20) and the extract was boiled for 15 min. The thermonuclease test was carried out on toluidine blue DNA agar medium (11). The thermonuclease-positive samples were tested for the presence of enterotoxins by the microslide gel diffusion technique (4). The standard antisera of the enterotoxins A, B, C and D were obtained from Dr. M. S. Bergdoll.

The isolates of staphylococci obtained from kulfi samples were characterized on the basis of anerobic glucose and mannitol fermentation (2), coagulase production (7), thermonuclease (11) and enterotoxin production (4).

RESULTS AND DISCUSSION

Of 40 samples of kulfi examined in the current study, four were positive for presence of thermonuclease (Fig. 2). The incidence was, however, more (15%) in kulfi samples obtained from push-cart vendors than from restaurants (5%). Data in Table 1 show that a fair correlation between detectable thermonuclease and viable counts of staphylococci in samples was not possible. The results of the present study indicate that kulfi samples from push-cart vendors had low viable counts of staphylococci (95 x 10³ per g) which are not suggestive of a potential danger but contained detectable levels of thermonuclease (zone diameter, 12.2 mm). Viable staphylococcal counts (14 x 10³ per g) were also less in thermonuclease-positive samples (zone diameter,...
Figure 1. Equipment and accessories used for preparation of kulfi. a. Plastic cones before filling. b. Milk mixture. c. Cones containing milk mixture. d. Sealing material. e. Sealed plastic cones. f. Freezing of kulfi contained in sealed plastic cones in an earthen pot filled with ice-salt mixture. g. Lid of earthen pot.

8.5 mm) obtained from restaurants. Similar findings were reported (3, 19) for butter, cheese, non-fat dry milk, dried malted milk and baby food samples. In an earlier survey conducted on several samples of kulfi from the Karnal market, Ghosh (9) observed staphylococcal counts ranging between $43 \times 10^2$ and $59 \times 10^3$ per g with an average of $139 \times 10^2$ per g in the samples.

Among the types of enterotoxins encountered in the market samples of kulfi, enterotoxin A predominated, followed by enterotoxin B. Both the types of enterotoxins (A and B) were detected in kulfi samples which also contained thermonuclease. In the present study, the incidence of enterotoxins A or B in kulfi samples from both the sources was about 10%. Enterotoxins C and D were not detected in any of the samples examined during this study (Table 1).

### Table 1. Incidence of thermonuclease and enterotoxins in kulfi samples.

<table>
<thead>
<tr>
<th>Source of kulfi sample</th>
<th>No. of samples</th>
<th>Incidence of thermonuclease positive</th>
<th>Zone diameter (mm)</th>
<th>Staphylococcal counts/g in thermonuclease positive samples</th>
<th>Types of enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percent</td>
<td>Range</td>
<td>Average</td>
<td>Range</td>
</tr>
<tr>
<td>1. Push-carts</td>
<td>3/20</td>
<td>15</td>
<td>9.5 - 14.5</td>
<td>12.2</td>
<td>$63 \times 10^8$ - $11 \times 10^4$</td>
</tr>
<tr>
<td>2. Restaurants</td>
<td>1/20</td>
<td>5</td>
<td>8.5</td>
<td></td>
<td>$14 \times 10^3$</td>
</tr>
</tbody>
</table>

- The numerator indicates number of positive samples and the denominator indicates total number of samples.
- This includes the diameter of well (4 mm).
- + = Present; - = Absent
- All three samples were positive for enterotoxin. (Sensitivity of microslide assay = 0.5 µg/ml)

A total of 50 isolates of staphylococci was collected from 20 samples of kulfi obtained from push-cart vendors and 31 isolates from 20 samples received from restaurants. Among 81 isolates examined, seven were positive for thermonuclease, coagulase and enterotoxins. In regard to the incidence of thermonuclease-producing strains of staphylococci from different sources tested, 10% (5/50) of the isolates were positive; only 6.5% (2/31) of the isolates from restaurant samples were positive. It is possible that enterotoxigenic staphylococci might have gained entry into milk at some stage of processing. According to Ghosh (9), incidence of enterotoxigenic staphylococci was more in frozen milk products like ice-cream (10.2%) and kulfi (6.7%) than in other dairy products.

Data in Table 2 indicate that each of the seven thermonuclease-producing isolates of staphylococci showed production of either enterotoxin A or B. The incidence of enterotoxin A produced by staphylococcal isolates in kulfi samples from push-cart vendors and restaurants was 8% and 3.2%, respectively. One isolate was positive for enterotoxin B from each source of sample. Enterotoxins C and D were not detected in any of the thermostable DNase-producing isolates examined during the current study.

One (out of three) of the coagulate positive isolates obtained from restaurant sample failed to produce either
thermostable DNase or enterotoxins. According to Rayman et al. (117), some staphylococcal strains capable of producing coagulase did not produce thermonuclease and enterotoxins. In contrast, Omori and Kato (14) demonstrated clearly certain coagulase-negative S. aureus strains which could produce enterotoxins. Hence the sole character of coagulase production in staphylococci cannot be considered as an indicator of enterotoxigenicity.

During the manufacture of kulfi, milk is subjected to a substantial heat treatment (boiling for 1 h); staphylococci are not known to survive such drastic heat treatments. The possibility of these organisms gaining entry into milk before freezing through fingers and other sources cannot be ruled out. These organisms, after attaining several millions, might have produced both the thermonuclease and enterotoxins in milk at ambient temperatures. In a similar study, Ghosh (9) found that enterotoxin A and B were detectable in milk when staphylococci reached a population of about 42 to 43 million per ml. Very recently, several other workers (8, 18) have indicated that staphylococci grow well in milk and produce thermonuclease and enterotoxins under favorable conditions.

In the light of the above reports, the findings of the present investigation suggest that market kulfi can serve as a vehicle of enterotoxigenic staphylococci causing food poisoning outbreaks, and thermonuclease test can be used as a rapid and reliable screening method for indicating the likely presence of enterotoxins, thus warranting enterotoxin analysis.

ACKNOWLEDGMENTS

The authors are indebted to Dr. M. S. Berdell, Food Research Institute, The University of Wisconsin, Madison, Wisconsin 53706, U.S.A. for supplying staphylococcal enterotoxins and their respective antisera. Sincere thanks are also due Dr. D. Sundaresan, Director, Dr. R. A. Srinivasan, Head and Dr. B. Ranganathan, Head, Southern Regional Station, Bangalore for their kind interests, encouragements and valuable suggestions.

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JOURNAL OF FOOD PROTECTION, VOLUME 43, JAN., 1980