Enterotoxin Production by Staphylococcal Isolates from Nigerian Fermented Milk Products

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ABSTRACT

A total of 369 samples of nono, furanono and manshanu were purchased from four different markets around Zaria, Nigeria. Five hundred and sixty-eight staphylococcal isolates were obtained from the three products of which a total of 37 (6.5%) were enterotoxigenic comprising 21 (10.7%) from nono samples and 16 (17.2%) from fura samples. The staphylococcal count, pH and titratable acidity of all the samples that contained enterotoxigenic staphylococci ranged from $5.4 \times 10^3$ to $6.3 \times 10^6$ CFU/ml, 3.89 to 4.21 and 0.47 to 0.74, respectively. Of all the staphylococcal isolates, 6.5% produced enterotoxin. Of the enterotoxigenic strains encountered 73.0% produced enterotoxin A, 10.8% B, 10.8% C and 2.7% produced enterotoxin. Of the enterotoxigenic strains encountered 73.0% produced enterotoxin A, 10.8% B, 10.8% C and 2.7% produced combinations of A and B and A and C each. Statistical analysis revealed no correlation between either coagulase, thermonuclease, hemolysin production and enterotoxin production.

Staphylococcal food-poisoning caused by enterotoxigenic staphylococci in milk and milk products has been reported (16). For Staphylococcus aureus to grow and produce enterotoxins the food environments and nutritional requirements must be adequate and without competitive growth by other microorganisms (13).

Staphylococcal enterotoxin is a toxic extracellular metabolite produced by staphylococcal cells. There are six serologically distinct types designated A, B, C, D, E and F, and all have been implicated in foodborne intoxication (7). It is also known that not all staphylococci produce these toxins but the percentage that produce them is unknown due to the inadequacy of methods of detection of the enterotoxin.

Many attempts have therefore been made to relate enterotoxin production and pathogenicity to other metabolic products of staphylococci like coagulase, thermonuclease, pigmentation and hemolysin production (21,22,23). Despite the amount of work done in this area, no one has come out completely with pathogenicity and enterotoxigenicity.

Adesiyun (3) has used hemolysin production along with thermonuclease and coagulase to differentiate staphylococci from ready-to-eat foods into animal and human biotypes.

This work reports the incidence of enterotoxin production by staphylococcal isolates from fermented milk (nono), fermented milk-cereal mix (fura) and local butter (manshanu). Attempts were made to relate toxin production to the ability of the isolates to produce certain metabolites and to the staphylococcal counts, pH and titratable acidity of the samples contaminated with enterotoxigenic staphylococci.

MATERIALS AND METHODS

Description of Nigerian milk products

The three products studied were nono (fermented milk), fura (fermented milk and cereal) and manshanu (local butter). Nono and manshanu. A portion of a previously fermented product is added as starter to raw milk in a calabash and allowed to ferment overnight. After fermentation it is shaken vigorously to remove fat. The fat is called manshanu and the milk nono. Nono is sold from a calabash covered with mat using scoops made from calabash. Manshanu is usually made into balls by hand and left in the nono or in a separate calabash for sale.

Fura. A fermented millet or sorghum is cooked, spiced, pounded and molded into balls by hand. In the market it is mixed in a bowl with nono for consumers. This is fura-da-nono, commonly referred to as fura. Usually one bowl is used in mixing for all the customers, without cleaning between use. Depending on the consistency, fura is used as food, refreshing drink and as weaning food for infants. These products are always in very high demand especially in the months of November to July.

Sampling

Ten samples of nono and five each of fura and manshanu were purchased from four markets, DanGiwa (Mkt I), Giwa (Mkt II), Wussasa (Mkt III) and Zaria City (Mkt IV) around Zaria using sterile containers. Each market was sampled once in two months between April and December, 1983. All samples were stored in an ice-packed cooler and brought to the laboratory for analysis.

Isolation of staphylococci

A ten-fold dilution of each sample using 0.1% buffered peptone water was prepared. Appropriate dilutions were made and 0.1 ml of the diluted samples was surface-plated on Baird-Parker agar (BPA). The plates were incubated at 37°C for 48 h.

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Identification of staphylococci

Typical black colonies on BPA which were gram-positive, in clusters and catalase-positive were stored on heart infusion agar slants at 4°C for biochemical characterization.

Coagulase production

The tube coagulase test was done using freshly prepared human, rabbit and bovine plasmas on an overnight culture in brain heart infusion broth and incubated at 37°C. The procedure described by Baer et al. (6) and test interpretation of Sperber and Tatini (19) were used. S. aureus strain F265 and S. epidermidis strain ATCC 14990 were used as the positive and negative controls, respectively.

Enterotoxin production

The control antigen and antisera were kindly supplied by M. S. Bergdoll of the Food Research Institute, University of Wisconsin, Madison, WI.

Thermonuclease production

The method described by Lachica et al. (15) was used. S. aureus (F265) and sterile brain heart infusion broth were used as the positive and negative controls, respectively.

Carbohydrate fermentation

Anaerobic fermentation of glucose and mannitol was carried out as modified by the subcommittee on Taxonomy of staphylococci and micrococci (20).

Enterotoxin production

The cellophane-over-agar method of Robbins et al. (17) was used to grow the isolates for enterotoxin production. Enterotoxin was detected by the double gel diffusion (microslide) technique of Casman and Bennett (10). The control antigen and antisera were kindly supplied by M. S. Bergdoll of the Food Research Institute, University of Wisconsin, Madison, WI.

Statistical analysis

The relationship between coagulase, thermonuclease, hemolysin and enterotoxin was analyzed using the correlation coefficient (11).

RESULTS AND DISCUSSION

One hundred and ninety seven samples of nono were collected from the four markets, 178 (90.4%) were contaminated with staphylococci. Manshanu was not contaminated with enterotoxigenic staphylococci. Similarly, 30 (38.0%) of the 79 samples of manshanu collected contained staphylococci and none was contaminated with enterotoxigenic staphylococci. On the whole, 95-100% of fura 82.5-94.3% of nono and 20-50% of manshanu were contaminated with staphylococci (Table 1).

According to Kosikowski (14), the standard pH and titratable acidity for yogurts ranges from 4.0 to 4.2 and 0.9 to 1.4%, respectively. Like yogurt, nono and fura are locally fermented milk products and had pH values that conformed with this standard (range 3.89 to 4.20) though the titratable acidity was lower, ranging from (0.47 to 0.74%). The low titratable acidity of these products could be attributed to low lactic acid development and the uncontrolled nature of the fermentation process. The low pH and high number of microbial competitors (as seen by the total bacterial count and high yeast cells on BPA) probably contributed to the low staphylococcal counts (Table 1). Although the counts were low, a high percentage of the samples were contaminated with staphylococci. This means that if there is a starter failure, resulting in poor acid development in the presence of few competitors, the enterotoxigenic strains present can grow to a level that could make the food hazardous to consumers. Tatini (21) also observed a decline in staphylococcal count in fermented food, whereas enterotoxin remained stable despite the low pH. It was therefore concluded that foods may contain enterotoxin without any viable staphylococci.

Five hundred and sixty-eight staphylococcal strains were isolated from all the products, of which 37 (6.5%) were enterotoxigenic, 21 (6.1%) were isolated from nono, 16 (8.1%) from fura and none from manshanu (Table 2). The enterotoxigenic staphylococci isolated from these samples produced enterotoxins A, 27 (4.7%); B, 4 (0.7%); C, 4 (0.7%); 51 (8.8%) contained enterotoxigenic strains. Of 93 samples of fura, 92 (98.9%) were contaminated with staphylococci and 16 (8.1%) contained enterotoxigenic staphylococci. Similarly, 30 (38.0%) of the 79 samples of manshanu collected contained staphylococci and none was contaminated with enterotoxigenic staphylococci.

<table>
<thead>
<tr>
<th>Market</th>
<th>Food type and No. of samples</th>
<th>Positive for staphylococci</th>
<th>Mean staphylococci count</th>
<th>Mean pH</th>
<th>Mean TA</th>
<th>No. (%) of enterotoxigenic strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(CFU/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Nono</td>
<td>50 (99.0)</td>
<td>1.3x10^6</td>
<td>4.04</td>
<td>0.64</td>
<td>6 (10.6)</td>
</tr>
<tr>
<td>II</td>
<td>Nono</td>
<td>52 (95.3)</td>
<td>2.7x10^6</td>
<td>4.21</td>
<td>0.49</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>III</td>
<td>Nono</td>
<td>34 (87.6)</td>
<td>7.2x10^6</td>
<td>4.04</td>
<td>0.63</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>IV</td>
<td>Nono</td>
<td>49 (89.0)</td>
<td>5.4x10^5</td>
<td>3.89</td>
<td>0.47</td>
<td>8 (16.7)</td>
</tr>
<tr>
<td>I</td>
<td>Fura</td>
<td>25 (100.0)</td>
<td>2.0x10^4</td>
<td>4.00</td>
<td>0.74</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>II</td>
<td>Fura</td>
<td>25 (100.0)</td>
<td>6.3x10^4</td>
<td>4.15</td>
<td>0.53</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>III</td>
<td>Fura</td>
<td>20 (95.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>IV</td>
<td>Fura</td>
<td>25 (100.0)</td>
<td>1.6x10^6</td>
<td>4.02</td>
<td>0.58</td>
<td>10 (40.0)</td>
</tr>
<tr>
<td>I</td>
<td>Manshanu</td>
<td>4 (20.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Manshanu</td>
<td>18 (94.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Manshanu</td>
<td>17 (63.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IV</td>
<td>Manshanu</td>
<td>24 (50.0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mean staphylococci count of samples containing enterotoxigenic strain. Manshanu was not contaminated with enterotoxigenic staphylococci.
TABLE 2. Incidence of enterotoxin production amongst staphylococcus strains isolated from nono and fura.

<table>
<thead>
<tr>
<th>Product</th>
<th>No. (%) of strains enterotoxigenic</th>
<th>No. (%) of strains producing enterotoxins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Nono</td>
<td>197</td>
<td>346</td>
</tr>
<tr>
<td>Fura</td>
<td>93</td>
<td>197</td>
</tr>
<tr>
<td>Manshanu</td>
<td>79</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>369</td>
<td>568</td>
</tr>
</tbody>
</table>

TABLE 3. Relationship between coagulase, thermonuclease hemolysin and enterotoxin production by staphylococcal strains isolated from nono, fura and manshanu.

<table>
<thead>
<tr>
<th>Coagulase production</th>
<th>Rabbit plasma</th>
<th>Human plasma</th>
<th>Bovine plasma</th>
<th>Thermonuclease production</th>
<th>Hemolysin production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolates</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>No. (%) of strains</td>
<td>166</td>
<td>377</td>
<td>239</td>
<td>304</td>
<td>99</td>
</tr>
<tr>
<td>Percent of total isolates</td>
<td>30.6</td>
<td>69.8</td>
<td>44.0</td>
<td>56.0</td>
<td>18.3</td>
</tr>
<tr>
<td>No. of enterotoxigenic strains</td>
<td>18</td>
<td>19</td>
<td>30</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Percent of enterotoxigenic strains</td>
<td>10.8</td>
<td>11.9</td>
<td>12.6</td>
<td>2.9</td>
<td>8.1</td>
</tr>
</tbody>
</table>

2Percent of test positive strains.

and combined enterotoxins A and B, I (0.2%) and A and C, I (0.2%). None of the strains produced enterotoxins D and E. Most of the enterotoxigenic strains encountered produced enterotoxin A as 73% of the enterotoxigenic strains elaborated it while 2.7% elaborated combined toxins AB and AC each. On the whole, there was a low isolation rate of enterotoxigenic strains from the different foods and the predominantly encountered enterotoxin from these isolates was staphylococcal enterotoxin A (SEA). This is in accordance with earlier work in the Zaria area where Adekeye and Adesiyun (2) found that 57 of 86 enterotoxigenic strains from breast milk produced SEA, 31 (12.5%) of the isolates from ready-to-eat food produced SEA (3), 46.7% of the enterotoxigenic isolates from human beings produced SEA (1) and 55 (26.6%) of 207 strains from food handlers in dining halls were enterotoxigenic (4). From these findings one is concerned with the extent to which this enterotoxin contributes to a possible cause of foodborne staphylococcal intoxication in this area. Simikovicova and Gilbert (18) had stated that toxicogenic S. aureus isolated from cases of food-poisoning usually produce enterotoxin A while isolates from milk samples usually produce either enterotoxin C or D or both.

Of the three plasmas used, human plasma was best in detecting coagulase production by staphylococci. Of 568 isolates, 239 (44.0%) were coagulase-positive using human plasma, 166 (30.6%) using rabbit plasma and only 99 (18.3%) using bovine plasma (Table 3). Most of the enterotoxigenic strains were coagulase-positive using human plasma, followed by rabbit plasma and lastly by bovine plasma. A slightly higher percentage of the enterotoxigenic strains produced thermonuclease, 22 (10.2%) and alpha hemolysin, 14 (8.9%). The incidence was low among beta, 4 (7.1%) and gamma, 19 (5.8%) hemolysin producers. However, the enterotoxigenic strains were coagulase and/or thermonuclease-positive using any of the plasmas.

Although the counts obtained in this study were slightly lower than the hazardous level of 1 × 10⁶ CFU/ml (22) and the incidence of enterotoxigenity by strains was also low, staphylococci are also important in many human infections. This concern is even greater when 49.6% produced coagulase irrespective of type of plasma, 39.8% produced thermonuclease and 28.9% produced alpha-hemolysin. Therefore more than 28% of the staphylococcal strains encountered were human biotypes. This is because animal biotypes of staphylococci produce predominantly beta-hemolysin (12) and coagulate bovine plasma better than that of human or rabbit origin (5). Again, a slightly higher incidence of enterotoxigenic production was observed among the alpha-hemolysin producers. Enterotoxigenic S. aureus has been implicated in cases of mastitis in humans and animals and in cases of infantile diarrhea (9). Nono and fura are consumed by both adults and infants. A large number of people in Northern Nigeria use fura as a weaning food for infants. The extent to which these products could contribute to infantile diarrhea and infection is presently unknown.

Although the staphylococcus counts and incidence of enterotoxigenicity were low, the rate of contamination of the samples with staphylococci was very high. The enterotoxigenic S. aureus encountered were both human biotypes and alpha-hemolysin producers. Therefore there is a need to make the food producers aware of the use of pure starter cultures and hygienic methods of handling dairy products especially at point of sale. This would help reduce the incidence of human contamination of these products and increase the chance of enough lactic acid development.

REFERENCES

Abbey et al., con't. from p. 533

Gardner L, a and b values for unwrapped and wrapped watermelon slices stored at 5°C are listed in Table 4. All three values decreased with increased storage time. The rate of change was more rapid in the unwrapped watermelon, becoming significant at 2 d. Since color change was slower in the wrapped slices, it is highly probable that this change is stimulated by surface oxidation, and the foil wrap, although not forming an hermatic seal, did reduce exposure of the watermelon to air. The peak change in color occurred in unwrapped slices between 4 d and 6 d, and in wrapped slices between 6 d and 8 d. The rapidly decreasing L, a and b values decreased with increased storage time. The rate of change was more rapid than changes in chromaticity and hue. Although force required to penetrate the unwrapped and wrapped slices was changing significantly between each measurement, the sensory panel did not rate the wrapped slices significantly poorer in texture for each successive measurement (Table 3); subjective ratings of texture in unwrapped slices, however, were significantly lower after 2 d storage compared to initial ratings. Therefore, texture does not appear to be as critical an attribute as does flavor and color in the deterioration of sliced watermelon.

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REFERENCES