Phage Susceptibility and Enterotoxin Production by
Staphylococcus aureus Strains Isolated from Nigerian Foods

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ABSTRACT

The sensitivity of Staphylococcus aureus strains isolated from Nigerian foods to phages in the international phage sets for typing human and bovine strains of staphylococci was determined. The enterotoxigenicity of the strains was also determined using the avidin-biotin enzyme-linked immunosorbent assay and the reversed passive latex agglutination test (for staphylococcal enterotoxin D only). One hundred and five (67.7%) of 155 strains tested were susceptible to phages in both typing sets. Phages for staphylococci of human origin lysed all 105 typeable strains while those for staphylococci of bovine origin were responsible for the lysis of 92 strains. Phages in the different phage groups (mixed) were most frequently responsible for lysis, 26 (24.8%) strains susceptible. Of the 155 strains tested, 122 (78.7%) were enterotoxigenic producing staphylococcal enterotoxins A, B, C, D, or a combination. Dried beef isolates were most enterotoxigenic (100.0%) and those from fermented milk least (68.8%). Staphylococcal enterotoxins C, B, and A were elaborated either singly or in combination by 71 (58.2%), 69 (56.6%), and 62 (50.8%) strains, respectively. It was concluded that a majority of staphylococcal strains isolated from Nigerian foods originated from humans and their high enterotoxigenicity could be a health risk to consumers.

The present study employs the susceptibility of strains of S. aureus isolated from foods to phages for typing staphylococci of human and bovine origin to indicate their possible sources and reports the enterotoxigenicity of the strains using highly sensitive enzyme-linked immunosorbent assay (ELISA) and reversed passive latex agglutination (RPLA) test.

MATERIALS AND METHODS

Nigerian foods studied

Descriptions of the Nigerian food products which served as sources of the strains in the present study, listed in Table 1, had been made elsewhere (4,22). In addition, raw market meat was sampled.

Sample collection

Local markets served as sources of food products with a collection of 50 samples each per product. Fermented milk products were collected into sterile universal bottle, while units of ready-to-eat foods were put into sterile aluminium foil wraps. Approximately, 50-g units of raw meat were randomly purchased from sellers.

Isolation of staphylococci

Surfaces of all meat samples, approximately 100 sq cm area, were swabbed with sterile cotton moistened in saline and streaked for isolation on Baird-Parker agar (BPA) plates. For fermented milk samples, 0.1 ml was spotted on BPA and streaked for isolation. Inoculated plates were incubated for 48 h at 37°C.

Identification of staphylococci

Typical black colonies were picked on BPA plates, Gram-stained and tested for catalase activity using standard methods (11). An isolate was picked per food sample. All catalase-positive, gram-positive cocci were stored at 4°C until needed.

Fermentation of mannitol

The ability of strains to utilize mannitol anaerobically was determined as recommended by the Subcommittee on Taxonomy of Staphylococci and Micrococci (20).

Coagulase detection

The tube assay of Baer et al. (5) with rabbit plasma (bioMerieux, France) and test interpretation guideline of Sperber and Tatini (19) were used.
TABLE 1. Susceptibility of S. aureus isolates from foods to human and bovine phages.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of isolates tested</th>
<th>Human phage set</th>
<th>Bovine phage set</th>
<th>No. (%) of isolates typeable with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RTD^1</td>
<td>100-RTD^2</td>
<td>Total</td>
<td>RTD</td>
</tr>
<tr>
<td>Fried chicken</td>
<td>18</td>
<td>6 (33.3)</td>
<td>1 (5.6)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>Fermented milk</td>
<td>32</td>
<td>6 (18.8)</td>
<td>15 (46.9)</td>
<td>21 (65.6)</td>
</tr>
<tr>
<td>Dried fish</td>
<td>25</td>
<td>5 (20.0)</td>
<td>11 (44.4)</td>
<td>16 (64.0)</td>
</tr>
<tr>
<td>Fried fish</td>
<td>18</td>
<td>10 (55.6)</td>
<td>8 (44.4)</td>
<td>18 (100.0)</td>
</tr>
<tr>
<td>Raw meat</td>
<td>40</td>
<td>17 (42.5)</td>
<td>14 (35.0)</td>
<td>31 (77.5)</td>
</tr>
<tr>
<td>Roast beef</td>
<td>15</td>
<td>2 (13.3)</td>
<td>6 (40.0)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Dried beef</td>
<td>7</td>
<td>1 (14.3)</td>
<td>3 (42.9)</td>
<td>4 (57.1)</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
<td>47 (30.3)</td>
<td>58 (37.4)</td>
<td>105 (67.7)</td>
</tr>
</tbody>
</table>

^1 Routine test dilution.
^2 Hundredfold routine test dilution.
^3 Typeable with human or bovine phage set or both.

Thermonuclease detection

The test protocol of Lachia et al. (14) was used.

Isolates that were catalase-positive, gram-positive cocci coagulating rabbit plasma with or without thermonuclease production were regarded as S. aureus.

Phage typing of S. aureus strains

Phage typing of all strains was done according to standard methods (8). All isolates were tested at the routine test dilution (RTD), but in cases of weak or negative reactions, the 100-RTD was also used. Each strain was typed with the 23 phages in the human phage set and with 11 additional phages in the bovine phage set. The following were the bovine phages used: 116 (group II); 42D, 102, 107, 117 (group IV); 42F, 78, 111, 118, 119, and ACI (miscellaneous group). All phages were obtained from the collection of the German Reference Laboratory for Phage Typing of Staphylococci at the University of Bonn, Germany.

Enterotoxin production

The cellophane-over-agar method of Robbins et al. (17) was used for enterotoxin production by strains. Harvested cells were centrifuged at 20,000 x g for 30 min and the supernatant stored at -20°C until needed. Staphylococcal enterotoxins A (SEA), B (SEB), and C (SEC) were assayed for in the supernates using the avidin-biotin ELISA as described by Hahn et al. (13). Staphylococcal enterotoxin D (SED) was detected with the RPLA test as described by Oda et al. (15) using RPLA test kits (Oxoid, United Kingdom). The avidin-biotin ELISA for SED was not available at the time of the investigation.

TABLE 2. Distribution of lysis of S. aureus isolates from foods by phages in various groups.

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of typeable isolates</th>
<th>No. of isolates lysed by phages in groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Fried chicken</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Fermented milk</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>Dried fish</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Fried fish</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Raw meat</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>Roast beef</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Dried beef</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>22</td>
</tr>
</tbody>
</table>

RESULTS

The sensitivity of S. aureus strains isolated from foods is shown in Table 1. Overall, 105 (67.7%) of 155 strains tested were lysed by a combination of phages in both human and bovine phage sets, at RTD and/or 100-RTD. Susceptibility ranged from 38.9% found among isolates from fried chicken to 100% detected among dried fish isolates. Phages for typing staphylococci of human origin lysed 105 (67.7%) strains representing all the typeable strains, while phages for staphylococci of bovine origin lysed 92 (59.4%). Except for isolates from fried chicken where phages for staphylococci of human and bovine origin lysed the same number of strains (18), isolates from all other sources were more sensitive to phages for staphylococci of human origin.

Table 2 shows the phage groups active on S. aureus tested. Lysis by phages in the different groups (mixed) was most frequent with 29 (27.6%) strains sensitive. A comparable number of strains were lysed by groups III, II, and I phages with 26, 24, and 22 isolates, respectively, sensitive. The predominant patterns observed were 3A by 11 (10.5%) strains and 83A/84/85 pattern exhibited by 9 (8.6%) of the 105 typeable strains. Overall, a total of 49 phage patterns were observed.

The enterotoxigenicity of S. aureus isolates from foods is displayed in Table 3. In all, 122 (78.7%) strains were...
An earlier study on Nigerian products, dried beef and dried fish, with staphylococci by extensively in epidemiological investigations, and susceptibilities of strains of \textit{S. aureus} have been documented to have lower frequencies of enterotoxigenicity, ranging from 10 to 62.5\% (10,24). An earlier study on Nigerian enterotoxigenic elaborating SEA, SEB, SEC, SED singly or in combination. SEC was most frequently elaborated singly with 14 (9.0\%) strains being producers, while SEB and SEA were produced singly by 13 (8.4\%) and 8 (5.2\%) strains, respectively. Only three (1.9\%) strains produced SED singly. Overall, the frequency of production of the three toxins was similar with 72 (58.2\%), 69 (56.6\%), and 62 (50.8\%) strains being positive for SEC, SEB, and SEA singly or in combination.

**DISCUSSION**

The higher susceptibility of \textit{S. aureus} strains from Nigerian foods to phages for typing staphylococci of human origin compared to those for staphylococci of bovine origin, developed mainly for animal strains, is suggestive that these strains may have been introduced by human handlers. A majority of the food products are ready-to-eat and, therefore, subjected to handling by human sellers and prospective buyers alike. In a similar study on \textit{S. aureus} strains isolated from human beings in the same environment, 72.2\% of the isolates were lysed, while only 17.0\% of animals isolates were susceptible to a combination of human and bovine phages (Adesiyun and others, unpublished). Phage patterns of \textit{S. aureus} strains have been used extensively in epidemiological investigations, and susceptibility to certain phages has given indication as to their biotypes and possible sources (16).

A possibility of human contamination of two of the products, dried beef and dried fish, with staphylococci by handlers existed was suggested in an earlier study (2). Similarly, Umoh et al. (22) used physiological characteristics of strains \textit{S. aureus} isolated from fermented milk products to infer that these isolates probably originated from human handlers. The data obtained from phage typing of food staphylococcal isolates in the present study further support these contentions.

The detection of a rather high frequency of enterotoxin production by the strains tested is of public health significance. Studies on \textit{S. aureus} strains isolated from foods not implicated in foodborne intoxication have been documented to have lower frequencies of enterotoxigenicity, ranging from 10 to 62.5\% (10,24). An earlier study on Nigerian ready-to-eat foods found only 39.4\% of the strains tested to be enterotoxigenic for staphylococcal enterotoxins A-E. Umoh et al. (22) also using isolates from Nigerian fermented milk products detected only 6.5\% of the strains to be producers of SEA-SEE. Twenty-two (68.8\%) of 32 strains of \textit{S. aureus} isolated from fermented milk in the present study were enterotoxigenic despite assaying for only four enterotoxins, SEA-SED. The fact that all previous studies on Nigerian strains of \textit{S. aureus} for enterotoxigenicity have employed the double gel immunodiffusion assay (microslide) with rather low sensitivity compared to the very sensitive ELISA used in this study appears to be responsible, to a large extent, for the higher number of enterotoxigenic strains detected in the present study. The advent of the use of ELISA has been claimed to have significantly increased the frequency of enterotoxigenicity reported for \textit{S. aureus} strains (6). It was, therefore, obvious that earlier studies on the enterotoxigenicity of \textit{S. aureus} strains isolated from Nigerian foods underestimated the potential for staphylococcal intoxication in consumers of these products as the factors that allow staphylococcal growth and enterotoxin production exist (12).

It is, therefore, imperative that appropriate sanitary measures be taken to minimize contamination of these products, particularly by human handlers. It is hoped that such actions will reduce the risk of staphylococcal intoxication that may result from their consumption.

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**REFERENCES**


STORAGE OF VACUUM PACKAGED BEEF


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