LABORATORY ASPECTS OF STAPHYLOCOCCAL FOOD POISONING FROM COLBY CHEESE

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Outbreaks of food poisoning in Wisconsin in 1958 were traced to Colby-type cheese, as were the outbreaks of greater magnitude in Indiana and Michigan. Laboratory examinations were made on a total of eighty-seven samples from cheese, milk from producer farms and from swabs from factory equipment. Seventy-eight cultures of staphylococci were isolated. Seventy-two of these were coagulase positive, and forty-five of the coagulase-positive cultures had phage patterns.

During the summer and fall of 1958 there were outbreaks of food poisoning in three families in Wisconsin, and outbreaks of greater magnitude in Michigan and Indiana. Epidemiological investigation revealed that cheese was the food involved in these outbreaks, and all of it was produced in one cheese factory in Wisconsin between May 15 and Nov. 6 of that year. A single type of cheese called Colby cheese was involved. This is a washed curd product of low-acid cure, and in this particular factory it was made from raw milk. The laboratory investigation confirmed the nature and source of the outbreak; Staphylococcus aureus was isolated from cheese, from milk from the producer farms and from swabs taken in different areas in the factory. This report relates the laboratory aspects of the investigation.

MATERIALS AND METHODS

Cheese

Small portions were cut from the center of each specimen, emulsified with saline and a drop plated on Litmus Lactose Agar (LL), SS Agar (Difco), blood agar made with ten percent defibrinated sheep blood in nutrient agar containing 0.85% salt, and on Staphylococcal Medium No. 110 (Difco). Plates were incubated 48 hours at 37°C. When there were crowded colonies on the LL Agar and blood agar suggestive of being staphylococci, colonies were isolated from the No. 110 Agar plates. In each case a number of colonies were picked which showed some pigment. These were pooled and replated on blood agar for purification purposes. Again, several colonies were picked, pooled and inoculated on nutrient agar (containing salt) slants.

Milk

Each milk sample represented a pooled sample from each producer farm. These samples were collected at the cheese factory as each producer brought in this milk. They were brought to the laboratory under refrigeration the same day. Each specimen, undiluted and diluted 1:10, was streaked on the above media and treated in the same fashion. Each culture spoken of in the body of this paper is a pool of several isolated colonies.

Swabs

Swabs were moistened with veal infusion broth and rolled across the above media.

Cultural characteristics

Pigmentation. No attempt was made to tabulate the degree of pigment. As stated above colonies with some color were picked from LL Agar, blood agar or No. 110 medium. The pooled cultures after twenty-four hours at 37°C showed some degree of pigmentation, and the color was enhanced in some cultures after sitting at room temperature for awhile.

Hemolysis. Blood Agar Base (Difco) containing five per-cent blood (sheep, human, rabbit) was used for streak plates to demonstrate alpha, beta and delta lysins. Hemolysis was read at eighteen hours.

Mannitol fermentation. Mannitol broth (Difco) containing 0.004% bromcresol purple was used. Production of acid was recorded after twenty-four hours at 37°C.

Coagulase. Two drops of a twenty-four hour trypticase soy broth culture were mixed with 0.4 ml. of rehydrated plasma (Difco) and incubated three hours at 37°C. Clotting was checked at one hour as well as three hours, and any degree of clotting was considered a positive test. Known positive and negative controls were included.

Phage typing. The twenty-four phages recommended by the National Reference center on phage typing at the Communicable Disease Center, Georgia, were used. Four to six hour trypticase soy broth cultures were used to inoculate trypticase soy agar plates, and drops of the appropriate dilutions for each phage were dropped on these plates within the hour. After overnight incubation at 30°C, lysis was read. The routine test dilution for each phage was checked on its own propagating strain whenever typing was done. One-plus reactions were recorded, but only two-plus and three-plus reactions were reported.

Antibiotic sensitivity. Pour plates were made with nutrient agar (containing 0.85% salt) and 0.1 ml. of a
Table 1—Characteristics of Cultures of Staphylococci Isolated from Various Sources

<table>
<thead>
<tr>
<th></th>
<th>No. of specimens</th>
<th>No. of cultures</th>
<th>Coagulate</th>
<th>Mannitol</th>
<th>Hemolysis</th>
<th>Phage:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Milk</td>
<td>31</td>
<td>24</td>
<td>24</td>
<td></td>
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<tr>
<td>Cheese samples A</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese samples B</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cheese samples C</td>
<td>32</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swabs</td>
<td>8</td>
<td>32</td>
<td>31</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>87</td>
<td>78</td>
<td>72</td>
<td>6</td>
<td></td>
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</tr>
</tbody>
</table>

Legend:
Staphylococci isolated from 89.6% of the samples
Coagulate positive cultures 92%
Cultures with phage patterns 62.5%
Samples A cheese - Misc. cheese samples for presumptive food poisoning.
Samples B cheese - Received from northern hospital. Later found to be from same source as Samples A.
Samples C cheese - Collected from factory which produced Samples A and B.

RESULTS

The overall results are summarized in Table 1. Gram stains of the cheese emulsions showed a predominance of small Gram positive cocci, and the majority of plates showed a heavy seeding of cocci with some streptococci, a few Gram negative bacilli and some spreaders. No colonies picked from the SS agar belonged to the Salmonella group. Direct smears were not made from the milk samples.

Five specimens of cheese received at different times during the fall showed Staphylococcus aureus (designated as Samples A). These cultures were hemolytic and coagulase positive. Mannitol fermentation was not done. Three of these cultures showed phage patterns (see Table 2).

There were eleven samples of cheese sent in by a northern hospital for examination, though no one was reported to have eaten any of this cheese (designated as Samples B). Coagulase positive staphylococci were isolated from each of the eleven cheese samples, and eight of these cultures had phage patterns. Nine of the eleven cultures were hemolytic and fermented mannitol, two cultures were not checked.

Thirty-two samples of cheese were collected at the cheese factory (designated as Samples C). Staphylococci were isolated from each sample. Of thirty-two cultures, (a) all were hemolytic, (b) all fermented mannitol, (c) one failed to coagulate plasma, (d) all were sensitive to antibiotics, and (e) twenty-three showed phage patterns.

Eight swabs were submitted from areas in the factory. Two, from the gate valve on the weighing can and the raw milk pump and pipe, showed no growth. Four cultures from inside the bottom of a cheese vat, conductor trough, sides of deep tank and filter, and the interior of the electric cooler showed no hemolysis, were coagulase negative, fermented mannitol and were non-typable. A culture from the gate valve on the cheese vat was hemolytic, coagulase negative, failed to ferment mannitol, and it was not typable. The eighth culture, from the rennet container, was hemolytic, coagulase positive and had a phage pattern.

Thirty-one samples of milk were examined from the producer farms of this cheese factory. Coagulase positive staphylococci were isolated from twenty-four of the milk samples. Of twenty-four cultures tested, eighteen were hemolytic and twenty-one fermented mannitol. One culture which showed evidence of hemolysis did not ferment mannitol. The ten cultures of this group which showed phage patterns were hemolytic, coagulase positive and fermented mannitol. All twenty-four cultures were sensitive to antibiotics with one exception, one culture was resistant to terramycin.

Throughout this study 87 samples (cheese, milk and swabs) were examined, and 78 showed staphylococci. Seventy-two of the 78 cultures examined were coagulase positive (47 from cheese, 24 from milk, and one from a swab), and 62 of the 78 cultures showed evidence of alpha, beta or delta lysins or combinations of them. The combination of the three lysins appear-
ed most frequently; however, alpha-delta lysins were seen in combination more than the alpha-beta lysin combination.

Phage patterns were demonstrated in 45 (62.5%) of the coagulase positive cultures; 42-D appeared twenty-one times and 44-A appeared eighteen times among these forty-five cultures. Representatives of the phage lytic Group III appeared twenty-one times, but it must be remembered that this is the group with the greatest number of phages. Lytic Groups I and II appeared less frequently.

### DISCUSSION

In the early fall of 1958 five specimens of cheese were received. These have been spoken of as Sample A cheese. There was no particular significance attached to them, and after they were examined, the cultures isolated were discarded.

Sample B Cheese came from a hospital laboratory early in November, and they were examined in the same manner. It was later learned that this group of cheese samples came from the same factory as those
in Sample A. That same week we learned of the outbreak of food poisoning in Indiana and Michigan from Wisconsin Colby-type cheese which had been produced in the same factory that supplied the cheese to the outbreak of food poisoning in Indiana and Michigan and also to California.

Retrospective inquiry revealed three family outbreaks of food poisoning (3). Three families had purchased cheese from the same country store which in turn had purchased it from the cheese factory in question: Case histories were as follows:

(a) Mr. R ate cheese at noon one day in July and by 4:30 p.m. he became ill characterized by vomiting and diarrhea. He was hospitalized 24 hours. Two days later he again ate some of the cheese in a sandwich and became ill again with milder symptoms.

(b) In August the L family purchased six pounds of cheese. Mr. and Mrs. L, their five children and the handyman, ate some of the cheese for dinner, and all eight became ill with vomiting and diarrhea occurring. Six days later Mrs. L, her daughter and the handyman again ate a small portion of the cheese, and all three became ill again.

(c) In September Mrs. S and her two children ate some of the cheese and within four hours developed the same symptoms.

By this time an investigation of this particular cheese factory was undertaken as well as the cheese produced there. Our district health officer submitted thirty-two samples of cheese which were selected at random from cheese made in this particular factory between May 15 and Nov. 6, 1958. These specimens have been referred to as Sample C. At this time milk samples from the producer farms of the cheese factory were collected with the cooperation of a representative from the Department of Agriculture.

Coagulase positive staphylococci were found among 72 of the 78 cultures isolated, and phage patterns were demonstrated in 45 of the 72 cultures. These phage reactions indicated cultures of bovine origin. Williams-Smith (9) in England found 42-D to be the most common type from milk, and Thatcher and Simon in Canada (6) found it the predominate type in butter and cheese. However, in Australia, McLean (2) showed the predominate type to be 44-A, and that was the experience of Seto and Wilson (5) in Wisconsin. These phage patterns among the milk and cheese cultures were not identical but there was a similarity. However, they did not point out the enterotoxigenic strains. Blair (1) has stated that the staphylococcus cultures from food poisoning usually fit into the lytic Group III, and this was the experience of Williams and Rippon in England (8) as well as Saint-Martin in Canada (4). However, Thatcher and Simon in their work in 1957 (7) found enterotoxigenicity in phage Groups II, III, IV and Misc., as well as among some of the non-typable cultures.

In retrospect it is likely that enterotoxigenic strains occurred among the cultures isolated from milk, and since this Colby-type cheese was made from raw milk, these cultures undoubtedly continued to produce enterotoxin. Colby-type cheese is a washed curd cheese resulting in a low-acid cure, and it has an open texture; therefore it might be a good vehicle for the maintainence of staphylococci. Furthermore, it was said that this factory had trouble with cheese starters which failed to produce their normal acidity.

Cultures were requested by the Robert A. Taft Sanitary Engineering Center, and six from Sample C were sent. Two of these six cultures, No. 17 and No. 24, were positive for enterotoxin assay by the cat procedure. Thus it was shown that there were enterotoxin producing staphylococci among the cheese cultures.

REFERENCES


