THE SIGNIFICANCE OF STAPHYLOCOCCI IN MEATS*

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The presence of staphylococci in meats and other foods continues year after year to be a major problem to food processors, handlers, and to consumers. A great number of papers have been published describing research on the micro-organism and staphylococcal enterotoxin. The fact that enterotoxigenic strains of Staphylococcus aureus are the major cause of food poisoning outbreaks in the United States seems sufficient reason for mass research efforts to better understand the characteristics and behavior of this microorganism.

My talk today is not a review of the literature nor is it an in-depth study of a single aspect of the overall problem. It is a mixture of what I feel are pertinent facts from the literature and some of my thoughts and questions on the significance of staphylococci in meats. I have also included very brief statements on enterotoxin detection.

Staphylococci are widely distributed in nature and have been found in the nasal passages and skin of about one-half of the healthy human adult population. Moore and Bower (1971) examined fifty normal healthy food handlers in fifteen public restaurants and found forty individuals harbored staphylococci in their noses and on their hands. Of the sixty-six total isolates obtained from the forty carriers, nineteen were coagulase-positive and three produced enterotoxin. Casman (1965) studied 212 strains of staphylococci obtained from noses of healthy individuals. Twenty-three percent of the isolates were enterotoxigenic. Enterotoxin A was produced by 80% of the enterotoxigenic isolates, while enterotoxins B and A and B were each produced by about 10% of the isolates. Staphylococci are also found, often in concentrated numbers, in skin lesions, boils and abscesses in both man and animal. In view of these carrier frequencies and sources of contamination, it is not surprising that staphylococci are present in meats, particularly those products which receive extensive handling during processing. And there is little doubt that so long as man is intimately involved in the manufacture of meats the staphylococci will be common contaminants of these products.

Now, any discussion on the significance of staphylococci in foods must eventually include numbers. Since I have already presented data to show the inevitability of staphylococci in meats, the question becomes, "What numbers of Staph are significant?" The standard microbiological answer is, "It depends!" It depends upon the type of product and the type of Staphylococcus.

A recent survey of fresh pork sausage (Surkiewicz et al., 1972) showed that 75% of all samples studied contained 100 or less S. aureus per gram. The highest S. aureus count obtained was 6,500 per gram. Our own surveys over the past year at a plant producing a whole-hog pork sausage has shown total staphylococci to routinely be less than 5000 per gram, while coagulase-positive staphylococci are consistently less than 100 per gram. Other surveys confirm the presence of low numbers of staphylococci in raw market meat products, so we may conclude that the presence of high numbers of this organism in fresh meats is a sign that plant sanitation is not what it should be or process controls are inadequate. Consistently high staphylococci counts in hot-boned pork for example, are usually a result of poor sanitation and/or inadequate refrigeration. There are some raw meats that frequently have high coagulase-positive staphylococci counts with no apparent relationship to processing techniques or plant sanitation. One example of this type of meat is pork head meat, e.g. cheeks and jowls. A survey conducted by our laboratories indicated this by-product could contain millions of staphylococci per gram. Casman and associates (1963) presented data that showed raw pork meat to be more conducive to enterotoxin A production than cooked pork, canned ham, and raw or cooked beef. The raw pork was handled aseptically so very few competing bacteria were present and the authors emphasized that their data was not conclusive. However, the fact that pork was incriminated in about 20% of the 1970 staphylococcal food poisoning outbreaks, twice as high as statistics for beef, may be an indication that pork is indeed an unusually susceptible meat substrate for enterotoxin production.

Cooked products such as bologna and frankfurters should be free of staphylococci or, as commonly stated, should contain less than 10 per gram. The presence of staphylococci in these products is a strong indication that there is improper cleaning of equipment or the product is coming into intimate contact with personnel after the heat treatment. Raw materials carrying staphylococci in lesions or abscesses can be a great problem even though the product is given a heat treatment and is supposed to be held under refrigeration through the marketing channels. The Morbidity and Mortality Weekly Report, Vol. 21, No. 20, describes a food poisoning incident caused by the ingestion of fried bacon. The bacon had an abscess which contained 12 million coagulase-positive staphylococci per gram. Regardless of how they got there, coagulase-positive staphylococci in precooked meat products is particularly serious since these foods are highly susceptible to consumer abuse and growth of the organism.

Some meat products are likely to contain relatively high levels of staphylococci due to the very nature of the manufacturing process. Most notable of these products are fermented meats which rely upon the growth of lactic-type organisms to produce the desired organoleptic characteristics and receive little or no heat treatment. If desirable competitors are not initially present at the millions per gram level, and if the initial coagulase-positive staphylococci level is in the vicinity of 10,000 to 100,000 per gram, there can be significant multiplication to the point that a public health problem exists.
Fortunately there are several steps that can be taken to prevent the unreasonable growth of coagulase-positive staphylococci in these products, such as high quality raw materials, chemical acidulants, and starter cultures. There are some indications that initial numbers of staphylococci in meat products which undergo fermentation are of less importance than the source of the organism. In reviewing the literature, for instance, one cannot help but notice the high frequency of staphylococci isolates from human sources that produce enterotoxin A, and the relatively low number of isolates from animal sources that yield enterotoxin A. And yet, in the United States, Staphylococcus food poisoning is most commonly caused by Type A enterotoxin. Casman (1965) studied the distribution of enterotoxins A and B in staphylococci isolated from healthy individuals and found it similar to that from strains isolated in food poisoning outbreaks. Recently, Siems et al. (1971) found that staphylococci of animal source were dominant in the raw material used in producing a fermented meat product. However, as the processing continued, the animal-specific organisms decreased while numbers of human-specific staphylococci increased. At the end of the process, the human-specific organisms completely dominated the microbial flora. Certainly more work of this nature is needed.

The second factor to be considered in judging the significance of staphylococci in meats is the type of Staphylococcus. In routine laboratory analyses, the staphylococci are separated into two groups: coagulase-positive and coagulase-negative. Coagulase is an enzyme which clots plasma and is secreted by the great majority of staphylococci which are capable of producing enterotoxin. Unfortunately, the reverse relationship, e.g. the coagulase-positive isolates which are enterotoxigenic, is not as good. Casman et al. (1967) presented data on the incidence of enterotoxin A, B, C and D production by coagulase-positive staphylococci from various sources. The percent positive ranged from a low of 2% for mastic cows, to 96% for isolates from food-poisoning outbreaks. The average incidence was about 30%. The relationship between coagulase production and enterotoxin production may improve somewhat as additional types of enterotoxin are identified and antisera are developed for their detection. Several other characteristics which have been proposed as indices of potential enterotoxigenicity have been described in an excellent review series published by Miner and Marth (1971-1972). The coagulase test, however, remains the most common screening procedure used today. A Staphylococcus isolate found positive for coagulase must also be considered capable of producing enterotoxin unless found negative in this respect by specific analysis for the toxin. And this leads us into a brief discussion of enterotoxin detection methodology.

There are at present five identified staphylococcal enterotoxins: A, B, C, D and E. Enterotoxin A accounts for the great majority of all food poisoning outbreaks in the United States that are attributable to staphylococci. Thus, in testing for enterotoxin in meats one is fairly safe in considering only enterotoxin A. However, if the presence of enterotoxin is likely and no A toxin is found, further testing for B, C, D or E obviously must be carried out.
There are two types of assays that are used in detecting enterotoxin. Prior to about ten years ago, the only method available was animal assay. The best animal subject was man himself, but success was obtained in using kittens and the rhesus monkey. The animal assay is still of value in determining emetic doses, but it has been replaced by serological assays in the analyses of foods and staphylococci isolates for enterotoxin.

Staphylococcal enterotoxins are antigenic and specific antibodies have been produced in rabbits, cats, monkeys, burros and horses. Several methods using the antigen-antibody reaction have been developed to detect specific enterotoxins. The most widely used at this time are the agar gel-diffusion precipitin methods. While these methods work quite well, they often require an extensive extraction, concentration, and purification of the enterotoxin from food materials. Based on our experience, one man using the slide gel-diffusion test can conduct about twelve assays a week on samples requiring treatment to concentrate and purify the toxin.

A second assay method we have used in our laboratory with success is the reverse passive hemagglutination test developed by Silverman (1968). The main advantage of this method is rapidity. One man can easily conduct ten assays a day using this procedure since extensive sample treatment is not required.

Other methods have been developed to detect staphylococcal enterotoxin, including a fluorescent antibody technique (Genigeorgis and Sadler, 1966a, b) and solid-phase radioimmunoassay (Johnson et al., 1971).

All of the assay methods based upon serological reactions suffer in common from one major problem. The supply of antisera used in these tests is limited. To my knowledge there are only two commercial suppliers, one in Germany and the other in Israel.

The United States Department of Agriculture has, over the last year, become extremely interested in the presence of staphylococci in meat products, particularly fermented meats. An example of their interest is a letter sent to all manufacturers of dry sausage from the Field Operations Division, Meat and Poultry Inspection Program, C&M, USDA. It requests information on ten specific points concerning dry sausage manufacture. Several of these points would, in my opinion, make challenging and highly useful subjects for M.S. and Ph.D. dissertations. I am hopeful that those of you representing the academic profession will seriously consider developing research programs around one or more of the following points:

1. A complete processing procedure for each style and type of Genoa prepared (ingredients, quantities, times and temperatures).

2. The speed of the initial pH drop and the level to which it drops.

3. The times during the procedure at which pH readings are taken and the results.
4. If growth of staphylococci occurs, the point in the process at which the number is maximum.

5. The usual maximum number for coagulase positive staphylococci.

6. The disposition of lots that exceed the usual maximum for coagulase positive staphylococci.

7. The disposition of lots in which the pH fails to drop at the usual rate.

8. The disposition of lots in which the pH fails to drop to the level specified in (2) above.

9. If rework or "backslopping" is used, the points in the processing procedure at which it is collected, it is reintroduced and into which products.

10. The warning zones, if any, and the actions taken when they are reached.

I think any academic program revolving around these ten points should include the artificial inoculation of raw materials with enterotoxin A producing cultures. The growth and toxin production should be measured against levels of staphylococci and production techniques.

In summary, staphylococci will no doubt be a common contaminant of meat and meat products for years to come. The staphylococci owe most of their eminence to the fact that a few of them can produce enterotoxin. In order to accurately assess their significance in meats and foodstuffs, we need to know more about the conditions under which staphylococci will multiply and not produce enterotoxin in these products. In my opinion, the relationship between coagulase activity and enterotoxin production is not satisfactory. A rapid screening test is needed that will more effectively determine the enterotoxin production capability of Staphylococcus isolates. Also, the current enterotoxin assay methods must be refined and simplified to the degree that they can be used routinely as quality control tools.
REFERENCES


