Bacteria in Chilled Delicatessen Foods

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MARKET OFFERINGS of chilled, ready-to-serve foods have been increasing along with the growing use by the American consumer of prepared foods in general. Because these chilled foods may be prepared by hand with no subsequent heat treatment and stored in refrigerated cabinets in open dishes, the possibility of a public health hazard seemed sufficient to warrant additional bacteriological investigation. Therefore, we undertook this investigation to find out whether selected organisms were present in chilled delicatessen-type foods offered for sale in shops in Madison, Wis., and, if so, in what numbers.

The literature concerning the microbiological quality of chilled prepared foods is limited. Shiffman and Kronick (*1*) investigated the microbiology of chilled chicken and tuna salads and concluded that the total bacterial counts and the numbers of coliforms and of staphylococci were sufficiently high to warrant a question as to the bacteriological safety of the food. Adame, Post, and Bliss (*2*) examined the sanitary quality of commercially prepared and wrapped sandwiches. The data obtained suggested that sandwiches with moist fillings were most prone to permit the growth of contaminating organisms, and these could be regarded as a potential food-poisoning hazard. Other investigators have been concerned with a comparison of specific organisms as indicators of fecal pollution (*3–6*) or with the role of psychrophilic organisms in determining food quality (*7*, *8*). No bacteriological standards for chilled prepared foods comparable to those proposed for frozen foods could be found in the literature.

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Procedure

To obtain samples of delicatessen food typical of that commonly available in the market, we chose at random a number of retail stores offering such merchandise. Each was visited on one or more occasions, and 85 samples were obtained in the manner of a usual retail purchaser. Temperature readings of each sample were made and recorded within 3 to 4 minutes of purchase time. The sample was then transported to the laboratory in an insulated bag containing cans of frozen refrigerant. Further testing was begun immediately.

The food samples purchased were classified, and the results are reported under the following groupings:

1. Protein-rich salads. In this group were chicken, ham, tuna, shrimp, egg, kidney bean, and baked bean salads.
2. Vegetable salads. This group was comprised of potato salad (mayonnaise base), German potato salad, marinated green bean salads, and coleslaw.
3. Gelatin salads incorporating only fruits or vegetables.
4. Gelatin desserts characterized by the addition of whipped cream.

To prepare the homogenate to be used for bacterial analysis, 50 grams of the food sample

were transferred by a sterile spoon into a sterile blender jar. An equal amount of 0.1 percent peptone water (*9*) was added aseptically to the jar, and the contents were homogenized at high speed for three 1-minute intervals. The pH of the food suspended in the peptone water was determined and then adjusted to 7.0±0.2 with a 1 percent solution of either sodium hydroxide or hydrochloric acid. The slurry was then placed in an ice-water bath, where it remained throughout the time when the various selective media and the tubes for establishing MPN (most probable number) counts were being inoculated.

Tryptone glucose extract agar (*A*) was used to establish a total bacterial count for each food sample. Pour plates in duplicate were made for each of several dilutions of the individual food samples. After 3 days of incubation at 35° C., colonies were enumerated and the counts recorded.

The food samples were analyzed for the MPN of coliforms (*10*); in additional tests, attempts were made to confirm the number of organisms in the original estimate which could be designated as *Escherichia coli*. For determination of the MPN coliform count, we followed the method described by Kelly (*11*), using a series of five fermentation tubes per dilution. The confirmation tests to differentiate *E. coli* were suggested by Raj and Liston (*12*) and entailed the use of selected media in the following order: E.C. medium (*A*), Levine's eosin methylene blue agar (*B*), and again E.C. medium. During the 24-hour incubation period in which the organisms were held in E.C. medium, we tried to keep the water-bath temperature at 45.5° C. Fishbein and Surkiewicz (*13*) reported that this temperature permitted greater specificity in recovering *E. coli*. Unfortunately, however, the temperature of the water bath varied on occasion by about 1° C.

For the determination of enterococci, two enumeration techniques were used—the MPN count and the direct plate method utilizing Barnes thallous acetate-tetrazolium medium (*14*, *15*). The media for the MPN determination for enterococci consisted of azide dextrose broth (*B*) with 0.003 percent bromthymol blue added, and ethyl violet azide broth (*B*) described by Raj and associates (*5*). The Barnes medium was made according to the formula he described in 1956 except that bacto peptone and bacto beef extract (*A*) replaced comparable British products. Before inoculation, the plates were surface-dried for 1 to 3 hours at 35° C. and then stored overnight. One-tenth of a milliliter of each appropriate decimal dilution of slurry was evenly spread over the agar surfaces with a sterile bent-glass rod. The plates were then incubated at 35° C. for 3 to 5 days.

To determine the portion of the enterococci MPN values which presumably was *Streptococcus faecalis*, the Barnes medium was used as a confirmatory step, as suggested by Raj and associates (*5*). In keeping with their recommendation, only colonies with a red center and white periphery, with a pink center and white periphery, or which were all pink were enumerated as *S. faecalis*.

Spread-plate techniques were used for the determination of the pseudomonads and *Staphylococcus aureus*. Before the experiment, all plates were surface-dried in the same manner as the Barnes plates. The portion of each dilution added to the plate and the manner of spreading the suspension was the same as that described for the final *S. faecalis* confirmatory test.

The medium used for detection of the pseudomonads was the one recommended by Mosurovsky and co-workers (*16*). Erythromycin and chloramphenicol were added aseptically to the medium immediately before pouring. The plates, after being spread with the food-sample dilutions, were incubated at room temperature for 3 days to 1 week.

For a direct count of *S. aureus*, the staphylococcus medium 110 (*A*), modified by the addition of sodium azide according to the method of Smuckler and Appleman (*17*) was chosen. The drop-plate technique recommended by Mallman and Broitman (*18*) was not feasible in this situation, and the spread-plate technique was used. After preparation, the plates were incubated at 35° C. and checked for growth at 24 hours and periodically thereafter for about 1 week. The white, yellow, and orange colonies were counted and recorded. At least 20 percent

Table 1. Microbial populations in chilled delicatessen foods

Organisms	Viable cells per gram (range of observed values)			
	Protein-rich salads (46 samples)	Vegetable salads (27 samples)	Gelatin salads (6 samples)	Gelatin desserts with whipped cream (6 samples)
Total	10–6, 000, 000	600–8, 350, 000	10–895, 000	10–47, 500
Pseudomonads	10–620, 000	0–1, 000, 000	10	10–3, 000
Enterococci—				
Plate count	10–3, 385, 000	0–6, 000, 000	10–129, 000	10–3, 000
MPN	20–24, 000	5–24, 000	0–24, 000	0–24, 000
Coliforms (MPN)	0–24, 000	0–24, 000	0–500	0–20
Staphylococci	10–34, 900	0–600	10–15	10–300

of the colonies were chosen from plates on which growth occurred on the selective medium, and each was streaked on a slant of tryptone glucose extract agar. The slants were incubated at 35° C. until good growth was attained and then refrigerated for future coagulase testing. Before the coagulase test, the staphylococci, which had been picked from the staphylococci count medium, were revitalized by being passed twice through tryptose phosphate broth (*A*). Chapman (*19*) reported that this broth enhanced the clotting ability of staphylococci cultures.

The 18-hour staphylococcus cultures in the tryptose phosphate broth were tested for the presence of coagulase with rabbit coagulase plasma (*A*) by the tube method. One milliliter of a 10 percent solution of the plasma in sterile physiological saline solution was layered onto 1 milliliter of the tryptose phosphate suspension. After 3 hours of incubation at 35° C. and subsequent holding on the laboratory bench overnight, those tubes with a clot formation were deemed positive.

Results

The data derived from total bacterial counts and the enumeration of selected organisms in 85 samples of delicatessen foods are presented in table 1. The range in numbers for total counts and for selected groups of organisms varied widely. The number of total viable bacteria was found to have been from 10 to 8,350,000 per gram of food sample. While the highest values for bacterial counts in individual food samples were frequently encountered among the vegetable salads, calculation of average values indicated that the protein-rich salads, as a group, supported the greatest microbial load.

The enterococci were usually found in greater numbers than the coliforms. The average MPN values for the enterococci were in the approximate magnitude of 10,000 per gram of food, whereas the average MPN values for the coliforms were about 2,500. Similar results have been reported by other investigators (*5*, *20*, *21*). The overall range of pseudomonads per gram for all foods was 10 to 1 million cells. The number of staphylococci observed in all food groups was in the range of zero to approximately 35,000 cells per gram.

Attempts were made to define that portion of total enterococci MPN value which could be regarded as *S. faecalis*. The percentage of enterococci identifiable as *S. faecalis* appeared to be consistent (table 2), 55 for the protein-rich salads and 50.3 for the vegetable salads;

Table 2. Percentage of total enterococci in delicatessen foods confirmed as *Streptococcus faecalis* [1]

Kinds of food	Number of samples	Percentage confirmed
Protein-rich salads	46	55.1
Vegetable salads	27	50.3
Gelatin salads containing fruits or vegetables	6	29.3
Gelatin desserts with whipped cream	6	27.3

[1] Barnes thallous acetate tetrazolium agar was used in the confirmation procedure.

the gelatin salads and the gelatin desserts had lower percentages, of 29 and 27, respectively.

Identification of *E. coli* from the coliform MPN counts was attempted by use of two steps of confirmation. The first step indicated that from 3 to 20 percent of the presumptive values were *E. coli;* the second confirmatory step lowered this number to less than 1 percent.

Table 3 records the results of the coagulase tests. Only 1.7 percent of the staphylococci recovered from the vegetable salads were coagulase positive. The protein-rich foods as a group contained the highest average numbers of staphylococci; 26 percent of the 244 colonies tested were coagulase positive. It should be emphasized that the average counts of staphylococci as reported in table 3 reflect only the numbers found present in the 35 food samples on which coagulase testing was done. The range of values for this organism in table 1 is derived from observation on all 85 samples.

The relationship of the pH of the samples of chilled delicatessen foods to the number of organisms was considered. As expected, a trend toward an increased number of organisms per gram of food was noted as the pH values became less acidic. At pH 5.4 and above, the numbers of staphylococci and of enterococci showed some tendency to decrease in numbers.

Table 3. Staphylococci colonies, from selected food samples, which were found to be coagulase positive

Food types	Number of samples	Total staphylococci count (average per gram of food)	Staphylococci colonies tested	
			Total number	Number coagulase positive
Total	35	--------	309	67
Protein-rich salads	27	62,340	244	64
Vegetable salads	7	850	59	1
Gelatin desserts with whipped cream	1	300	6	2

The number of organisms present in the food samples seemed to be influenced to some degree by the types of stores in which the delicatessen services were located. The average number of cells for all organisms in the chilled delicatessen-type food samples obtained from the large chain stores was lower than in foods purchased from a local chain outlet and from independent shops.

Discussion

The total count of viable aerobic bacteria is the criterion most commonly used in assessing microbiological quality. From the standards enumerated for frozen precooked foods, a total viable count of 100,000 cells per gram of food appears to be most generally indicated as acceptable and as attainable in industry when adequate processing procedures are employed (*20–24*). When the chilled delicatessen foods analyzed in our study were compared with the frozen food standards for total viable count, however, the average values in counts per gram for the gelatin salads with fruit or vegetables, for the gelatin desserts with whipped cream, and for the vegetable salads were found to be lower than the proposed standard for frozen precooked food. The protein-rich salads had average total viable counts more than six times that of the proposed standard.

Chilled chicken and tuna delicatessen salad samples obtained in Philadelphia, Pa., were reported (*1*) to have an average total viable count in the order of 10 million cells per gram, while the protein-rich salads in our study had a total count more nearly approaching 1 million cells per gram. The range of organisms per gram of food was great for both studies, but the uppermost limit for protein-rich salads in Madison was 6 million cells per gram as contrasted with 810 million cells per gram for the Philadelphia study.

In the study reported by Adame and associates (*2*), which was concerned with commercially prepared and wrapped egg and tuna sandwiches, the range of counts for total viable bacteria (5.8 to 9.0 × 10 million cells per gram) was higher than in our study. Considering the staphylococci count as well as the total count of bacteria, these investigators expressed the

opinion that the sandwiches could have presented a potential food-poisoning hazard. On the basis of their standards, the average values for the protein-rich salads analyzed in our study would also qualify as being potentially hazardous.

This situation suggests the advisability of considering bacterial standards for delicatessen-type foods. Should the trend toward greater production and use of chilled prepared foods continue, as seems likely, the need for bacteriological control would seem urgent.

In the various food groups, different organisms appeared to have been predominant (table 1). Foods high in protein and the vegetable salads favored the growth of staphylococci, enterococci, and pseudomonads. Except for the enterococci, the gelatin salads exhibited very low average numbers of organisms for all species studied. In addition to a low concentration of nutrients in the gelatin salads, the low pH values (ranging from 3.0 to 3.9) may have prevented the growth of many bacteria. The reason for the relatively high numbers of enterococci in the gelatin salads is not clear. When whipped cream was added to the gelatin, some difference in the bacterial flora was observed.

Before the experiment proper, preliminary tests were conducted to determine the degree of selectivity of the Barnes medium (*14*, *15*) when foods were present as a significant part of the inoculum. After being inoculated in this manner, many replicates of each different type of colony growing in the medium were tested with several biochemical reactions characteristic of *S. faecalis*. These identification tests revealed that when the selective medium was used with food, it had a 72 percent accuracy in detection of *S. faecalis* colonies.

By the plate count method, enterococci were usually found to be present in far greater numbers than by the MPN technique. There apparently was little correlation between the results of the two methods. Although the medium proposed by Barnes (*14*, *15*) contained the inhibiting agent thallous acetate, when used for a direct plate count, it seemed not to permit a selectivity as great as that of the MPN technique. Raj and Liston (*5*) reported that they found the presumptive and confirmatory tests as we used them to be specific for the detection and enumeration of enterococci in frozen seafoods.

The greatest number of pseudomonads were found in the protein-rich salads and in the potato salads. At no time did the numbers reach a slime- or odor-producing level. A preliminary study similar to the one used to test the accuracy of the isolation medium for the identification of *S. faecalis* was carried out for the pseudomonads. The selective medium used to identify and enumerate pseudomonads present in food samples was found to have an approximate accuracy of 80 percent.

In our study, the staphylococcal count was very low for most of the food groups examined, the one exception being the protein-rich foods. A possible explanation may be the competition offered by the enterococci and the coliforms. A ratio of other microbial species acting as inhibitors to staphylococci of 100 to 1 was found to have been effective for preventing the attainment of hazardous numbers of staphylococci (*25*, *26*). In most of the food groups, except the protein-rich ones, this ratio was attained. Lactic streptococci have been reported to inhibit the growth of *S. aureus* by depletion of a vital nutrient (*27*). From the consistently high enterococci counts it appeared likely that lactic streptococci were present in many foods tested. Furthermore, all of the food groups had been held in the stores at temperatures of 15° C. or less, the temperature range found most effective in inhibiting staphylococci (*25*, *28*).

Like Iandolo and associates (*27*), we noted the stimulating effect on the growth of staphylococci of an environment in which the pH was approximately 5.2.

Shiffman and Kronick (*1*) found a much smaller population of staphylococci in protein-rich foods (delicatessen chicken and tuna salads) than we did. The mean values were 160 cells per gram for the chicken salads and 510 for the tuna salads. With an average staphylococci count double the highest values that Shiffman and Kronick found, the protein-rich foods examined in our investigation could conceivably be a public health problem. An average of 26 percent coagulase-positive staphylococci colonies in salads is even more convincing evidence of a possible sanitary problem presented by the high-protein foods.

Summary

The numbers of selected organisms in 85 samples of chilled delicatessen foods purchased in the Madison, Wis., area were determined. Protein-rich salads were found to contain relatively high numbers of total viable bacteria, enterococci, pseudomonads, and staphylococci. More than one-quarter of the staphylococci colonies tested were coagulase positive. The vegetable salads contained considerable numbers of pseudomonads, enterococci, and total bacteria. The staphylococci count in the vegetable salads was low; only 1.7 percent of the colonies tested were coagulase positive. The gelatin salads and gelatin desserts had low counts for all groups of organisms studied except the enterococci.

The type of retail store appeared to influence the size of bacterial populations in the foods offered for sale. Samples from large chain stores showed a lower average number of cells for all organisms than samples which came from a local chain outlet or from independent shops.

In the delicatessen foods, the enterococci were consistently present and in greater numbers than the coliforms. Confirmatory steps were used to distinguish *Streptococcus faecalis* and *Escherichia coli* from their respective groups. *S. faecalis* was identified in 27 to 55 percent of the significant dilutions of tubes used to determine the enterococci MPN value. Results of attempts to identify *E. coli* through the use of additional confirmatory media indicated values much lower than the original coliform MPN values.

The study results suggest the need for bacterial standards for chilled, commercially prepared delicatessen foods.

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(A) Difco Laboratories, Detroit 7, Mich.: Coagulase plasma, tryptone glucose extract agar, E.C. medium, staphylococcus medium 110, lauryl tryptase broth, tryptase phosphate broth, bacto peptone, and bacto beef extract.
(B) Baltimore Biological Laboratories, Baltimore, Md.: Levine's eosin methylene blue, azide dextrose broth, and ethyl violet azide broth.

Effectiveness of Veterinary Drugs

The National Academy of Sciences-National Research Council is evaluating the effectiveness of approximately 1,100 veterinary drugs introduced on the market between 1938 and 1962. Undertaken at the request of the Food and Drug Administration, this new study is conducted along lines established for a massive National Research Council study, now in progress for the Food and Drug Administration, of the medical effectiveness of drugs for human use.

The veterinary drug study was prompted by the 1962 Kefauver-Harris Amendments to the Federal Food, Drug, and Cosmetic Act which authorize evaluation of the efficacy of drugs marketed between 1938 and 1962, when only proof of safety was required. In the amendments no distinction is made between drugs intended for human use and veterinary drugs.

The study was organized by the Agricultural Board of the National Research Council's Division of Biology and Agriculture, and it is directed by a 12-member committee under the chairmanship of Dr. C. M. Stowe, professor of veterinary medicine, University of Minnesota.

The committee has established 12 categories of drug usage for grouping drugs identified by the Food and Drug Administration for review. The categories are (1) antihelminthic, (2) antibacterial, (3) antiprotozoan, (4) cardiac, hematologic, and renal, (5) dermatologic, (6) endocrinic, (7) gastroenteric-respiratory, (8) genital-mammary, (9) insecticidal, (10) metabolic, (11) neural, and (12) ophthalmic.

Each drug is to be evaluated on the basis of information obtained from the scientific literature, records of the Food and Drug Administration, data submitted by the drug manufacturer to the Food and Drug Administration, and other appropriate sources.

In judging the validity of therapeutic claims on drug labels, the reviewers will first classify the drugs in broad categories ranging from clearly effective to clearly ineffective, corresponding to the criteria adopted for the larger study of drugs used in human medicine. After the initial classification, the reviewers will seek additional data on those drugs in the intermediate categories in order to classify as many as possible as either effective or ineffective.

Program Notes

Children in Need of Medical Care

The Philadelphia School Board, under a Public Health Service contract, is establishing a program to bring school children with known ailments or defects in contact with medical resources.

Under the contract, the board will add a nursing superintendent and other nurses to its school health program in one school district. These nurses are to implement a system for identifying children who have not had their detected disorders attended to. They will counsel parents and conduct followup checks to see if treatment was obtained.

Adolescents in Mental Hospitals

More than 400 adolescents (12–17 years) were treated last year in Maryland's mental hospitals. This number is equal to 1 per 1,000 Maryland residents in this age group.

Half of the adolescents were diagnosed as having transient situational personality disturbances; schizophrenia was diagnosed in one of six; personality disorders were diagnosed in one of eight.

A shortage of residential beds for this age group meant that the majority had to be treated on the same wards with other patients.

Improved Blood Test for Rubella

A new, highly sensitive, rapid, and reliable test for the presence of rubella (German measles) antibody is now part of the routine examination of all blood specimens from pregnant women at the Maryland State Department of Health's bureau of laboratories.

Scientists in the Division of Biologics Standards at the National Institutes of Health developed the new procedure, known as the hemagglutination inhibition test. It was recently released to all State laboratories through the Public Health Service.

The new procedure takes only a few hours, as compared to the 2 weeks required for a previous method of comparable accuracy. It is also able to demonstrate immunity acquired many years previously. When rubella is suspected, two tests 10 days apart will show whether antibodies represent old immunity or active disease.

Behavior of Tuberculosis Patients

The National Tuberculosis Association will conduct studies on the behavioral characteristics of tuberculosis patients, using representative samples in Baltimore, Md., St. Louis, Mo., and San Francisco, Calif.

Dr. Robert E. Farber, Commissioner of Health of Baltimore, in announcing the inclusion of his city, expressed the hope that the studies "will help us remove the barriers between patients and clinics and reduce the present significant extent of noncooperation by tuberculosis sufferers . . ."

Fluoridation Saved Parents Money

Since 1956, Chicago's flouridated water supply has saved parents more than $2 million in dental costs for children under age 14, Dr. John G. Bergman, city dental director, has estimated.

The Chicago Board of Health announced results of 10 years of fluoridation. A survey of 12,000 children in the first and fifth grades revealed a 67 percent reduction in tooth decay, the board reported.

The fluoridation process now takes place in one of the world's most modern water filtration plants. It costs each Chicagoan 12.8 cents annually. Operating expenses are offset by purchase of fluoridated water by 58 suburbs.

Arizona's Flying Psychiatrist

The community guidance clinic serving the Verde Valley of Arizona has as its medical director, Dr. Maier Tuchler, a psychiatrist, who flys one Saturday a month 90-odd miles to Cottonwood, Ariz. William Peckham, the executive director, drives 50 miles. Dr. Richard Parry, a psychologist, drives about 40 miles.

The people to whom these men and other staff members supply counseling and other services include children referred by the schools and courts and persons sent by welfare workers, ministers, and family physicians. An increasing number of clients also come on their own.

The clinic, now more than a year old,was financed in its first year by donations from private citizens, business and civic organizations, and a grant from a private foundation. Even so, according to Sam F. Ciulla, editor of *Arizona's Health*, it could not have kept its doors open without additional help from the Arizona State Department of Health.

Abnormalities After Age 40

Half of the U.S. Senate employees over 40 years of age turned up with one or more abnormalities when screened by the mobile unit of the District of Columbia Department of Health in September 1966.

Among menial employees at other Government agencies and among the poor and unemployed, three of every four persons over 40 who have been tested had it least one abnormality.

Health department officials try to encourage better participation of the poor by locating the mobile unit in poor neighborhoods, advertising at the U.S. Employment Service office, and providing free buses from public housing projects for the aged.

Since the health department instituted the screening program in 1963, as a free service for District residents over 40 years of age, 45,000 persons have been tested. Results are sent to the patient's physician or to the health department clinic.—*Washington Post*, February 26, 1967.

Items for this page: Health departments, health agencies, and others are invited to share their program successes with others by contributing items for brief mention on this page. Flag them for "Program Notes" and address as indicated in masthead.
