Subcutaneous Bacteria in Turkey Carcasses

JOHN S. AVENS AND BYRON F. MILLER

Department of Animal Sciences, Colorado State University, Fort Collins, Colorado 80521

Received for publication 25 October 1972

Two methods were employed to quantitate the subcutaneous bacteria in fresh, refrigerated, and frozen turkey carcasses. Relatively few bacteria were detected in the skin-flesh interface and in the flesh as compared with the number of bacteria on the skin surface and in the skin layer. No subcutaneous bacteria were detected in 49% of the skin-flesh interface and flesh samples. The number of bacteria detected in skin samples from carcasses chemically disinfected to kill skin surface bacteria was smaller than that in nondisinfected skin samples. These results indicate that the skin blending method used to quantify microorganisms on poultry carcass skin measures the skin layer flora and that the number of subcutaneous membrane or flesh bacteria measured is not normally large enough to have a significant influence on the results.

The flesh of a live animal is essentially sterile; however, during processing, bacteria on the skin surface may contaminate the flesh and skin membrane through severed blood vessels or skin cuts and tears. It has been suggested that most bacterial growth is confined to the skin surface of dressed and eviscerated poultry and that very few bacteria are present in the adjoining flesh (3, 6). A review of live human skin structure and its bacterial flora by Price (5) further supports the above assumptions. Several changes occur in skin after death, however, which could affect its function as a barrier to bacterial migration into the inner tissues. The extent of such changes partially depends on the elapsed time after death, the carcass temperature, and the way the carcass was handled.

The presence of a significant number of viable bacteria beneath the skin of processed poultry carcasses would be of concern for a number of reasons. It might indicate bacterial migration through the skin enhanced by time-temperature abuse or unsanitary processing. Subcutaneous bacteria in poultry meat might present a potential public health hazard and shorten the shelf-life of the product. Methods employed to reduce the skin surface bacteria level during processing might not necessarily affect subcutaneous bacteria. Analytical methods based on quantifying skin surface bacteria by blending and plating skin samples (1, 2) might be including a significant number of subcutaneous bacteria in the skin "surface" count. This last possibility was what prompted the study reported herein.

It has been reported that a skin sample "blending" method for quantifying the estimated bacterial population on poultry carcass skin yields significantly higher counts than the "cotton swabbing" technique (1, 4) or the "carcass rinse" technique (4). However, if a significant number of subcutaneous bacteria were present, the skin "blending" method (2) would reflect both surface and subcutaneous bacteria, whereas the "swab" and "rinse" methods would enumerate primarily surface bacteria. Therefore, it was necessary to determine the relative numbers of subcutaneous bacteria in freshly eviscerated, refrigerated, and frozen-thawed poultry carcasses as compared with the numbers of bacteria in the skin tissue and on the skin surface.

MATERIALS AND METHODS

Two methods were used to determine subcutaneous bacteria in turkey carcasses. In one method, the right half of the carcass was chemically disinfected by swabbing the skin with 5% phenol for 5 min followed successively by 70% ethyl alcohol, 2.5% sodium hypochlorite, and finally with 70% ethyl alcohol again, each for approximately 1 min. The final application of ethyl alcohol was allowed to evaporate. Skin samples were cut on this half with a sterile brass cutting tool (2.54 cm in diameter), removed from the carcass with sterile forceps (scissors were used to facilitate removal), and homogenized in a Waring blender containing 100 ml of

1Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Series Paper No. 1904.
Butterfield’s buffered phosphate diluent for 2 min. Three skin samples were removed from the breast and three from the leg. The skin homogenate was plated in plate count agar (Difco). Flesh samples, 1.27 cm in diameter and approximately 1.27 cm deep, were cut below each skin sample that was removed from the disinfected side. These flesh samples were removed, blended, and plated in a similar manner. Carcasses were from three different treatment groups: freshly eviscerated, refrigerated (4 C) for 7 days, or frozen (−29 C) and thawed (8 C for 16 h). Six skin and six flesh samples were removed from the disinfected side from each of duplicate carcasses from each treatment group. Thus, a total of 12 skin and 12 flesh samples were taken from the disinfected side, per carcass group.

Six skin samples were removed from the nondisinfected half of each carcass in a similar way. These samples were taken immediately after the samples from the disinfected side of any one carcass had been plated. They were blended and plated as previously described to quantitate the bacteria in the skin tissue. Flesh samples were also removed, blended, and plated as described in the previous paragraph. All plates were incubated at 37 C for 48 h and at 20 to 25 C for 48 h before colonies were counted.

The second method used to determine subcutaneous bacteria involved cutting and lifting a portion of carcass skin and swabbing the skin-flesh interface. The skin in the region of the incision was disinfected with 70% ethyl alcohol, which was allowed to evaporate. A V-shaped incision approximately 2.54 cm long was made in the skin with a sterile scalpel. The skin was pulled away from the flesh with sterile forceps. One sterile calcium alginate swab (Calgiswab; Consolidated Laboratories, Inc.) was moistened with one-fourth strength Ringer solution containing 1% Calgon (Calgon Consumer Products Company, Inc.) and was used to puncture the skin membrane and swab the skin-flesh interface. An area of approximately 1 cm² was swabbed. The swab was then agitated until dissolved in 10 ml of the modified Ringer solution. All 10 ml of the Ringer solution with the dissolved swab was plated (three plates) in plate count agar (Difco). Plates were incubated at 37 C for 62 h before colonies were counted. A total of 60 samples was taken; 10 samples were removed from each of the duplicate carcasses for each treatment condition. Bacterial counts per square centimeter of skin surface were estimated on each carcass by the same “swab” method.

All turkey carcasses used in this study were processed by conventional methods in a laboratory pilot plant. For reasons of availability, male turkey carcasses were used with the first method and female turkey carcasses were used with the second method.

RESULTS

The ratios of flesh samples yielding any viable bacteria to the number of samples taken by the first sampling method are presented in Table 1, as are the average aerobic plate counts per square centimeter in the flesh samples. Average aerobic plate counts per square centimeter of skin are shown for comparison. Among the 36 flesh samples from the disinfected sides of the turkeys, 27 were positive for bacteria; 24 of 36 from the nondisinfected sides were positive. The replicate averages of the flesh sample aerobic plate counts per square centimeter from fresh, refrigerated, and frozen-thawed carcasses were 10, 36, and 280, respectively, on the nondisinfected side, and 32, 150, and 35, respectively, on the disinfected side. The “disinfected” skin layer of the carcasses yielded replicate average aerobic plate counts per square centimeter of 160, 290, and 110 for fresh, refrigerated, and frozen-thawed carcasses, respectively.

The ratios of skin-flesh interface swab samples yielding viable bacteria to the number of samples taken are presented in Table 2, as are the average aerobic plate counts per sample. The average aerobic plate counts per square centimeter of skin surface determined by swabbing are shown for comparison. No bacteria were isolated from 44 of 60 skin-flesh interface swabblings. Each of the three carcass conditions averaged fewer than 10 bacteria per swab sample. The replicate averages of the skin surface aerobic plate counts per square centimeter from fresh, refrigerated, and frozen-thawed carcasses were 4,000, 11,000, and 1,100, respectively.

Table 1. Skin layer and flesh bacteria counted from untreated and disinfected sides of freshly eviscerated, refrigerated (4 C, 7 days), and frozen (−29 C)-thawed (8 C, 16 h) turkey carcasses

| Carcass treatment | Skin surface treatment | Sample | Fraction positive | Avg APC/cm² * |
|-------------------|-----------------------|--------|------------------|---------------|
| Fresh             | Untreated             | Skin   | 3/12             | 3,500         |
|                   |                       | Flesh  | 10               |               |
| Disinfected       |                       | Skin   | 8/12             | 180           |
|                   |                       | Flesh  | 32               |               |
| Refrigerated      | Untreated             | Skin   | 12/12            | 1,400         |
|                   |                       | Flesh  | 36               |               |
| Disinfected       |                       | Skin   | 10/12            | 290           |
|                   |                       | Flesh  | 150              |               |
| Frozen-thawed     | Untreated             | Skin   | 9/12             | 1,300         |
|                   |                       | Flesh  | 290              |               |
| Disinfected       |                       | Skin   | 9/12             | 110           |
|                   |                       | Flesh  | 35               |               |

* Fraction of flesh samples that were positive for bacteria; (number of samples from which bacteria were isolated)/(total samples).

APC = aerobic plate count; values recorded are the arithmetic means of the six samples taken from each carcass side, averaged over two replicate carcasses.
TABLE 2. Skin surface and skin-flesh interface bacteria counted from freshly eviscerated, refrigerated (4 C, 7 days) and frozen (-29 C)-thawed (8 C, 16 h) turkey carcasses

| Carcass treatment | Swab sample | Fraction positive* | Avg APC/cm** |
|-------------------|-------------|--------------------|--------------|
| Fresh             | Skin surface Interface | 8/20                | 4,000 7      |
| Refrigerated      | Skin surface Interface | 5/20                | 11,000 6     |
| Frozen-thawed     | Skin surface Interface | 3/20                | 1,100 1      |

*Fraction of interface samples that were positive for bacteria; (number of samples from which bacteria were isolated)/(total samples).

**APC = aerobic plate count; interface values recorded are the arithmetic means of the 10 samples taken from each carcass, averaged over two replicate carcasses.

DISCUSSION

By the skin blending method used (100 ml of blending fluid) and the agar plate count technique, 100 bacteria per square centimeter of skin is the lowest number that can be determined with any accuracy. Aerobic plate counts are only reported to an accuracy of the first two left-hand digits of the calculated average count. Aerobic plate counts greater than 1,000/ square centimeter of skin, determined by blending the skin, would be very slightly, if at all, affected by fewer than 100 subcutaneous bacteria per square centimeter.

When one considers the possible sources of contamination of subcutaneous tissue during sampling, the data indicate that there were very few bacteria below the skin tissue in the fresh, refrigerated, or frozen-thawed turkey carcasses tested. There is, however, a possibility that bacteria are firmly imbedded in the rough skin surface or perhaps just beneath the surface layer. Even if this is true, it would be desirable to include them as part of the carcass bacteria count.

It appears that subcutaneous bacteria are negligible compared to the level of skin surface bacteria present in fresh, refrigerated, or frozen turkey carcasses. Any significant number of bacteria in the flesh of a turkey carcass would indicate severe time-temperature abuse or unusual contamination through skin cuts and tears, or both. Since turkey carcass skin seems to be a rather effective barrier to bacterial migration into the tissues, an intact eviscerated carcass would be expected to have a longer shelf life than carcass parts or a carcass with large skin cuts or tears.

The results of this study indicate that with freshly eviscerated, refrigerated, or frozen-thawed turkey carcasses, skin “surface” bacteria counts determined by blending skin samples will not be significantly affected by subcutaneous membrane or flesh bacteria. Skin “surface” bacteria counts are probably only slightly affected, if at all, by subsurface bacteria in the skin layer.

LITERATURE CITED

1. Avens, J. S., and B. F. Miller. 1970. Quantifying bacteria on poultry carcass skin. Poultry Sci. 49:1309-1315.
2. Avens, J. S., and B. F. Miller. 1970. Optimum skin blending method for quantifying poultry carcass bacteria. Appl. Microbiol. 20:129-132.
3. Frazier, W. C. 1967. Food microbiology, p. 311. McGraw-Hill Book Co., Inc., New York.
4. Fromm, D. 1959. An evaluation of techniques commonly used to quantitatively determine the bacterial population of chicken carcasses. Poultry Sci. 38:887-893.
5. Price, P. B. 1957. Surgical antiseptics, p. 399-421. In G. F. Reddish (ed.), Antiseptics, disinfectants, fungicides, and chemical and physical sterilization, 2nd ed. Lea & Febiger, Philadelphia.
6. Sharf, J. M. (ed.). 1966. Recommended methods for the microbiological examination of foods, 2nd ed., p. 119-125. American Public Health Association, Inc., New York.