Bruised Poultry Tissue as a Possible Source of Staphylococcal Infection

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Bacteriological analyses were made on 45 swab samples secured from hands of poultry workers on processing line, on 31 bruised and 15 normal poultry tissue samples, and on 15 swabs obtained from infected lacerations and exudates of abcesses on hands, arms, chest, and abdomen of poultry workers. A total of 170 Staphylococcus cultures were isolated from samples examined. These cultures were characterized morphologically and biochemically and then grouped into six distinct patterns. S. aureus was found in 86.6% of swab samples obtained from infected workers, in 40% of swabs from hands of workers who handle bruised birds, and in 38.7% of bruised tissues, and was absent from all samples obtained from hands of workers who do not handle bruised birds. All the coagulase-positive staphylococcal isolates were bacteriophage-typed, and the results showed that the same phage-type S. aureus was found in many poultry bruises and in infected lesions of poultry workers as well as on hands of workers who handle bruised birds. These results indicate that poultry bruises are a source of staphylococcal infection encountered among poultry workers.

Microbiological examination of poultry bruises by McCarthy et al. (10) revealed that these tissues harbor large numbers of various types of bacteria, particularly Staphylococcus aureus. It was also reported that bruised tissue supported and stimulated the growth of Escherichia coli and S. aureus in vivo (5, 6). The latter organism was able to persist in injured tissues for 18 days, even in the absence of noticeable infection (5). Although higher incidence of staphylococcal infections occurred in the wounds of poultry workers in the Clarke County areas as compared to the population at large (5), no epidemiological studies had been conducted to ascertain a definite source. These infections appeared among the employees of the poultry processing plants who came in contact with live chickens and carcasses during the initial stages of dressing. To substantiate the existence of a health hazard to these workers, the log book kept by the company nurse at a large poultry processing plant in Clarke County was examined. Analyses of 252 complaints recorded over a 4-day working period revealed that requests for treatment of staphylococcal infections occurred 35 times during this interval (Table 1). The majority of the infections were characterized as "oozing sores" resulting from cuts and scratches that became infected and failed to heal properly. These areas were most prevalent on the hands, arms, chest, and abdomen of poultry workers who came in contact with live chickens, particularly during the initial stages of dressing, thus confirming previous observations (5). Therefore, the present investigation was designed to examine the role of poultry bruises as a possible source of the staphylococcal infection encountered among poultry workers. This problem causes economic losses to poultry industry in terms of decreased manpower production as well as presenting a possible health hazard.

MATERIALS AND METHODS

Collection of samples. Forty-five hand swabs were collected on sterile cotton-stick applicators, and each was placed in a tube containing 10 ml of sterile saline. Thirty of these hand swabs were taken from processing line workers (trimmers) who handle the carcass birds prior to trimming of bruises and 15 hand swabs were obtained from those who handle normal healthy birds as well as birds that were trimmed of either bruised tissues or condemned parts of the carcass. The hands (back and palm) were swabbed thoroughly, including the fingernails and between the fingers. Fifteen other swab samples, obtained from sores, abcesses, and infected lacerations and lesions, were collected by the company
nurse. All samples were immediately cooled and subjected to bacterial analyses within 1 hr after collection.

Poultry tissues were obtained from individual birds that were removed from the processing line at trimming stations. The skin over the tissue (normal or bruised) was swabbed with 70% alcohol, and the desired tissues were excised under aseptic conditions and immediately placed in sterile precooled petri dishes. Thirty-one bruised and 15 normal (control) tissue samples were collected and held at 2 to 4 C to prevent further growth of the organisms present. Each tissue sample was then aseptically minced and thoroughly homogenized (16,000 rev/min) with saline for 2 min in a precooled sterile Sorvall Omnimixer.

Isolation and screening. Dilution measurements and bacterial isolation were executed by using standard bacteriological procedures. All tissue homogenates and hand swab samples were analyzed for both aerobic bacterial counts and staphylococcal population. The former was obtained by plating the proper dilution on tryptone-glucose extract-agar (TGEA), and the latter was made by plating on mannitol-salt-agar (MSA). Colonies appearing circular (1 to 2 mm in diameter), smooth, convex, glistening at the surface, with the entire edge surrounded by yellow or red zones on the MSA and exhibiting the morphological characteristics of staphylococci were examined and counted. Examination of Gram-stained smears from suspected or typical colonies was always conducted to aid in the differentiation between *Staphylococcus* and *Micrococcus*. Enrichment techniques were employed to detect the presence of small numbers of staphylococci in samples. In this technique, 1 ml of saline suspension of the hand swabs or the swabs of the infected lacerations or lesions was inoculated into mannitol-salt broth containing 7.5% NaCl (to enrich selectively the staphylococci), and the tubes were incubated for 24 hr at 37 C. Growth from mannitol-salt broth was streaked onto MSA plates. The colonies on the plates were examined after incubation for 24 to 48 hr, and each staphylococcus isolate was again subcultured and stained (Gram-stained smears) to assure purity. All cultures were carried on TGEA slants and stored at refrigerator temperature until further analyses.

The staphylococcal isolates were characterized by colony pigmentation on *Staphylococcus* medium 110 (Difco) after incubation at room temperature for 48 hr, by mannitol fermentation (anaerobically), by coagulase, lysozyme, deoxyribonuclease, gelatinase, and alpha hemolysin activities, and by bacteriophage typing. Mannitol fermentation was performed by stabbing freshly heated and cooled phenol red mannitol-agar tubes and observing for color changes after 12 and 18 hr of incubation at 37 C. Determination of deoxyribonuclease was conducted on deoxyribonuclease test agar (Difco) by using the technique of Jefries et al. (9). After incubation of the inoculated plates for 24 hr at 37 C, the plates were flooded with 1 N HCl and a clear zone (3 to 5 mm in diameter) was considered positive for deoxyribonuclease. The coagulase tube test was performed with reconstituted coagulase plasma (Difco) on all gram-positive staphylococci isolated and the tubes examined after 4 hr of incubation at 37 C. Any degree of clotting, however slight, was considered positive. Liquefaction of gelatin was tested by inoculating Chapman stone medium and examining for clear zones surrounding colonies after 48 hr of incubation at 30 C. The alpha hemolysin was detected by using rabbit blood-agar plates containing 2% blood. Lysozyme activity was determined by the plate method using a modified procedure previously reported by Grossgebauger et al. (4) and Jay (8). A 100-ml suspension of lyophilized cells of *M. lysodeikticus* (1 mg/ml) in 0.06 M phosphate buffer (pH 6.2), and 400 ml of Brain Heart Infusion agar (Difco) were prepared. The agar and suspensions were autoclaved separately at 15 psi for 15 min, cooled to 55 C, immediately mixed, and poured into sterile petri dishes. The plates were then inoculated with the test organism and incubated for 24 hr at 37 C. A clear zone (5 to 10 mm in diameter) surrounding the growth was considered positive for lysozyme activity.

**Bacteriophage typing.** Coagulase-positive staphylococci were phage-tested by the method recommended by Blair and Williams (2) as modified by Blair and Parker (1). Each culture was streaked onto a Trypticase soy agar (TSA) plate and incubated overnight to check purity. A typical colony was then picked into Trypticase soy broth and incubated at 37 C for 4 to 5 hr. The broth culture was then spread over the surface of a sterile TSA plate and excess broth was removed. After the surface had dried, one drop of each phage at its routine test dilution (RTD) was placed on the seeded plate in a standard pattern. The plates were dried again (at room temperature) and then incubated overnight at 30 C. They were examined for lysed areas, and lytic reactions of 50 or more plaques were recorded. Weaker reactions were not reported unless they were contributory to establishing identity of patterns. If a culture was not lysed by RTD phage, it was retested with phages at 1,000 X RTD. Only strong (50 plaques or more) reactions were recorded. Phage patterns which differed by two or more strong lytic reactions were generally considered as different patterns. Phage typing was performed by P. B. Smith (Center for Disease Control, Atlanta, Ga.).

**RESULTS AND DISCUSSION**

A total of 170 cultures was isolated from the
106 samples collected. These cultures were characterized for colony pigmentation and other biochemical activities and then grouped into 6 distinct patterns (Table 2). Isolates exhibiting the reaction patterns of types 1 and 4 (42 cultures, 24.7% of total) represent S. aureus; types 2, 3, and 5 are characteristics of various strains of S. epidermidis (120 cultures, 70.6% of total); type 6 (8 cultures, 4.7% of total) may be considered a potentially pathogenic strain of S. epidermidis. Bronson (3), Grossgebauer et al. (4), and Hawiger (7) reported that coagulase-negative staphylococci which produced lysozyme were considered pathogens and are able to cause infection. Table 3 summarizes the results of bacterial counts (on TGEA and MSA) for all samples except those obtained from sores, abscesses, and infected lacerations. The data revealed that these samples contained high bacterial populations, the majority of which were salt-tolerant as indicated by the viable counts on MSA plates. McCarthy et al. (10) showed that bruised tissues, secured randomly from broilers on a commercial processing line and from experimentally inflicted bruises, contained a microbial count relatively high both aerobically and anaerobically compared to control samples. Many gram-positive micrococci were encountered on these plates. However, the predominant staphylococcal organism isolated from all samples (pattern 3) had white-pigmented colonies on staphylococcal medium 110, had no alpha hemolysin, did not ferment mannitol anaerobically, and exhibited no enzyme activities tested except deoxyribonuclease. These bacteria were found in 55 samples (51.9% of total) and are not considered

**Table 2. Reaction patterns of 170 isolates: their colony pigmentation, biochemical characteristics, and incidence**

| Reaction pattern | No. of isolates | Pigment* | Mannitol fermentation | Alpha hemolysin | Coagulase | Enzyme activities | Incidence* (%) |
|------------------|----------------|----------|----------------------|-----------------|-----------|-------------------|----------------|
|                  |                |          |                      |                 |           | Deoxy-ribonuclease |                |
|                  |                |          |                      |                 |           | Gellanase          |                |
|                  |                |          |                      |                 |           | Lysozyme          |                |
| 1                | 36             | G        | +                    | +               | +         | +                 | 21.2           |
| 2                | 35             | W        | -                    | -               | -         | -                 | 20.6           |
| 3                | 76             | W        | -                    | -               | +         | -                 | 44.7           |
| 4                | 6              | G        | +                    | -               | +         | +                 | 3.5            |
| 5                | 9              | W        | -                    | +               | -         | -                 | 5.3            |
| 6                | 8              | W        | -                    | -               | +         | +                 | 4.7            |

*G, golden; W, white.
* Based on reactions in this table.

**Table 3. Average aerobic bacterial counts (on TGEA and MSA), frequency, and incidence of various staphylococcal patterns in samples**

| Source of sample | No. of samples | Viable counts | No. of samples with pathogenic staphylococci | No. of samples containing pattern |
|------------------|----------------|---------------|---------------------------------------------|----------------------------------|
|                  |                | TGEA*         | MSA                                         | 1*                              |
|                  |                |               |                                             | 2                               |
|                  |                |               |                                             | 3                               |
|                  |                |               |                                             | 4*                              |
|                  |                |               |                                             | 5                               |
|                  |                |               |                                             | 6*                              |
| Hand swabs       |                | 75.55         | 4.49                                        | 12                              |
|                  |                | 4.96          | 4.05                                        | 0                               |
|                  |                |               |                                             | 5                               |
|                  |                |               |                                             | 11                              |
|                  |                |               |                                             | 10                              |
| Tissue           |                | 4.57          | 3.55                                        | 12                              |
|                  |                | 4.46          | 3.92                                        | 3                               |
|                  |                |               |                                             | 1                               |
|                  |                |               |                                             | 10                              |
| Infections       |                |               |                                             | 13                              |
|                  |                | 4.05          | 3.55                                        | 12                              |
|                  |                | 4.46          | 3.92                                        | 3                               |
|                  |                |               |                                             | 1                               |
|                  |                |               |                                             | 10                              |
| Total            |                | 75.55         | 4.49                                        | 12                              |
|                  |                | 4.96          | 4.05                                        | 0                               |
|                  |                |               |                                             | 5                               |
|                  |                |               |                                             | 11                              |
|                  |                |               |                                             | 10                              |

* TGEA, tryptone-glucose extract-agar; MSA, mannitol-salt-agar.
* Pathogenic staphylococci.
* Log no./hand swab.
* Log no./g of tissue.
* From symmetrically located areas of the bruises on the same bird.
* Samples were not assayed.
pathogens. The only other bacterium found in considerable numbers was a large gram-positive Bacillus which was encountered in eight of the samples examined. On the other hand, it was observed that 40 samples (37.7% of the 106 samples) contained large populations of pathogenic staphylococci (patterns 1, 4, and 6). Twenty-eight (70%) of these pathogenic were S. aureus cultures which exhibited golden-pigmented colonies, fermented mannitol, and produced alpha hemolysin, coagulase, deoxyribonuclease, gelatinase, and lysozyme (pattern 1). It is of interest that pathogenic staphylococci (patterns 1, 4, and 6) were consistently isolated from 13 swab samples (86.6%) obtained from infected lacerations, abscesses, and sores, from 12 hand swab samples (40%) secured from workers who handle bruised birds and from 12 bruised tissue samples (38.7%). They were found in only 20% of samples obtained from normal tissue located symmetrically to the bruise on the same bird. None of the hand swab samples obtained from poultry workers who did not handle bruised birds contained staphylococcal organisms with the aforementioned characteristics. These results indicated that pathogenic staphylococci designated as patterns 1, 4, and 6 could be transmitted from bruised tissue to poultry workers who handle bruised birds and possibly to other healthy birds.

To substantiate these findings, all the coagulase-positive staphylococcal isolates were phage-typed, and the results are recorded in Table 4. It was observed that three of the isolates obtained from poultry workers with infected lacerations, sores, and abscesses were of the same phage type as nine cultures isolated from hand swabs of poultry workers who handle bruised birds. The same phage-type strains were noted in six S. aureus cultures secured from bruised tissue and from one normal tissue sample that was symmetrical to a bruise on the same bruised bird. Ten other S. aureus cultures isolated from various samples gave no typical reaction with any of the phages tested. Further study of these nontypable strains may show that many of them were the same.

The results verify that bruised tissue can harbor S. aureus of the same phage type as found in infections and from hand swabs of workers who handle birds with bruises. Such staphylococci were not detected in hand swab samples obtained from workers who handle poultry only after the bruises have been trimmed away. The results strongly suggest that poultry bruises comprise a significant source of staphylococcal infections occurring among poultry workers. The finding of S. aureus in bruised tissue also may present a potential health-hazard to the consumer and suggests that more stringent measures may be required with respect to the further processing of bruised birds.

When the authorities of one poultry processing plant in Clarke County were informed of the results obtained in this investigation, a new protocol was initiated among poultry workers. The protocol consisted of a thorough washing with sudsing antibacterial skin cleanser (pHisOnHex, Winthrop Lab.) to all cuts, wounds, and scratches followed by Merthiolate painting and application of a triple antibiotic ointment (polymyxin B, bacitracin, and neomycin). When this treatment was followed, the incidence of staphylococcal infection was drastically reduced as evidence by the decreased number of infected lacerations and cuts among poultry workers examined during that time. Nine staphylococcal infections were reported among poultry workers of this plant during a 5-month period following the initiation of this protocol as compared to 35 cases during the 4-day period prior to this treatment.

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