Antibiotics in Poultry Meat Preservation: Development of Resistance Among Spoilage Organisms

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Chlortetracycline (CTC) and oxytetracycline (OTC) have been approved by the Federal Food and Drug Administration for use in extending the market life of poultry meat. These antibiotics are now being widely used in this country for this purpose on eviscerated broilers. During the course of carrying out some experiments on antibiotics in a commercial processing plant, the authors had occasion to observe conditions under which the Acronize\(^2\) process was being used. It seemed quite clear to us that a real possibility existed whereby resistant forms of the spoilage organisms could develop in this plant. Accordingly, some studies were initiated to determine whether, in fact, resistance to CTC among spoilage organisms did develop under conditions of commercial use.

MATERIALS AND METHODS

Eviscerated whole and cut-up tray-packed broilers were obtained directly from a processing plant as well as from several retail stores. In the former case it was known by direct observation whether or not the birds had been treated with CTC. In the latter case, it was assumed that only those birds carrying a label declaration had been treated with CTC.

Samples of birds were collected from the processing plant and the retail stores over a period of several months during 1956 and 1957. They were brought to Davis and stored in a cooler at 7.2 C (45 F) until spoiled (as determined by the development of typical “dirty-dish-rag” odor and slimy condition). Swabs were taken and the swabbings plated out onto nutrient agar; the plates were kept at 7.2 C (45 F) until colonies formed. Pure cultures from the predominant types of colonies were obtained by usual isolation techniques. The isolates were maintained as slant cultures on nutrient agar.

The resistance of the isolated cultures was determined by a modification of the method of Beech et al. (1955): A multiple-needle inoculation disc was constructed of metal. This device is 3 in. in diameter and has mounted on it 24 needles in two concentric circles (plus a 25th needle in the center). A similarly shaped disc of wood was constructed containing 25 holes, positioned to fit the 25 needles of the metal disc.
Both discs possess a marker that permits identification of each needle and corresponding hole. Suitable vials of such size (1.2 ml) as to fit the holes in the wooden disc were used to hold suspensions of the test organisms.

In operation, the resistance test was conducted as follows: Suspensions of the isolates (approximately 10^6 cells per ml) were prepared and placed in the vials. The multiple-needle inoculating disc was then positioned over the disc containing the vials and lowered until all needles were evenly immersed in the suspensions. The disc was then removed and lowered onto a Petri dish containing sterile nutrient agar. It was then withdrawn and the cover replaced on the Petri dish. This procedure was repeated with nutrient agar containing CTC. All inoculations (with and without CTC) were made in duplicate in an aseptic inoculation room.

The plates were then incubated at 7.2 C (45 F). Observations for growth were made daily. Control plates were compared with those containing antibiotic; readings were considered significant (for each test culture) only at the time when growth first was observed on the control plates. Isolates were called sensitive when duplicate plates failed to exhibit colony formation on agar containing CTC.

RESULTS AND DISCUSSION

Table 1 summarizes the data for isolates obtained from three types of birds: 1) those obtained from spoiled poultry which had not been treated with CTC and from plants not known to be using the Acronize process, 2) those obtained from spoiled poultry which had not been treated with CTC but from a plant in which a regular portion of the poultry was treated with CTC, and 3) those obtained from spoiled poultry which had been treated with CTC and from plants in which at least a portion of the poultry was treated with CTC. In the latter two situations, approximately half of the isolates were from whole birds and half from cut-up tray-pack birds.

It may be seen that none of the unexposed cultures were resistant to CTC at concentrations of 5, 10, or 15 ppm. On the other hand, a large proportion of those previously exposed to CTC (whether directly or indirectly) were resistant. The presence of resistant forms on birds treated directly with CTC is to be expected since these organisms originally developed in the presence of some residual CTC (estimated to be 2 to 5 ppm; Food and Drug Administration regulations permit a tissue residual of 7 ppm).

The presence of resistant forms among isolates from birds not treated directly with CTC could be explained only if opportunity existed for growth of the organism in the plant (on birds, equipment or elsewhere) in the presence of CTC. Critical appraisal of operations in the plant revealed that such an opportunity existed. The following facts were ascertained: The CTC formulation was added to the ice-slush in which the eviscerated whole birds were chilled. The birds remained in the slush for 1 to 4 hr. After removal of the birds and water from the chill tank, the chill tank and the remaining ice is were rinsed and returned to service. (The tanks are thoroughly washed only once a day.) Birds to be sold as a whole-bird pack are hung on a draining conveyor line; then ice-packed in wirebound shipping containers and placed in a cooler pending shipment.

In the cut-up operation, the whole carcasses were also drained on a conveyor line; they were then removed from the line and cut up on a meatcutting band saw. Pieces were assembled, by hand, in a fiber-board tray and overwrapped with transparent plastic film. These tray-packaged birds were then packed in a fiber-board shipping container and placed in the cooler.

In the latter operation, there was ample opportunity for bacterial growth to occur during the cutting and packaging operations. For example, counts on the saw platform and blade after a few hours' use ran as high as 150,000 psychrophiles per square inch. When the Acronize process was being used, such growth obviously occurred in the presence of CTC leading to the development of resistant forms. The construction of the saw is such that it is virtually impossible to keep it in a sanitary condition; even periodic cleaning as practiced in this plant certainly does not eliminate the possibility of a day-to-day carry-over of contamination from this source.

It is not so easy to explain how resistant forms develop in the whole bird operations. Still it is clear that they do. Perhaps the bacteria grow on the surfaces of the chill tanks between use, or the whole pack birds become contaminated by transfer of bacteria from the cut-up operations. Inasmuch as there is an exchange of equipment and personnel between the two opera-

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**Table 1. Resistance of spoilage organisms to chlorotetracycline (CTC)**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Isolates*</th>
<th>Unexposed</th>
<th>U-CTC</th>
<th>A-CTC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>No. resistant</td>
<td>Per cent</td>
<td>No. resistant</td>
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<td>ppm</td>
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<td>0</td>
<td>6</td>
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<td>15</td>
<td></td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

* Unexposed = twenty-five isolates from spoiled birds not treated with antibiotic from plant not using antibiotic.

U-CTC = Nine isolates from spoiled birds not treated with antibiotic but from plant using CTC regularly on part of pack.

A-CTC = Thirty-eight isolates from spoiled birds treated with a tetracycline antibiotic and from plant using CTC regularly.
Drying Conditions on Survival of *S. marcescens*

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Most of the literature on drying of microorganisms has dealt with lyophilization. While freeze drying is a satisfactory method of preserving stock cultures, other processes, for example, spray drying, offer considerable economic advantage for drying large quantities of cells. This study, therefore, was performed to observe the behavior of a nonsporeforming bacterium when dried from a nonfrozen state by evaporation of water in an air stream.

The drying method consisted of suspending cells on nylon cloth held in an air stream of controlled temperature, relative humidity, and flow rate. While this procedure has no practical application, drying conditions could be varied easily and therefore some of the conditions which might be encountered in a commercial process could be simulated.

Experimental Methods

Preparation of cells for drying. *Serratia marcescens* strain 8 UK was chosen as the principal test organism because it is moderately sensitive to dehydration and its pigmentation makes it readily differentiable from contaminants. The cultures were grown in a synthetic medium containing per L of distilled water: glucose, 2 g (autoclaved separately); ammonium sulfate, 2 g; sodium citrate dihydrate, 2 g; dibasic potassium phosphate, 1 g; MgSO₄·7H₂O, 40 μg; NaCl, 2 μg; FeSO₄·7H₂O, 2 μg. The medium after sterilization and addition of glucose had a pH of 6.5.

Twenty-five ml of the synthetic broth in a 125-ml Erlenmeyer flask was inoculated with 0.5 ml of a 24-hr nutrient broth culture and incubated at 30 C for 24 hr on a New Brunswick rotary shaker at 360 rpm. A portion of the culture was centrifuged, diluted to the original volume with 0.1 per cent peptone solution, centrifuged again, and then diluted in the drying menstruum to a concentration of about 10⁶ cells per ml.

The composition of the various drying menstruums are included with the experimental results. A stock solution of each ingredient was prepared and held at 5 C. Immediately before use, the ingredients were mixed and adjusted to pH 6.2 to 6.5 with KOH. Sterilization was avoided to prevent any changes that might be caused by heat. Chance microbial contaminants in the drying menstruum were successfully diluted out during subsequent plating.

1 Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

2 Present Address: 6th U. S. Army Medical Laboratory, Ft. Baker, California.

Summary

A study was made of organisms isolated from commercially processed eviscerated chickens when deliberately spoiled at 7.2 C (45 F). The data collected clearly show that, where the chickens had been processed in the presence of chlortetracycline, resistant forms prevail. Such forms were not demonstrated on spoiling poultry which had been processed in plants not using chlortetracycline.

References
