Streptomycin Resistance of Coliform Bacteria from Turkeys Fed Streptomycin

MORTIMER P. STARR AND DONALD M. REYNOLDS

Division of Bacteriology, College of Agriculture, University of California, Davis, Calif.

DURING an investigation of the microbiological aspects connected with the use of streptomycin and other antibiotic supplements in turkey nutrition, we observed cases of considerable drug fastness in the intestinal microflora. Since the development of antibiotic-resistant strains of microbes has a bearing on the successful use of antibiotics as nutritional supplements, as well as on the use of these agents for chemotherapy, an examination was made of the streptomycin resistance of coliform bacteria from turkeys which had been on diets containing streptomycin. It is, of course, recognized that results with streptomycin cannot be equated completely with those expected using other antibiotics because of the relative ease with which streptomycin-resistant mutants arise.

Our first efforts were directed toward determining whether inclusion of streptomycin in the diet of poults results in the appearance of an intestinal microflora containing drug-resistant coliforms. Cultures for this purpose were isolated from two types of material.

In one type of experiment, intestinal contents were obtained at autopsy from turkey poults * which for about a month had been fed an adequate, high-soybean-oil meal ration (Kratzer, 1951) to which was added 50 mg. of streptomycin † per kg. of ration. This concentration is approximately twice the amount currently used in commercial poultry feeds containing streptomycin. Immediately after killing, the intestinal contents were removed aseptically from the region immediately anterior to the ileo-caecal junction.

In a second type of experiment, the intestinal microflora was followed by periodic culturing from hatching until 5 to 6 weeks. Fecal samples were obtained from such turkeys by collecting droppings immediately after defecation, using aseptic precautions. In both cases, control birds on the adequate basal diet, but lacking streptomycin, were cultured in the same way.

Samples from both sources were treated in a similar fashion. Since this work is one phase of a general study of the over-all effect of antibiotics on the intestinal microflora of poultry, quantitative platings of intestinal contents and fecal material were carried out on a variety of culture media. The cultures with which we are presently concerned originated on quantitative pour plates of Bacto eosin-methylene-blue agar. After

* The generous coöperation of the Poultry Husbandry Division and especially of Professor F. H. Kratzer is gratefully acknowledged.

† The 50 mg. of streptomycin per kg. were added in the form of the calculated amount of Merck streptomycin mixture for oral veterinary therapeutic use, each gram of which was equivalent to 0.39 gm. streptomycin base.

18 to 48 hours' incubation at 37° C., typical coliform colonies were picked from these plates. These were replated as often as necessary to insure purity, via suspensions in sterile water.

Only cultures with typical coliform characteristics were retained for this study, i.e., Gram-negative, aerobic, nonsporeforming rods, which ferment lactose with the production of acid and gas. *Escherichia, Aerobacter,* and intermediate types were present. Stock cultures were maintained on yeast extract, glucose, and $CaCO_3$ agar, and stored at 4° C. Such storage procedure should have little effect on the drug fastness of highly streptomycin-resistant strains (Klein and Kimmelman, 1946).

Sensitivity to streptomycin was determined by the agar-streak method of Waksman and Reilly (1945). The bacterial cultures were grown on nutrient agar slants at 37° C. for 18 to 24 hours and then suspended in approximately 10 ml. of sterile water for testing. Nutrient agar plates were prepared containing graded amounts of streptomycin * (calcium chloride complex, Merck) as follows: 1.0 or 0.3 ml. of an appropriate aqueous streptomycin solution was placed in a 10 cm. Petri dish, 9 ml. of nutrient agar were added, and mixed thoroughly by swirling. The final streptomycin levels were 60,000, 30,000, 3,000, 1,000, 300, 100, 30, 10, 3, and 1 μg. per ml. of agar. After 20 to 24 hours' incubation at 37° C., results of growth were recorded and compared with control plates lacking streptomycin. The inhibitory endpoint was taken as that concentration of streptomycin which completely, or nearly completely, inhibited growth of the organism.

A total of 317 coliform cultures were tested for streptomycin resistance. One hundred and ninety-five of these (see Figure 1) originated from poults which had been on the basal control diet devoid of streptomycin. Most of the cultures from these control birds were sensitive to low concentrations of streptomycin, 74 per cent being inhibited by 10 μg./ml., and 86 per cent by 30 μg./ml. The occurrence in normal birds of streptomycin-resistant coliform cultures can be explained on several bases, such as: (1) the normal existence of a minority of resistant cells in a population (English and McCoy, 1951); or (2) the carriage of occasional bits of streptomycin-laden feed or feces by air currents or other agents to the cages of these control birds, followed by ingestion of such material, which might result in an unpredictable appearance of resistant forms.

On the other hand, the cultures isolated from birds on a streptomycin-containing diet were, as one would ex-

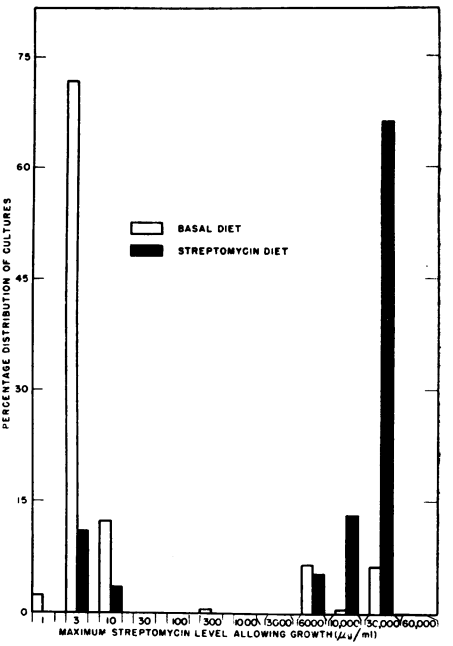

FIGURE 1—Distribution of streptomycin resistance in coliform cultures isolated at random from intestines of turkey poults, calculated as percentage of total number of cultures in each of the following categories: □ = 195 cultures from poults on basal diets; ■ = 122 cultures from poults on streptomycin diet.

* A generous gift of streptomycin from Merck and Company, Rahway, N. J., is hereby gratefully acknowledged.

pect, generally highly resistant to the drug. Figure 1 shows that of the 122 such cultures examined, 66 per cent required a concentration of 60,000 μg. of streptomycin per ml. to prevent growth. The absence of forms intermediate in resistance is typical for streptomycin. Demerec (1948) considers the existence of slightly and highly streptomycin-resistant mutants to involve resistance gene loci of unequal "potency"; thus mutation of a low potency gene results in a slight increase in resistance, and mutation of a high potency gene gives a highly resistant strain.

Our experimental technique did not enumerate those streptomycin-resistant mutants which had also become streptomycin-dependent, inasmuch as they had little bearing on the public health problem which motivated this work. If these had been counted, the relative percentage of streptomycin-resistant coliforms in streptomycin-fed birds would be even higher than shown above.

In view of the fact that resistance to streptomycin develops in the intestinal coliform bacteria following the feeding of streptomycin to poults, it would be instructive to learn how quickly the change to a resistant flora takes place. Data on this point are available as a result of periodic quantitative bacteriological plating, from the day of hatching until about 6 weeks of age, of feces from poults on both the adequate basal diet and on the streptomycin diet. Fecal samples were obtained from such poults by collecting droppings, using aseptic precautions; these were then plated quantitatively on eosin-methylene-blue agar (EMB), and on EMB plus 100 μg. per ml. of streptomycin (EMBS). Figure 2 shows that the addition of streptomycin to the basal ration caused a rapid shift to a drug-resistant coliform population. The control birds remained relatively free of streptomycin-resistant coliform bacteria for several weeks, after

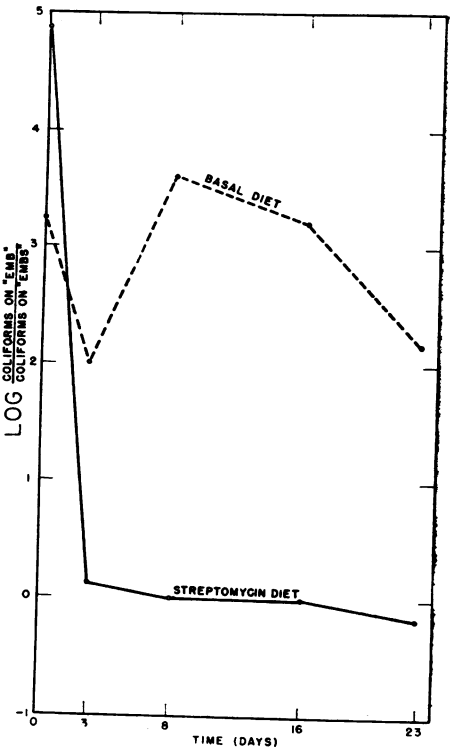

FIGURE 2—Numbers of streptomycin-resistant coliforms in the intestines of turkey poults on adequate diets containing or lacking added streptomycin. The coliforms were enumerated on eosin-methylene-blue agar (EMB) and on EMB plus 100 μg. per ml. of streptomycin (EMBS).

which there was a progressive increase in the proportion of drug-resistant component of the flora, probably caused by the inadvertent transfer of streptomycin to the nearby cages containing the control birds. The rapid development of streptomycin resistance in the coliform component of fowl intestinal flora shown here corresponds with the results obtained when streptomycin is administered orally to human beings to reduce the microflora of the lower gut prior to surgery (Miller and Bohnhoff, 1950).

It appears worthwhile to point out that the mass feeding of antibiotic drugs in routine diets of poultry and other domestic animals, with its concomitant development of drug-fast strains of in-

testinal bacteria, presents potential hazards to both agricultural industry and the public health. Since the economics of the poultry industry generally preclude the use of antibiotics for bird chemotherapy, the existence of drug-fast strains of pathogens in fowl is of little immediate practical interest from this viewpoint.

However, one should not overlook the potential utility of these drugs, such as reported by Kirkpatrick, Moses, and Baldini (1950), who used streptomycin to treat ulcerative enteritis in bobwhite quail.

A problem which is of a more serious, immediate nature is the very distinct hazard that drug-fast intestinal pathogens can develop in a flock of poultry and then be ingested by large farm animals in the same establishment, with the consequence that effective chemotherapy in those animals would be made more difficult. Salmonellosis provides the classic type of relationship between fowl and large animal diseases. It is widely believed that while certain species of *Salmonella* (e.g., *S. typhosa, S. paratyphi, S. abortus-equi*) are particularly adapted to specific hosts, a great number of other species (e.g., *S. typhimurium, S. enteriditis*) parasitize a broad spectrum of animal hosts. Fowl are considered to be a principal reservoir of the latter type of *Salmonella* (Hinshaw and McNeil, 1951). The large dosage generally required to cause these enteric infections is provided under barnyard conditions. The spread of antibiotic-resistant strains of *Salmonella* to larger animals on the same farm could indeed become a serious problem, for the increasing relative value of meat animals is making chemotherapy more and more important in the treatment of livestock diseases.

Larger than the agricultural problems noted is one concerned with public health itself and involving the greater question as to just how much indiscrimination should be permitted in the use of new chemotherapeutic agents.

As mentioned in the foregoing, numerous species of *Salmonella* capable of infecting a large variety of different hosts are widely distributed through the animal kingdom. Swine and poultry are outstanding among *Salmonella*-infectable animals which serve as human food, with respect to the high frequency with which they are found to harbor these enteric pathogens. In fact, Edwards, Bruner, and Moran (1948) have stated that birds still constitute the greatest reservoir of potential *Salmonella* infection. In any case, a fair consensus of informed opinion is that fowl and swine vie with each other for the position of the largest reservoir of human salmonellosis.

A cursory scrutiny of *Bergey's Manual* (Breed, *et al.*, 1948) will reveal that a large number of *Salmonella* species pathogenic to man are found in poultry. Outbreaks of food poisoning have been traced to fowl infected with typical food poisoning types; even those species commonly thought of as restricted to fowl (e.g., *S. pullorum* and *S. gallinarum*) have on occasions been isolated from human carriers and enteritis cases (Hinshaw and McNeil, 1951). Examination of over 6,000 samples of American dried eggs shipped to Britain during World War II showed that 10 per cent were infected with *Salmonella*; a number of British food poisoning outbreaks were ascribed to this source by extremely strong circumstantial evidence (Medical Research Council, 1947). Unequivocal proof of the virulence to man of *Salmonella* which originate in poultry is provided by the work of McCullough and Eisele (1951). Using three cultures each of *S. meleagridis* and *S. anatum* isolated from spray-dried egg powder, these workers produced carrier states or clinical cases or both ranging in severity from mild brief enteritis to serious prostrating ill-

ness, in 32 human volunteers with all six cultures.

It is indeed fortunate that outbreaks of food poisoning, enteritis, and typhoidal infections caused by *Salmonella* are not more common in man when one considers the size of the natural reservoir among poultry, swine, and other animals and the numerous routes by which the organisms are constantly contaminating the public food supply. A partial explanation for this low incidence is found in the work of McCullough and Eisele (1951), who found that large doses, on the order of 25 million cells per person, were necessary to bring about *Salmonella* infections in human volunteers.

The public health implications of the development of antibiotic-resistant flora in the poultry population are obvious. Though mutants tend to revert gradually to sensitivity in the absence of the drug, the route of infection from poultry to man can be so direct that the physician called upon to administer antibiotic chemotherapy for the more severe typhoidal types of human salmonellosis may be gravely handicapped by having to combat a drug-fast strain. With the sulfa drugs, and to some extent with penicillin, we have seen how drug fastness has in some instances seriously damaged the chemotherapeutic effectiveness of these agents (Miller and Bohnhoff, 1950). In these cases, development and spread of resistant pathogens was inevitable even with the best planned scheme of drug administration. It would be unfortunate if a large reservoir of drug-fast enteric pathogens potentially harmful to man accumulated unchecked in the poultry population. We hope that those charged with the protection of the public health will objectively evaluate this situation.

Another aspect of this public health question which merits investigation is the marked relative increase in numbers of yeasts or yeastlike organisms which arise in the bird gut under the pressure of antibiotic feeding (Kratzer, *et al.*, 1951). It is possible that some of these organisms may be pathogenic to man and may give rise to problems of antifungal human chemotherapy, for which efficacious drugs are lacking at present.

We grant that the poultry industry cannot readily forego the great economic advantages which accrue from the growth promoting effect of antibiotics on young birds. But it is strongly felt that a few years of research are likely to elucidate the fundamental mechanism underlying this growth promoting effect and that this information will permit agriculturalists to secure more rapid animal growth without inflicting potential hazards on the public health.

SUMMARY

The use of streptomycin as a growth promoting supplement in turkey poults results in the appearance within three days of streptomycin-resistant coliform bacteria. In 66 per cent of the drug-fast strains, resistance to the antibiotic rose to 60,000 μg. per ml. The potential hazard to veterinary and public health stemming from the indiscriminate use of antibiotic poultry-feed supplements is discussed.

BIBLIOGRAPHY

Breed, R. S., Murray, E. G. D., and Hitchens, A. P. *Bergey's Manual of Determinative Bacteriology*. Baltimore: Williams & Wilkins, 1948.

Demerec, M. Origin of Bacterial Resistance to Antibiotics. *J. Bact.* 56:63–74, 1948.

Edwards, P. R., Bruner, D. W., and Moran, A. The Genus *Salmonella:* Its Occurrence and Distribution in the United States. *Kentucky Agr. Exper. Sta. Bull.* 525, 1948.

English, A. R., and McCoy, E. A Study of Streptomycin Resistance in *Micrococcus pyogenes* var. *aureus*. *J. Bact.* 61:51–56, 1951.

Hinshaw, W. R., and McNeil, E. *Salmonella* Infection as a Food Industry Problem. *Advances in Food Research* 3:209–240, 1951.

Kirkpatrick, C. M., Moses, H. E., and Baldini, J. T. Streptomycin Studies in Ulcerative Enteritis in Bobwhite Quail. I. Results of Oral Administration of the Drug to Manually Exposed Birds in the Fall. *Abstracts of Papers at the 39th Annual Meeting of the Poultry Science Association*, Madison, Wis.

Klein, M., and Kimmelman, L. J. The Role of Spontaneous Variants in the Acquisition of Streptomycin Resistance by the Shigellae. *J. Bact.* 52:471–479, 1946.

Kratzer, F. H. The Growth Response to Vitamin B_{12} and Antibiotics in the Diet of Turkey Poults from Hens Fed Rations Containing no Animal Protein. *Poultry Science* (in preparation).

Kratzer, F. H., Grau, C. R., Starr, M. P., and Reynolds, D. M. The Growth-promoting Activities of Antibiotics and Yeast Cultures for Chicks and Turkey Poults. Unpublished report presented to the 1951 Meeting of the American Institute of Nutrition.

McCullough, N. B., and Eisele, C. W. Experimental Human Salmonellosis. I. Pathogenicity of Strains of *Salmonella meleagridis* and *Salmonella anatum* Obtained from Spray-dried Whole Egg. *J. Infect. Dis.* 88:278–289, 1951.

The Bacteriology of Spray-dried Egg, with Particular Reference to Food Poisoning. Medical Research Council (Great Britain). *Special Report Series* No. 260, 1947.

Miller, C. P., and Bohnhoff, M. The Development of Bacterial Resistance to Chemotherapeutic Agents. *Ann. Rev. Microbiol.* 4:201–222, 1950.

Waksman, S. S., and Reilly, H. C. Agar-streak Method for Assaying Antibiotic Substances. *Indust. & Engin. Chem. (Anal. Ed.)* 17:556–558, 1945.

ACKNOWLEDGMENT: The competent technical assistance of Marian Wells Thomas is gratefully acknowledged.

Interstate Milk Shipments

The Second National Conference on Interstate Milk Shipments, attended by representatives from more than 30 states, was held in St. Louis, Mo., June 4–6, 1951. "The Best Possible Milk Supply for All Our People" was given by J. L. Rowland, chairman, as the theme of the meeting. Among the more than 100 in attendance were 29 representatives of industry. Task groups considered certification and supervision, laboratory procedures, and education both of the industry and the public, among others. The 1939 edition of the Public Health Service's Recommended Milk Ordinance and Code was again accepted as the basic standard. A minimum rating of 90 per cent was agreed upon for certification of an area. A brief history of the interstate milk shipment problem and the background of the 1951 conference are an important part of the report.

The conference gave special attention to:

- Preparation of uniform shipping tags and bills of lading
- Recognition of inspection by industry under official supervision
- Formulation of standards for Grade A supplemental milk fats, concentrated and dry milk products, and manufacturing and processing

Complete report of the conference available from J. L. Rowland, Division of Health of Missouri, Jefferson City.