

Resistance of *Salmonella typhimurium* to Tetracyclines. (23801)

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The problem of development of resistance by bacteria to antibiotic substances is so well known as to need no comment. As concerns development of resistance by enteric bacteria as a result of prophylactic and therapeutic use of antibiotics, the observations of LeMinor *et al.*(1), Buttiaux *et al.*(2), Smith(3,4) and Smith and Crabb(5,6) are pertinent. These workers observed the emergence of resistant strains of *Escherichia coli* in man and animals following prophylactic and therapeutic use of the agents. Further, Smith and Crabb(5) noted rapid emergence of tetracycline resistant strains of *E. coli* in fowls and swine maintained on feeds which contained these substances.

Materials and methods. Since the inclusion of antibiotic substances in feeds has become such a general practice it was thought desirable to examine the reaction of *Salmonella* cultures to these substances. *Salmonella typhimurium* is the serotype most frequently found in man and animals, and therefore, was chosen for test. Advantage was taken of a large collection of *Salmonella* cultures isolated prior to 1948, *i.e.*, before inclusion of antibiotic substances in animal feeds and before the broad spectrum antibiotics came into general therapeutic use. These cultures had been stored in infusion agar stabs in tubes stoppered with paraffined corks and had not been transferred since they were received and identified. They had been kept in the dark at room temperature for periods varying from 10 to 18 years. From these cultures were selected 100 strains of *S. typhimurium* isolated from animals and an equal number isolated from man. Of the animal strains, 90 were derived from fowls and 10 from swine. From cultures isolated subsequent to Jan. 1956, were selected 100 cultures of *S. typhimurium* isolated from fowls and 100 cultures isolated from man. In all instances an effort was made to select strains of wide geographic distribution, so that cultures from all sections of the

country were included. The antibiotic substances tested were tetracycline, oxytetracycline, chlortetracycline, chloramphenicol, dihydrostreptomycin, polymyxin B, penicillin, and erythromycin. In screening the cultures, blood agar base plates were streaked with individual cultures and antibiotic discs of intermediate concentration placed on the surface as soon as the inoculum was dry. Results were read after overnight incubation at 35°C. Early in the work it became apparent that significant results would not be obtained with penicillin, erythromycin, dihydrostreptomycin, and polymyxin B. All cultures tested with penicillin and erythromycin were resistant to the action of these substances and equal numbers of old and new isolates were resistant to the action of streptomycin. All cultures tested with polymyxin B gave a narrow clear zone of inhibition. With the exception of dihydrostreptomycin, which was used in examination of one-half of the cultures, the above substances were used in the examination of only a quarter of the strains. All cultures tested also were susceptible to the action of chloramphenicol, but since this substance has been employed so widely in the treatment of enteric infections, its use was continued throughout the work. After cultures had been screened with discs, all tetracycline resistant cultures and an equal number of susceptible strains were examined in tube dilution tests with chlortetracycline. Susceptible cultures were made up of equal numbers of old and new isolates. Tests were done in the usual manner with serial dilutions of the antibiotic which ranged in final concentrations from 0.10 µg to 200 µg/ml. To 0.5 ml of the antibiotic dilutions in distilled water were added 1.5 ml portions of 18-hour cultures which had been diluted 1 to 400 in trypticase soy broth. Results were read after overnight incubation.

Results. The only significant differences noted in reactions of cultures were obtained with tetracyclines. Among cultures isolated

prior to 1948, no resistant cultures were found. Among recently isolated strains, 9% from fowls and 5% from man appeared completely resistant as judged by their reactions toward the discs containing these substances. As would be expected, cultures which were resistant to one of the tetracyclines were resistant to all.

In every instance the results obtained by the disc method were confirmed. Among the susceptible strains, no differences were apparent between the old and new isolates. With the exception of one culture which produced visible growth in 6.25 $\mu\text{g/ml}$, the cultures which appeared susceptible by the disc method were inhibited by concentrations of chlortetracycline higher than 3.125 $\mu\text{g/ml}$. On the contrary, with one exception which grew only in 25 $\mu\text{g/ml}$, the resistant strains grew well in 50 μg concentration of the antibiotic and half grew in a concentration of 100 $\mu\text{g/ml}$.

From these results it is apparent that *Salmonella* cultures which are resistant to tetracyclines occur more frequently today than they did before these agents were used. Had a larger number of cultures isolated prior to 1948 been examined, some resistant cultures might have been found. However, the fact that no resistant cultures were found among 200 strains of *S. typhimurium* indicates that resistant cultures previously occurred very rarely. The fact that a significant increase in occurrence of resistant strains was found only in tetracyclines probably is a reflection of the widespread use of these substances in animal feeds. With their continued inclusion in feedstuffs and their continued therapeutic use, an increase in the proportion of resistant strains may be expected. The demonstration of resistance in twice as many cultures derived from fowls as in cultures derived from man also probably is related to the inclusion of tetracyclines in

poultry feeds, and may be contrasted with the lack of resistance to chloramphenicol which is not so used.

Although an increasing proportion of resistant strains may be expected in years to come, the rather slow emergence of such strains is somewhat surprising when one considers results obtained in the study of *E. coli*. Smith and Crabb(6) noted very high proportions of tetracycline resistant *E. coli* in pigs fed the substances. Buttiaux *et al.*(2) noted that among cultures of *E. coli*, type 111:B₄, isolated from children admitted to one hospital the percentage of resistant strains rose from zero to 96.2 over a 2 year period. These findings suggest that *S. typhimurium* develops resistance to tetracyclines much more slowly than does *E. coli*.

Summary. Cultures of *Salmonella typhimurium* isolated from man and animals prior to 1948 and cultures of the same serotype isolated from man and fowls after Jan. 1956 were compared for resistance to several antibiotic substances. Significant results were obtained only with tetracyclines. Whereas none of the 200 older cultures examined was resistant to tetracyclines, 9% of cultures recently isolated from fowls and 5% of cultures recently isolated from man were resistant to the substances. Attention is called to the possible relationship of these findings to the inclusion of tetracyclines in poultry feeds.

1. LeMinor, S., LeMinor, L., Nicolle, P., Buttiaux, R., *Atti VI Congr. Internaz. Microbiol.*, 1949, v4, 240.
2. Buttiaux, R., Nicolle, P., LeMinor, L., LeMinor, S., Gandier, *Arch. Malad. l'Appar. Diges.*, 1956, v45, 225.
3. Smith, H. W., *Vet. Rec.*, 1954, v66, 42.
4. ———, *ibid.*, 1957, v69, 749.
5. Smith, H. W., Crabb, W. E., *ibid.*, 1956, v68, 274.
6. ———, *ibid.*, 1957, v69, 24.

Received December 16, 1957. P.S.E.B.M., 1958, v97.