Resistance of Salmonellae Isolated in 1959 and 1960 to Tetracyclines and Chloramphenicol

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ABSTRACT

Ramsey, Carolyn H. (U. S. Public Health Service, Atlanta, Ga.), and P. R. Edwards. Resistance of salmonellae isolated in 1959 and 1960 to tetracyclines and chloramphenicol has been examined periodically. Although none of 200 cultures isolated prior to 1948 was resistant to the antibiotics, 5% of 100 cultures from man and 9% of 100 cultures from fowls which were isolated in 1956 and 1957 were resistant to tetracyclines. Among 158 cultures isolated from man and 100 cultures isolated from fowls in 1959 and 1960, 13.9 and 29%, respectively, were resistant to tetracyclines. In the last series, cultures resistant to chloramphenicol were found for the first time. Among 150 cultures of other Salmonella serotypes from man and 137 similar cultures isolated from fowls in 1959 and 1960, 5.3 and 8%, respectively, were found resistant to tetracyclines. There is no obvious explanation for the higher percentage of resistant strains occurring in S. typhimurium than in other serotypes.

In view of the widespread use of tetracyclines as therapeutic agents and as additives to livestock feeds, we have attempted to survey the resistance of salmonellae isolated at different periods to these substances. In this work Salmonella typhimurium largely has been used as a model since it is the serotype most frequently isolated from man and animals and large numbers of this type isolated during a restricted period could be collected without difficulty. In the current series of tests, a number of other serotypes isolated during the same period were examined to compare their resistance with that of S. typhimurium.

MATERIALS AND METHODS

All duplicate cultures from individuals and epidemics were eliminated and cultures from widely separated geographic areas in the United States were chosen for examination. The cultures tested were isolated from man and from domestic fowls and were made up of strains received for diagnosis. During the course of the studies the resistance of the organisms to a number of chemotherapeutic substances was tested but results obtained in previously reported series indicated that of the substances used, only the tetracyclines and chloramphenicol were likely to yield significant results. Thus, those were the only substances used to test all of the cultures studied. At the beginning of the work, tetracycline, oxytetracycline, and chlortetracycline were used but, since results obtained with all were invariably similar, only oxytetracycline and chlortetracycline were used throughout.

The methods used were described by Huey and Edwards (1958). All cultures were screened by the use of discs of intermediate concentration and those which appeared resistant were examined in tube dilution tests using chlortetracycline or chloramphenicol as indicated. Equal numbers of apparently susceptible strains were included for comparison. In every instance the results obtained by the disc method were confirmed.

RESULTS

The results of the tests are given in Table 1 and the earlier series are included for comparison. It will be noted that the percentage of cultures of S. typhimurium resistant to tetracyclines roughly has trebled within a 3-year period and this applies both to cultures isolated from man and from fowls. This increase in resistant strains was apparent in all sections of the country but was particularly noticeable among cultures isolated from man in the New England area where 24% of 41 cultures were resistant. Among the strains isolated from fowls, resistant cultures isolated from turkeys in the midwestern states were quite numerous.

Although the percentage of resistant cultures increased markedly in the 3-year period that elapsed between the second and third series of cultures, the resistance of individual strains remained unchanged. The susceptible strains were inhibited by 3.1 or 6.25 µg/ml of chlortetracycline, whereas the resistant strains were inhibited by 100 to 200 µg/ml. These levels of resistance corresponded closely to those found in the 1956-1957 series of cultures. Three cultures of S. typhimurium from man were resistant to chloramphenicol, whereas no resistant cultures appeared previously. These three cultures also were resistant to tetracyclines.
In addition to the *S. typhimurium* cultures isolated in 1959-1960, 150 cultures of other serotypes from man and 137 similar cultures from animals isolated during the same period were examined. These series of cultures were made up of a variety of serotypes distributed among the various O antigen groups roughly in the proportion that those groups occurred in diagnostic material. Here also duplicate cultures from individuals and epidemics were eliminated. Among the 150 human cultures, 8, or 5.3% were resistant to tetracyclines and of the 137 cultures from animals, 11, or 8% were resistant. Only one culture of *Salmonella choleraesuis* isolated from a hog was resistant to chloramphenicol. The writers have no explanation for the higher percentage of resistance among cultures of *S. typhimurium* than that found among other serotypes.

**Discussion**

One finds many random observations in the literature concerning antibiotic resistance of salmonellae but few studies on sizable series of cultures have appeared. Eisenberg et al. (1958) reported that 50% of 62 salmonellae of various serotypes were resistant to tetracyclines. It should be noted that these tests were done by a "wet disc" method and resistance apparently was not confirmed by titrations in fluid medium. Further, the cultures tested were isolated from patients in one hospital and presumably the majority of the infections was contracted in the same general geographic area. These facts may account for the unusually large number of resistant strains reported. On the contrary, Joachin, Mayes, and Olarte (1959) reported that 9% of 32 cultures of *S. typhimurium* and 6.4% of 78 cultures of other serotypes were resistant to tetracycline. These cultures were isolated during 1956 and 1957 and the results coincide quite closely with the results obtained in this laboratory with strains isolated at that time. Smith (1960) in England found that none of 106 cultures of salmonellae isolated from hogs was resistant to tetracycline. In a study of 12,014 Salmonella cultures, Mainten, Kampelmacher, and Guinée (1960) found only 2.08% resistant either to tetracycline or chloramphenicol, or to both substances. In this series of tests also the percentage of *S. typhimurium* resistant to antibiotics (2.5%) was somewhat higher than that of other serotypes (1.7%). It should be noted that these cultures were tested on agar media which contained, respectively, 30 μg/ml of chloramphenicol and 25 μg/ml of tetracycline. Bokkenheuser and Richardson (1960) reported that among 99 Salmonella cultures isolated from Bantu children who attended a rural school, 10.1% were resistant to chlorotetracycline but none resistant to chloramphenicol or oxytetracycline. Among 64 cultures isolated from residents of Johannesburg, 70.3% were found to be resistant to chlorotetracycline, 1.6% to oxytetracycline, and 6.3% to chloramphenicol. The pronounced differences in the reactions of the cultures to chlorotetracycline and to oxytetracycline is an unusual finding.

The resistant strains from fowls were derived almost exclusively from acute fatal infections in young birds although a few of them were isolated from the internal organs of older birds that died of salmonellosis. The resistant cultures from man were isolated almost exclusively from stools of acutely ill, hospitalized patients, largely young children although a very few were from acute localized infections in surgical cases or from the stools of family contacts of acute cases of gastroenteritis. In neither the cultures from fowls nor from man was there any history of the organisms having been isolated from cases or carriers after prolonged, intensive antibiotic therapy which might have led to development of resistance within the immediate host.

It must be realized that the vast majority of human salmonellosis is made up of apparently sporadic infections and most of the cultures examined were derived from cases not known to be related. Although not included in the data presented, multiple cultures from a single individual were examined in several instances. Without exception all cultures from the same person were either uniformly susceptible or uniformly resistant. In four instances cultures from related cases were examined, two groups of which were from small outbreaks of *S. typhimurium* infection in which all cultures examined were susceptible. The third and fourth epidemics occurred simultaneously in the same locality on the Texas-New Mexico border and were due respectively to *S. ser. saintpaul* and *S. ser. newport*. Both types were isolated in local health laboratories of each state. In both epidemics all strains received for diagnosis were resistant to tetracyclines.

From the results obtained in this study it is evident that salmonellae are rather slow to develop resistance to tetracyclines and chloramphenicol when compared with *Escherichia coli* and shigellae which, according to published reports (Buttiaux et al., 1956; Smith and Crabb, 1957; Olarte and de la Torre, 1959; Olarte, 1960), develop resistance more readily. Nevertheless,

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**Table 1. Resistance of Salmonella typhimurium to tetracyclines and chloramphenicol**

<table>
<thead>
<tr>
<th>Date of isolation</th>
<th>Source</th>
<th>No. tested</th>
<th>Per cent resistant to Tetracyclines</th>
<th>Chloramphenicol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1948</td>
<td>Man</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1948</td>
<td>Animals</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1956-1957</td>
<td>Man</td>
<td>100</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1956-1957</td>
<td>Fowls</td>
<td>100</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1959-1960</td>
<td>Man</td>
<td>158</td>
<td>13.9</td>
<td>1.9</td>
</tr>
<tr>
<td>1959-1960</td>
<td>Fowls</td>
<td>100</td>
<td>29</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ninety cultures from domestic fowls, ten from swine.
if the present trend continues, within a few years the percentage of salmonellae resistant to tetracyclines will reach sizable proportions. The appearance of cultures resistant both to tetracyclines and chloramphenicol is of more than academic interest. The sensitivity of salmonellae to antibiotics should be determined periodically and such studies should include sufficient numbers of cultures and serotypes to determine trends in the development of resistance.

LITERATURE CITED


Comparison of Tellurite Resistance and Tetracycline Resistance among the Enterococci¹,²

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Abstract

Appleman, M. D. (University of Southern California, Los Angeles), and I. M. Heinmiller. Comparison of tellurite resistance and tetracycline resistance among the enterococci. Appl. Microbiol. 9:391-394. 1961.—A correlation was found to exist between tellurite and tetracycline resistance in the enterococci.

Fleming (1932) reported that species of bacteria which were susceptible to penicillin were resistant to the action of potassium tellurite and vice versa. Enterococci, common molds, and yeasts were resistant to both agents.

Utilizing the observation of Fleming that potassium tellurite had a selective effect on bacteria resistant to penicillin, Bornstein (1940) found that 27 strains of enterococci would grow in the presence of both penicillin and potassium tellurite. The lactic group was inhibited only by the latter agent, whereas the viridans group was completely sensitive to both the antibiotic and the tellurite.

Watson (1944) tested the sensitivity of various serological groups of streptococci to penicillin, finding that organisms of group M were the most sensitive, whereas those of group D were the most resistant.

Nyman (1949) reported that of 449 strains of fecal streptococci tested, 89% were resistant to 1:1,700 potassium tellurite. He concluded that high potassium tellurite resistance was characteristic of the fecal streptococci and believed that media containing tellurite were suitable for the isolation of enterococci from urinary tract infections.

The enterococci have been divided into two well-defined groups, namely, tellurite-resistant and tellurite-sensitive strains, by Skadhauge (1950), which meet the major requirements of Sherman and Stark (1931) and Sherman, Mauer, and Stark 1937 for the enterococci, i.e., growth at 10 C and 45 C, tolerance to