ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1976, p. 89-95

Vol. 10, No. 1
Printed in U.S.A.

Influence of Subtherapeutic Levels of a Combination of Neomycin and Oxytetracycline on *Salmonella typhimurium* in Swine, Calves, and Chickens

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Received for publication 14 January 1976

Subtherapeutic levels of oxytetracycline plus neomycin in animal feeds did not bring about increases in the quantity, prevalence, or shedding of *Salmonella typhimurium* in swine, calves, or chickens. In fact, the medication generally reduced the proportion of animals carrying *S. typhimurium*. The medicated groups were fed rations containing oxytetracycline plus neomycin commencing 5 days prior to oral inoculation with *S. typhimurium* and continuing through a 28-day postinoculation period. Colonization of *S. typhimurium* occurred in all three animal species, as evidenced by clinical signs of infection and/or colony counts in feces. Only from swine and on only one occasion was a single resistant colony isolated. It is concluded that no evidence has been obtained which would implicate the continuous low-level feeding of oxytetracycline and neomycin for a 4-week period to a potential increased incidence of disease in animals or as a hazard to humans.

There has been general concern over the feeding of subtherapeutic levels of antibiotics to animals primarily because of a possible hazard to human health (4, 5). Recently (2) we reported on the influence of oxytetracycline on *Salmonella typhimurium* in animals. Generally the subtherapeutic use of oxytetracycline brought about a decrease in the percentage of animals carrying *S. typhimurium* and did not bring about any increase in the quantity, prevalence, or shedding of *S. typhimurium* in swine, calves, and chickens.

The studies reported here were undertaken to ascertain whether the continuous feeding of a combination of neomycin and oxytetracycline at subtherapeutic levels increases the quantity, prevalence, and duration of shedding of *S. typhimurium* in swine, calves, and chickens over that observed in nonmedicated controls. In addition, the susceptibility of *S. typhimurium* to neomycin, oxytetracycline, and several other antimicrobial agents used in human clinical medicine was determined before and during the course of these studies.

MATERIALS AND METHODS

Organism(s). The strains of *S. typhimurium* used were the same as those previously reported (2). All strains produced an acid butt with H_2S and an alkaline slant in triple sugar iron agar (TSI; Difco Laboratories, Detroit, Mich.), were urease negative, agglutinated with *Salmonella* group B antiserum (BBL, Cockeysville, Md.), and were susceptible to oxytetracycline, neomycin, kanamycin, gentamicin, ampicillin, cephalothin, streptomycin, and chloramphenicol.

Salmonella challenge. Swine and calves were fed 0.5 lb (approximately 226 g) of basal feed to which was added a 40-ml suspension of *S. typhimurium* containing ~1.1×10^{11} to 1.4×10^{11} organisms. The chickens were given the inoculum by delivering a slurry of *S. typhimurium* suspension in feed into the gullet. A plate count of the inoculum revealed that each chicken received ~2.92×10^{11} organisms.

Animals. All animals were purchased from commercial sources. At the start of the experiments, the Hampshire-Yorkshire Cross swine weighed an average of 9.1 kg, whereas Holstein calves' average weight was 85.7 kg, and the chicks, at 8 days of age, averaged 0.0798 kg.

Experimental diets. Groups designated T-1 were fed a ration of nonmedicated feed throughout the experimental period, whereas the T-2 groups were fed the same feed containing neomycin and oxytetracycline, commencing 5 days prior to *S. typhimurium* inoculation and throughout the remainder of the experimental period. No other medication was administered over the experimental period. Finished feeds were bioassayed for neomycin and oxytetracycline at the start and termination of each study according to established procedures (3). The levels of neomycin and oxytetracycline in each animal feed are presented in Table 1.

General. The methods and materials used in the maintenance of the animals and clinical records, as well as performance of pretest *Salmonella* screening, quantitation of *S. typhimurium* from inoculated animals, quantitation of pretest oxytetracycline-resistant *Escherichia coli*, antibiotic susceptibility,

and statistical analysis, were the same as those previously reported (2). The oxytetracycline resistance in *E. coli* was examined after random lotting of animals but before the start of treatment.

RESULTS

Swine. Just prior to the start of the experiment, oxytetracycline-resistant *E. coli* represented an average of 5.4% of the total *E. coli* population in the infected controls and 19.7% in the medicated animals.

TABLE 1. *Levels of oxytetracycline and neomycin in each animal feed*

Animal	Nonmedicated feed (basal)	Amount of each antimicrobial added to feed (g/ton)
Swine	Pig grower ration	150[a]
Calf	Rough cattle finisher ration	94.9[b]
Chicken	Chick starter ration	200

[a] A 150-g portion of oxytetracycline plus 150 g of neomycin sulfate.

[b] Calculated to provide a level equivalent to 350 mg of each compound/head per day at the start of the experiment.

The viable *Salmonella* counts from the fecal samples are presented in Table 2. It is apparent that *S. typhimurium* colonized the swine gut in both the medicated and nonmedicated groups. Logarithms of the colony counts obtained over the seven sampling periods were averaged for each animal, and comparison (two-sample *t* test) showed that the quantity of *Salmonella* in the medicated group was significantly less than that in the nonmedicated controls having a value of $P < 0.001$.

The medicated group exhibited more *Salmonella*-free fecal specimens, 56/70 (10 swine/group × 7 samplings = 70 samples) compared to 17/70 for the control group. A statistical analysis of prevalence was performed by examining the overall gross patterns of shedding. A score was assigned to each animal using the number of days (out of 7) on which shedding occurred. For instance, animal no. 18 had a score of four, animal no. 36 had a score of three and animal no. 37 (in whom an apparent reinfection may have occurred on day 14) had a score of four (Table 2). Two-sample *t* tests were then run on these scores, and the rate of decrease in shedding was significantly ($P < 0.001$) more rapid in the neomycin-oxytetracycline group than in the nonmedicated controls.

TABLE 2. *S. typhimurium counts per gram[a] of swine feces*

Swine no.	Days (postinoculation)						
	2	4	7	10	14	21	28
T-1 group[b]							
1	4.19×10^7	1.90×10^6	9.17×10^3	5.99×10^4	4.04×10^3	8.02×10^3	2.50×10^2
4	2.45×10^7	4.59×10^6	2.25×10^4	0	0	0	0
9	2.76×10^6	1.38×10^5	1.96×10^5	4.81×10^4	1.69×10^4	5.59×10^3	2.36×10^2
12	5.34×10^4	3.39×10^4	2.24×10^3	0	1.39×10^3	0	1.86×10^2
18	3.38×10^5	2.84×10^3	1.64×10^4	1.00×10^4	0	0	0
23	2.60×10^6	7.14×10^3	4.07×10^4	1.85×10^3	2.25×10^3	9.96×10^5	0
27	2.50×10^5	1.25×10^5	4.43×10^4	4.81×10^4	2.25×10^4	2.25×10^4	7.07×10^3
29	3.10×10^5	6.96×10^3	3.46×10^4	2.48×10^4	2.67×10^4	1.54×10^3	1.20×10^5
36	3.38×10^5	1.14×10^4	0	0	0	0	3.32×10^4
37	9.77×10^6	7.77×10^4	2.02×10^3	0	1.13×10^3	0	0
T-2 group[c]							
1	6.49×10^4	0	0	0	0	0	0
8	1.52×10^7	0	1.90×10^5	4.24×10^4	0	0	0
13	0	0	0	0	0	0	0
15	7.78×10^5	4.20×10^4	4.07×10^2	0	1.70×10^4[d]	0	0
21	0	0	0	0	0	0	0
26	0	2.17×10^3	0	0	0	0	0
32	1.35×10^3	2.63×10^4	0	0	0	0	0
34	5.46×10^5	5.13×10^3	0	0	0	0	0
39	2.92×10^7	0	0	0	0	0	0
43	0	0	0	0	0	0	0

[a] Dry weight.

[b] Control nonmedicated group. The results of all control groups were previously published (2) and are presented here for the benefit of the reader.

[c] Neomycin- and oxytetracycline-medicated group.

[d] Resistant colony (one colony of five examined from a 10^{-3} dilution; see text for discussion).

The percentage of swine shedding *Salmonella* as a function of time is plotted in Fig. 1A, which shows a decrease in both groups with time. The rate of decrease in shedding is faster in the oxytetracycline-neomycin group. Since all animals were inoculated initially, the statistical analysis dealt with the rate at which *S. typhimurium* disappeared. A rate of change in the probability of shedding per day was computed across all 28 days of the trial for each animal. These rates were then compared with a two-sample t test. The rate of decrease in shedding was significantly ($P < 0.01$) more rapid in the medicated group than in the nonmedicated controls.

Susceptibility tests were performed with 57 isolates from the treated group (T-2) over the sampling periods during the 28-day postinoculation period. The only instance of antibiotic resistance was with one isolate (pig no. 15) that was found to be resistant to kanamycin, neomycin, and streptomycin, as defined in the Federal Register (1). There were no changes in the susceptibility of *S. typhimurium* to the other antibacterial agents tested. This resistant colony (Table 2) was one of five isolated from a 10^{-3} dilution of sample spread on an antibiotic-free medium. The resistant count then was $\sim 3.4 \times 10^3$. These results demonstrate that the subtherapeutic level of neomycin and oxytetracycline did not cause a significant increase in resistant *S. typhimurium*. No resistance was detected in the 232 *S. typhimurium* isolates recovered from the nonmedicated group.

Clinical signs of disease, body temperature, and diarrhea scores are plotted in Fig. 2A. The observations demonstrate that clinical signs of infection were established in swine. In fact, the animals were on the border line of a severe illness that would normally be treated with therapeutic doses of the antibiotic. The medicated group demonstrated a decrease in the severity of diarrhea but no reduction in temperature elevation. The performance values for feed conversion (Table 5) indicate that the medicated feed was better than the control nonmedicated feed in its efficiency.

Calves. During the first postinoculation week, disease symptoms were so severe that there was concern for the survival of the animals. By the second week the animals had a generally healthy appearance. It seemed then that the challenge was near the lethal dose and the illness acute in nature, since recovery and disappearance of *S. typhimurium* was rapid and complete. The fecal counts observed during the course of this study were not as high as anticipated, but a higher challenge may have been fatal. In fact, one animal in the nonmedicated group died on day 6 postinoculation. The necropsy report indicated the presence of *Pasteurella hemolytica* and *S. typhimurium*. *S. typhimurium* was cultured from the liver, spleen, lung, a lymph node, and the ileocecal junction. *P. hemolytica* was isolated from the lungs. Gross lesions were present in the lung probably due to pasteurellosis with the additional stress of the *S. typhimurium* challenge.

The oxytetracycline-resistant *E. coli* examined before the start of the experiment represented an average of 65.6% of the total *E. coli* population in the infected controls and 54.8% in the medicated animals.

The viable *Salmonella* counts obtained from the fecal samples are shown in Table 3. It is clear that the *S. typhimurium* population in the nonmedicated group decreased with time. *S. typhimurium* was detected in only two ani-

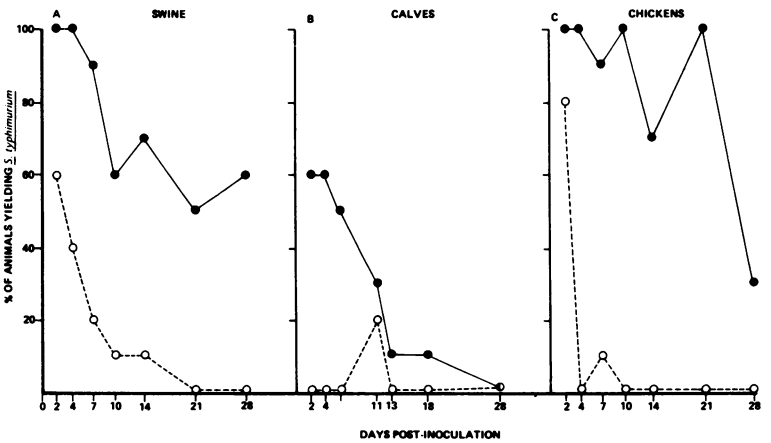

FIG. 1. *Percentage of animals shedding S. typhimurium. Symbols: ●, Nonmedicated controls; ○, oxytetracycline-neomycin treated.*

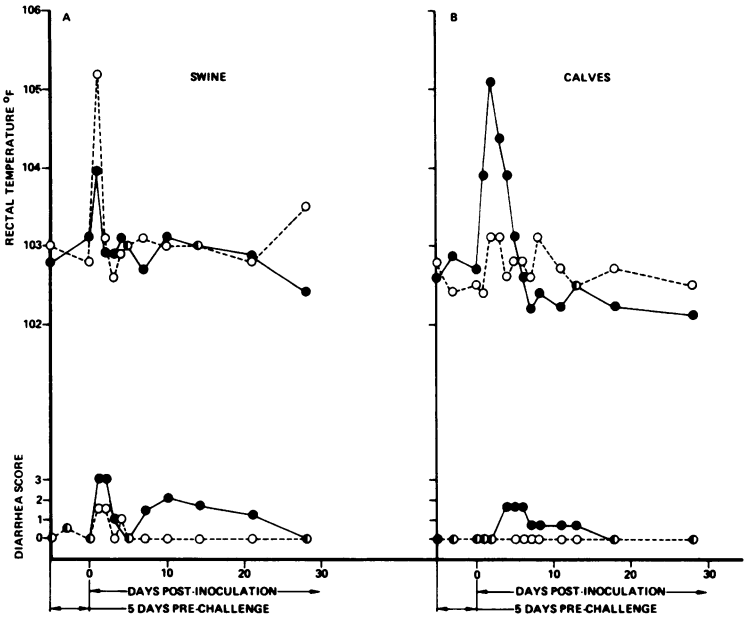

FIG. 2. *Temperature and diarrhea measurements (median values). Symbols: ●, Nonmedicated controls; ○, oxytetracycline-neomycin treated. Diarrhea was scored according to stool consistency with 0 as normal and 3 being a very loose consistency.*

TABLE 3. *S. typhimurium counts per gram[a] of calf feces*

Calf no.	Days (postinoculation)						
	2	4	6	11	13	18	28
T-1 group[b]							
12	8.89×10^6	2.60×10^7	6.96×10^7[c]				
26	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0
31	2.16×10^2	0	0	0	0	0	0
34	2.43×10^2	4.96×10^7	5.67×10^4	0	0	0	0
36	5.46×10^5	6.90×10^6	8.32×10^5	8.60×10^3	6.93×10^3	4.79×10^3	0
39	1.69×10^4	3.37×10^4	7.39×10^3	3.94×10^2	0	0	0
41	0	0	0	0	0	0	0
42	2.93×10^4	8.16×10^7	5.00×10^5	2.29×10^2	0	0	0
44	0	1.31×10^4	0	0	0	0	0
T-2 group[d]							
14	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0
19	0	0	0	4.78×10^4	0	0	0
24	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0
37	0	0	0	5.86×10^3	0	0	0
43	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0

[a] Dry weight.
[b] Control nonmedicated group.
[c] Died on postinoculation day 6.
[d] Neomycin- and oxytetracycline-medicated group.

mals of the medicated group and this detection was limited to one sampling period. A comparison of logarithms of the colony counts with a two-sample t test showed that the quantity of *Salmonella* in the medicated group was significantly ($P < 0.05$) less than that in the nonmedicated controls.

The medicated group exhibited many more *Salmonella*-free cultures (68/70) over the duration of the experiment than did the nonmedicated controls (44/66). A statistical analysis of the prevalence of *S. typhimurium* in the medicated versus the nonmedicated group revealed that there was a significant ($P < 0.05$) decrease in the medicated animals.

The percentage of calves shedding *S. typhimurium* as a function of time is plotted in Fig. 1B. Whereas the rate of decrease in shedding was faster in the neomycin-oxytetracycline group, statistical analysis of the rate of change in probability of shedding yielded no significant difference. A 95% confidence interval on the mean difference suggests that the difference in probability of shedding per day lies between 1.6% less to 0.10% more for the medicated group compared to the nonmedicated group.

There were no resistant organisms detected in the *S. typhimurium* population recovered from either the medicated or nonmedicated group.

Rectal temperature measurements and diarrhea scores are plotted in Fig. 2B. These observations demonstrate that clinical signs of infection were established in the nonmedicated calves and, as noted previously, the challenge was a near lethal one. The neomycin-oxytetracycline-medicated group showed a reduced temperature rise and better control of diarrhea compared with nonmedicated controls.

Chickens. The oxytetracycline-resistant *E. coli* examined before the start of the experiment represented an average of 30.0% of the total *E. coli* population in the infected controls and 14.9% in the medicated animals.

The viable *Salmonella* counts obtained from the fecal samples are shown in Table 4. A com-

TABLE 4. *S. typhimurium counts per gram[a] of chicken feces*

Calf no.	Days (postinoculation)						
	2	4	7	10	14	21	28
T-1 group[b]							
7	1.56×10^5	3.13×10^5	1.36×10^5	2.92×10^4	7.69×10^2	1.17×10^4	0
8	4.02×10^7	4.52×10^5	3.63×10^4	1.57×10^4	3.52×10^4	2.42×10^3	0
14	1.21×10^5	2.24×10^6	1.88×10^8	2.50×10^4	1.64×10^3	1.22×10^4	0
19	2.18×10^4	1.06×10^4	4.37×10^2	2.89×10^2	0	1.32×10^4	0
31	1.03×10^7	7.79×10^5	6.61×10^5	1.94×10^5	1.87×10^3	3.33×10^4	0
34	3.06×10^5	1.36×10^5	5.21×10^3	7.61×10^3	0	3.39×10^3	0
37	3.47×10^6	5.56×10^4	4.38×10^3	4.97×10^3	1.52×10^4	1.23×10^3	2.55×10^4
56	1.99×10^6	3.15×10^4	7.43×10^3	8.85×10^2	2.18×10^4	1.00×10^4	1.65×10^3
73	3.47×10^5	1.88×10^4	0	4.82×10^3	0	2.08×10^4	5.08×10^2
81	2.92×10^6	7.21×10^3	3.25×10^2	8.57×10^2	2.08×10^3	5.47×10^3	0
Environmental group							
18	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0
T-2 group[c]							
3	3.13×10^2	0	0	0	0	0	0
21	0	0	0	0	0	0	0
25	2.15×10^4	0	0	0	0	0	0
49	1.43×10^4	0	0	0	0	0	0
57	0	0	0	0	0	0	0
71	4.95×10^3	0	0	0	0	0	0
83	1.35×10^5	0	0	0	0	0	0
87	3.74×10^3	0	0	0	0	0	0
90	5.71×10^3	0	1.86×10^2	0	0	0	0
92	1.47×10^6	0	0	0	0	0	0

[a] Dry weight.
[b] Control nonmedicated group.
[c] Neomycin- and oxytetracycline-medicated group.

parison between the two groups demonstrates the beneficial effects of this subtherapeutic dosage regimen of oxytetracycline and neomycin. The quantity of *S. typhimurium* was considerably less in the medicated group than in the control group. This observation was shown to be a statistically significant decrease, having a value of $P < 0.001$. The medicated group also yielded many more *Salmonella*-free cultures (61/70) over the duration of the experiment than did the nonmedicated controls (11/70) (Table 4). This was also shown to be statistically significant, having a value of $P < 0.001$.

The percentage of chickens shedding *S. typhimurium* as a function of time is plotted in Fig. 1C. All chickens in the nonmedicated group were shedding *S. typhimurium* on day 21; a dramatic decline was observed only on the final day of the experiment. In contrast, 8/10 animals of the medicated group were shedding *S. typhimurium* on day 2 postinoculation, and there was only one more occurrence of shedding in the medicated group during the remainder of the trial. The results demonstrate that the oxytetracycline-plus-neomycin treatment decreased the extent of colonization. The rate of decrease in shedding was significantly ($P < 0.001$) more rapid in the oxytetracycline-neomycin group than in the nonmedicated controls.

There was no increase in the development of resistance of *S. typhimurium* in the medicated animals (no resistant colonies among the 33 recovered from the medicated group).

The values calculated for feed conversion (Table 5) suggest that the medicated feed was approximately equal to the control nonmedicated feed in its efficiency.

DISCUSSION

Some of the difficulties involved in determining the proper inoculum size and route of challenge allowing colonization without death were presented in an earlier report (2). Clinical signs of disease were apparent in swine and calves when using *S. typhimurium* at a concentration of approximately 10^{11} cells/ml. Temperature elevation and/or diarrhea of varying degree were evident in both medicated and nonmedicated groups. The viable counts of *S. typhimurium* obtained from all the swine and calves provided evidence that the organism successfully colonized the intestines. No clinical signs of disease were observed in the two groups of chickens, even though colonization by *S. typhimurium* was also successful, utilizing an inoculum of $\sim 2.9 \times 10^{11}$/chicken.

The challenges used in these model studies were intentionally much more severe than would be found under practical field conditions. Animals with symptoms of disease, as observed in the swine and calf experiments, would normally be treated with larger doses of antibiotic (therapeutic rather than subtherapeutic drug levels).

The numbers of *S. typhimurium* in medicated swine, calves, and chickens did not increase over those observed in the nonmedicated controls. On the contrary, the quantity of *S. typhimurium* isolated from the medicated swine, calves, and chickens was significantly less than that of their nonmedicated counterparts. The degree of prevalence and shedding of *S. typhimurium* was not increased in the medicated swine and chickens. If one assumes that an increase in the animal *Salmonella* reservoir is a hazard to man, these data suggest that the use of subtherapeutic levels of oxytetracycline plus neomycin actually reduces the potential hazards of *S. typhimurium* to human and animal health.

Except for one isolate obtained from swine, there was no change in the susceptibility of the 97 *S. typhimurium* colonies isolated from all medicated animals. The results demonstrate that the subtherapeutic level of neomycin and oxytetracycline did not cause a significant increase in resistant *S. typhimurium*.

Accepting that food animals are usually fed antibiotic for more than 28 days, we feel that the results would not have been significantly

TABLE 5. *Weight, feed consumption, and feed conversion*

Animal	Treatment	Average daily gain (kg/animal)	Gain relative to control (%)	Feed efficiency (feed consumed/wt gain)	Feed efficiency relative to control (%)
Swine	Nonmedicated	0.328	100.0	2.342	100.0
Swine	Medicated	0.497	151.5	1.971	118.8
Calves	Nonmedicated	0.678	100.0	5.244	100.0
Calves	Medicated	1.193	176.0	3.604	145.5
Chickens	Nonmedicated	0.030	100	1.744	100.0
Chickens	Nonmedicated[a]	0.028	92.2	1.758	99.2
Chickens	Medicated	0.030	100	1.729	100.9

[a] This group (environmental controls) was not inoculated.

different had we extended the experimental period, since all treated and nontreated animals of all species demonstrated a general decline in shedding *Salmonella* as a function of time. The medicated animals included in these experiments were fed antibiotic throughout the studies, although there is usually a withdrawal period prior to marketing. The inclusion of a withdrawal period conceivably could have removed any differences observed between the treated and nonmedicated groups with regard to antibiotic resistance, because of the reduction of antibiotic selective pressure.

A significant increase in the reservoir of *Salmonella* in food animals is speculated to constitute an increased risk to human health. The results reported here indicate there was no significant increase in the quantity, prevalence, and shedding of *S. typhimurium* in the medicated group compared to nonmedicated inoculated controls. Although only one strain was employed, the data generally demonstrate that the subtherapeutic use of oxytetracycline and neomycin actually decreases the quantity, prevalence, and shedding of *S. typhimurium* in animals. No evidence that would associate the continuous low-level feeding of oxytetracycline plus neomycin with an increased incidence of salmonellosis in animals or humans has been obtained.

ACKNOWLEDGMENTS

We are grateful to William Babcock and the staff at the Pfizer Animal Health Center at Terre Haute, Ind., for performing the bioassays on the feed, collecting the fecal samples, and generally assisting in the logistical support of this study. We wish to thank David S. Salsburg, who did the statistical analysis of the data, and R. J. Borovoy and F. A. Meyer for their technical assistance.

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