

## Acquired Resistance of Autochthonous *E. coli* in Controls and Orchardists Engaged in the Spraying of Oxytetracycline

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The use of antibiotics as food supplement for livestock can ultimately produce resistance in autochthonous enteric flora of the resident farming family. LEVY *et al* (1976) studying the ecology of human fecal flora in response to tetracycline feeding of chickens on a farm showed that passage of resistance occurred within five months in the majority of the farm family members. Possibly a more obvious means of developing resistance of enteric flora might be exposure to the spraying of an antibiotic used as a bactericide by orchardists. Such a retrospective study is the subject of this report which involved sprayer workers in various parts of the United States and their wives and children who represented controls.

### MATERIALS AND METHODS

A section 18 exemption of Federal Insecticide, Fungicide, Rodenticide Act (1974) (FIFRA) was secured by the participating groups to use Myco-shield (oxytetracycline-Pfizer) for spraying orchards of stone fruit in South Carolina, New Jersey, Michigan, and California. The use of masks was advocated for the sprayer worker but how rigidly their use was enforced is not known. The spraying in all cases occurred two to four mos. prior to the time when stool samples were acquired and cultured. All workers and family members volunteering stools ranged in age from 18 to 56 and presumably had not been exposed to tetracyclines previously in the last year nor had received this antibiotic as medication during this period.

Stool samples were quickly frozen with dry ice on receipt and kept in the frozen state until they reached our laboratory. Each specimen was thawed and cultured immediately on Eosin-methylene blue agar (EMB) and MacConkey's agar to isolate *Escherichia coli*, the organism chosen for study of resistance to oxytetracycline. Attention was given to other enteric flora but only to the extent that non-lactose fermenting pathogens were identified as a means of noting possible incipient diarrhea that could be interpreted as responsible for a nonphysiologic state in the subjects.

After overnight incubation on the primary isolation media, three to four colonies of E. coli of different morphology were selected from the EMB and MacConkey plates to serve as inoculum for trypticase soy broth culture which was then incubated at 35°C for 3-4 h. or for the length of time where the turbidity was measurable on the nephelometer scale as approximately  $9 \times 10^8$  organisms per milliliter. Two swabs of this material were then used to streak Mueller-Hinton plates after which oxytetracycline discs of 30 mcgm, 1.2 mcgm, and 0.5 mcgm were applied at various intervals on the inoculum lawn. Plates were incubated at 35°C overnight and reading of growth inhibition performed the next morning using Kirby-Bauer technique (BAUER et al. 1966). Locally, 75% of patient E. coli isolations show an average 19 mm level of inhibition to oxytetracycline (BANNISTER 1977) so that an inhibition zone of 18 mm or greater was used to be evidence of sensitivity to this antibiotic for the 30 mcgm disc and 9 mm or greater inhibition for the 1.5 mcgm disc. Any zone of inhibition around the 0.5 mcgm disc was considered to be sensitivity. When some difficulty was experienced in reading lower concentration inhibition, the more readily readable higher concentration reading was used. All readings were made in terms of bacteriostasis as evidenced by clear zones of growth inhibition. No second generation growth was observed but tube inhibition was done on two or three cultures to insure that the Kirby-Bauer readings were interpretable as realistic levels of bacteriostasis.

## RESULTS

Table 1 (combined column) shows that of the 62 test and control specimens received, all but 9 cultures grew E. coli on the primary isolation plates. (Seven of these cultures were from oxytetracycline exposed workers; two were from controls.) In the South Carolina group (22 persons - 14 exposed, 8 controls), 20 individuals (12 exposed, 8 controls) grew cultures of E. coli that were very sensitive to oxytetracycline. One of the thirteen exposed individual's culture (7.7%) in this group grew E. coli that was completely insensitive at all three levels of disc concentration. One exposed subject's culture failed to grow.

The New Jersey cohort of 17 grew 12 cultures (5 controls, 7 exposed) of E. coli that were sensitive to oxytetracycline. Five cultures (3 exposed, 2 controls) were Kirby-Bauer resistant. Three of 10 exposed individuals in this group had E. coli cultures (30%) which demonstrated no zones of inhibition for all three discs employed. Two controls, the wives of two of the three exposed subjects mentioned above similarly demonstrated complete refractoriness to all three concentrations of oxytetracycline.

TABLE 1

Source of Culture from Exposed (E) and Control (C) Subjects and Sensitivity of Their Autochthonous E. coli to Oxytetracycline.

	S.C.			N.J.			MI			CA			Combined (62)		
	E	C	T	E	C	T	E	C	T	E	C	T	E	C	T
No Growth of <u>E. coli</u>	1	0	1	0	0	0	3	0	3	3	2	5	7	2	9
Sensitive to oxytetracycline	12	8	20	7	5	12	3	3	6	5	1	6	27	17	44
Resistant to oxytetracycline	1	0	1	3	2	5	0	3	3	0	0	0	4	5	9
% Resistant **	7.7	0.0	4.8	30.0	28.6	29.4	0.0	50.0	33.3	0.0	0.0	0.0	12.9	22.7**	17.0**

++ Significantly ( $P < .01$ ) less than 50%.

+ No significant difference ( $P > .05$ ) in percent resistance between exposed and control either within location or between location by Fisher's Exact Test.

\* % based on total of sensitive and resistant

\*\* Corrected to 19% due to prior medication of 1 control.

In the Michigan group of 12 specimens, three specimens failed to grow any E. coli organisms. Six specimens (3 exposed, 3 controls) grew E. coli which were sensitive at all concentrations of the antibiotic. An additional 3 specimens from three controls (50%) grew E. coli cultures that were completely resistant to all disc concentrations.

The California specimens (11 in number) had five cultures which failed to grow any enteric flora at all. Five specimens from individuals exposed to the sprayed antibiotic grew E. coli that were sensitive at all concentrations of oxytetracycline discs. The one remaining specimen from a control grew E. coli that was similarly oxytetracycline sensitive.

Cultures of E. coli that proved resistant then amounted to a total of nine or 17% when all specimens culturing E. coli are considered. Four of these resistant specimens were from exposed individuals; five were from household or other controls.

Resistance in all exposed individuals (4/31) amounted to 12.9%; resistance in the controls (5/22) was higher 22.7%.

#### DISCUSSION

In an attempt to explain the higher control resistance figures, a further check was made by phone to determine if any controls had tetracycline as medication during the past year. It was found that one control in the Michigan group did have such treatment in January for influenza of approximately 1 g per day for 10 days. This would be the basis for dropping this subject from the study and changing the control resistance figure to 19% from the original 22.7%. That this individual's presumed resistance of her E. coli was due to medication is based on the work of GIBSON and THOMPSON (1956) with chlorotetracycline and LEPPER et al (1954) with erythromycin. These investigators showed regression of antibiotic resistance within a population following withdrawal of an antibiotic to require almost 6 mo. to substantially decrease. Our control subject's stool was obtained approximately three mo. after medication which makes it doubtful if resistant strains due to medication had been eliminated.

The other two cases of resistance in the controls of the Michigan group cannot readily be explained except as normal incidence. In one case, the husband's stool failed to grow E. coli while the wife's E. coli strains showed no sensitivity at all to any of disc concentrations employed. In the other case, the husband showed considerable sensitivity of his E. coli strains to oxytetracycline while the wife's strains were refractory to all concentrations of this antibiotic.

It is in the New Jersey group that resistance in control subjects seems easily explainable. In this group (Table 1) the two controls demonstrating resistance to oxytetracycline are the wives of two exposed subjects with oxytetracycline resistant

E. coli. Both husband and wife subjects demonstrated complete resistance of their E. coli strains to all concentrations of disc tetracycline. (The third exposed subject showing resistance was unmarried.)

In the California group, no resistance was encountered among exposed subjects or controls. In the South Carolina group, only one of the exposed subjects demonstrated resistance. Interestingly, five of the control stools used in the South Carolina studies were provided by local Medical University health personnel (doctors, nurses, etc.) and these E. coli strains showed excellent sensitivity to all concentrations of oxytetracycline, which is generally the case for hospital strains at our Medical University hospital.

The total incidence of resistant E. coli in the specimens from the four states for both exposed and control group appears to be low when compared with figures worldwide. DATTA (1969) reported isolating 52% resistant coliforms to tetracycline in fecal specimens collected before admission from patients awaiting elective surgery. MOOREHOUSE (1969) similarly detected R factor carrying strains to tetracycline in 81% of all coliforms from healthy infants under age five. Thus it would appear that antibiotic resistance, particularly broad spectrum resistance, is very common among gut coliforms and it is likely that everyone, well or ill, carries some R<sup>+</sup> coliforms at all times. Certainly as RICHMOND (1972) insists selection increases their abundance in the individual but even in the absence of the known antibiotic contact the organisms are there and constitute a potential source of refractory infection.

In this limited study, resistance of autochthonous E. coli to oxytetracycline in exposed subjects did not differ from that observed for control subjects. The use of an antibiotic spray for stone fruit trees does not appear to constitute an occupational hazard when respiratory masks are worn.

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