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Frequency of Four Classes of Tetracycline Resistance Determinants in *Salmonella* and *Shigella* spp. Clinical Isolates

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The frequencies of tetracycline resistance determinants of the classes A, B, C, and D were determined in 53 non-lactose-fermenting clinical isolates. The most frequent class of determinant in *Salmonella typhimurium* and *Shigella flexneri* strains was class B; however, the predominant determinant in *Shigella sonnei* strains was class C.

In gram-negative bacteria, resistance to tetracycline is generally encoded on plasmids (3). In these bacteria, four distinct classes of tetracycline resistance exist (A, B, C, and D), all inducible by tetracycline but distinguishable by resistance to tetracycline and tetracycline analogs (8). DNA sequence analysis of the *tet* determinants of classes A, B, and C has revealed significant homologies among the *tet* structural genes (7), although the different classes of *tet* determinants are distinguished by DNA-DNA hybridization. The prototype plasmids or transposons for the four tetracycline classes are RP1 or pRSD1 (Tn*1721*) for A, R100 (Tn*10*) for B, pSC101 or pBR322 for C, and RA1 for D (1, 8). Marked differences in the frequencies of these determinants in a population of lactose-fermenting coliforms have been reported (7), the class B determinant being the one most frequently encountered (73.3%) (7).

In this work, we determined the frequency of distribution of the four tetracycline resistance determinants among strains of a relatively homogeneous bacterial population. This population consisted of 53 non-lactose-fermenting coliforms: 20 *Salmonella* sp. (16 *Salmonella typhimurium*, 2 *S. enteritidis*, 1 "*S. london*," and 1 "*S. poona*") and 33 *Shigella* sp. (25 *Shigella sonnei* and 8 *S. flexneri*) strains. These strains were isolated from different patients in four hospitals in Mexico City, Mexico, during the years 1978 and 1979. During those years, there were no reports of *Salmonella* or *Shigella* epidemics. Strains presenting the same pattern of antibiotic resistance to tetracycline, streptomycin, and sulfonamide were selected for this study. The plasmid content of these strains was determined by agarose gel electrophoresis of clear alkaline lysates (6). All the strains exhibited extrachromosomal DNA bands (data not shown). The plasmid patterns obtained were heterogeneous. A major fraction (94%) of the strains contained one large plasmid. The sizes of these plasmids varied from 40×10^6 to 80×10^6 daltons, depending upon the strain. In 45% of the *Salmonella* strains and 100% of the *Shigella* strains, several DNA bands with molecular masses of less than 10×10^6 daltons were also present. A high percentage (74%) of the strains transferred by conjugation the tetracycline resistance marker to *Escherichia coli* HB94 (*hsdR*).

To determine the frequency of tetracycline resistance determinant classes in the *Salmonella* and *Shigella* strains, all the strains were tested by colony hybridization (4) (total cellular DNA) and by dot blot hybridization (5) with DNA enriched for plasmid DNA from clear alkaline lysates (6). Strains containing no tetracycline resistance determinants, *E. coli* NK5561 *lacZ*::Tn*10* (class B), and strains carrying the low-copy-number plasmids RP4 (class A), pSC101 (class C), and RA1 (class D) were used as controls. The restriction fragments used as DNA probes were the following: for class A, the 5.48-kilobase *Eco*RI-*Hin*dIII fragment from Tn*1721* (1) present in plasmid pMS02; for class B, the 1.27-kilobase *Hin*cII fragment from plasmid pRT29 (11); for class C, the 0.92-kilobase *Eco*RII fragment from pBR322 (2, 10); and for class D, the 3.05-kilobase *Hin*dIII-*Pst*I fragment from pSL101 (7). To construct plasmid pMS02, we transposed Tn*1721* from plasmid pRSD1 (1) to plasmid pMS01, a derivative of plasmid pBR322 which contained a deletion of the major portion of the *tet* gene. To obtain the DNA probes, we purified plasmid DNA from clear alkaline lysates by centrifugation through cesium chloride-ethidium bromide gradients (6). Restriction enzyme digestions were performed in accordance with the instructions of the manufacturers (New England BioLabs, Inc., and Amersham Corp.). The DNA fragments were separated by electrophoresis in 1% agarose gels, and the restriction fragments bearing the tetracycline resistance determinants were cut from the gels and then isolated by electroelution (6). These fragments were labeled by nick translation (9) with [α-^{32}P]dCTP (Amersham Corp.). Colony (4) and dot blot (5) hybridizations were performed by incubation of the nitrocellulose filters containing the DNA in prehybridization and hybridization buffers (4). Both buffers contained 50% formamide. The hybridization buffer contained approximately 10^6 dpm of denaturated, nick-translated probe DNA. The hybridizations were carried out at 42°C for 18 h. The filters were washed (4) and dried at 70°C for 1 h. For autoradiography, Kodak XK-1 X-ray films were exposed for 1 to 3 days to the filters; the films were then processed by standard procedures.

In general, colony and dot blot hybridizations yielded the same results (Table 1). Colony hybridization yielded unclear results with eight *S. sonnei* strains; however, these strains showed clear positive dot blot hybridization with the class C probe. The majority of the *S. typhimurium* and *S. flexneri* strains analyzed carried the class B determinant. Classes C and A were detected in fewer *S. typhimurium* and *S. flexneri*

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TABLE 1. Frequencies of classes of tetracycline resistance determinants in *Salmonella* and *Shigella* strains[a]

Bacterial strains (n)	% of strains with determinant(s) of classes(es):					
	A	B	C	D	A and B	C and D
Salmonella typhimurium (16)	0.0	75.0	25.0	0.0	0.0	0.0
Shigella sonnei (25)	4.0	24.0	72.0	8.0	0.0	8.0
Shigella flexneri (8)	25.0	87.5	12.5	0.0	25.0	0.0

[a] The *Salmonella* species tested were as follows: *S. enteritidis* (n = 2), "*S. london*" (n = 1), and "*S. poona*" (n = 1). Class A was present in *S. enteritidis*, class B was present in *S. enteritidis*, and "*S. poona*," and class C was present in "*S. london*."

strains. Class D was not detected in these strains (Table 1). The frequencies of the *tet* determinants among these non-lactose-fermenting coliforms were similar to those reported for lactose-fermenting coliforms (7), except that the frequency of the class C *tet* determinant in *S. typhimurium* was higher (25%) than that (8%) reported for lactose-fermenting coliforms (7). However, the *S. sonnei* strains presented a different frequency of distribution of the *tet* classes; the majority of these strains carried the C determinant, and fewer carried the other determinants (Table 1). A relatively high percentage of the strains (7.5%) carried two determinants; combinations of A and B and of C and D were encountered in *S. flexneri* and *S. sonnei* strains, respectively (Table 1).

The tetracycline resistance levels of all the clinical isolates were determined as described by Mendez et al. (8). The tetracycline resistance phenotype was inducible in all but six of the strains. *E. coli* strains containing the determinants A, B, C, or D are resistant to 75 to 150, 150 to 200, 25, or 100 μg of tetracycline per ml (8). The tetracycline resistance levels shown by the majority of the strains tested corresponded to the *tet* determinant present in these strains. However, in general, the *Salmonella* sp. strains carrying the class B or C determinant showed a higher level of tetracycline resistance than did the *Shigella* sp. strains bearing the same determinant (data not shown).

The results presented in this work showed that there was an unequal distribution of the four classes of tetracycline resistance determinants between the two genera of non-lactose-fermenting coliforms (*Salmonella* and *Shigella*) and also between the two *Shigella* species (*S. sonnei* and *S. flexneri*). The most frequent determinant present in the *Salmonella* and *S. flexneri* strains analyzed was class B, as has been reported for other gram-negative bacteria (7); however, we found that the predominant determinant in *S. sonnei* was class C. An unequal distribution of the *tet* determinants among different *Salmonella* species was also suggested by our results. However, since *S. typhimurium* was the only species for which a sufficient number of strains was studied, a greater number of other *Salmonella* strains will have to be analyzed to support this possibility. Our results, like others (7), suggest the presence of multiple dynamic factors affecting the frequency of each class of determinant in different genera and species of gram-negative bacteria.

The presence of two different determinants in several strains suggests the possibility of the generation of new recombinant variants among the determinants. This possibility would contribute to the heterogeneity of genes coding for resistance to tetracycline and therefore to the variability of the tetracycline phenotype.

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