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Changing Patterns of Plasmid-Mediated Drug Resistance During Tetracycline Therapy

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The patterns of drug resistance and the frequency of conjugative R plasmids in intestinal *Escherichia coli* from 88 patients treated for a skin disease (*acne vulgaris*) with low oral doses of tetracycline are reported. The proportion of patients with resistant bacteria was progressively greater in patients who received tetracycline for 1 week, 4 weeks, or longer (from 50 to 88%). No multiply drug-resistant bacteria were detected before treatment or after 1 week of treatment. After more than 4 weeks of treatment, multiply drug-resistant *E. coli* were isolated from about 50% of the patients. The origin and selection of R plasmid-determined multiple drug resistance are discussed.

The problems related to the increasing prevalence of infectious multiple-drug resistance presently have serious implications on human health all over the world (9). The infectious multiple-drug resistance is mediated by conjugative R (resistance) plasmids, which are extrachromosomal deoxyribonucleic acid molecules possessing genes generally determining the production of enzymes that are able to inactivate the effect of the antibiotics. The pathogenic enteric bacteria, which may become multiply resistant include the organisms that cause bacillary dysentery, typhoid fever, cholera, and plaque. R plasmids are particularly common in *Shigella* and *Salmonella* with frequencies of about 50% as reported from many countries (7, 9, 14, 21, 25). The frequencies of R plasmids in the common enteric bacteria such as *Escherichia coli, Proteus* and *Klebsiella* presently seem to be at least 10 to 20% in populations at large (4, 9, 17, 24) and at least 20 to 30% in hospitals (1, 9, 17).

Extrachromosomal-determined antibiotic resistance is also well known in *Staphylococcus* (18) and is currently reported in species within the genera *Streptococcus* (5, 16), *Haemophilus* (11), *Mycobacterium* (13), *Clostridium* (19), and *Bordetella* (23).

The R plasmid-mediated resistance is often, and presumably with increasing frequency, multiple, which means that the R plasmid-carrying bacteria are resistant to 3 to 5 or even more antibiotics. There are also some indications of an increasing frequency of multiple resistance in especially virulent strains (E. S. Anderson, E. J. Threfall, J. M. Carr, and J. A. Frost, Proc. Soc. Gen. Microbiol., no. 1, p. 3, 1974) of different bacteria and in bacteria that produce plasmid-determined pathogenicity factors (9, 10, 26).

Certain antibiotics, notably tetracycline, have a great potential for selection of bacteria with R plasmid-determined drug resistance (8). Such bacteria may also be resistant to other drugs than the one used for selection of the R plasmids (8). The treatment of severe cases of *acne vulgaris* with low doses of tetracycline for long periods is widely used and generally accepted to be without serious side effects (20). In this paper, we report on the drug resistance patterns and the frequency of R plasmids in *E. coli* isolated from a group of otherwise healthy people that had been treated for different periods with low oral doses of tetracycline for a skin disease (*acne vulgaris*).

MATERIALS AND METHODS

Patients. All patients (88) were seen in the outpatient clinic at the Department of Dermatology, Marselisborg Hospital, Aarhus, for treatment with tetracycline of *acne vulgaris* or *rosacea*. Apart from this treatment, none of the patients had been treated with antbiotics or had been admitted to a hospital for the last 6 months.

The treatment consisted of a single oral dose of tetracycline (250 to 500 mg/day) for the first 2 weeks. Thereafter, the dose generally was 250 mg/day. The patients were divided into four groups. One group consisted of patients (18) not yet treated. A second group (13 patients) had been treated for 7 days, and a third group (16 patients) had been treated for 1 month. The patients in the fourth group (41) had been treated for more than 1 month, some of them even for several years.

Fecal culture. Specimens of feces were collected

from the patients at the outpatient clinic on charcoal-imbibed cottonwool swabs. The swabs were sent to our laboratory in Stuart medium and inoculated on a bromothymol blue agar plate with 1% lactose and on a blood agar plate without peptones and incubated overnight at 37°C. On the blood agar plate were placed nine different paper disks containing antibiotics, and bacteria showing inhibition zones corresponding to a minimal concentration (per ml of agar) of trimethoprim, 10 μg, streptomycin, kanamycin, gentamicin, or tetracycline, 15 μg, chloramsulfonamide, 100 μg, were considered resistant. A secondary susceptibility test was performed on a colony of *E. coli* from the lactose plate. The results of the primary and secondary susceptibility tests were compared with the purpose of excluding loss of resistance in the bacterial strain purified for further examination and were always identical. The lactose plates were examined, and the colonies with the colonial appearance of *E. coli* were streaked separately on a lactose plate with 15 μg of tetracycline per ml of agar. The proportion of the ten colonies able to grow on the plates were scored. Identical results for the ten colonies were obtained in all cases but one. All swabs were inoculated in a tube with 5 ml of brain heart infusion broth (Difco Laboratories) containing 15 μg of tetracycline per ml. If growth appeared in the tubes but not on the plates containing tetracycline, bacteria from the tubes were examined following the procedure described above. This happened in four instances.

Identifications of strains were performed by means of standard bacteriological methods.

Resistance transfer. All resistant strains of *E. coli* were tested for transfer of resistance to the *E. coli* K-12 strain J53-1 according to methods described previously (17). All the J53-1 strains were able to transfer their acquired resistance pattern to another recipient *E. coli* K-12 strain J 62-2. This was done separately for each marker of the resistance pattern.

RESULTS

The 88 patients were divided into four groups as described in Materials and Methods. The results are given in Tables 1, 2, and 3. It is seen that the proportion of patients with resistant *E. coli* increases from 50% before treatment to 69% after 1 week of treatment, to 75% after 4 weeks, and to 88% in the group treated for more than 4 weeks. The figures for the frequencies of R plasmids in the resistant bacteria in the four groups were 63, 67, 75, and 83%. No multiple-resistant *E. coli* (resistant to 3 or more antibiotics) was found in patients before or after 1 week of treatment. However, in the course of the treatment multiple-resistant *E. coli* could be isolated with increasing frequency (from 38% of the patients treated for 4 weeks and from 49% in the group treated for more than 4 weeks). Resistance against gentamicin and nalidixic acid did not occur among the strains investigated. The resistance patterns of these multiply drug-resistant bacteria were partially or completely transferable in 83 and 85% of the cases, respectively. The patterns of resistance transferred are shown in Table 3.

A few points for explanation of the results are needed. It is known that some resistance plasmids are not self-transmissible. Because of that and limitations in the method for determination of transfer, the figures for the fraction of resistant bacteria containing R plasmids are minimum values.

Although only semiquantitative methods are used, it seems safe to conclude that in patients treated for a few weeks or longer, the majority of the population of *E. coli* had the resistance pattern stated in Table 2.

The rather unexpected finding of only susceptible *E. coli* in some of the patients treated for 4 weeks or longer needs an explanation. One would certainly have expected these bacteria to be resistant to tetracycline. It appeared however, that the majority of bacteria isolated in these cases were either mono- or multiple-resistant *Proteus mirabilis* or *Streptococcus faecalis*, while *E. coli* accounted for only a minor

TABLE 1. *Frequency of drug resistance and occurrence of R plasmids in E. coli from patients with acne vulgaris treated with tetracycline*

Duration of treatment	No. of patients	No. (%) of:			
		Resistant[a] strains	R plasmids[b] in resistant strains	Multiply[c, d] resistant strains	R plasmids in multiply resistant strains
Before treatment	18	9 out of 18 (50)	5 out of 9 (63)	0 out of 18 (0)	
1 week	13	9 out of 13 (69)	6 out of 9 (67)	0 out of 13 (0)	
4 weeks	16	12 out of 16 (75)	9 out of 12 (75)	6 out of 16 (38)	5 out of 6 (83)
More than 4 weeks	41	36 out of 41 (88)	30 out of 36 (83)	20 out of 41 (49)	17 out of 20 (85)

[a] t (Rank test) = 2.85, 2p = 0.44%.
[b] t (Rank test) = 1.64, 2p = 10.1%.
[c] t (Rank test) = 4.02, 2p < 0.01%.
[d] Strains resistant to 3 or more of the following drugs: tetracycline, sulfonamide, streptomycin, trimethoprim, chloramphenicol, kanamycin, nalidixic acid, ampicillin, and gentamicin.

TABLE 2. *Patterns of resistance in E. coli from patients before and after treatment with tetracycline*

Before treatment	Treatment for:		
	1 week	4 weeks	More than 4 weeks
Sensitive (9)[a]	Sensitive (4)	Sensitive (4)	Senstitive (5)
Su[b] (1)	Su (1)	Tc (4)	Tc (9)
Tc (6)	Tc (6)	Tc-Su (1)	Tc-Su (2)
Tc-Su (1)	Tc-Ap (2)	Tc-Sm (1)	Tc-Ap (3)
Su-Sm (1)		Tc-Su-Sm (4)	Tc-Sm (3)
		Tc-Su-Sm-Ap (1)	Tc-Su-Sm (10)
		Tc-Su-Sm-Cm-Ap (1)	Tc-Su-Sm-Ap (2)
			Tc-Su-Cm-Ap (1)
			Tc-Su-Sm-Cm-Ap (3)
			Tc-Su-Tp-Sm-Ap (1)
			Tc-Su-Tp-Sm-Cm-Km (1)
			Tc-Su-Sm-Cm-Km-Ap (1)

[a] Number in parentheses is number of patients.
[b] Tc = tetracycline, Su = sulfonamide, Tp = trimethoprim, Sm = streptomycin, Cm = chloramphenicol, Km = kanamycin, Ap = ampicillin, Nx = nalidixic acid, Gm = gentamicin.

TABLE 3. *Patterns of resistance transferred from the drug resistant E. coli strains*

Before treatment	Treatment for:		
	1 week	4 weeks	More than 4 weeks
Su (1)[a]	Tc (5)	Tc (4)	Tc (13)
Tc (4)[b]	Tc-Ap (1)	Tc-Sm (2)	Tc-Su (1)
		Tc-Ap (1)	Tc-Sm (6)
		Tc-Su-Sm (2)	Tc-Ap (1)
			Tc-Su-Sm (4)
			Tc-Su-Sm-Cm (1)
			Tc-Su-Sm-Km-Ap (1)
			Tc-Su-Sm-Cm-Ap (2)
			Tc-Su-Cm-Tp-Km (1)

[a] Number in parentheses is number of patients.
[b] Tc = tetracycline, Su = sulfonamide, Tp = trimethoprim, Sm = streptomycin, Cm = chloramphenicol, Km = kanamycin, Ap = ampicillin, Nx = nalidixic acid, Gm = gentamicin.

fraction of the aerobic organisms in the intestines of these patients.

DISCUSSION

The results clearly illustrate that long-term tetracycline treatment has two main effects on the drug resistance of *E. coli* in the intestinal flora. One is the occurrence of an increasing frequency of R plasmid-determined resistance and the other is the progressive tendency for this resistance to be multiple.

It is known that tetracycline very effectively selects for R plasmid-determined resistance from studies of R plasmids in enteric bacteria from animals given tetracycline in their feed (9) and from patients treated for a shorter time (10 days) with tetracycline (2, 8, 12). It is important to realize that the R plasmid-carrying bacteria, once selected to constitute the majority of the aerobic intestinal flora, disappear slowly only after withdrawal of the antibiotic treatment (2, 6). It is also known that R plasmid-carrying bacteria are, to a great extent, disseminated to other people in the patient's environment (6).

Although it has been reported that treatment with certain antibiotics such as tetracycline, may sometimes select for bacteria that are multiply resistant (8), there is no obvious explanation why this should happen with the frequency and apparent regularity that was seen in this study. The fact implies that the additional resistance genes or other factors somehow make these bacteria more fit in an environment containing tetracycline than are bacteria with R plasmid-determined tetracycline resistance only. It must be remembered that multiply drug-resistant bacteria were not demonstrated at all in patients before or after 1 week of treatment, whereas bacteria with R plasmid-determined tetracycline resistance were detected fairly often in these groups. Therefore,

the question is not only why the multiply drug-resistant bacteria came to dominate, but also where they came from.

It is known that R plasmid-carrying bacteria are transferred to humans, either from food contaminated with animal bacteria (3), from other human beings directly (6), or via contaminated food or water (15, 25). Before treatment, the patients may already harbor multiply drug-resistant bacteria at a concentration below the detectability of the methods used, or they may acquire them during the treatment. A third possibility that might play a role in the development of the resistance patterns could be the transfer of drug resistance from the anaerobic bacteria that constitute the majority of the enteric bacteria in the bowel flora.

Finally, we wish to emphasize the need for an intensified research of the factors responsible for the increasing frequency of bacteria that are multiply drug resistant. It is possible that only certain antbiotics, as shown here for tetracycline, have the ability for a preferential selection of multiple resistance. If this is true, a limitation in the use of these drugs should be recommended.

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