Tetracycline Resistance in *Escherichia coli* Isolates from Hospital Patients

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Hospital isolates of *Escherichia coli* resistant to tetracycline (TC) were studied to identify mechanisms which regulate TC resistance levels and ability to transfer TC resistance. Antibiotic resistance patterns, resistance levels to TC, and ability to transfer TC resistance were determined for the isolates. Similar data were obtained for the transferable plasmids after transfer to several new host strains of *E. coli*. Of the 110 isolates, 50% were able to transfer TC resistance by conjugation. There was a nearly linear relationship between the minimum inhibitory concentration (MIC) of TC for the hospital strains and the percentage of strains at a given MIC that could transfer TC resistance. The strains that were simultaneously resistant to tetracycline, streptomycin, and ampicillin had relatively high MICs of TC and high ability to transfer TC resistance. These results and surveys of TC-resistant *E. coli* by others suggest that TC resistance levels and transmissibility may be influenced by other resistance markers. The isolates which did not transfer TC resistance by conjugation were tested for the presence of TC resistance plasmids by mobilization or by transformation with deoxyribonucleic acid from the isolates. Evidence for plasmid-mediated TC resistance was found in 92 (84%) of the 110 hospital strains.

When drug resistance is mediated by a plasmid both the resistance level and the transfer of resistance are important clinical parameters. A review of the early literature and a discussion of these parameters has been presented by Watanabe (35) and more recently by others (8, 26). For penicillin, chloramphenicol (CM), and the aminoglycosides, the plasmid mediates production of an enzyme which catalyzes the inactivation of the antibiotic. In the case of tetracycline (TC), the plasmid product probably interacts directly with the cell membrane (2, 13, 20, 33) to block active uptake of the antibiotic (10, 18, 27). Thus, although both pilus formation and TC resistance can be described by the accumulation of a specific protein at or in the membranes of plasmid-bearing bacteria, little is known about possible interrelationships between these two phenomena. It is not known at present why the various plasmids produce different levels of TC resistance when transferred into a common host strain. It is known, however, that TC resistance is induced by incubation of cells with subinhibitory concentrations of TC (11, 19), suggesting repressor control (12), and that induction is a common feature of TC resistance whether it is transferable or not (manuscript in preparation).

This paper reports the results of a study of 110 TC-resistant *Escherichia coli* hospital isolates. The minimum inhibitory concentration (MIC) of TC, the antibiotic resistance pattern, and the ability to transfer TC resistance were examined. There appeared to be a relationship between the level of TC resistance of the various isolates and their ability to transfer resistance by conjugation. In addition, the ability to transfer TC resistance appeared to be influenced by the other resistance determinants present in the host cell.

Evidence is presented that in at least 92 of the 110 isolates TC resistance is plasmid mediated. In the other isolates, studies of TC uptake and minocycline resistance demonstrate that the mechanism of TC resistance is the same as for plasmid-mediated resistance.

MATERIALS AND METHODS

Cultures and chemicals. Clinical cultures of *E. coli* that were resistant to TC were provided over a 6-month period by K. Wicher, E. J. Meyer Memorial Hospital (63 cultures); N. O'Connell, Sisters of Charity Hospital (30 cultures); and E. Neter, Children's Hospital (17 cultures), all located in Buffalo, N.Y. Laboratory bacteria and plasmids used are listed in Table 1. TC hydrochloride and streptomycin sulfate (SM) were obtained from Nutritional Biochemicals

Corp.; ampicillin trihydrate (AM) was a gift of R. Sgroi, Bristol Laboratories; kanamycin sulfate (KM) was from Sigma Chemical Co. and CM was from Calbiochem. Nalidixic acid (NA) was a gift from F. Nachod, Sterling Winthrop Research Institute. 1-Methionine was obtained from Nutritional Biochemicals Corp. Penassay agar, Penassay broth, and MacConkey agar were purchased from Difco. Minimal medium was prepared according to Davis and Mingoli (9).

Incubations. All liquid incubations and growth were carried out in Penassay broth at 37 C unless otherwise stated.

Mating. The standard conjugation procedure consisted of incubating 2.0 ml of stationary-phase recipient (*E. coli* J6-2N, C600N, or J5-3) with 0.2 ml of log-phase donor culture in 7.8 ml of Penassay broth for 60 min. The recipient-donor cell ratio varied, but in most cases it was approximately 10:1. Selection and counterselection were accomplished with the appropriate antibiotics or supplemented minimal agar plates. All hospital cultures that did not transfer TC resistance to J6-2N or C600N were also mated with J6-2N, using a different procedure in which equal volumes of donor and recipient cultures (both in stationary phase) were incubated for about 24 h at 37 C. This procedure increased the sensitivity of detectable transfer from a transfer frequency of approximately 10^{-7} to about 10^{-9} . The hospital strains which did not transfer TC resistance were tried in mobilization (co-transfer) tests using a modification of the procedure of Anderson (1). Tubes containing 2.0 ml each of stationary-phase ($\sim 10^9$ cells/ml) hospital strain (the potential donor) and *E. coli* C600 (R1-19KM⁺) (the transfer unit-bearing strain) were incubated for 2 h. Two milliliters of warm Penassay broth and 2.0 ml of log-phase ($\sim 10^9$ cells/ml) *E. coli* J6-2N (the recipient) were added, and the incubation was continued overnight. Colonies were selected on MacConkey plates containing TC, KM, and NA, each at 20 μ g/ml. When *E. coli* J6-2(RTEM) was used as the transfer unit-bearing strain, the same procedure was used except that selection was on plates containing TC and NA only. The J6-2N cultures that received TC resistance were further mated with *E. coli* J5-3 as described above.

MIC. The MIC of TC was determined, using a Steers replicator (32), by inoculating Penassay agar plates (about 10^8 cells) containing TC at 50 to 350 μ g/ml in 50- μ g/ml intervals. The cultures prepared for inoculation were grown to log phase (3 h). During the last 30 min of log-phase growth, TC at 2 μ g/ml was added so that all cultures would be fully induced for TC resistance. The MIC was judged to be the concentration at which no or very few cells were seen to be growing after 18 h at 37 C. The results were reproducible to within 50 μ g of TC per ml. The MICs of AM, CM, KM, or SM were similarly determined, except that the concentration of antibiotics used in the Penassay plates was 12.5, 25, 50, 100, 200, 300, and 400 μ g/ml.

Patterns of antibiotic resistance. Penassay agar plates containing 12.5 μ g of AM, CM, KM, or SM per ml, or 20 μ g of NA per ml, were inoculated using a Steers replicator, with cultures grown to log phase.

TABLE 1. *Bacterial strains and plasmids*

Code	Relevant markers	Source
Strains		
J6-2	F ⁻ , <i>his</i> ⁻ , <i>pro</i> ⁻ , <i>trp</i> ⁻ , <i>lac</i> ⁻	J. T. Smith
J6-2N	J6-2 trained to chromosomal resistance to nalidixic acid at 200 μ g/ml	
C 600	<i>thr</i> ⁻ , <i>leu</i> ⁻ , B ₁ ⁻ , <i>hsr</i> ⁻ , <i>hsm</i> ⁻ , <i>lac</i> ⁻	M. Meselson
C 600N	C 600 trained to chromosomal resistance to nalidixic acid at 200 μ g/ml	
J5-3	F ⁻ , <i>met</i> ⁻ , <i>lac</i> ⁺	J. T. Smith
Plasmids		
R1-19	F-like (<i>fi</i> ⁺), derepressed transfer unit	S. Falkow
R1-19KM ⁺	R1-19 segregant with KM resistance only	
RTEM	I-like (<i>fi</i> ⁻), AM, SM	J. T. Smith

TC uptake. Uptake of TC was done as previously described (27), with the following alterations. Cells grown overnight were diluted and grown to mid-log phase and then induced for maximal TC resistance by addition of TC at a final concentration of 0.5 μ g/ml for 30 min. After 20 min of uptake with [³H]TC (10 μ Ci/ml; 0.25 μ mol/ml), duplicate 1-ml samples were added to 9.0 ml of saline at room temperature and filtered with membrane filters (Millipore Corp.). As previously described the A600 of the uptake suspension was measured, and the TC uptake was calculated as nanomoles per milliliter of cell water.

RESULTS

Survey of susceptible and resistant *E. coli*.

The bacteriology laboratory at the E. J. Meyer Memorial Hospital determines antibiotic susceptibilities by the Kirby-Bauer procedure (4) on several thousand *E. coli* cultures per year. Of these, the resistance patterns of 759 cultures randomly selected from the hospital records of 1973 were surveyed. Three hundred and sixty-two (48%) were resistant to one or more of the following antibiotics: AM, CM, KM, SM, TC, and sulfonamide. Multiple resistance accounted for 53% of the resistant strains. TC-resistant strains accounted for 25% of all cultures recorded and 53% of resistant cultures. Of the TC-resistant strains, 40 (21%) were resistant to TC alone and thus 79% exhibited multiple-drug resistance. The TC resistance in the multiple resistant strains was most frequently associated with SM resistance (119 out of 153 cultures or 78%), followed by association with sulfonamide (65%) and then by AM (49%).

Resistance patterns of TC-resistant *E. coli* isolates. One hundred and ten TC-resistant isolates from three hospitals were examined in more detail concerning their resistance characteristics. Resistance patterns are shown in Table 2. Thirteen percent of the strains were resistant to TC alone, whereas 87% had multiple drug resistance. TC resistance was associated with SM resistance in nearly all cases of multiple resistance (91 of the 97 isolates), whereas TC, SM, AM multiple resistance was found in 47 of the isolates. A comparison of resistance patterns of the 110 TC-resistant isolates examined in this laboratory with those strains in the 759 cultures from E. J. Meyer Memorial Hospital that were TC resistant showed that they were similar. The comparison was made with respect to TC, SM, KM, CM, and AM only.

MIC and transfer patterns of TC-resistant *E. coli* isolates. The MIC of TC was determined for the isolates, all of which had been induced for maximal TC resistance. Table 3 shows that the resistance levels are distributed around a median of 200 $\mu\text{g/ml}$, with only five isolates at 50 $\mu\text{g/ml}$ and two at 350 $\mu\text{g/ml}$.

All were tested for their ability to transfer TC resistance to *E. coli* J6-2N. Fifty-one transferred TC resistance at frequencies ranging between 8×10^{-2} and 3×10^{-7} . When standard conjugations were extended to 24 h two more isolates were found to transfer TC resistance. An additional two isolates were found to transfer at a frequency of $\sim 10^{-8}$ when all the remaining isolates were tested using equal volumes of stationary-phase recipient and donors in a 24-h incubation. These results suggest that in 55 of the hospital isolates (50%) the TC resistance was borne on a transferable plasmid.

The transfer of TC resistance from the hospital isolates to *E. coli* J6-2N resulted in an

increase of resistance level of 50 to 100 $\mu\text{g/ml}$ for 32 of the plasmids, a decrease for nine plasmids, and no change for 14 of the plasmids. Subsequently, TC resistance was transferred from *E. coli* J6-2N to *E. coli* J5-3, which resulted in decreases in TC resistance level of 50 to 100 $\mu\text{g/ml}$ for 43 plasmids whereas 12 remained at the same level.

Table 3 shows that there is an approximately linear relationship between the MIC of the hospital isolates and the fraction of isolates at each MIC which transferred TC resistance by conjugation. For example, none of the five isolates with an MIC of 50 $\mu\text{g/ml}$ transferred TC resistance, whereas the 10 strains at 300 $\mu\text{g/ml}$ or over all transferred TC resistance. The correlation also holds at intermediate MICs where there were more isolates. There was no apparent relationship between MIC and transfer frequency for the isolates which did transfer.

The transfer of TC resistance from hospital strains was more prevalent when TC resistance was accompanied by both SM and AM resist-

TABLE 2. Resistance patterns of TC-resistant strains of *E. coli* used in this study

Resistance pattern	No. of isolates
TC, SM	37
TC, SM, AM	23
TC, SM, AM, KM	15
TC	14
TC, SM, AM, CM	7
TC, SM, KM	4
TC, AM	3
TC, SM, CM	2
TC, SM, AM, KM, CM	2
TC, SM, KM, CM	1
TC, AM, KM	1
TC, AM, CM	1

TABLE 3. Tetracycline MIC and transfer patterns of hospital isolates

MIC ($\mu\text{g/ml}$)	No. of isolates	Transfer of TC resistance by conjugation		No. that transfer TC resistance by mobilization ^a	No. that yielded plasmid DNA which transformed susceptible strain to TC resistance ^b
		No.	%		
50	5	0	0	0	4
100	7	1	14	1	3
150	26	8	31	10	3
200	33	16	49	5	4
250	29	20	69	4	3
300	8	8	100		
350	2	2	100		

^a Mobilization was attempted with those isolates that did not transfer TC resistance by conjugation.

^b Transformation tests were tried with the isolates that did not transfer TC resistance by conjugation or mobilization.

ance (in the presence or absence of other markers) than when it was combined with either SM or AM but not both. Thus 66% (31 out of 47 total) of the strains whose multiresistance included TC, SM, and AM transferred TC resistance compared to 39% (17 out of 44 total) with TC and SM and 40% (2 out of 5 total) with TC and AM. While 81% of the TC, SM, AM ($\pm$ other) strains showed MIC levels of 200 $\mu\text{g/ml}$ or higher, 57% of the TC, SM ($\pm$ other) group and 60% of the TC, AM ($\pm$ other) group were at this level. Of the isolates which had only TC resistance, 36% (5 out of 14 total) transferred the resistance and 36% were scored at MICs of 200 $\mu\text{g/ml}$ or greater.

Further attempts to transfer TC resistance. The 55 isolates which did not transfer TC resistance in matings with *E. coli* J6-2N were used in attempts to demonstrate transfer with other conditions.

(i) **Transfer into a nonrestricting host.** Since *E. coli* J6-2N is a K-12 strain, transfer was also tried using *E. coli* C600N, which is a mutant with no known restriction/modification system. None of the 55 isolates transferred TC resistance to *E. coli* C600N either, suggesting that the reason for lack of transfer was not restriction by the host of the entering plasmid.

(ii) **Selection with other antibiotics.** All of the strains which carried SM resistance (43 of the 55) were tested for transfer with *E. coli* J6-2N using SM as the selecting agent. Of these, three transferred SM resistance to *E. coli* J6-2N. Similarly all the isolates which were resistant to AM (19 isolates), KM (11 isolates), and CM (5 isolates) were mated using the individual drug as the selecting agent. Four transferred AM resistance, two transferred KM, including one strain which had also transferred the SM marker, and two transferred CM, including one which had transferred AM. None of the recipients that had acquired the SM or AM marker in this series were TC resistant, showing that even though a transferable plasmid was present in the original hospital cultures these cultures did not contain a transferable TC resistance determinant. Furthermore, of the nine hospital isolates which transferred markers other than TC, seven were resistant to three or more drugs (including TC) but only two transferred resistance to more than one drug.

(iii) **Alteration or induction of TC resistance to higher levels.** Because of the finding that a greater percentage of strains transferred TC resistance when the resistance level (MIC) was high (Table 3), eight of the nontransferable cultures were altered to higher levels of resistance (450 $\mu\text{g/ml}$) by serial subculture in Penassay broth containing increasing concentra-

tions of TC. None of these cultures transferred TC resistance in a standard 60-min mating. There was no appreciable change in transfer frequency when five strains with transmissible TC resistance were altered to higher levels of TC resistance. Again, because of the relationship between TC resistance level and transfer of TC resistance, and because it was observed that TC resistance in hospital isolates was always inducible (unpublished data), transfer was tried after induction of TC resistance in the donor cultures. None of the induced isolates transferred TC resistance.

(iv) **Possible colicin interference.** To insure that colicin production by the hospital strains did not destroy the recipient cells and thereby invalidate the conjugation studies, *E. coli* J6-2N (recipient) was incubated for 24 h with each of 20 nontransferring hospital isolates. The colony count of *E. coli* J6-2N remained approximately the same in the presence or absence of the hospital strains.

(v) **Mobilization.** Mobilization experiments were conducted with all 55 isolates, which did not transfer TC by direct mating with *E. coli* J6-2N. The strain C600(R1-19KM⁺) bears an F-like KM resistance plasmid with a transfer factor derepressed for pilus synthesis. In a test in which this transfer factor donor, the TC resistance donor (hospital strain), and the recipient *E. coli* J6-2N were incubated together it was found that 20 strains contained mobilizable TC resistance, including three of the nine strains which had transferred resistance determinants other than TC. This suggests that TC resistance in these strains is located on a plasmid and that the plasmid did not have a functional transfer factor. The MICs of the strains which were mobilized can be seen in Table 3. All of the TC-resistant *E. coli* J6-2N that were recovered in these co-transfer matings were subsequently mated with *E. coli* J5-3 in standard 60-min matings. In all but two cases the TC resistance was transferred to J5-3. When the two J6-2N hosts with nontransferable resistance were further mobilized with C600(R1-19KM⁺), TC resistance was recovered in the *E. coli* J5-3 recipient.

The 35 hospital isolates which had not shown transmissible TC resistance plasmids either in conjugation or the co-transfer tests described above were then tested for mobilization using *E. coli* J6-2(RTEM), a strain containing an I-like plasmid which conjugates with J6-2N at a transfer frequency of 3×10^{-3} . None of the isolates were found to transfer TC resistance.

(vi) **Transformation with isolated plasmid deoxyribonucleic acid (DNA).** The 35 cultures that did not transfer TC resistance either by

direct conjugation or by mobilization were grown in K medium (34) containing deoxyadenosine (33) labeled with [*methyl*-³H]thymidine (0.04 to 0.06 μ g/ml; specific activity, 83 μ Ci/ μ g; New England Nuclear Corp.) and were lysed with Brij 58 (6) or with sodium dodecyl sulfate (W. D. Rupp, personal communication), and the cleared lysates were centrifuged in 5 to 20% alkaline sucrose (34). Fractions of high activity were pooled, dialyzed, concentrated, and examined for ability to transform competent *E. coli* C600N cells (33). It was found that 17 of the 34 strains yielded DNA fractions which could donate TC resistance to the C600N host (Table 3). None of the C600N recipient hosts were subsequently able to transfer TC resistance to *E. coli* J5-3 in overnight mating tests. A control host strain containing one or another of two known transferable TC resistance plasmids was also examined in this study. After isolation of DNA and transformation of *E. coli* C600N to TC resistance, only one of the plasmids was subsequently able to transfer TC resistance to *E. coli* J5-3. This suggests that the isolated DNA fractions from one of the plasmids carried a TC resistance determinant but not a transfer unit under the conditions of our tests. One of the isolates in the series had previously been found to transfer SM but not TC resistance. This strain produced DNA fractions which could transform C600N to SM resistance only, TC only, or both. Only the SM-resistant hosts could transfer SM resistance (only) to *E. coli* J5-3.

Of the 110 hospital strains which were studied, plasmid-mediated TC resistance was shown to be present in 92 (84%): 55 by conjugation, 20 by mobilization, and 17 by transforming DNA. An additional three were found to have plasmids that transferred drug resistance other than TC.

TC uptake by hospital isolates. Uptake of TC by the strains in which TC resistance was demonstrably plasmid mediated was not significantly different from the strains in which plasmid-mediated TC resistance could not be demonstrated (Table 4). The uptake of the resistant strains is significantly lower than that of nine TC-susceptible strains.

Susceptibility to minocycline. Susceptibility to minocycline for all 110 isolates ranged from less than 12.5 up to 25 μ g/ml. There was no apparent difference between the cultures with plasmid-mediated TC resistance and with no demonstrable plasmid-mediated TC resistance.

DISCUSSION

Several recent surveys of *E. coli* resistance patterns presented data which were quite simi-

lar to some of the data found by us. Slocombe and Sutherland (30) examined enteropathogenic *E. coli* in the United Kingdom from 1957 to 1960 (200 cultures) and 1967 to 1968 (200 cultures). With respect to the same antibiotics surveyed in this report, they found 33 to 39% of all strains were resistant to one or more antibiotics (this study 48%); 18 to 22% of their total strains were TC resistant (this study 25%); 52 to 53% of the resistant strains were TC resistant (this report 53%); in 60 to 68%, TC resistance was transferable (this study 50%); and 53 to 67% of the TC-resistant strains were associated with SM resistance (this report 61%). In a 1968 study, Lewis (21) found 24% of 300 *E. coli* cultures from a healthy population had resistance to one or more antibiotics, whereas 16% of the total strains were resistant to TC. TC resistance was present in 69% of the drug-resistant strains and 61% of these also had SM resistance. Lewis also reported 100 cultures isolated from hospital patients in which 81% of TC-resistant strains had SM resistance. In either group (healthy or hospital) about 60% of the TC-resistant strains was transferable. The percentage of strains with self-transmissible TC resistance from the three Buffalo hospitals appears to be lower than that reported by the investigators cited, as well as by Babcock et al. (3) and possibly by others (22) whose data could not be accurately analyzed with reference to TC resistance only.

Transfer of the TC resistance plasmids from all hospital isolates occurred at low frequencies, like most R factors. Subsequent transfer from a common host, J6-2N, to J5-3 resulted in a variety of changes in frequency, some being similar to the original mating and others vary-

TABLE 4. TC uptake by strains with transferable and nontransferable TC resistance

Strain	Resistance transfer by conjugation ^a	TC uptake (mean $\pm$ SEM; nmol/ml) ^a
TC susceptible		335 $\pm$ 45
TC resistant	+	37 $\pm$ 13
TC resistant	-	36 $\pm$ 8

^a The nine TC-susceptible strains did not carry resistance plasmids. +, Five TC-resistant strains that transferred TC resistance by conjugation, -, Nine TC-resistant strains that had no demonstrable TC resistance plasmid.

^b Significance of difference between means calculated by Student's *t* test. For TC-susceptible strains versus either group of TC-resistant strains, *P* < 0.08; for TC-resistant groups transferable versus nontransferable, *P* > 4.5. SEM, Standard error of the mean.

ing by as much as 10^4 . No distinction can be made between plasmid-controlled factors and host-controlled factors since no attempt was made to identify the possible presence of plasmids other than the TC resistance plasmids in the original hosts, which could contribute to the results.

In 20 of the 55 strains which did not have self-transmissible TC resistance, the F-like plasmid R1-19KM⁺ was able to mobilize TC resistance. This shows that the resistance markers were borne on plasmids that were unable to promote conjugation. It is possible that some of the other 35 strains have plasmids that may be mobilized by other types of transfer factors (1, 15, 31). In addition, other factors in the hospital strains may contribute to an inability to demonstrate the presence of plasmids by mobilization. For example, some hospital strains were shown to accept R1-19KM⁺, a plasmid derepressed for pilus synthesis, and yet its transfer to a subsequent host was at relatively low frequencies or not at all under the conditions examined. In nine of the hospital strains that did not transfer TC resistance by conjugation, there were other markers that did transfer by conjugation. This indicates that these strains had transfer factors that could not be utilized by the TC resistance determinant. In six of these strains a TC resistance plasmid was demonstrated by mobilization or transformation.

The preliminary DNA isolation study used for rapid screening was conducted in rather harsh conditions for survival of a transforming non-denatured extrachromosomal DNA (preparative alkaline sucrose gradient). In these tests 17 out of 24 isolates, which did not transfer TC resistance in either conjugation or mobilization tests, were able to donate TC resistance to a second host. It is possible that a modification of the technique may show an even higher proportion of strains bearing plasmid-mediated TC resistance. Resistance to TC has been produced in *E. coli* by serial subculture in TC-containing media (5, 7) or as a result of selection for CM resistance (25). Resistance has also been observed as a result of integration of TC-resistance plasmids into the chromosome (14, 16, 17). It seems likely that most clinical TC resistance is now plasmid mediated, although prior to the upsurge of plasmid-mediated resistance in the late 1950s most TC resistance was probably chromosomal (30). We have observed (manuscript in preparation) that TC resistance is inducible whether or not it is transferable, suggesting a common basis for transferable and nontransferable resistance.

An interesting and previously unreported re-

sult of this study is the correlation found between the MICs of TC for the hospital isolates and the percentage of cultures at each MIC that could transfer TC resistance (Table 3). There was no apparent relationship between MICs of the cultures and their transfer frequencies.

The isolates having a resistance pattern that included TC·SM·AM exhibited relatively high MICs, and a relatively high percentage of these isolates transferred TC resistance. Otaya and Machihara (24) found a direct correlation between MIC and the number of antibiotics to which *E. coli* (and *Staphylococcus aureus*) strains were resistant, but their studies did not discriminate TC (or other) MICs at levels over 100 µg/ml. It is possible that since multiple (three or more) resistance in the isolates examined during the period of this study were for the most part TC·SM·AM, the specific antibiotic markers carried along with the TC marker may be irrelevant and a similar result could be obtained with a different pattern. It has been reported (29) that *E. coli* strains that became resistant to TC after administration of subtherapeutic doses of oxyTC also gained concurrent resistance to SM (and also a sulfonamide). It may be that relationships between transferability, resistance pattern, and resistance level are unique for the TC resistance systems in which a plasmid product may interact directly with the bacterial membrane rather than with the antibiotic.

Evidence that mutation of a transmissible plasmid to higher levels of antibiotic (AM·CM·SM·sulfonamide) resistance resulted in increased functional efficiency of genes involved in pair formation and gene transfer has been reported by Nordstrom (23). In our study, strains that were altered to high levels of TC resistance did not change either the transmissibility of nontransferable strains or the transfer frequency of strains that transferred TC resistance. At present it is not known if bacteria other than *E. coli* show a similar correlation between TC MIC and conjugation as is reported here.

Decreased TC uptake appeared to be the basis for TC resistance whether or not a TC resistance plasmid could be demonstrated. Furthermore, all 110 isolates had MICs for minocycline of 12.5 to 25 µg/ml, indicating that the resistance mechanism for TC had relatively little effect on minocycline. It had previously been shown (28) that plasmid-mediated TC resistance produced relatively low minocycline resistance.

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