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Bacterial Resistance to Tetracycline: Mechanisms, Transfer, and Clinical Significance

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CURRENT CLINICAL USES OF TETRACYCLINE: IS TETRACYCLINE IN DANGER OF BECOMING OBSOLETE AS A CLINICALLY USEFUL ANTIBIOTIC?

During the 1950s and 1960s, tetracycline was one of the most widely used antibiotics in the United States. It had a broad spectrum of activity against a variety of different bacteria and was effective against intracellular and extracellular pathogens (68). Tetracycline has been particularly useful for outpatient therapy because it is relatively cheap, can be taken orally, and has a relatively few side effects (59, 99). It does, however, have some important limitations. It is bacteriostatic rather than bactericidal, and it cannot be used for treatment of pregnant women or small children because it causes depression of skeletal growth in premature infants and discoloration of teeth in children (99). There is also a problem with patient compliance because treatment generally involves multiple doses. Nonetheless, the combination of low toxicity and broad spectrum of activity has far outweighed any drawbacks tetracycline might have.

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Today tetracycline and its various derivatives have only limited use in treatment of clinical infections because tetracycline resistance has appeared in many groups of medically important bacteria (47). For example, as late as the 1980s tetracycline was used to treat sexually transmitted diseases. With the appearance of resistant strains of *Neisseria gonorrhoeae*, however, tetracycline has been discontinued as the first line of therapy (17, 57, 60). However, oxytetracycline and tetracycline are still used to treat nongonococcal urethritis and other chlamydial infections (106). Similarly, because resistance to tetracycline is now widespread in gram-positive cocci and has begun to appear in the mycoplasmas, tetracycline cannot be used as the primary treatment for bacterial infections of the lower respiratory tract (39, 51, 75, 99). Although tetracycline is used less frequently as a drug of choice, it is still used to treat brucellosis, rickettsial infections, tularemia, early Lyme disease, and typhus (99). Doxycycline is used to treat exotic diseases such as plague (99). Additionally, tetracycline is used by dentists to treat periodontal disease (92) and by dermatologists to treat acne. Nonetheless, further spread of tetracycline resistance could render tetracycline obsolete for treatment of even these infections.

Since tetracycline was such a useful antibiotic in the past,

it is worth considering whether an effort should be made to reclaim the tetracyclines as broad-spectrum antibiotics. This has been done recently with other classes of antibiotics, e.g., development of β-lactamase-resistant β-lactam antibiotics and new quinolones. These successes encourage optimism about possible new derivatives of tetracycline that would be effective against bacteria that are currently resistant. Development of such drugs would have to be based on a thorough understanding of the mechanisms of resistance. One goal of this review is to survey what is known about the mode of action of tetracycline, the various mechanisms of tetracycline resistance, and regulation of resistance genes. A second goal is to survey information about elements that transfer tetracycline resistance genes, including some novel types of gene transfer elements that are not plasmids. These elements have an unusually broad host range and also carry genes encoding resistance to macrolides, lincosamides, and chloramphenicol. The ramification of the spread of these elements is that other clinically significant antibiotics besides tetracycline could become useless.

OTHER USES OF TETRACYCLINE

Antiparasitic Activity

Although tetracycline is used clinically as an antibacterial agent, it also has activity against some protozoal parasites. Tetracycline derivatives inhibit the growth of *Giardia lamblia*, *Trichomonas vaginalis*, *Entamoeba histolytica*, *Plasmodium falciparum*, and *Leishmania major* (40). Tetracycline derivatives differ with respect to their effectiveness in inhibiting the growth of these parasites, and these differences are correlated with how readily the derivatives are taken up by the parasites. The most effective derivatives are the lipophilic ones, such as thiatetracycline, which readily cross the cytoplasmic membrane (4). Although tetracycline derivatives have not been used clinically as antiparasitic drugs, such an application is conceivable in the future.

Additives to Livestock Feed

Oxytetracycline is widely used as an additive to livestock feed because it stimulates weight gain in some domestic animals (17, 22). It has been fed routinely to calves, chickens, turkeys, sheep, and pigs (22). Tetracycline is also used to improve the health and promote the growth of fish in commercial fisheries (22). These uses of tetracycline have been controversial because of fears that such widespread nonclinical use would increase the incidence of tetracycline-resistant strains. These fears are often dismissed as groundless by the agriculture industry because the levels of tetracycline used are lower than those needed to prevent growth of bacteria in laboratory medium. However, bacteria growing in natural environments are not growing under the optimal conditions used to measure susceptibility to antibiotics in clinical laboratories, and low levels of tetracycline could well have deleterious effects on an organism trying to survive in a competitive, nutrient-limited environment. Additionally, as will be described below, exposure to low levels of tetracycline stimulates the transfer of some transmissible elements. Thus, long-term low-level use of tetracycline could enhance the spread of resistance transfer elements as well as select for strains that acquire resistance genes. For this reason, regular use of tetracyclines in animal feed should be seriously reviewed.

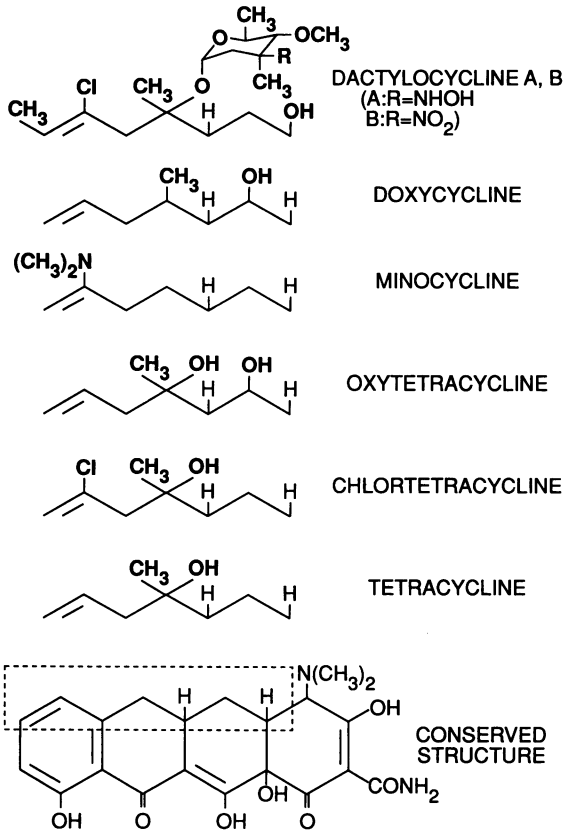

FIG. 1. Structures of tetracycline derivatives currently used to treat bacterial infections and structure of the new class of tetracyclines, dactylocyclines. The basic tetracycline structure is shown at the bottom of the figure. In the different tetracycline derivatives, only residues within the boxed region are changed. Accordingly, only this region is shown for the tetracycline derivatives listed above the conserved tetracycline structure. The moieties that are different from those in the conserved structure at the bottom of the page are shown in boldface type.

STRUCTURES AND MODE OF ACTION OF TETRACYCLINE AND ITS DERIVATIVES

Structures

The structures of tetracycline and some of its derivatives are shown in Fig. 1. All the derivatives shown, except for the dactylocyclines, have been used in the treatment of clinical infections in humans. The dactylocyclines are an interesting new group of tetracycline derivatives produced by *Dactylosporangium* spp. (21, 111). They differ from other tetracycline derivatives in that they are glycosides of tetracycline. They appear to be effective only against bacteria carrying tetracycline resistance genes of class K (71). However, some tetracycline-susceptible bacteria are not inhibited by dactylocyclines. The failure to inhibit growth of gram-negative bacteria and some gram-positive bacteria is most probably due to the failure of these bacteria to take up dactylocyclines. In a linked transcription-translation system, dactylocyclines inhibit protein synthesis at a level comparable to that of tetracycline (71). These results are encouraging in that they indicate the possibility that new tetracycline derivatives effective against known resistance mechanisms can be found.

Modes of Action

Tetracycline is thought to inhibit the growth of bacteria by entering the bacterial cell, binding to bacterial ribosomes, and stopping protein synthesis (26). Despite years of study, however, it is not clear exactly how tetracycline exerts its effect. It has been shown that tetracycline binds strongly to a single site on the 30S ribosomal subunit, and the 7S ribosomal protein appears to form part of the binding site (26). A highly conserved region of 16S rRNA may also be part of the binding site (74), a feature that would explain the broad spectrum of tetracycline. There are many weaker tetracycline-binding sites on the ribosome, but their significance is unclear. The direct effect of tetracycline binding to ribosomes is that aminoacyl-tRNAs do not bind productively to the A site on the ribosome (23). This could, by itself, be responsible for the ability of tetracycline to inhibit bacterial growth. However, interference with the binding of aminoacyl-tRNAs to the A site could also induce the stringent response and thus trigger numerous secondary effects (16). These secondary effects would include effects on tRNA stability, rRNA synthesis, and amino acid metabolism, in addition to inhibition of protein synthesis indirectly via the stringent response (16).

Although tetracycline and most of its derivatives have been shown to bind to ribosomes and selectively inhibit protein synthesis, a few of the derivatives may not act this way. Rasmussen et al. (74) have recently shown that although chelocardin and thiatetracycline have good antibacterial activity against *Escherichia coli* and *Bacillus subtilis*, they are very poor inhibitors of protein synthesis and appear to bind ribosomes ineffectively or not at all. Also, unlike other tetracyclines, they inhibit both DNA and RNA synthesis as well as protein synthesis. Rasmussen et al. (74) suggest that chelocardin and thiatetracycline may be exerting their effects on the cytoplasmic membrane of the bacteria. Recently, Olivera and Chopra (70) proposed that tetracyclines be divided into two types on the basis of their modes of action: those that inhibit protein synthesis (e.g., tetracycline, chlortetracycline, minocycline) and those that interact with the cytoplasmic membrane (e.g., chelocardin, anhydrotetracycline, thiatetracycline). Further work is necessary to determine the exact effect of chelocardin-type tetracyclines on the cytoplasmic membrane.

MECHANISMS OF RESISTANCE TO TETRACYCLINE

Bacteria could use three strategies to become resistant to tetracycline: limiting the access of tetracycline to the ribosomes, altering the ribosome to prevent effective binding of tetracycline, and producing tetracycline-inactivating enzymes (Fig. 2). All three types of resistance have been found in clinical isolates. With the discovery of so many tetracycline resistance genes in recent years, a classification scheme had to be devised (50). The current convention is to assign a resistance gene to a particular class on the basis of DNA-DNA hybridization with members of that class (47). Two genes that cross-hybridize on Southern blots under relatively high-stringency conditions are considered to be members of the same class. The different classes have been given letter designations, and an attempt has been made to keep classes with the same mechanism grouped together in the alphabet. A list of the accepted classes of tetracycline resistance genes is given in Table 1. Tetracycline resistance genes have also been classified according to whether they confer resistance to minocycline. However, as can be seen in Table 1, this is not a very useful classification tool. Although tetracycline resistance genes from a wide variety of bacteria have been studied and classified, there are still reports of genes that do not cross-hybridize with any of the genes used as hybridization probes (83). Therefore, the list of classes in Table 1 will undoubtedly expand as the screening of tetracycline-resistant strains continues.

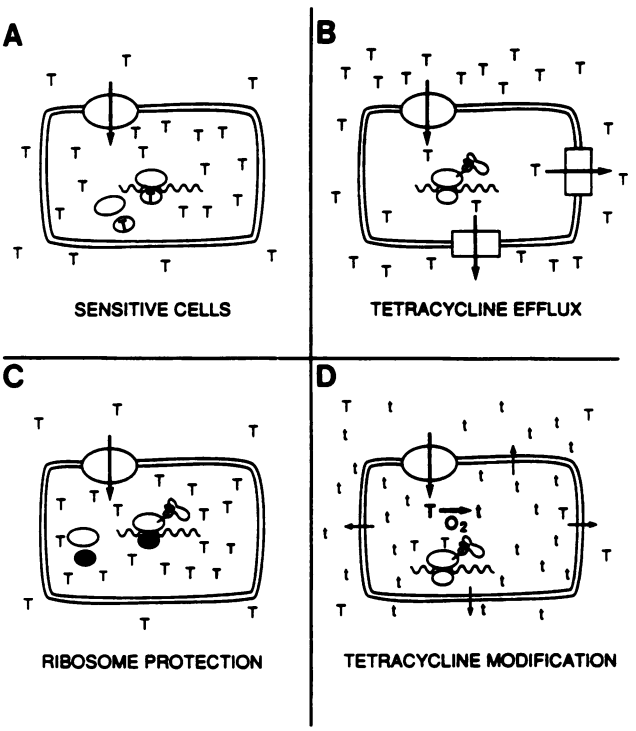

FIG. 2. Different mechanisms of tetracycline resistance. (A) Susceptible bacteria accumulate tetracycline (T) to an internal concentration high enough to allow tetracycline to bind to ribosomes and stop protein synthesis. (B) Bacteria carrying an efflux type of resistance gene produce a cytoplasmic membrane protein (rectangular box), which pumps tetracycline out of the cell as fast as it is pumped in. This keeps the intracellular level low enough to allow protein synthesis to proceed. (C) Bacteria carrying a ribosome protection type of resistance gene produce a 72-kDa cytoplasmic protein (not shown) that somehow interacts with the ribosomes and allows the ribosomes to proceed with protein synthesis even in the presence of high intracellular levels of the drug. Although the effect of the 72-kDa protein is indicated graphically by shading one of the ribosomal subunits, it is not known whether the resistance protein binds to the ribosome. (D) Bacteria carrying a tetracycline modification resistance gene produce a 44-kDa enzyme that chemically modifies tetracycline (T) to an inactive form (t), which diffuses freely out of the cell. The enzymatic reaction requires oxygen and NADPH. Reprinted from *Molecular Microbiology* (83) with permission of the publisher.

Limiting Tetracycline Access to Ribosomes

Reduced uptake. For tetracycline to inhibit protein synthesis, it must enter the bacterial cell and bind to the ribosome. The protonated form of tetracycline diffuses through the cytoplasmic membrane (35, 112). However, simple diffusion would not explain the observation that susceptible bacteria accumulate tetracycline in their cytoplasm. McMurry et al. (55) showed that there was an energy-dependent phase of tetracycline uptake in addition to diffusion. Argast and Beck

TABLE 1. Classification of known tetracycline resistance genes

Class(es)[a]	Location	Resistance[b]	Mechanism	Protein	Family and genus[c]	Reference(s)
A to E	Plasmid	Tc^r Mn^r (A, B, E), Tc^r Mn^s (C, D)	Efflux	43 kDa, membrane	*Vibrio*, *Aeromonas*, *Haemophilus*, *Moraxella*, *Enterobacteriaceae*	14, 47, 49, 53, 80
K	Plasmid	Tc^r Mn^r	Efflux	35 kDa, membrane	*Staphylococcus*, *Bacillus*	10, 34, 56
L	Plasmid	Tc^r Mn^s	Efflux	50 kDa, membrane	*Streptococcus*, *Enterococcus*, *Bacillus*	7, 34, 56
M	Chromosome,[d] plasmid	Tc^r Mn^r	Ribosome protection	72 kDa, cytoplasmic	Many Gm^- Gm^+ (see text)	8, 28, 38, 61, 83
O	Plasmid, chromosome[e]	Tc^r Mn^r	Ribosome protection	72 kDa, cytoplasmic	*Campylobacter*, *Streptococcus*, *Enterococcus*, *Peptostreptococcus*, *Mobiluncus*	1, 76, 81, 104
P	Plasmid	?	Efflux[f]	?	*Clostridium*	1, 76, 81
Q	Chromosome[g]	Tc^r Mn^r	Ribosome protection	72 kDa, cytoplasmic	*Bacteroides*	24, 66
X	Plasmid	Tc^r Mn^r	Detoxification	44 kDa, cytoplasmic	*Bacteroides* (cryptic)	96

[a] Classes are defined by DNA-DNA hybridization. Classes F and N have been shown not to exist (32, 97).
[b] Tc, tetracycline; Mn, minocycline.
[c] Genera in which the resistance class is commonly found. Some resistance classes, such as K and L, have only rarely been found in genera other than those listed. A full distribution of these classes is still not established.
[d] In most cases, *tet*(M) is found on a Tn*916*-type conjugative transposon (75).
[e] Chromosomal *tet*(O) determinants have so far not been shown to be self-transmissible.
[f] Tentative assignment based on DNA sequence analysis (81).
[g] Virtually all *tet*(Q) determinants are associated with a conjugative transposon.

(3) disputed the existence of energy-dependent uptake because they could not show saturation of tetracycline uptake, as would be expected if a tetracycline transporter existed. Recently, Yamaguchi et al. (112) resolved this controversy by showing that there is energy-dependent uptake of tetracycline but that it involves ΔpH and not a transport protein. Tetracycline can exist in a protonated form (TH_2) and a magnesium-chelated form (THMg). The TH_2 form diffuses readily through phospholipid bilayers, whereas the THMg form does not. Since the proportion of tetracycline in the THMg form increases at higher pH, tetracycline is trapped inside the bacteria as THMg because the internal pH is higher than the external pH (112).

If diffusion were the sole mechanism of tetracycline uptake, it would be virtually impossible for bacteria to become resistant by blocking the movement of tetracycline across the cytoplasmic membrane. As expected, this type of resistance has not been seen. Alteration of porin proteins (e.g., OmpF) to limit the diffusion of tetracycline into the periplasm is a possible mechanism of resistance in gram-negative bacteria. This type of resistance, which can decrease susceptibility 6- to 18-fold, has been found in a number of gram-negative bacteria (19, 20, 85, 86). Strains that acquire this type of resistance also become resistant to other antibiotics such as β-lactams and fluoroquinolones (19, 85, 86). Recently, Cohen et al. (19) have shown that the multiple antibiotic resistance of some *E. coli* strains is due not only to changes in OmpF but also to changes in other outer membrane proteins.

Tetracycline efflux. A second way to limit access of tetracycline to ribosomes is to reduce intracellular concentrations of tetracycline by pumping the antibiotic out of the cell at a rate equal to or greater than its uptake. This resistance mechanism, tetracycline efflux, is the best-studied and most familiar mechanism of tetracycline resistance (55). The resistance gene product is a cytoplasmic membrane protein that is an energy-dependent tetracycline transporter (Fig. 2). It is not known how this efflux protein pumps tetracycline out of the cell, nor is it clear how this pump protects the cells. Although efflux clearly prevents the degree of tetracycline accumulation seen in susceptible cells (41), the level of intracellular tetracycline is still relatively high compared with levels that inhibit protein synthesis. One possible explanation is based on the ability of tetracycline to exist in several ionic forms (35). It is possible that one form binds more readily to ribosomes than the other. If so, it is conceivable that preferential pumping of the active form might permit intracellular concentrations of tetracycline to remain high and still allow protein synthesis. Another possibility is that the repressor protein that is coinduced with the Tet protein acts as an initial sink for tetracycline and thus accounts for the high internal levels (103). Alternatively, ribosomes in living cells may for some reason be more resistant to tetracycline than ribosomes in in vitro systems.

To date, eight classes of tetracycline efflux genes have been identified. Classes A to E are found among the members of the family *Enterobacteriaceae* and the genera *Haemophilus*, *Vibrio*, *Aeromonas*, and *Moraxella* (14, 47, 53, 80). Class P, recently shown to encode a tetracycline efflux-type resistance, has been found only in *Clostridium* spp. (1, 81). Classes K and L have been found only in gram-positive bacteria (10, 34, 41, 56). Initially, it appeared that class K was most often found in *Staphylococcus* spp. whereas class L was more likely to be found in *Streptococcus* and *Enterococcus* spp. (10). However, recent reports of strains of gram-positive cocci and *Bacillus* spp. that carry both class K and class L resistance determinants have raised questions about whether such a clear-cut segregation actually exists (7, 10, 34).

Although classification by DNA-DNA hybridization is now the preferred method, efflux resistance genes have also been classified by susceptibility to minocycline, a lipophilic analog. Bacteria carrying resistance genes from classes A, B, E, and K are more resistant to minocycline than are other efflux classes, although class B exhibits much higher resistance than classes A, E, and K. This difference in minocy-

cline susceptibility indicates that there may be two functional classes of efflux-based resistance, but the basis for the difference is not understood.

Some efflux-type resistance genes are cryptic. Ives and Bott (36) have recently shown that a region of the *B. subtilis* chromosome near the origin of replication can confer resistance to tetracycline when the copy number is increased. The original strain that had this region in a single copy was susceptible to tetracycline, whereas the strains carrying multiple tandem duplications of this chromosomal region were resistant. Cloning of the region on a multicopy plasmid produced the same tetracycline resistance (36). Apparently, expression of the gene is too low to confer resistance if the gene is present in single copy. Sequence analysis of the gene has shown that it belongs to class L (37). This cryptic tetracycline efflux gene is not a general feature of *B. subtilis* and is found in only a few strains (2). Although *B. subtilis* is not a human pathogen, the finding of a cryptic resistance gene in this strain raises the possibility that such genes also occur in pathogenic species.

Efflux resistance structural genes from different classes share considerable DNA sequence similarity. Classes A and C share 74% DNA sequence identity, while class B shares 45% identity with class A and class C (52, 108). Similarly, the two gram-positive efflux structural genes, *tet*(K) and *tet*(L), share 69% DNA sequence identity (34, 44). Phylogenetic analysis of classes A through C and classes K and L has shown that these two families probably represent separate lines of descent from a common ancestor. A resistance gene from *Streptomyces rimosus* may represent a third lineage (88). The tetracycline efflux proteins also share homology with other proton-dependent transport proteins such as sugar transporters, especially in their amino-terminal regions (82, 88). Thus, the efflux-type resistance genes may have evolved from transport genes.

Ribosome Protection

A less familiar type of tetracycline resistance mechanism than tetracycline efflux is ribosome protection. Although this mechanism is less familiar, it is probably more widespread than tetracycline efflux (83). The resistance gene product migrates as a 68-kDa protein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis; however, DNA sequence analysis reveals that the size of the protein is actually 72 kDa (12, 18, 45). This cytoplasmic protein interacts or associates with the ribosome, making it insensitive to tetracycline inhibition (Fig. 2) (11, 12, 51). The exact mode of interaction of the resistance protein with the ribosomes is not understood. Burdett (12) has purified one of the ribosome protection resistance proteins (TetM) and has shown that it can bind to ribosomes. Manavathu et al. (51) showed that in the presence of TetM, binding of tetracycline to ribosomes is not altered. TetM appears not to catalyze covalent modification of a ribosomal component, as seen with erythromycin (12, 33). The exact role of the ribosome protection protein is still subject to much conjecture and will not be resolved until further work has been done to determine its binding sites on the ribosome and its role in protein synthesis.

Three classes of the ribosome protection resistance genes have been characterized and sequenced: *tet*(M), *tet*(O), and *tet*(Q) (45, 83, 93). Burdett et al. originally identified a second class of ribosome protection genes in streptococci and designated it class N (13), but this class has subsequently been shown not to exist (32). *tet*(M) was originally discovered in gram-positive cocci (11) but has now been found in a wide variety of bacteria, including *Neisseria*, *Haemophilus*, *Mycoplasma*, *Ureaplasma*, *Streptococcus*, *Staphylococcus*, *Peptostreptococcus*, *Bacteroides*, *Kingella*, and *Bacillus* spp. (5, 9, 28, 39, 43, 61, 83). *tet*(O), originally found in *Campylobacter* spp. (45), has now been found in *Streptococcus*, *Peptostreptococcus*, *Enterococcus*, *Lactobacillus*, and *Mobiluncus* spp. (75–77, 113). So far, *tet*(Q) has been found only in *Bacteroides* species (24, 66). These distributions may be misleading. Hybridization probes for identifying *tet*(M) and *tet*(O) have been available for some time and have been used in many surveys, whereas probes identifying *tet*(Q) have only recently become available. Thus, *tet*(Q) may well be found in many more species than indicated above when surveys including this class are made of tetracycline-resistant strains.

TetM and TetO are very closely related and share 75% amino acid sequence identity (45). TetQ is much more distantly related and shares only 40% amino acid identity with TetM and TetO (66). All three resistance classes confer resistance to minocycline as well as to other tetracycline derivatives (Table 1). An interesting feature of the ribosome protection resistance proteins is that they share considerable amino acid homology to elongation factor G (12, 84). This homology is concentrated in the region of elongation factor G that contains the GTP-binding site. Recently, Burdett (12) has shown that TetM, like elongation factor G, has ribosome-dependent GTPase activity. Thus, the ribosome protection genes may have evolved from genes encoding bacterial elongation factors.

Strains of bacteria carrying combinations of efflux and ribosome protection resistance genes have been found (7, 113). Bismuth et al. (10) reported that many strains of *Staphylococcus aureus* carried both *tet*(K) and *tet*(O), while some isolates carried *tet*(K), *tet*(L), and *tet*(M). Roberts (76) found various combinations of classes K, M, and O in some strains of *Streptococcus* spp. and *Peptostreptococcus* spp. It would be interesting to determine whether these resistance classes work in synergy or whether the level of resistance is equivalent to that conferred by a single class.

Tetracycline Inactivation

A third type of tetracycline resistance mechanism has been discovered recently: enzymatic inactivation of tetracycline (Fig. 2) (95, 96). The gene encoding this resistance was found on two closely related *Bacteroides* transposons that also carry a gene for erythromycin resistance. The tetracycline resistance gene was first identified by its ability to confer resistance on *E. coli*. However, the gene worked only in aerobically grown *E. coli* cells and did not confer resistance on anaerobically grown *E. coli* or on *Bacteroides* spp. The gene product is a 44-kDa cytoplasmic protein that chemically modifies tetracycline in a reaction that requires oxygen and NADPH. Sequence analysis has shown that this gene shares amino acid homology with a number of NADPH-requiring oxidoreductases, particularly in the region containing the NADPH-binding site (94). This resistance gene has been classified as *tet*(X). Originally, Park et al. (72) had suggested the classification *tet*(F) (an efflux classification) because one of the transposons, Tn*4400*, conferred weak energy-dependent efflux activity on *E. coli*. However, Speer and Salyers (97) showed that this efflux activity was not linked with the resistance phenotype and that increasing the efflux activity did not make the strain tetracycline resistant. The finding that a strain exhibiting tetracycline efflux was not tetracycline resistant raises fur-

ther questions about how efflux of tetracycline confers resistance.

The clinical significance of *tet*(X) is unclear. Not only does it not confer resistance on the *Bacteroides* strains in which it was originally found, but it requires such high levels of aeration to function as a resistance factor in *E. coli* that it probably could not confer meaningful levels of resistance in the microaerophilic environment found in most sites on the human body. At this point, the possibility cannot be ruled out that some interaction with hemoglobin or other oxygen-bearing molecules allows it to function in the human body. It will be interesting to see whether further examples of this class of antibiotic resistance gene are found in aerobic or facultative clinical isolates.

Multiple Resistance Systems

There have been a number of reports of chromosomal mutations that confer resistance to more than one type of antibiotic, including tetracycline (19, 20, 25, 38, 85, 86). One case involved some strains of *N. gonorrhoeae* that were resistant to penicillins and cephalosporins but did not produce β-lactamase. These strains were also resistant to tetracycline, erythromycin, and dyes. Several genes involved in this multiple resistance (*penB*, *tet*, *mtr*) were identified (38) and appear to be able to act singly or in combination. These genes have not yet been characterized in enough detail to ascertain the mechanism of action.

Multiple antibiotic resistance in gram-negative enteric bacteria has also been reported (19, 20, 25, 85, 86). George and Levy (25) showed that exposing *E. coli* to increasing concentrations of tetracycline or chloramphenicol selected for mutations that made bacteria resistant to a variety of antibiotics including penicillins, cephalosporins, rifampin, nalidixic acid, and quinolones, as well as tetracycline and chloramphenicol. Recently, a locus that appears to control this multiple-antibiotic-resistance (MAR) system has been identified. The locus has been designated *marA* (27). Loss of *marA* renders *E. coli* unable to mutate to the multiple-resistance phenotype. An interesting feature of *marA* is that the amount of *marA* transcript was found to increase severalfold after exposure to tetracycline or chloramphenicol, two antibiotics that selected for mutations leading to the MAR phenotype. One possible explanation of the MAR effect is that an outer membrane porin is being altered in such a way as to prevent diffusion of antibiotics through the outer membrane. A connection between the MAR phenotype and changes in outer membrane proteins has been noted (19, 20, 85, 86). MAR mutants appear to have an active efflux system (25), but the actual mechanism of this multiple resistance has not yet been determined.

REGULATION OF TETRACYCLINE RESISTANCE GENES

Many tetracycline resistance genes are regulated. The best-understood type of regulation is that of *tet*(B) the gene found on Tn*10* (9, 29). *tet*(B) is transcribed divergently from *tet*(R), a gene encoding the repressor protein TetR(B). (The convention for naming regulatory proteins is to use R and give the class of the structural gene in parentheses.) The two promoters are overlapping and share a common regulatory region (30, 31, 42). In the absence of tetracycline, TetR(B) binds as a dimer to two operator sites. This binding blocks transcription of both the *tet*(B) and *tetR*(B) genes. The affinity of TetR(B) for tetracycline is much greater than its affinity for the operator sites. When tetracycline enters the cell, it binds TetR(B) and changes the conformation of TetR(B) so that it no longer binds to the operator. Thus, transcription of the resistance gene is derepressed. Transcription of *tet*(R) is also derepressed, but TetR(B) inhibits transcription only when there is no longer enough tetracycline present to bind all of the repressor in the cell.

A different type of regulation is seen in the gram-positive efflux genes *tet*(K) and *tet*(L) (37). This mechanism, attenuation, is similar to the mode of regulation described previously for erythromycin resistance genes (33). The mRNA transcript, which starts 123 bp upstream of the start codon of the *tet* gene, contains two ribosome-binding sites (RBS). Ribosomes binding to the first RBS, RBS1, translate a short peptide that ends shortly before the second RBS, RBS2. If RBS2 is exposed, ribosomes bind at this site and begin to translate the resistance gene. The region of mRNA containing RBS2 can form one of two stem-loop structures. One of these structures covers RBS2 and prevents the ribosomes from binding. This is the structure that is thought to form in the absence of tetracycline. The ribosome binds to RBS1 and moves rapidly along the mRNA until it encounters a stem-loop structure that blocks RBS2 and the start for the Tet protein (34, 41). When tetracycline is present, however, ribosomes stall and thereby allow the formation of an alternate, more stable stem-loop structure that does not mask RBS2. Under these conditions, the resistance gene is translated (34, 41).

Recent work has focused on the mechanism of regulation of ribosome protection genes. Expression of the *Bacteroides* gene *tet*(Q) is regulated by tetracycline at the level of transcription (100). However, sequence analysis of the upstream region has shown that there is no repressor gene and no stem-loop structures such as those usually seen upstream of the genes regulated by attenuation (65). Thus, the regulation of *tet*(Q) is probably different from that of the efflux genes. There is some disagreement about whether expression of *tet*(M) and *tet*(O) is regulated. Wang and Taylor (110) have identified a 300-bp region upstream of the *tet*(O) coding region that is essential for full expression of the gene. They describe expression of *tet*(O) as constitutive. However, Burdett (12) reported that the amount of TetM increased when streptococci were exposed to tetracycline. Also, Nesin et al. (63) reported that preexposure of an *S. aureus* strain carrying *tet*(M) to subinhibitory concentrations of tetracycline not only doubled the tetracycline MIC but also caused an increase in the level of *tet*(M) mRNA transcripts seen on Northern (RNA) blots. Subsequently, Su et al. (102) reported tetracycline enhancement of *tet*(M) transcription from Tn*916*. They detected a short transcript as well as the full-length transcript in Northern blots. Because they detected stem-loop structures in the region upstream of the structural gene, they suggested that TetM expression was regulated by attenuation. Thus, it seems clear that expression of *tet*(M) is regulated, although the precise mechanism is still uncertain. Since *tet*(M) and *tet*(O) have virtually identical upstream regions, they would be expected to exhibit the same type of regulation. The sequence of the upstream region of *tet*(Q) differs considerably from those of the upstream regions of *tet*(M) and *tet*(O), so that the regulation of *tet*(Q) could well be different from that of *tet*(M) and *tet*(O).

TESTING FOR SUSCEPTIBILITY TO TETRACYCLINE

Because many tetracycline resistance genes are regulated, it may be desirable to include an induction step in the procedure for testing susceptibility to tetracycline. Nesin et al. (63) found that preexposure of an *S. aureus* strain carrying *tet*(M) to low levels of tetracycline increased the tetracycline MIC from 25 to 60 μg/ml. No systematic studies of the impact of preexposure to tetracycline on MIC have been performed, and the effect would probably vary from species to species. On the basis of experience with other inducible resistances, it is possible that such an induction step is not required to obtain accurate MICs, because failure to induce usually results in a lag in growth rather than in a failure to grow. Thus, if incubation times are relatively long, the difference in MICs between induced and uninduced cultures is not great. However, the effect of eliminating the induction step could well be substantial for some types of bacteria, particularly those that are difficult to grow. For this reason, a comparison of induced versus uninduced inocula should be done when procedures for MIC testing on any new group of bacteria are being established.

A little-appreciated problem in tetracycline susceptibility testing is that different genera of bacteria have different basal levels of susceptibility to tetracycline. For example, tetracycline MICs for *E. coli* strains not harboring resistance genes are generally 1 to 2 μg/ml. In comparison, MICs for *Haemophilus* strains not harboring resistance genes are usually 10-fold lower than those for *E. coli*. Thus, a resistance gene in *E. coli* that increases the tetracycline MIC 50-fold will give MICs of at least 50 μg/ml, whereas a gene that produces a similar increase in the MIC for *Haemophilus* may give an MIC of only 4 to 8 μg/ml. To make matters worse, there can be considerable strain-to-strain variability in MICs associated with the same gene in different organisms. This kind of variability has been seen in *Corynebacterium* strains (79). Much of the variability is found in the intermediate range of susceptibility. One possible reason for the variability in MICs is that the medium has not been sufficiently adjusted to support optimum growth for all strains of the genus. Other reasons include the presence of cryptic genes that can be activated during exposure to tetracycline or resistance genes whose expression has been increased by insertion of an insertion element upstream of the gene. Further investigation of this phenomenon is needed. It is clear that the susceptibility and resistance breakpoints must be established separately for each genus and species and not simply inferred from results obtained with a few well-studied species.

A potential solution to the problems described above, which reflect difficulties in interpreting the phenotypic expression of a resistance gene, is to base resistance determinations on the presence of a gene rather than on the level of its expression in laboratory medium. There are now enough DNA sequences of different genes representing efflux and ribosome protection types of resistances to permit the design of polymerase chain reaction (PCR) primer sets on the basis of DNA sequences conserved in the different classes of resistance genes that would recognize virtually all efflux or all ribosome protection genes. At present, PCR technology would be cost prohibitive in the clinical setting and the assay does not always yield interpretable data. Recently, however, Tokue et al. (105) reported the use of PCR to detect methicillin-resistant *S. aureus*. In this study, clinical isolates were used to test for the presence of methicillin resistance by both MIC and PCR techniques. Although the study proved PCR to be effective in detecting methicillin-resistant strains that MIC assays failed to detect, PCR technology has not been sufficiently developed for routine use in hospitals and clinical-testing laboratories. Whether PCR will prove as powerful a tool in the detection of resistant strains as it has in the diagnosis of diseases such as Lyme disease has yet to be determined (105).

HAVE WE SEEN IT ALL? PROSPECTS FOR APPEARANCE OF NEW TYPES OF TETRACYCLINE RESISTANCE

Each of the three well-characterized types of resistance gene (efflux, ribosome protection, and antibiotic inactivation) appears to have evolved from housekeeping genes such as those encoding transport proteins (efflux), elongation factors (ribosome protection), and oxidoreductases (antibiotic inactivation). Mutations that affect porins should probably be added to this list, since porins could be considered to have a housekeeping function in the gram-negative bacteria. In the case of efflux- and ribosome protection-type resistance genes, it is clear from the sequence divergence seen when different classes of genes are compared that these genes have been evolving for a long time. If other housekeeping genes could evolve into tetracycline resistance genes, we should have seen them by now, particularly since information on tetracycline resistance genes from virtually all known phylogenetic groups of bacteria that cause human disease is now available. However, it is important to remember that human clinical isolates represent only a small portion of bacteria in nature. Thus, surveys of bacteria isolated from sites other than the human body could conceivably uncover new resistance mechanisms.

The tetracycline-producing bacteria must protect themselves from the antibiotic they produce, and antibiotic resistance genes are frequently linked to genes for antibiotic production (109). Since there has presumably been a strong continuous selection for resistance genes in tetracycline-producing bacteria, the variety of resistance genes found in these bacteria should give an indication of the possible varieties of tetracycline resistance mechanisms that might arise as a result of tetracycline selection. *Streptomyces rimosus* (an oxytetracycline producer) has two types of tetracycline resistance genes (69). One type appears to encode a resistance similar to TetM, TetO, and TetQ. That is, the resistance protein is loosely associated with ribosomes and protects them from tetracycline without any apparent covalent modification. A second gene encodes a protein that is responsible for decreased accumulation of tetracycline and is probably an efflux-type resistance protein. In *Streptomyces aureofaciens* (a producer of tetracycline and chlortetracycline), ribosomes appear to be covalently modified (58). Thus, it is possible that a covalent-modification type of ribosome protection mechanism will eventually be seen in clinical isolates. It is interesting that tetracycline producers have oxidoreductases similar to TetX that participate in the biosynthetic pathway, although these have not been linked to protection of the antibiotic producer.

TRANSFER OF TETRACYCLINE RESISTANCE GENES AMONG HUMAN CLINICAL ISOLATES

Types of Gene Transfer Elements

Surveys of tetracycline-resistant strains of bacteria have repeatedly found the same efflux or ribosome protection

genes in many different bacterial genera. These findings suggest that extensive horizontal transfer of these resistance elements has occurred. Conjugation is probably responsible for most of this horizontal transmission. Two types of conjugal element have been described: conjugative plasmids and conjugative chromosomal elements called conjugal transposons (83).

Efflux genes and ribosome protection genes have been found both on plasmids and in the chromosome. Conjugative plasmids have undoubtedly contributed to the spread of efflux gene classes A to E within the gram-negative bacteria and of classes K and L within the gram-positive bacteria (34, 44, 46). *tet*(M) has been found on plasmids in *Neisseria* spp. but is also frequently located in the chromosome of other bacteria (77, 78). The chromosomal *tet*(M) gene is frequently transmissible, and transmissible chromosomal *tet*(M) determinants are associated with conjugative transposons (87, 107). The *tet*(M)-carrying conjugative transposons have a remarkably wide host range and can transfer from gram-positive to gram-negative bacteria as well as between gram-positive bacteria (8). Chromosomal *tet*(Q) determinants are frequently transmissible and have so far been found exclusively on conjugative transposons (89).

Since conjugative transposons have only recently been studied in detail and are not as familiar as conjugative plasmids, a summary of their properties will be given here. Three types of conjugative transposons have been characterized. Their structures are compared with that of Tn*10* in Fig. 3. Tn*10* and most other transposons are flanked by large (1- to 1.5-kb) repeats (insertion sequences). This is not the case with the conjugative transposons. The only identity at the ends is a short, imperfect inverted repeat less than 15 bp long (6, 73). It is unfortunate that the conjugative chromosomal elements have been called transposons and in many cases have been given transposon designations (e.g., Tn*916*), because the so-called conjugative transposons have almost nothing in common with transposons such as Tn*10*. Aside from the fact that the conjugative transposons, unlike Tn*10*, are capable of precise excision and do not have large flanking repeats, they do not duplicate the target site when they insert (6, 15, 73). In fact, they have more in common with the lambdoid phages than with transposons such as Tn*10* (73).

Conjugative transposons range from 16 to more than 150 kb in size. Members of the Tn*916* family, which were originally found in streptococci, are the smallest examples (87). Much larger conjugative transposons are found in *Streptococcus pneumoniae* and *Bacteroides* species (6, 46, 62, 89). The streptococcal conjugative transposons carry *tet*(M), and the *Bacteroides* conjugative transposons carry *tet*(Q). The larger streptococcal elements, such as Tn*3701*, are composite elements that contain an inserted copy of a Tn*916*-type transposon (4, 46). Another example of a composite element has recently been found in *Bacteroides* spp. A 150- to 200-kb *Bacteroides* conjugative transposon (Tc^r Em^r-12256) consists of one of the smaller (70- to 80-kb) *Bacteroides* transposons inserted into another element (6). Many of the conjugative transposons carry not only *tet*(M) or *tet*(Q) but also genes encoding resistance to macrolides and lincosamides (*erm*) and resistance to chloramphenicol (*cat*) (4, 89).

The conjugative transposons can do more than transfer themselves from the chromosome of a donor to the chromosome of a recipient. First, they can insert into plasmids (107), making the plasmid self-transmissible. Second, conjugative transposons in the chromosome can mobilize coresident plasmids in *trans* (62, 89). Recently, the *Bacteroides* conjugative transposon was shown to be capable of excising discrete, unlinked chromosomal segments (101) and transferring these segments to a recipient, where the segments were integrated into the chromosome (6). Although the excised segments thus far studied appear to be cryptic, some may turn out to carry resistance genes. Thus, the presence of a conjugative transposon in a bacterial strain makes that strain capable of transferring not only resistance genes on the conjugative transposon but also resistances located on other elements such as plasmids and chromosomal segments that are not self-transmissible.

An important feature of conjugative transposons is that they do not exclude other conjugative transposons or plasmids (67). Therefore the same strain can acquire multiple conjugative transposons and plasmid combinations. This observation should be kept in mind by those who screen clinical isolates for different tetracycline resistance classes.

Regulation of Transfer Genes by Tetracycline

An interesting feature of the *Bacteroides* conjugative transposons is that their transfer frequency is enhanced 100- to 1,000-fold by preexposure to tetracycline (100). This characteristic may not be limited to the *Bacteroides* elements. Torres et al. (107) have now found that transfer of Tn*925*, one of the Tn*916* family of *tet*(M) conjugative transposons, is enhanced by preexposure to tetracycline, although the enhancement was only 10-fold. Stevens et al. (100) recently showed that *tet*(Q) is the first gene in a three-gene operon that contains two genes with amino acid homology to components of known two-component regulatory systems. This three-gene operon appears to be essential for transfer and other activities of the *Bacteroides* elements. Thus, *tet*(Q), unlike most resistance genes found on conjugative elements, is not simply a hitchhiker that has been picked up by the self-transmissible element but instead appears to play an important role in the regulation of element transfer. It is possible that the failure to detect the transfer of chromosomal *tet*(O) genes has been because transfer is tetracycline inducible (45).

Most investigations to date have focused on transfer of the conjugative transposons themselves or on cotransfer of discrete elements such as plasmids. Torres et al. (107) have shown that Tn*925* was able to transfer chromosomal genes from one *B. subtilis* strain to another. The interesting feature of this transfer was that the transferred chromosomal genes were not closely linked to the Tn*925* insertion in the donor but were scattered around the *B. subtilis* chromosome. On the basis of this observation, Torres et al. (107) suggested that mating between donor and recipient actually involves a type of cell fusion rather than formation of a selective pore as seen in plasmid conjugation. These results raise the interesting possibility that the tetracycline resistance conjugative transposons are capable of transferring chromosomal virulence factors as well as antibiotic resistances, but such transfer has not yet been shown.

THE NORMAL MICROFLORA AS A RESERVOIR FOR RESISTANCE GENES

It has long been suspected that the bacteria that normally colonize the human body (the resident microflora) could act as reservoirs for resistance genes, which could then be transferred to pathogens during their temporary colonization of the same site (48). *tet*(M), the resistance determinant found in many pathogens that transiently colonize the human

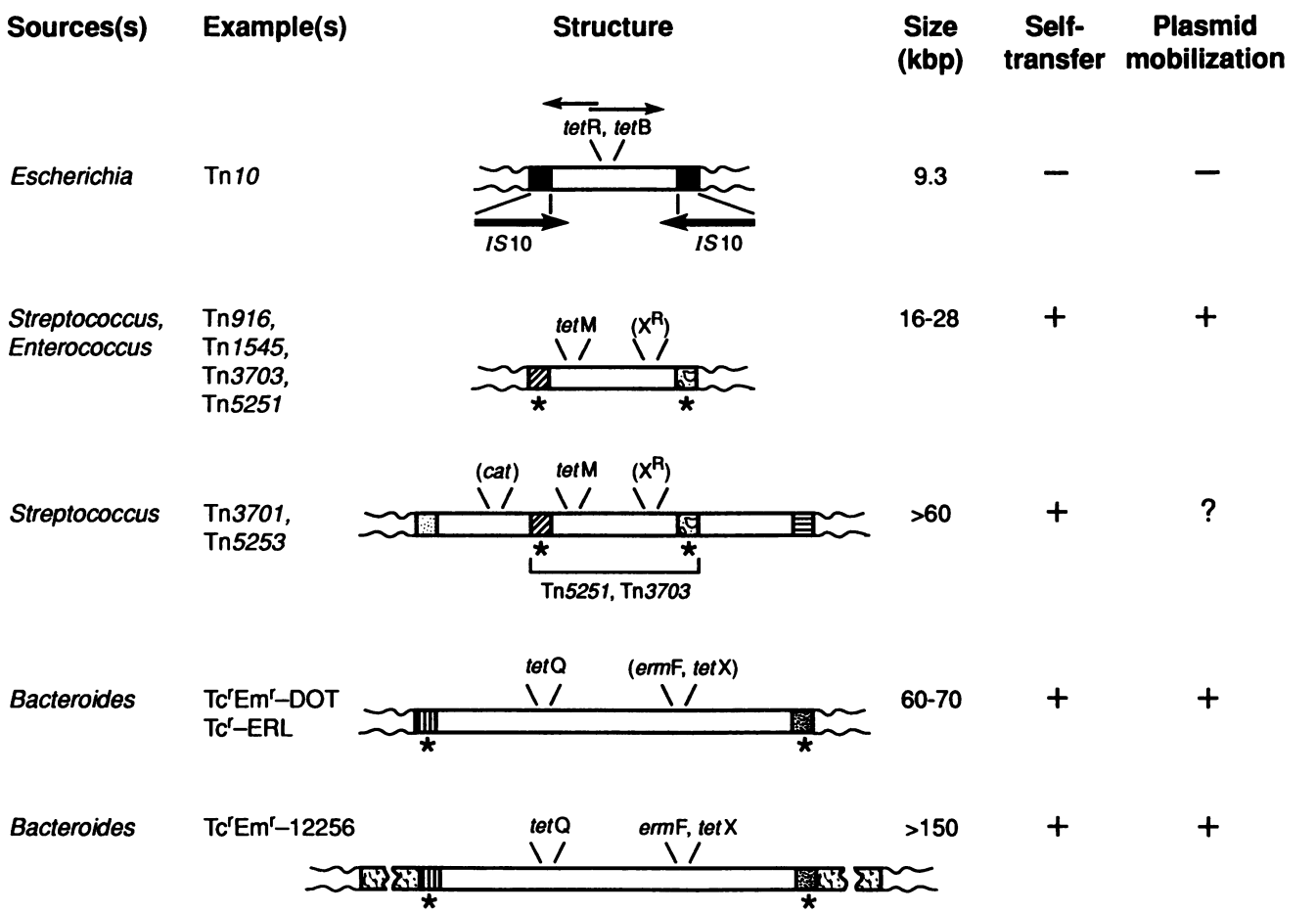

FIG. 3. Structures of known conjugative transposons compared with that of Tn*10*. Tn*10* and other well-studied transposons are flanked by large direct or inverted repeats (heavy arrow) called insertion sequences (IS). Wavy lines on either side indicate the replicon (chromosome, plasmid) into which the transposon is inserted. In Tn*10*, the resistance gene (*tet*B) and its repressor (*tet*R) are transcribed divergently from the same promoter-operator region (thin arrows). The Tn*916* class of conjugative transposon does not have large repeats at the ends (indicated by different patterns) but rather has short 15-bp imperfect indirect repeats (*) (18). These conjugative transposons all carry *tet*(M) and sometimes other resistance genes (X^r), where X can be erythromycin or kanamycin. The larger streptococcal elements, exemplified by Tn*3701* and Tn*5253*, also appear not to have large repeats at the end. They have a composite structure with an inserted Tn*916* type of conjugative transposon and frequently carry a *cat* gene as well as other resistance genes. Most *Bacteroides* elements so far studied resemble Tc^r Em^r-DOT (shown here). Like Tn*916*, they have short, imperfect inverted repeats at their ends (*) but not large direct or indirect repeats (6). All these elements carry *tet*(Q), and many carry *ermF* and *tet*(X). The *tet*(X) gene is cryptic in this case. One *Bacteroides* element, Tc^r Em^r-12256, consists of a Tc^r Em^r-DOT-type element inserted into another element. Self-transfer means the ability to excise from the donor chromosome, transfer to a recipient, and integrate into the recipient chromosome. Plasmid mobilization means that the element can mobilize coresident plasmids in *trans*.

body, is also found in *Enterococcus faecalis*, a component of the normal microflora of the colon, and in *S. aureus* and streptococci that colonize the oropharynx. *tet*(O) has been found in *Lactobacillus* strains that colonize the vaginal tract. Thus, it is possible that horizontal transfer between members of the normal microflora and pathogens occurs readily in the various microniches provided by the human body. Despite the importance of transfer between members of the resident microflora and pathogens that transiently colonize the human body, few studies of the incidence of *tet* genes in the normal microflora have been done.

To date, the most comprehensive study of common commensals has been a survey in which Roberts and Hillier (77) tested 71 random *Peptostreptococcus* isolates from pregnant women, who had not taken any antibiotic for 2 weeks prior to sampling, for the presence of different tetracycline resistance determinants. Ninety-six percent of the strains were resistant. Most of this resistance was associated with TetM, but classes K, L, and O were also detected. The discovery in common commensals of the same resistance genes now found frequently in many genera of pathogenic bacteria thought to have humans as their only host provides strong evidence that the normal microflora does in fact exchange DNA with pathogens that transiently colonize the same site. Recently, transfer of the resistance plasmid pAMβ1 between *Enterococcus* and *Lactobacillus* spp. was shown to occur in the gastrointestinal tract of mice (54). This finding provides further evidence that conjugal transfer of broad-host-range resistance transfer elements can actually occur under conditions existing in a mammalian host.

TRANSFER OF RESISTANCE GENES BETWEEN THE NORMAL MICROFLORAS OF HUMANS AND DOMESTIC ANIMALS

It is perhaps not surprising that extensive transfer of tetracycline resistance genes has occurred between different species of bacteria that colonize the human body either permanently as members of the normal microflora or transiently as pathogens, because these bacteria are in frequent contact within the same host. Also, the widespread use of tetracycline for treatment of human diseases, even when it is administered in subtherapeutic doses, tends to select for strains that have acquired resistance genes. It is not so obvious whether the use of tetracycline in animal feed might favor the rise of tetracycline resistance genes in animals and the subsequent transfer of these genes to human pathogens. Reports of antibiotic-resistant *Salmonella* strains that were apparently transmitted from farm animals to humans have appeared (98), but until recently there was no evidence that transfer of genes between bacteria that normally colonize humans and bacteria that normally colonize domestic animals had occurred. The most insidious type of transfer would be transfer between the normal microflora of humans and the normal flora of animals, because once a gene on a transmissible element has entered the normal microflora of humans, it has the potential to be spread to any pathogen that comes into contact with the microflora. Thus, the spread of resistance genes would not be confined to zoonoses such as *Salmonella* spp., which colonize both domestic animals and humans.

It has now been shown that naturally occurring conjugative transposons from the *Bacteroides fragilis* group can transfer to *Prevotella ruminicola*, a species found in high numbers in the normal microflora of the rumen of cattle and sheep, under laboratory conditions (91). Similarly, a transmissible plasmid originally found in a strain of *P. ruminicola* isolated from the rumen of sheep could be transferred to members of the *B. fragilis* group under laboratory conditions. Evidence that this type of transfer may actually have occurred in nature came from the finding that the tetracycline resistance gene on the plasmid from *P. ruminicola* was virtually identical to the *tet*(Q) gene found on the *Bacteroides* conjugative transposon (90). Recently, the *tet*(Q) gene from the *Bacteroides* conjugative transposon has also been found in some other *Prevotella* strains isolated from animals in a different location (64). Finding the same gene in two distantly related species strongly suggests that horizontal transfer has occurred in the recent past.

CONCLUSIONS AND RECOMMENDATIONS

The desirable properties of the tetracyclines make them prime candidates for future rehabilitation. The recent discovery of a new class of tetracycline derivatives, the dactylocyclines, indicates that despite the limited number of groups on the tetracycline molecule that can be modified without abolishing activity, new classes of tetracycline may yet be found. The findings by Rasmussen et al. (74), which indicate that some tetracycline derivatives may not act by stopping protein synthesis, raise further hope that new tetracycline derivatives may be forthcoming. Presumably, the ribosome protection type of tetracycline resistance genes would not confer resistance to tetracycline derivatives that did not act at the level of the ribosome. The mechanisms of tetracycline efflux and ribosome protection are still not fully understood, and further work is required to elucidate them. Once they are fully understood, the information could aid in the design of new tetracycline derivatives. In particular, it would be of interest to know whether only one form of tetracycline, e.g., the zwitterionic form, is effective in stopping protein synthesis and, if so, whether this form is preferentially ejected by the efflux pump. Such a preference would explain why efflux pumps confer resistance without dropping the intracellular tetracycline concentration to very low levels.

Oddly enough, there does not seem to be a standard battery of resistance classes that is used to evaluate new antibiotics. For example, the dactylocyclines have so far been tested only on strains carrying efflux resistances. The much more widespread and problematic ribosome protection resistance genes were not included. Any new tetracyclines should be tested at a minimum on strains carrying classes A (or B), C (or D), K, L, M, and O. It would also be wise to test new tetracycline derivatives for effectiveness against strains carrying the *Streptomyces* gene that appears to covalently modify ribosomes, since this type of resistance might appear in clinical isolates in the future. Finally, new tetracycline derivatives should be tested for ability to induce resistance gene expression and transfer frequency of the conjugative chromosomal elements. Even if new antibiotics are effective against strains carrying *tet*(M), they could still contribute to the transfer of resistances linked to *tet*(M) on conjugative transposons if they induce transfer.

Tetracycline resistance determinants may prove to be useful tools for studying transmission of resistance genes from resistant microfloras to pathogens and from one pathogenic species to another, since some tetracycline resistance genes and the conjugative elements that carry them have extraordinarily broad host ranges. The genes can thus be monitored in a variety of different types of bacteria. Also, since more than one conjugative element and more than one resistance class can coexist in a single strain, the incidence of multiple transfer events to the same strain can be investigated. Several questions must be answered. How widespread is carriage of known tetracycline resistance genes in nonhospitalized people, and which genes are most common? Although there have been a number of surveys of the incidence of tetracycline resistance in these populations (48), the resistance genes themselves have rarely been identified. Do low levels of tetracycline in foods, especially fish, affect the number of resistant bacteria in the resident microflora? Is there any evidence that conjugative transposons, whose transfer is stimulated by tetracycline, spread more or less frequently than elements exhibiting constitutive transfer? Does cessation of exposure to tetracycline reduce the resistance level of the resident microflora?

The advisability of continuing to add tetracycline to livestock and fish feed must be reevaluated, especially if new tetracycline derivatives return the tetracycline family to the category of broad-spectrum antibiotics. There is no point in losing tetracycline twice. Unfortunately, there is still relatively little information on which to base such a reevaluation. For example, there is no information about the incidence of resistant bacteria in animals being fed tetracycline. It is also not clear to what extent bacteria colonizing animals that are not directly receiving tetracycline-supplemented feed will be affected by feeding tetracycline to subpopulations of animals on the same farm. Conceivably, aerosols may spread tetracycline more widely than is now realized. In particular, there is virtually no information on the effect of long-term exposure to low levels of tetracycline on the incidence and spread of antibiotic resistance genes. On the

positive side, recent advances in knowledge about some of the numerically predominant groups of bacteria in the normal microflora of animals now make it possible to address such questions on a scientific basis.

Even if tetracycline becomes obsolete as a clinically useful antibiotic, tetracycline resistance transfer elements will continue to be a cause for concern because they can carry other resistance genes. Also, it is interesting that tetracycline is one of the antibiotics that selected for the multiple-resistance phenotype (MAR) described by George and Levy (25). Tetracycline exposure could increase resistance to other antibiotics by this route as well.

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