Resistance Gene Transfer in Anaerobes: New Insights, New Problems

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Investigations of antibiotic-resistance gene transfer elements in *Bacteroides* species have generated some new insights into how bacteria transfer resistance genes and what environmental conditions foster gene transfer. Integrated gene transfer elements, called conjugative transposons, appear to be responsible for much of the transfer of resistance genes among *Bacteroides* species. Conjugative transposons not only transfer themselves but also mobilize coresident plasmids and excise and mobilize unlinked integrated elements. Less is known about resistance gene transfer elements of the gram-positive anaerobes, but there are some indications that similar elements may be found in them as well. An unusual feature of the *Bacteroides* conjugative transposons is that transfer of many of them is stimulated considerably by low concentrations of antibiotics. Thus, antibiotics not only select for resistant strains but also can stimulate transfer of the resistance gene in the first place. This finding raises questions about whether use of low-dose tetracycline therapy may have a greater effect on the resident microflora than had been previously thought. Finally, investigations of resistance genes in *Bacteroides* species and other genera of bacteria have begun to provide evidence that the resident microflora of the human body does indeed act as a reservoir for resistance genes, which may be acquired from and passed on to transient colonizers of the site.

Multidrug Resistance: A Worsening Problem in Anaerobes

A considerable amount of press attention has been given to multidrug-resistant strains of *Mycobacterium tuberculosis, Streptococcus pneumoniae, Enterococcus* species, *Staphylococcus aureus,* and *Klebsiella* species. These bacterial pathogens certainly pose major public health threats, but it is important to realize that these are not the only cases in which multidrug resistance is becoming a steadily more serious problem. Anaerobes, especially *Bacteroides* species, are also becoming increasingly resistant to antibiotics [1–3]. Although treatment failures are still relatively uncommon in cases of anaerobic infection, multiply resistant strains of *Bacteroides* and other anaerobes could easily become a serious problem in the future.

Indicative of how widely resistance genes can spread among colonic anaerobes is the observation that nearly 100% of all *Bacteroides* clinical isolates are now resistant to tetracycline because of the acquisition of a single resistance gene, *tetQ* [4, 5]. This resistance gene encodes a ribosomal-protection-type tetracycline resistance that renders the bacteria resistant to all of the clinically used members of the tetracycline family. Tetracycline resistance was once uncommon in *Bacteroides* clinical isolates (<10%). Resistance to clindamycin and to third-generation cephalosporins is now being seen much more commonly [1–3] and could soon render these antibiotics ineffective for treating bacteroides infections.

Resistance to metronidazole is still rare, but it has appeared in clinical isolates and is known to be carried on transmissible elements [6, 7], so the possibility that metronidazole resistance could become more widespread in the future must be considered. Finally, as fluoroquinolones are used more frequently to treat anaerobic infections, it is reasonable to expect that resistance to those antibiotics will emerge and spread. To avert the clinical problem of untreatable anaerobic infections, it is necessary to understand how resistance genes are being spread among *Bacteroides* species and among other groups of anaerobic bacteria, as well as what conditions foster the transfer of antibiotic-resistance genes.

Another issue arises in connection with *Bacteroides* and other genera of anaerobes, which are prominent members of the microflora of the human body. These bacteria have the potential to serve as reservoirs of antibiotic-resistance genes. That is, they are in a position to acquire resistance genes from bacteria that transiently colonize the same site and later pass these genes on to human pathogens.

The question of whether the anaerobes that predominate in the resident microflora act as reservoirs for antibiotic-resistance genes is an important one because most antibiotic treatments of human infections are also treatments of the microflora. Thus, antibiotic treatment could select for anaerobes that have acquired resistance genes. The idea that members of the resident microflora could serve as reservoirs for resistance genes is an old one, but only recently has evidence to support that hypothesis begun to accumulate.

This review describes some novel gene transfer elements, called conjugative transposons, which are making a major contribution to the spread of antibiotic-resistance genes between

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strains of human colonic *Bacteroides* species and may be driving resistance gene transfer among other groups of anaerobes as well. It is well-accepted that antibiotic use selects for resistant bacteria in the microflora of people exposed to the antibiotic. The *Bacteroides* conjugative transposons illustrate yet another type of interaction between antibiotics and the microflora: antibiotic stimulation of resistance gene transfer.

The broad host range of the *Bacteroides* conjugative transposons and the fact that their transfer is stimulated by the use of at least some antibiotics fit well with the hypothesis that *Bacteroides* species could act as a reservoir for resistance genes. Evidence supporting the hypothesis that the microflora of the intestine is in fact capable of serving as a reservoir for antibiotic-resistance genes is also reviewed. For simplicity, references are to review articles rather than to primary research papers whenever possible.

More Than Plasmids: Chromosomal Gene Transfer Elements Play an Important Role in the Spread of Resistance Genes

Until recently, most of the available information about how resistance genes are transferred has come from studies of the gram-negative enteric pathogens and *Pseudomonas* species. As scientists have begun to pay more attention to antibiotic-resistance transfer mechanisms in the gram-positive bacteria and in gram-negative bacteria such as *Bacteroides* species, which are as distant genetically from the *Escherichia coli–Pseudomonas* group of gram-negatives as are the gram-positive bacteria [8], new types of gene transfer elements have been discovered.

One discovery has been that plasmids are not the only type of element that is transferred by conjugation. Conjugally transferred integrated elements are also widespread and are making a major contribution to resistance gene transfer in some groups of bacteria. These integrated elements have been called conjugative transposons [9]. This name is somewhat misleading because the conjugative transposons actually have at least as much in common with plasmids and phages as with conventional transposons and insertion sequence (IS) elements.

Conjugative transposons are self-transmissible DNA segments that are normally integrated into the chromosome [9]. The steps thought to be involved in their transfer are illustrated in figure 1 [9]. The integrated element first excises to form a nonreplicating circular transfer intermediate. A single-stranded copy of the circular form is then transferred to the recipient, where a double-stranded circle is regenerated. The circular form then integrates into the recipient chromosome.

It is important to note that this type of transfer is different from transfers mediated by integrated plasmids, e.g., Hfr strains, in which a portion of the integrated plasmid and a segment of chromosomal DNA adjacent to the insertion site are transferred to a recipient. Recipients in such a transfer event are not themselves able to retransfer the integrated DNA they receive because only a portion of the plasmid responsible for the transfer event is transferred. By contrast, a conjugative transposon that is transferred to a recipient has all the genes needed for retransfer and is thus fully transfer-proficient, assuming its transfer genes are expressed in the new host.

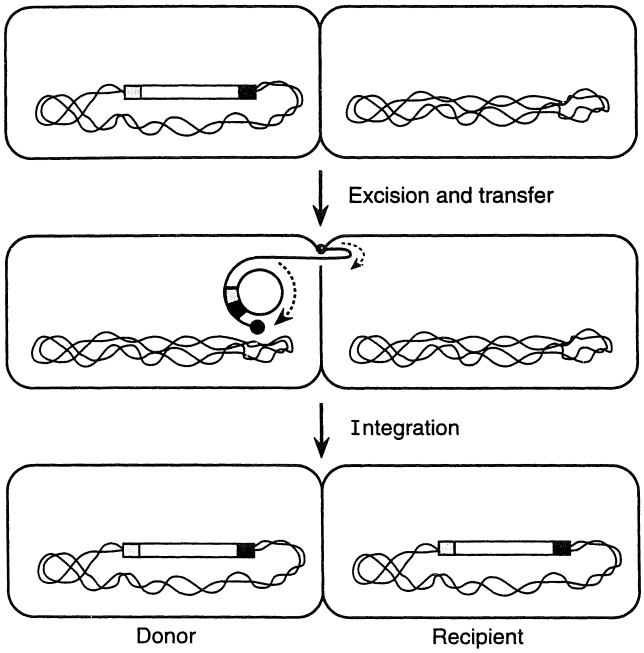

Figure 1. Steps thought to occur in the conjugal transfer of a conjugative transposon. The integrated element excises precisely or nearly precisely from the chromosome to form a covalently closed circle. A single stranded copy of this circle is transferred through a mating apparatus to the recipient cell. As it is transferred, the double stranded form is regenerated in the donor and recipient (*dashed lines*). The circles then integrate back into the chromosome. The ends of the conjugative transposon are shaded differently to emphasize the fact that they are not identical to each other.

The conjugative transposons found so far in *Bacteroides* species are large elements (>50 kbp in size), which frequently carry the tetracycline-resistance gene, *tetQ* [9, 10]. One *tetQ*-containing plasmid, pRRI4, has been found in a strain of *Prevotella ruminicola* that was isolated from the rumen of a sheep [11], but so far all of the *tetQ* alleles in clinical *Bacteroides* isolates have proved to be located on conjugative transposons. The association between *tetQ* and conjugative transposons is so commonly seen in *Bacteroides* species that finding a *Bacteroides* strain that is tetracycline-resistant is sufficient reason to suspect the presence of a conjugative transposon.

The fact that *tetQ* is now found in virtually all clinical isolates of *Bacteroides* species, whereas at one time tetracycline resistance was rare in this group, is an indication of how widely conjugative transposons have been spreading. Although tetracycline resistance can serve as a useful indicator of the presence of a conjugative transposon, this does not mean that a tetracycline-susceptible strain is necessarily free of conjugative transposons. One cryptic *Bacteroides* conjugative transposon family, XBU4422, has been found [9, 10], and there are likely

Table 1. Characteristics of various types of known integrating elements that carry resistance genes.

Type of element	Size range (kbp)	Self-transmissible?	Mobilizable?
Compound transposon (e.g., Tn*5*, Tn*10*)	5–15 (flanked by direct or inverted repeats)	No	Not mobilized *in trans* but can become part of a transmissible plasmid
Conjugative transposon	15–>150 (not flanked by direct or indirect repeats)	Yes	Can mobilize plasmids and unlinked integrated elements
NBU, NBU-like Tn*4555*	10–15 (not flanked by direct or indirect repeats)	No	Can be excised and mobilized by conjugative transposons

NOTE. NBU = nonreplicating *Bacteroides* unit.

to be others. The *tetQ* gene has also been found in human oral isolates of *Porphyromonas gingivalis,* one of the suspected agents of periodontal disease, and may be carried on conjugative transposons in this group as well [10]. Although tetracycline resistance is still relatively uncommon in oral isolates of *P. gingivalis,* the fact that it is being carried on a conjugative transposon suggests that tetracycline resistance might soon be much more common in this species. An increase in tetracycline resistance in *Porphyromonas* species and other oral anaerobes could impair the efficacy of tetracyclines as potentially useful therapeutic agents for periodontal disease.

Many of the *tetQ*-carrying conjugative transposons also carry genes that confer resistance to clindamycin and erythromycin (*erm* genes) [10]. The *erm* gene found most widely in clinical isolates of *Bacteroides* is *ermF* [12, 13]; *ermF* is found not only on conjugative transposons but also on conventional compound transposons, such as Tn*4351,* Tn*4400,* and Tn*4551* (table 1)[10, 12, 13]. All three of these conventional transposons were found originally on transmissible plasmids. The *ermF* genes carried on conjugative transposons did not result from integration of Tn*4351* or some related compound transposon into the conjugative transposon, because the insertion sequences that flank these transposons are not found on the *ermF*-carrying conjugative transposons [12]. In a later segment of this review, we will return to the question of how the *ermF* genes on *ermF*-carrying conjugative transposons entered these elements.

The *ermF* gene is not the only clindamycin-resistance gene found in *Bacteroides* clinical isolates. From time to time, there have been reports of clindamycin-resistant clinical isolates whose DNA did not cross-hybridize with *ermF.* Recently, we cloned and characterized the *erm* gene from one of these clinical isolates. It shared the highest level of sequence identity with *ermG,* an *erm* gene found originally in *Bacillus sphaericus* [4, 14]. It is not yet clear how widely *ermG* is distributed in *Bacteroides* clinical isolates, but since it was located on a conjugative transposon, Tc^rEm^r 7853, it certainly has the potential to be transferred to other *Bacteroides* strains.

The nomenclature used to designate conjugative transposons is very confusing to the uninitiated. Many groups have simply given these elements transposon designations, without differentiating them nomenclaturally from conventional compound transposons. Our group has used designations that indicate the resistances each conjugative transposon carries and the strain in which it was found. Thus, Tc^rEm^r 7853 carries a Tc^r gene and an Em^r gene and was originally found in *Bacteroides fragilis* 7853. Although this nomenclature has the advantage of alerting the reader that something different from a conventional transposon is being discussed, it is also not satisfactory. It is not clear whether the nomenclature problem will be addressed in the near future.

Other Activities of the Conjugative Transposons: Mobilization of Coresident Plasmids

It might seem at first glance that cryptic conjugative transposons, as well as the conjugative transposons that carry only *tetQ* and no other resistance genes, have no clinical importance because the cryptic ones do not carry resistance genes and tetracycline resistance is now so widespread among *Bacteroides* clinical isolates that tetracycline is no longer used to treat bacteroides infections. Nonetheless, these conjugative transposons can have important clinical effects because they are capable of mobilizing plasmids and integrated elements that do carry clinically important resistance genes [9, 10].

The *Bacteroides* conjugative transposons have proved to be unusually versatile in their ability to move DNA between different strains of bacteria. They can mobilize plasmids in two ways, which are illustrated in figure 2 [10]. First, they can mobilize plasmids *in trans* by providing the mating apparatus through which the plasmids move into a recipient. The *ermF* plasmid pBFTM10 is an example of a plasmid found in a clinical isolate that is incapable of self-transfer but can be mobilized *in trans* by conjugative transposons.

We have found that many of the small cryptic plasmids, which are widespread in *Bacteroides* clinical isolates, are mobilizable by conjugative transposons. Such plasmids, although cryptic at present, could easily become gene transfer elements in the future by acquiring one or more antibiotic-resistance genes. It is also worth noting that many *Bacteroides* plasmids are mobilizable not only by *Bacteroides* conjugative transposons but also by the IncP plasmids of *E. coli* and related bacteria

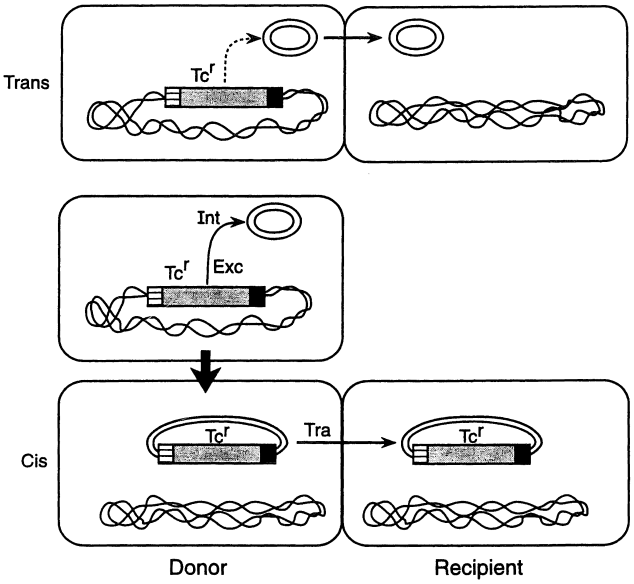

Figure 2. Two ways a conjugative transposon (integrated element; *Tc^r^*) can mobilize coresident plasmids. *Trans* mobilization occurs when proteins produced by the conjugative transposon (*dashed line*) create the mating pore through which the plasmid transfers. *Cis* mobilization occurs when the conjugative transposon excises (*Exc*) and integrates (*Int*) into the plasmid, and then the chimeric plasmid transfers itself (*Tra*) into the recipient.

[10]. Thus, *Bacteroides* plasmids have a broad range of transfer potential.

Only a few metronidazole-resistance genes have been cloned and characterized, but most of the genes found so far have been located on plasmids [6]. The mechanism by which these genes confer resistance to metronidazole and other 5-nitroimidazoles is not known. The plasmids carrying these genes are transmissible, but it is still not clear whether they are being mobilized by a conjugative transposon or are self-transmissible [7]. Since the plasmids are only 7–10 kbp in size, it seems unlikely that they are self-transmissible.

Chromosomally encoded metronidazole-resistance genes have also been reported, and in at least one case the resistance gene was transmissible [7]. This raises the possibility that some metronidazole-resistance genes are carried on conjugative transposons. The fact that at least some metronidazole-resistance genes are transmissible should serve as a warning to those who think that metronidazole will continue indefinitely to be universally effective against *Bacteroides* species.

Another interesting characteristic of the known metronidazole-resistance genes is that their expression is due to the integration of an IS upstream of the resistance gene [6, 7]. This is only one of several cases in which IS elements have activated cryptic resistance genes in *Bacteroides* strains; *ermF* on Tn*4351,* Tn*4400,* and Tn*4551* is expressed from an IS promoter [12, 13], and several cefoxitin-resistance genes are activated by insertions of IS elements upstream of the genes [15, 16]. Clearly, IS elements capable of activating resistance genes are widespread in *Bacteroides* species. Once a gene has been activated, it can easily accumulate further promoter mutations that increase its expression. Thus, once the process has started, a gene that confers only very weak resistance can evolve, under suitable selective pressures, into a gene that confers high-level resistance.

The metronidazole-resistance genes may well be in an early stage of such an evolutionary process. For this reason, reports of metronidazole-resistance strains should be taken seriously, even though in many cases the strains carrying them are still not resistant enough to cause treatment failures. Another point to be made in this connection is that although chloramphenicol resistance is uncommon in *Bacteroides* clinical isolates, a *cat* gene from *E. coli* functions in *Bacteroides* species if it is provided with a *Bacteroides* promoter, e.g., IS*4351* [17]. Thus, a *Bacteroides* IS element could activate a *cat* gene that happened to migrate into a *Bacteroides* clinical isolate.

A conjugative transposon can also mobilize plasmids *in cis,* by integrating into the plasmid and creating a hybrid plasmid that is then self-transmissible (figure 2). This has been demonstrated in the laboratory, where the cryptic conjugative transposon XBU4422 inserted itself into a nonmobilizable plasmid to form a chimera that was self-transmissible [18], but so far no examples of conjugative transposons inserted in plasmids have been found in *Bacteroides* clinical isolates.

Still Another Activity of Conjugative Transposons: Excision and Mobilization of Other Integrated Elements

Perhaps the most unusual activity of the *Bacteroides* conjugative transposons is the one illustrated in figure 3, which shows how the conjugative transposons mobilize unlinked integrated elements that are not self-transmissible. The conjugative transposon triggers the excision and circularization of the integrated element and then mobilizes the circular form into a recipient. In the recipient, the circular form integrates into the

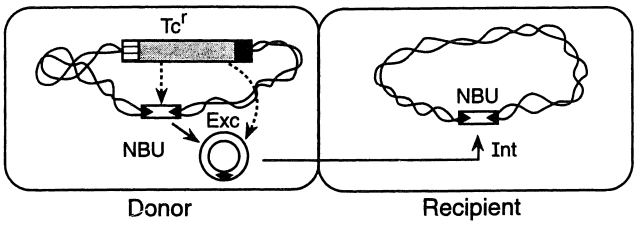

Figure 3. Excision and mobilization of nonreplicating *Bacteroides* units (NBUs). The integrated conjugative transposon (*Tc^r^*) provides proteins *in trans* (*dashed lines*) that cause the integrated form of the NBU to excise from the genome and form a covalently closed circle (*Exc*). The conjugative transposon also provides proteins *in trans* that allow the NBU circular form to be transferred by conjugation to the recipient. In the recipient, the circular form of the NBU integrates (*Int*) into the recipient's genome.

genome [9, 10]. Since the circular form is unable to replicate, we call these mobilizable elements NBUs (nonreplicating *Bacteroides* units).

The NBU about which most is known, NBU1, most closely resembles the integrating plasmids of *Streptomyces* species [19, 20] and the lambdoid phages [21]. NBU1, like these other integrating elements, integrates site-specifically at the end of a tRNA gene and its integrase is distantly related to lambda integrase [22]. NBU1 does not carry any antibiotic resistance genes, but a gene encoding resistance to cefoxitin has been found on an NBU-like element, which has been given a transposon designation: Tn*4555* (table 1)[23].

Recently, we have found a lincosamide-resistance gene on another NBU-like element, NBU2 (unpublished findings). These findings suggest that the NBU-type elements as well as plasmids are contributing to the spread of antibiotic-resistance genes within the *Bacteroides* group.

Since NBUs carry a transfer origin and a mobilization gene, which allow them to be mobilized by the conjugative transposons, the NBUs themselves can be viewed as portable mobilization regions. In theory, an NBU could integrate into a nonmobilizable plasmid, thus rendering the plasmid mobilizable. So far no examples of this have been seen in clinical isolates, but only a relatively small number of isolates have been screened for conjugative transposons or NBUs.

Still another type of integrated element that is mobilizable by the conjugative transposons is the mobilizable transposon, Tn*4399* [10, 24]. Unlike the NBUs, which are more phagelike than transposonlike, Tn*4399* appears to be a transposon that carries a mobilization region, although the possibility that it has a circular intermediate has not been ruled out. The mobilization genes of Tn*4399* are unrelated at the sequence level to the mobilization gene of the NBU-type elements. Tn*4399* carries no known resistance genes, but other elements of this type may prove to be resistance transfer elements. It is also worth noting that Tn*4399,* like the NBUs, could enter a nonmobilizable plasmid that carries resistance genes and render that plasmid transmissible. *Bacteroides* strains certainly have no shortage of ways in which they can exchange DNA.

Are *Bacteroides* Resistance Genes on Portable Cassettes That Could Facilitate the Development of Multiply Resistant Strains?

In the *E. coli–Pseudomonas* group of gram-negative bacteria, small DNA segments have been found that carry only one or two open reading frames and are capable of integrating into other DNA segments, called integrons. These portable DNA segments have been called gene cassettes and are proving to play a major role in the evolution of multidrug-resistance plasmids [25]. Each gene cassette contains, in addition to the resistance gene it carries, a short sequence that allows it to integrate site-specifically and orientation-specifically into an integron. The integron provides not only a site where multiple resistance genes can accumulate but also a promoter that causes the genes to be expressed.

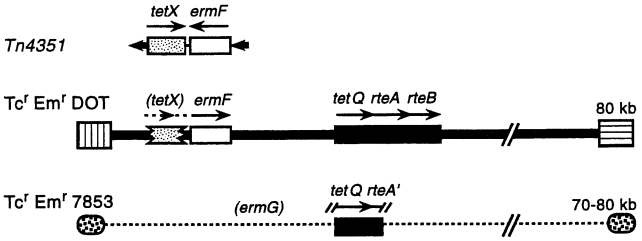

Figure 4. Examples of short segments containing antibiotic-resistance genes that appear to have been excised from one element and integrated into another one. The boxed areas indicate the region that is virtually identical at the DNA sequence level. Generally this includes the open reading frame that encodes the resistance gene and is <100 bp upstream and downstream of the open reading frame. In the case of *tetX,* a gene that encodes a resistance that confers resistance on *E. coli* but not on *Bacteroides* species, the edges of the region in the conjugative transposon Tc^{r}Em^r DOT are jagged to indicate that the gene has been fragmented and the fragments oriented in different directions. The *ermG* gene is also on a cassettelike segment, but its location on the conjugative Tc^{r}Em^r 7853 is not known. Tn*4351* is a compound transposon that is flanked by directly repeated insertion sequence elements (*heavy arrows*). The dotted line indicates that the element shares little or no homology with the other conjugative transposons exemplified by Tc^{r}Em^r DOT. rteA and rteB are regulatory genes that control transfer of the conjugative transposons.

Some evidence is accumulating that *Bacteroides* strains have resistance genes that can move as distinct segments, thus allowing the bacterium to mix and match resistance genes on transmissible elements. Most of the resistance genes found in *Bacteroides* clinical isolates act as if they are each located on mobile segments, which consist of the open reading frame encoding the resistance protein and <100 (sometimes only a few) bp of DNA upstream or downstream of the open reading frame. Some examples of this phenomenon are illustrated in figure 4.

The *tetQ* gene on conjugative transposon Tc^r DOT is identical to the *tetQ* gene on conjugative transposon Tc^{r}Em^{r}7853, but the identity ceases abruptly a short distance upstream and downstream of *tetQ* [5]. The *ermF* gene found on Tc^{r}Em^{r}DOT is virtually identical to the *ermF* gene on the compound transposons Tn*4351,* Tn*4400,* and Tn*4551,* but the identity between Tc^{r}Em^{r}DOT and these transposons ends a few bp upstream and a few bp downstream of the coding region of *ermF* [26]. The *tetX* gene of Tn*4351* and Tn*4400* is found on Tc^{r}Em^{r}DOT, but the copy on Tc^{r}Em^{r}DOT has been fragmented and rearranged internally (unpublished data). Finally, the *ermG* gene on Tc^{r}Em^{r}7853 is identical to an *ermG* gene in *B. sphaericus,* but the identity ends abruptly a few bp upstream and downstream of the open reading frame [14].

What all this suggests is that segments of DNA containing resistance genes are able to move readily from one element to another, providing the bacterium with tremendous flexibility in how it arrays its resistance genes. In particular, resistance

genes that arise in the chromosome can be moved onto conjugative transposons and conjugative plasmids, enabling them to be spread.

The gene cassettes that integrate in integrons are characterized by a sequence called the ''59 bp'' element—although it can be longer or shorter than 59 bp—which mediates integration into the integron attachment site [25]. So far, we have seen no indication of such a sequence near the ends of *Bacteroides* resistance genes that appear to have migrated as cassettes. In fact, different alleles of *tetQ* integrated into different sites do not have the same endpoints [4, 5]. Nonetheless, the important point is that there are are numerous examples of cases where a resistance gene has been moved from one type of transmissible element to another. The prediction from this is that once a resistance gene has appeared in a *Bacteroides* clinical isolate, even if it is not initially on a transmissible element, it could easily end up on a transmissible element.

This phenomenon is not limited to antibiotic-resistance genes. The NBUs appear to contain a DNA segment that could itself be a mobile integrating element. This is the segment that carries the transfer origin, a mobilization gene that enables the circular intermediate to move through the mating apparatus, and another gene of unknown function. This segment of NBUs may be mobile because even though this same mobilization region has been found on three NBU-like elements—NBU1, NBU2, and the cefoxitin-resistance transposon Tn*4555*—these elements are unrelated outside the shared mobilization region ([27] and unpublished data). In fact, one small *Bacteroides* plasmid that is otherwise unrelated to NBUs has also been found to carry this same mobilization region [28]. It is not clear how this mobilization region is moving from element to element. It is not part of a compound transposon because there is nothing resembling IS elements near its ends.

Transmissible Elements of the Gram-Positive Anaerobes

Little is known about gene transfer elements of the gram-positive anaerobes. The gene *tetM* has been found in peptostreptococci by DNA hybridization [2]. Since *tetM* has so far been found almost exclusively on conjugative transposons in the gram-positive bacteria [10], this suggests the possibility that conjugative transposons, which are widely distributed in the gram-positive facultative cocci, may be present in the gram-positive anaerobes as well. Bannam and co-workers [29] have found a mobilizable transposon, Tn*4451,* in a porcine isolate of *Clostridium perfringens.*

Tn*4451* resembles the NBUs in many respects. That is, it excises to form a circular intermediate, has an integrase that belongs to the lambda integrase family, and carries mobilization genes. Unlike the *Bacteroides* conjugative transposons and NBUs, Tn*4451* was found on a plasmid rather than in the chromosome. Tn*4451* carries a chloramphenicol-resistance gene. It is still not clear whether Tn*4451* is self-transmissible or is mobilized by some other element. Tn*4451* seems too small to be self-transmissible, but if there is another element mobilizing it, this element has yet to be identified.

Host Range—Broadening the Definition of ''Broad''

In much of the early literature on plasmid transfer, the term *broad host range* was used to indicate transfers between different species, e.g., *E. coli* and *Pseudomonas aeruginosa.* Yet this is not a very broad range of transfer from the phylogenetic point of view, because both *E. coli* and *Pseudomonas* species belong to the same phylogenetic group, the purple bacteria [8]. More recent examination of gene transfers between distantly related phylogenetic groups, e.g., between *E. coli* and gram-positive bacteria or between *Bacteroides* species and *E. coli,* have shown that such transfers can in fact occur in the laboratory at relatively high frequencies (10^{-4} to 10^{-6} per recipient).

Plasmids, such as the IncP plasmids, and conjugative transposons are also capable of interphylum transfers, although the IncP plasmids do not replicate in distantly related phylogenetic groups such as the gram-positive bacteria and the *Bacteroides* group [30]. Thus, there appear to be no barriers to the transmission of genes, at least under laboratory conditions.

The question is sometimes raised as to how relevant the interphylum gene transfers actually are, because a gene that is expressed in *E. coli* may not be expressed in a gram-positive bacterium or in distantly related gram-negative bacteria such as *Bacteroides* species. Some resistance genes are expressed and function effectively in a very wide range of hosts. For example, *tetM* confers tetracycline resistance on a wide variety of bacterial species, including gram-positive as well as gram-negative bacteria [30]. If a *Bacteroides* IS element or promoter is provided, *tetM* also confers tetracycline resistance on *Bacteroides* species [17]. The *ermG* gene of *B. sphaericus* is working well enough in *Bacteroides* species to make them clinically resistant. Furthermore, as pointed out earlier in this review, even if a resistance gene is not expressed initially in a new host, an IS element can activate it.

The Microflora as a Reservoir of Resistance Genes: New Evidence for an Old Idea

In order for the resident microflora to serve as a reservoir for resistance genes, it must be able to acquire resistance genes from microbes passing through the area and then transmit these genes to other microbes that later colonize the site. Since a variety of bacteria from soil, food, and the resident microflora of the mouth—not to mention upper respiratory or intestinal pathogens—pass through the human colon, transfers of resistance genes from these microbes to the *Bacteroides* species or gram-positive anaerobes in the colon would require very broad host range events that cross genus and even phylum lines. Although it is well-established that transfer of resistance genes can occur across genus and phylum lines under the idealized

Table 2. Examples of cases in which virtually identical alleles of the same antibiotic-resistance gene have been found in very distantly related genera of bacteria.

Reference(s)	Resistance gene	Found in:
[30]	*ermBC*	Gram-positive cocci, *E. coli*
[31, 32]	*tetM*	Gram-positive cocci, *Actinomyces* species, *Bifidobacterium* species, *Bacillus* species, *Streptomyces* species, soil mycobacteria, *Campylobacter* species, *Fusobacterium* species, *Neisseria* species, *Veillonella* species*
[4, 32]	*tetQ*	*Bacteroides* species, *Prevotella* species, *Porphyromonas* species
[14, 32]	*ermG*	*Bacillus* species, *Bacteroides* species

* This is only a partial listing of the species in which *tetM* has been found.

conditions provided by a laboratory setting, the question naturally arises as to how frequently such transfers occur in nature.

Laboratory experiments demonstrating very broad host range transfers not only employ conditions bacteria are not likely to encounter in a natural setting but also frequently use specially constructed gene transfer elements [30]. For example, plasmids have been engineered to contain more than one replication origin, so that they will survive in recipients that do not support replication of the wild-type plasmid, or new markers may have been engineered into the plasmid or conjugative transposon because the markers it normally carries are not expressed in the recipient. Thus, the elements that are being used to demonstrate very broad host range transfers are often artificial constructs.

The best way to answer these quite reasonable objections is to turn to the few studies that have looked for evidence of natural gene transfer events by searching for cases in which the same gene is found in two distantly related bacterial genera. If the resistance genes are much more closely related than the organisms that carry them, this suggests that the resistance gene was spread by horizontal transfer.

Some experimental findings that support widespread dissemination of resistance genes between distantly related bacteria in nature are summarized in table 2 [30, 32]. In considering these examples, one should keep in mind that very few surveys of this type have been conducted. The fact that examples have been found at all in these limited surveys suggests that very broad host range transfers occur quite commonly in nature. Evidence that gene transfers occur between gram-positive and gram-negative bacteria is the finding of *tetM* in both gram-positive bacteria and a variety of gram-negative bacteria in the *E. coli* phylogenetic group, as well as our finding of virtually identical versions of *ermG* (>99% DNA sequence identity [14]) in *Bacteroides* species and in *B. sphaericus.*

Finding *ermG* both in *Bacillus* species, which are soil microbes, and in *Bacteroides* species also supports the contention that ingested soil bacteria may be able to transfer their genes to members of the colonic microflora. A similar conclusion is suggested by the recent finding of *tetM* in soil microbes, such as *Streptomyces* species and nontuberculous mycobacteria, as well as in *Peptostreptococcus* species, which are components of the colonic microflora.

If ingested bacteria can pass genes to members of the microflora, a new safety question arises in connection with foods and probiotics that contain live bacteria. Perhaps the antibiotic-resistance profile of such bacteria—not just whether they cause human disease—should be considered as part of the safety assessment. Do we really want people ingesting a vancomycin-resistant isolate of *Lactobacillus* species on a daily basis?

The fact that *tetQ* has been found in oral isolates of *Prevotella* species as well as in colonic *Bacteroides* strains indicates that microflora from different body sites can exchange genes. The fact that *tetM* has been found in many pathogens as well as in members of the resident microflora supports the hypothesis that transfers occur between members of the microflora and human pathogens.

A New Reason for Concern About Low-Dose Tetracycline Use

A striking and unusual feature of the *Bacteroides* conjugative transposons is that their transfer functions are regulated by tetracycline [9]. That is, exposure to low concentrations of tetracycline stimulates self-transfer by 1,000- to 10,000-fold. Other activities such as plasmid mobilization and NBU excision and mobilization are stimulated by 100- to 1,000-fold. This means that not only does tetracycline select for strains that have acquired a conjugative transposon, but it increases dramatically the likelihood that the conjugative transposon will be transferred in the first place. All members of the tetracycline family of antibiotics that are currently in clinical use have this stimulatory capacity, and the concentrations required for stimulation (<1 μg/mL) are below the MIC for most bacterial strains, even those considered susceptible to tetracycline [9, 10].

Perhaps the reason why *tetQ*-carrying conjugative transposons are now found in virtually all of the clinical isolates of *Bacteroides* species, whereas tetracycline-resistant strains were relatively uncommon 30 years ago, is that use of low-dose tetracycline therapy by dermatologists has stimulated their dissemination. Oral tetracycline or doxycycline is routinely prescribed for young adults with acne and for adults with rosacea [33, 34]. Especially in the latter case, the antibiotics are taken for long periods (months or years).

Use of tetracycline in animal feed as a growth promoter [35] may also be having the same effect. Where tetracycline is being used as a feed additive, it is continuously present at low levels over prolonged periods in the animals and in the environment, not to mention the bodies of agricultural workers who handle the animals. Recently, we found the same *tetQ* gene, which is now widespread in human *Bacteroides* clinical isolates, in

P. ruminicola strains isolated from cattle, sheep, and pigs [4]. Oddly enough, the *tetQ* genes in the rumen isolates were not on any of the conjugative transposons described to date, although the genes had >95% DNA sequence identity with the genes found in human *Bacteroides* species.

The tetracycline stimulation phenomenon raises the question of whether other antibiotics may be able to have this effect. If so, antibiotics not only affect the resistance of the resident microflora by providing conditions that favor the growth of resistant strains but also can drive the spread of resistance genes that evolve. Clearly, the notion that concentrations of an antibiotic that are below the MIC have no effect on the resident microflora needs to be reassessed.

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