ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2000, p. 775–777 Vol. 44, No. 3
0066-4804/00/$04.00+0

Occurrence of the New Tetracycline Resistance Gene *tet*(W) in Bacteria from the Human Gut

KAREN P. SCOTT,* CLAIRE M. MELVILLE, TERESA M. BARBOSA,† AND HARRY J. FLINT

Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, United Kingdom

Received 9 August 1999/Returned for modification 2 November 1999/Accepted 16 December 1999

Members of our group recently identified a new tetracycline resistance gene, *tet*(W), in three genera of rumen obligate anaerobes. Here, we show that *tet*(W) is also present in bacteria isolated from human feces. The *tet*(W) genes found in human *Fusobacterium prausnitzii* and *Bifidobacterium longum* isolates were more than 99.9% identical to those from a rumen isolate of *Butyrivibrio fibrisolvens*.

The rapid increase in antibiotic resistance in human pathogenic bacteria is a major problem, particularly for nosocomial infections (5). In the past, antibiotic resistance genes have primarily been described either in clinical pathogens or in antibiotic-producing microorganisms, and comparatively little work has been done on the incidence of antibiotic resistance in the commensal gut flora, either of humans or of animals. A new ribosome-protection-type tetracycline resistance (Tc^r) gene, *tet*(W), (GenBank accession no. AJ222769), was recently identified in the rumen anaerobe *Butyrivibrio fibrisolvens* and was also found in rumen isolates of *Selenomonas* spp. and *Mitsuokella* spp. and in one *Mitsuokella* isolate from a Japanese pig (1). The high degree of homology between all of these *tet*(W) genes suggested that recent gene transfer events had resulted in the spread of the gene. *tet*(W) was shown to be chromosomally located in *B. fibrisolvens* and to transfer at frequencies of 10^{-3} to 10^{-5} per recipient between genotypically diverse *B. fibrisolvens* strains in vitro (10). The translated product of *tet*(W) shares only 68% amino acid homology with Tet(O) and Tet(M) proteins (1). Here, we describe for the first time the identification of *tet*(W) in anaerobic bacteria recovered from human feces.

Human fecal samples were resuspended in anaerobic 0.1 M sodium phosphate buffer (pH 7.2), and dilutions were plated out anaerobically either on M2GCS agar plates (6) containing 5 or 10 μg of tetracycline per ml or in M2GCS roll tubes (2) containing 10 μg of tetracycline per ml. Plates were inoculated in an anaerobic cabinet (55% CO_2, 40% N_2, and 5% H_2; Coy Laboratory Products Inc., Grass Lake, Mich.), and roll tubes were prepared under 100% CO_2 (2). Cultures were incubated at 37°C.

For one sample from a middle-aged male receiving daily tetracycline treatment over a 10-year period, more than 99% of the 8.3×10^{10} colonies growing anaerobically were Tc^r. Random colonies were picked from roll tubes and regrown in the presence of 10 μg of tetracycline per ml. Total genomic DNA was purified (10) and amplified by PCR, either using degenerate primers which identify all ribosome-protection-type Tc^r genes (1) or using a primer combination specific for *tet*(W) (tetW for [5′ AAGCGGCAGTCACTTCCTTCC 3′] and tet2 [see reference 1]). All 14 of the colonies tested yielded a product with the degenerate Tc^r primer set, while only one, isolate K10, yielded a product with primers specific for *tet*(W). Culturing of two additional samples from 25-year-old individuals who had not taken antibiotics for at least 10 years showed that less than 0.01% of the total anaerobic bacterial count was Tc^r. Total genomic DNA purified from 3 of 20 Tc^r colonies (F5, F8, and F10) from one individual yielded a PCR product when the primer set specific for *tet*(W) was used.

The PCR products obtained as described above were sequenced using the ABI 377 automated sequencing system and confirmed to be *tet*(W) products using a basic local alignment search tool search for database comparisons. This initial sequence analysis demonstrated that the *tet*(W) gene from the human isolates was very closely related to *tet*(W) genes from the rumen isolates (1). An extended region of the new *tet*(W) genes was amplified using primers corresponding to positions 165 to 185 and 2096 to 2113 in the database sequence AJ222769. Subsequent sequence analysis showed that the genes from K10 and F5 differed by a single nucleotide and, furthermore, differed by 0 or 1 nucleotides (nt), respectively, over 1,864 nt of the 1,917-nt coding sequence of the *B. fibrisolvens tet*(W) gene. Table 1 indicates the sequence divergence between the *tet*(W) genes we have identified so far. The degree of homology observed for *tet*(W) genes of diverse origin is much higher than that observed for other ribosome-protection-type Tc^r genes and indicates that the gene has not evolved greatly following acquisition by the divergent host bacteria, which therefore implies that transfer events resulting in the spread of *tet*(W) have been recent. A survey done to compare *tet*(Q) genes from *Bacteroides* or *Prevotella* isolates of animal and human origin indicated that an internal 407-nt segment differed by up to 59 nt between different isolates (8). Although this survey found that human isolates of *Prevotella intermedia* and *Bacteroides fragilis* contained *tet*(Q) genes which were identical across the region analyzed, the closest homology between genes from different hosts was 98%.

Bacterial isolates confirmed to contain *tet*(W) were partially characterized by Gram staining and by sequencing 16S ribosomal DNA fragments amplified by PCR using eubacterial primers (12). Searches for homologous sequences in the database showed that the K10 isolate was related to *Clostridium* spp. and that the F5, F8, and F10 isolates were related to *Bifidobacterium* spp. Further identification at the Scottish Anaerobe Laboratory (University of Edinburgh) confirmed the identity of K10 as *Fusobacterium prausnitzii* and the identities of F5, F8, and F10 as *Bifidobacterium longum. F. prausnitzii*, unlike other *Fusobacterium* spp., is related to gram-positive bacteria (11).

* Corresponding author. Mailing address: Rowett Research Institute, Greenburn Rd., Bucksburn, Aberdeen, AB21 9SB, United Kingdom. Phone: 44 (0) 1224 712751. Fax: 44 (0) 1224 716687. E-mail: k.scott@rri.sari.ac.uk.

† Present address: Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111.

TABLE 1. Characteristics of bacterial strains harboring *tet*(W)

Species and strain(s)	Source[b]	Gene location	Transferability test result	No. of differences[a]
Butyrivibrio fibrisolvens				
1.230 and 1.23	Bovine rumen, RRI (1993)	Chromosome	Yes	0
JK51 and JK214	Sheep rumen, UNE (1996)	Chromosome	ND[c]	0
Selenomonas ruminantium				
FB322	Bovine rumen, RRI (1989)	Chromosome	No[d]	1
FB32	Bovine rumen, RRI (1989)	Plasmid	No[d]	0
FB34	Bovine rumen, RRI (1989)	Plasmid	No[d]	0
Mitsuokella multiacidus				
46/5(2)	Sheep rumen, RRI (1987)	Chromosome	No[d]	0
P208-58	Pig feces, Japan (1974)	Chromosome	ND	7
Fusobacterium prausnitzii				
K10	Human fecal flora, RRI (1999)	Chromosome	ND	0
Bifidobacterium longum				
F5, F8, and F10	Human fecal flora, RRI (1999)	Chromosome	ND	1

[a] Number of nucleotide differences from 1.230 *tet*(W) found over a 1.25-kb internal fragment. PCR-amplified template DNA was sequenced; hence, *Taq* polymerase errors cannot be ruled out as possibly contributing to nucleotide differences.
[b] Data in parentheses indicate year of isolation of bacterial strain. RRI, Rowett Research Institute, Aberdeen, United Kingdom; UNE, University of New England, Armidale, Australia.
[c] ND, not yet determined.
[d] Transfer was tested using *Selenomonas ruminantium* HD4 as a potential recipient.

The genetic location of these closely related *tet*(W) genes from the different bacterial species was investigated. Total genomic DNA was purified from the human isolates F5, F8, and K10 and digested with *Eco*RI or *Bam*HI. Hybridization of the resulting Southern blot to a ^{32}P-labeled *tet*(W) probe indicated that different fragments contain the gene in different species, the hybridizing bands ranging in size from 7 kb (*B. fibrisolvens* 1.230) to 12 kb (*B. longum* F8 [Fig. 1]). The *tet*(W) probe also recognized a second, faint *Bam*HI fragment in *F. prausnitzii*. Attempts at PCR amplification using primers specific for regions of the transferable element Tn*B123O* flanking *tet*(W) did not yield products with the human isolates. The extent of the homology and the mobility of *tet*(W) genes from the different isolates are currently being investigated.

The results described here indicate that the newly identified *tet*(W) gene is widespread among anaerobic commensal gut bacteria. The identification of the gene in *F. prausnitzii*, the fifth most dominant human colonic anaerobe (7), indicates that, as is the case with rumen isolates, *tet*(W) occurs in some of the most abundant members of the gut flora. From a recent survey it was inferred that *tet*(Q) could be the most common Tc^r gene among anaerobic gram-negative bacteria (4), and the group conducting the survey also identified *tet*(Q) in gram-positive bacteria for the first time. They also found, however, that a number of the Tc^r isolates contain unknown Tc^r genes. Some, perhaps many, of these unknown genes could prove to be *tet*(W).

The extremely high level of sequence identity between the *tet*(W) genes found in bacteria of different genera isolated from different hosts implies recent gene transfer events. Although the *tet*(W) gene is located on a highly mobile chromosomal element, Tn*B123O* (10), in the *B. fibrisolvens* strain where it was first identified, the same mobile element does not appear to be present in all rumen isolates (1) or in human isolates that carry *tet*(W). Thus, the full range of mechanisms by which the *tet*(W) gene has spread remains to be elucidated. Interestingly, with the exception of *B. fibrisolvens* (DNA G+C content of 36 to 41%), *tet*(W) generally seems to be associated with higher-G+C-content bacterial species (*Fusobacterium* sp. G+C content, 52 to 57%; *B. longum* G+C content, 58%; *Selenomonas* sp. G+C content, 54 to 61%; and *Mitsuokella* sp. G+C content, 56 to 58%). *tet*(W) itself has a much higher G+C content (53%) than most ribosome-protection-type *tet* genes (1), and this may be reflected in its host range.

The occurrence of almost identical Tc^r genes in commensal bacteria from the animal and human gut is evidence of recent

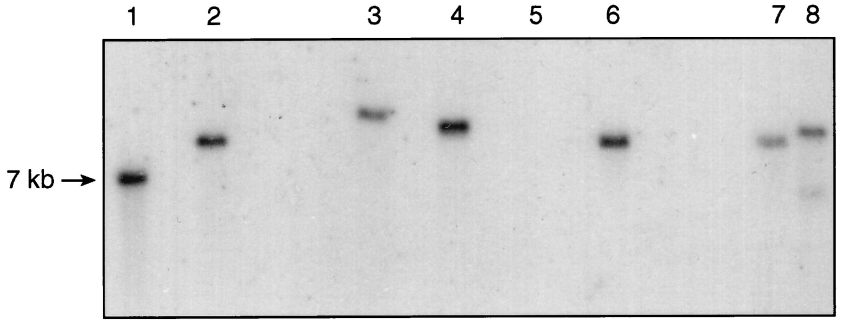

FIG. 1. Southern blot of total genomic DNA purified from the human isolates digested with either *Eco*RI (lanes 1 to 5) or *Bam*HI (lanes 6 to 8) hybridized to a PCR-amplified *tet*(W) probe corresponding to nucleotides 165 to 2113 in the database sequence. Lane 1, *B. fibrisolvens* 1.230 (positive control); lanes 2 and 6, *B. longum* F5; lanes 3 and 7, *B. longum* F8; lanes 4 and 8, *F. prausnitzii* K10; lane 5, *B. fibrisolvens* 2221 (Tc^s, negative control).

gene flow between these populations and leads to the important conclusion that obligate anaerobiosis is not a barrier to genetic exchange. The most likely route for transfer between hosts may be via intermediary facultative anaerobes that are capable of colonizing animals and man. Alternatively, it is also likely that transfer of obligately anaerobic gut bacteria between hosts occurs with sufficient frequency to mediate gene transfer events. It is of course impossible to conclude from the present evidence whether transfer of *tet*(W) has been predominantly to or from the human gut flora. This question is clearly central to the debate over the use of antibiotics as growth promoters in agriculture and the impact such use has on the clinical use of antibiotics in the treatment of human disease. Tetracyclines continue to be important as therapeutic antibiotics, but they are still employed in agriculture in many countries (3), making them overall the second most used group of antibiotics worldwide (9).

This work was supported by SERAD (Scottish Executive Rural Affairs Department) and by a studentship award to T. M. Barbosa (Sub-Programa Ciência e Tecnologia do 2° Quadro Comunitário de Apoio PRAXIS XXI/BD/3382/94).

B. fibrisolvens strains JK51 and JK214 were kind gifts from J. Kopecny.

REFERENCES

1. **Barbosa, T. M., K. P. Scott, and H. J. Flint.** 1999. Evidence for recent intergeneric transfer of a new tetracycline resistance gene, *tet*(W), isolated from *Butyrivibrio fibrisolvens*, and the occurrence of *tet*(O) in ruminal bacteria. Environ. Microbiol. **1:**53–64.
2. **Bryant, M. P.** 1972. Commentary on the Hungate technique for cultivation of anaerobic bacteria. Am. J. Clin. Nutr. **25:**1324–1328.
3. **Khachatourians, G. G.** 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Can. Med. Assoc. J. **159:** 1129–1136.
4. **Leng, Z., D. E. Riley, R. E. Berger, J. N. Krieger, and M. C. Roberts.** 1997. Distribution and mobility of the tetracycline resistance determinant *tetQ*. J. Antimicrob. Chemother. **40:**551–559.
5. **Levy, S. B.** 1992. The antibiotic paradox: how miracle drugs are destroying the miracle. Plenum Press, New York, N.Y.
6. **Miyazaki, K., J. C. Martin, R. Marinsek-Logar, and H. J. Flint.** 1997. Degradation and utilization of xylans by the rumen anaerobe *Prevotella bryantii* (formerly *P. ruminicola* subsp. *brevis*) $B_1$4. Anaerobe **3:**373–381.
7. **Moore, W. E. C., and L. H. Moore.** 1995. Intestinal floras of populations that have a high risk of colon cancer. Appl. Environ. Microbiol. **61:**3202–3207.
8. **Nikolich, M. P., G. Hong, N. J. Shoemaker, and A. A. Salyers.** 1994. Evidence for natural horizontal transfer of *tetQ* between bacteria that normally colonize humans and bacteria that normally colonize livestock. Appl. Environ. Microbiol. **60:**3255–3260.
9. **Roberts, M. C.** 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol. Rev. **19:**1–24.
10. **Scott, K. P., T. M. Barbosa, K. J. Forbes, and H. J. Flint.** 1997. High-frequency transfer of a naturally occurring chromosomal tetracycline resistance element in the ruminal anaerobe *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol. **63:**3405–3411.
11. **Wang, R. F., W. W. Cao, and C. E. Cerniglia.** 1996. Phylogenetic analysis of *Fusobacterium prausnitzii* based upon the 16S rRNA gene sequence and PCR confirmation. Int. J. Syst. Bacteriol. **46:**341–343.
12. **Weisberg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.