MICROBIAL ECOLOGY OF ANTIBIOTIC RESISTANCE GENES IN THE HUMAN COLONIC MICROBIOTA

A group project of the Microbial Diversity Course, 1996, led by Nadja Shoemaker

Introduction

Although a great deal has been written about the colonic microbiota as a reservoir for antibiotic resistance genes, all studies to date of resistance gene distribution among the colonic bacteria of healthy adults have focused exclusively on the facultlative bacteria such as *E. coli*. Yet, these bacteria account for less than 0.2% of the human colonic microbiota. The predominant bacterial genera are the gram-negative anaerobes, especially *Bacteroides* spp., and the gram-positive anaerobes, such as *Eubacterium*, *Peptostreptococcus*, *Bifidobacterium* and *Clostridium*. A few studies of antibiotic resistance genes and the elements that transfer them have been done with clinical isolates of *Bacteroides* spp., and it is clear from these studies that extensive transfer of antibiotic resistance genes has occurred within this group. It was not clear, however, whether this was true only of clinical isolates or whether widespread resistance gene transfer was also occurring in the colons of normal humans. We decided to do a small survey of anaerobic isolates obtained from healthy volunteers to determine if they carried known gene transfer elements.

Design of the Study

There are two types of gene transfer element in *Bacteroides* spp: plasmids and integrated elements known as conjugative transposons (Salyers et al., 1995, Microbiol. Rev. 59: 579-590). Conjugative transposons are normally integrated into the organism's genome but can excise themselves and transfer to other bacterial hosts. Excision and transfer of at least some *Bacteroides* conjugative transposons can be stimulated over 1000-fold by low concentrations of the antibiotic tetracycline. Since low-dose tetracycline is used widely as a therapy for acne, and tetracycline is one of the antibiotics used as a growth stimulant in livestock animals, it is conceivable that these elements might be spreading among different members of the colonic populations in people who are not hospitalized. The antibiotic resistance gene, *tetQ*, is commonly assiciated with *Bacteroides* conjugative transposons. It encodes a ribosome protection type of tetracycline resistance. Because of its association with conjugative transposons, we used a cloned internal region of *tetQ* as a hybridization probe in our experiments. We also used clones that contained part of the ends of the conjugative transposon that is most often found in clinical isolates, Tc^r Em^r DOT. Another type of integrated element, called NBUs, has also been found recently to be carrying antibiotic resistance genes in some *Bacteroides* clinical isolates. NBUs are about 10-12 kbp in size, much smaller than the conjugative transposon which range in size from 60 kbp to 150 kbp. NBUs are not self-transmissible, but they can be excised and transferred *in trans* by the conjugative transposons. NBUs are quite diverse, but many of them share a highly conserved mobilization region. We used a clone containing a portion of this region as a probe in our experiment.

Volunteers were asked to take swabs and obtain a rectal sample immediately after defecating. These swabs were used to inoculate prereduced BHI agar plates that contained gentamicin (200 ug/ml). The gentamicin selects against the facultative enterics. Although *Bacteroides* and other anaerobes far outnumber *E. coli* in the colon, the percentage of *E. coli* is higher in the rectum. Also, *E. coli* grows faster than the anaerobes and will overgrow a plate if its growth is not inhibited. *Bacteroides* spp. are uniformly resistant to gentamicin, but it is not known whether this is true of the other anaerobic species in colon contents, so the antibiotic may also have selected against the gram-positive anaerobes. *Bacteroides* spp. are much more aerotolerant than other colonic anaerobes and are easier to grow. Since we inoculated the plates on the bench before placing them in

GasPak jars, a further selection for *Bacteroides* could have occurred at this step. The plates were incubated anaerobically for 3 days. Isolated colonies were picked onto 3 BHI-gentamicin plates in an identical array. These replica plates were then incubated anaerobically for 2 days. The colonies were transferred to Nytran filters and the filters were subjected to a standard colony hybridization procedure. One filter of each set of three was hybridized with the *tetQ* probe, one was hybridized with the conjugative transposon end probe (CT), and the third was hybridized with the NBU probe. Probes were labeled with fluorescein and bound probe was detected using the Renaissance kit.

Results and Discussion

The results of the survey are summarized in the table below

Source	Number of Colonies Tested	Number of Colonies Positive for *tetQ*	CT	NBU
Group I	50	38 (76%)	37	38
Subject D	20	17 (85%)	7	0
Subject A	20	12 (60%)	4	5
Subject S	9	7 (70%)	1	0
Subject Am	31	25 (77%)	8	10
Subject R	16	10 (60%)	7	0
Subject WM	45	37 (75%)	30	31
Subject CP	47	46 (99%)	46	45
Subject E	11	9 (90%)	2	8
Subject DJ	50	7 (14%)	5	3
Subject MD	50	12 (24%)	3	33

The incidence of *tetQ* was very high in all but two of the subjects tested. A survey of isolates of *Bacteroides* submitted to culture collections before 1970 showed that *tetQ* and the conjugativet transposon detected by the probe used here were quite rare (<10% of isolates). This was true both for clinical isolates and isolates from the feces of healthy individuals (Nadja Shoemaker, unpublished data). In the same survey, NBU-hybridizing DNA was found in about 25% or the isolates tested. The incidence of *tetQ* and the CT have clearly increased dramatically, whereas the incidence of NBU-hybridizing DNA has remained at about this level or below it in most of the subjects tested in the present study. In studies of recent *Bacteroides* clinical isolates

(since 1990), *tetQ* was found in 100%of the isolates and was always associated with the CT detected in the present study with one exception (N. Shoemaker, unpublished results). A striking finding of the present study was that only about 60% of the strains that contained *tetQ* also contained DNA that hybridized with our CT probe. No attempt was made to determine whether the *tetQ* alleles in the strains that did not cross-hybridize with the CT probe were transmissible, but *tetQ* has so far been associated only with transmissible elements in *Bacteroides*. The findings of the present study thus raise the possibility that the CT which is so commonly found in clinical isolates may not be the only CT now circulating in *Bacteroides* strains. Taken together, the results of this simple survey suggest that there has been a lot of gene transfer activity in the human intestine among the obligate anaerobes and that the amount of gene transfer has increased dramatically during the past 2 decades.