Detection of antibiotic resistance genes in the feces of young adult Japanese

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Antibiotic resistance genes in the feces of healthy young adult Japanese were analyzed with polymerase chain reaction using specific primers. Antibiotic resistance genes against macrolides (ermB, ermF, ermX, and mefA/E), tetracyclines (tetW, tetQ, tetO, and tetX), β-lactam antibiotics (blaTEM), and streptomycin (aadE) were detected in more than 50% of subjects. These antibiotic resistance genes are likely widespread in the large intestinal bacteria of young adult Japanese.

Key words: antibiotic resistance gene, macrolide, tetracycline, human feces

Numerous bacteria that construct a complex microbial ecosystem with competition and symbiosis are present in the human large intestine [1, 2]. These intestinal bacteria immunologically and metabolically influence host health. Their health benefits have attracted attention.

Antibiotics have been used for the treatment and prevention of infectious disease caused by pathogenic bacteria. Use of antibiotics may seriously impact intestinal bacteria. Occasionally, disruption of the microbial composition in the large intestine by antibiotics may cause diarrhea. In the animal industry, antibiotics have been used not only for the treatment and prevention of infectious diseases but also for the promotion of growth in livestock animals [3]. Frequent use of antibiotics in medical care and the animal industry may lead to the emergence of antibiotic-resistant bacteria [4–6]. Mutation of antibiotic target genes or acquisition of antibiotic resistance genes that cause inactivation of antibiotics, modification of the antibiotic targets, or efflux of antibiotics allows bacteria to resist antibiotics [7, 8]. Furthermore, obtained antibiotic resistance genes can be transferred to other bacteria [8]. This may lead to the emergence of novel antibiotic-resistant bacteria. Thus, the spread of antibiotic resistance genes and the emergence of antibiotic-resistant bacteria, in particular antibiotic-resistant pathogens, have created serious problems for human beings. The transfer of antibiotic resistance genes likely occurs readily among bacteria in an environment in which large amounts of bacteria are present with a high density, such as in soil, sludge, and the large intestine [9]. It has been mentioned that the intestinal microbial ecosystem has the potential to preserve antibiotic resistance genes [7, 10, 11]. Indeed, some antibiotic resistance genes were detected in human feces by metagenomic and microarray analysis [6, 9, 12, 13]. It was reported that metagenomic analysis detected 1,093 antibiotic resistance genes among 162 individuals from three different countries and that ermB, blaTEM, and sul2, which are resistance genes against macrolides, β-lactams, and sulfonamides, respectively, were commonly detected by microarray analysis in saliva and feces collected from five European countries.

Polymerase chain reaction (PCR) using specific primers is an alternative method for detecting antibiotic resistance genes in intestinal bacteria. Although only previously known antibiotic resistance genes are detected with PCR, they can be more rapidly and easily detected with PCR using some specific primers in specimens than with metagenomic or microarray analysis. This method enables us to easily determine the presence of antibiotic resistance genes in individuals and the distribution of antibiotic resistance genes among a community. This information should be useful for preventing the further spread of antibiotic resistance genes and the emergence of novel antibiotic-resistant bacteria. In this study, feces collected from healthy young adult Japanese were analyzed for the presence of antibiotic resistance genes by PCR targeting previously known antibiotic resistance genes.

A total of 32 fecal samples from healthy female volunteers between 21 and 22 years old were analyzed. The volunteers consumed non-specific Japanese diets and had no antibiotics for one month prior to fecal collection. This study was performed in accordance with the Helsinki Declaration as updated in Brazil in 2013. The details of this study were explained to the volunteers, and an informed consent agreement was obtained from each. bacterial DNA was extracted from 0.1 g of feces in accordance with the
methods of Godon et al. [14]. Antibiotic resistance genes against tetracyclines (tetW, tetQ, tetO, tetM, tetS, tetB/P, tetT, tetL, tetK, and tetX), macrolides (ermA, ermB, ermC, ermF, ermT, ermX, ermA, erEB, mphA, mefA/E, and msrA/B), chloramphenicol (cat), β-lactam antibiotics (blaSHV, blaCTX-M, blaTEM, and blaOXA), fluoroquinolones (qnrA, qnrB, qnrS, qnrD, qepA, oqxA, oqxB, and aac(6’)-Ib-cr), ampicillin (ampC), methillin (mecA), streptomycin (aadA and aadE), sulfonamide (sulI and sulII), and vancomycin (vanA, vanB, and vanC1) were amplified with PCR using specific primers [15–23]. The PCR conditions were as follows: 10 μl of GoTaq Green Master Mix (Promega, Madison, WI, USA), 400 μmol/l of each primer, and 25 ng of extracted bacterial DNA in a total volume of 20 μl. The thermal program consisted of an initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 30 sec, primer annealing at the optimum temperature for 30 sec, and elongation at 72°C; and then final elongation at 72°C for 5 min. The annealing temperature and elongation time in each PCR were designed as described previously [15–23]. Aliquots of the PCR amplicons (10 μl) were analyzed by electrophoresis on 1.5% (w/v) agarose gels and visualized after staining with GelRed (Wako Pure Chemical Industries, Osaka, Japan). The detection of each antibiotic resistance gene was performed three times.

In this study, due to the sensitivity of PCR analysis [24], the number of antibiotic-resistant bacteria for which the target antibiotic resistance gene was not detected by specific PCR may be 10⁵ cells/g of feces. In other words, the detection of target antibiotic resistance genes in this study showed that bacteria that have the target resistance gene might be present in more than 10⁵ cells/g of feces.

The number of antibiotic resistance genes detected in a subject ranged 7 to 17. On average, 11 antibiotic resistance genes were detected in a subject. In human fecal bacterial genomes, antibiotic resistance genes against macrolides, tetracyclines, β-lactams, fluoroquinolones, vancomycin, and sulfonamides were commonly detected with the metagenomic and microarray analysis [6, 7, 9, 11, 12, 25]. In particular, antibiotic resistance genes against macrolides ermB and ermF, and against tetracyclines tetO and tetW were most commonly detected in fecal bacterial genomes. In this study, ermB, mefA/E, tetW, and tetO were detected in all subjects (Table 1), and ermF, ermX, tetO, and tetX were detected in more than 50% of subjects. In addition, tetL was detected in a few (20–30%) subjects. The other antibiotic resistance genes against macrolides and tetracyclines analyzed in this study were detected in few (less than 20% of subjects) or no subjects. These results indicate that antibiotic resistance genes against macrolides and tetracyclines are likely widespread in the fecal bacteria of young adult Japanese. The erm genes encode rRNA methylase, which methylates the 23S rRNA of bacteria to alter the macrolide-binding site in rRNA [21]. mefA and mefE genes encode efflux pumps of macrolides [16]. It was considered that rRNA methylase and efflux pumps should be major methods of macrolide resistance in the fecal bacteria of young adult Japanese. tetW, tetQ, and tetO genes encode ribosomal protection proteins to resist tetracycline [26]. tetX and tetL encode oxidoreductase to inactivate tetracycline [27] and efflux proteins [26], respectively. It was considered that ribosomal protection should be the main method of resistance to tetracyclines in the fecal bacteria of young adult Japanese.

Furthermore, the high detection rate of aadE (81.3%) that encodes adenylyltransferase to resist streptomycin [28] and blaTEM (62.5%) that encodes β-lactamase, which inactivates

| Antibiotic | Resistance gene | Number of positive subjects (%) |
|------------|----------------|---------------------------------|
| Macrolide  | ermA           | 0 (0)                           |
|            | ermB           | 32 (100)                        |
|            | ermC           | 0 (0)                           |
|            | ermF           | 31 (96.9)                       |
|            | ermT           | 5 (15.6)                        |
|            | ermX           | 20 (62.5)                       |
|            | ermA           | 1 (3.1)                         |
|            | erEB           | 0 (0)                           |
|            | mphA           | 5 (15.6)                        |
|            | msrA/B         | 0 (0)                           |
|            | mefA/E         | 32 (100)                        |
| Tetracycline| tetW           | 32 (100)                        |
|            | tetQ           | 32 (100)                        |
|            | tetO           | 29 (90.6)                       |
|            | tetM           | 3 (9.4)                         |
|            | tetS           | 5 (15.6)                        |
|            | tetB/P         | 0 (0)                           |
|            | tetT           | 0 (0)                           |
|            | tetL           | 7 (21.9)                        |
|            | tetK           | 0 (0)                           |
|            | tetX           | 17 (53.1)                       |
| Chloramphenicol | cat           | 0 (0)                           |
| β-Lactam   | blaSHV         | 7 (21.9)                        |
|            | blacTX-M       | 2 (6.3)                         |
|            | blaTEM         | 20 (62.5)                       |
|            | blaOXA         | 1 (3.1)                         |
| Fluoroquinolone | qnrA          | 0 (0)                           |
|              | qnrB           | 2 (6.3)                         |
|              | qnrS           | 7 (21.9)                        |
|              | qnrD           | 0 (0)                           |
|              | qepA           | 0 (0)                           |
|              | oqxA           | 7 (21.9)                        |
|              | oqxB           | 7 (21.9)                        |
|              | aac(6’)-Ib-cr  | 0 (0)                           |
| Ampicillin  | ampC           | 0 (0)                           |
| Methicillin | mecA           | 1 (3.1)                         |
| Streptomycin| aadA           | 12 (37.5)                       |
|              | aadE           | 26 (81.3)                       |
| Sulfonamide | sulI           | 9 (28.1)                        |
|              | sulII          | 13 (40.6)                       |
| Vancomycin  | vanA           | 0 (0)                           |
|              | vanB           | 0 (0)                           |
|              | vanC1          | 2 (6.3)                         |
β-lactam antibiotics, indicated that these antibiotic genes could be moderately spread among the intestinal bacteria in young adult Japanese.

The distribution of antibiotic resistance genes in the human microbiome differs among various countries [7, 12]. The results in this study agreed with previous reports in which ermB, ermF, tetO, tetQ, and tetW were frequently detected in the fecal microbiome of Japanese [12]. Notably, ermX, mefA/E, tetX, blaTEM, and aadE were also detected with high frequency in this study. It is likely that antibiotic resistance genes against macrolides (ermB, ermF, ermX, and mefA/E), tetracyclines (tetW, tetQ, tetO, and tetX), β-lactam antibiotics (blaTEM), and streptomycin (aadE) are widespread in the large intestinal bacteria of young adult Japanese. The composition of the intestinal microbiota differs among life stages. Therefore, it is likely that the antibiotic-resistant bacteria colonizing the large intestine are also different among infants, children, adults, and the elderly.

In the human large intestine, ermB was widespread among Enterococcus [25]. In addition, Bacteroides could receive ermB from Enterococcus [29]. ermF was found on mobile genes, such as the plasmids pBF4, pBI136, and pBFTM10, and the conjugative transposons CTnDOT and CTn7853 in Bacteroides [25, 29]. mefA and mefE were also detected in a wide range of bacteria, in particular Enterococcus [30, 31]. tetQ, tetW, tetO, and tetX were detected in bacteria dominantly colonized in the human large intestine, such as Bacteroides, Clostridium, Enterococcus, Lactobacillus, and Bifidobacteria [9, 12, 25, 27, 29, 32]. Shoemaker et al. [29] indicated that 80% of isolated Bacteroides strains from the human large intestine already have tetQ to resist against tetracycline. This gene was spread horizontally from Bacteroides to other bacteria with transposon CT'ns. tetX was found on the Bacteroides transposons Tn4351 and Tn4400 [27]. Sequence analysis of tetW indicated that tetW was among intestinal bacteria spread from rumen Butyrivibrio fibrisolvens [32, 33]. Spread of this gene among Bifidobacterium, in particular Bifidobacterium animalis subsp. lactis, has been identified [33–36]. aadE was found on transposon Tn5405 within the chromosomes of staphylococci [37] and was detected in Enterococcus faecium, which dominantly colonized the human large intestine [38]. In addition, aadA was also detected in Enterococcus [28]. Thus, it is possible that antibiotic resistance genes with high detection rates in this study are spread widely among human intestinal bacteria, in particular Bacteroides and Enterococcus, via mobile elements, such as plasmids and/or conjugative transposons. However, little is known about the distribution of ermX and intestinal bacteria harboring ermX, although ermX was detected in human skin bacteria [21].

In addition to the moderate detection of blaTEM, blaSHV was also slightly detected in 21.9% of subjects. These β-lactamase antibiotic resistance genes are widespread throughout the world and have been found in Enterobacteriaceae [39]. Our results suggest the possibility of modest spread of blaTEM and blaSHV among human intestinal bacteria, particularly Enterobacteriaceae.

Antibiotic resistance genes against sulfonamides (sulI and sulII) and quinolones (qnrS, oqxA, and oqxB) were slightly detected in this study. sulI and sulII encode alternative drug-resistant variants of dihydropteroate synthase, which is an important enzyme in the folate production of bacteria and is inhibited by sulfonamides [40]. Recently, sulfonamides have not been used frequently due to the wide spreading of sulfonamide-resistant bacteria with plasmids and transposons [41]. Although the detection rates of sulfonamide resistance genes were low, this suggests that sulfonamide-resistant bacteria are likely present in the human large intestine. qnrS, oqxA, and oqxB are quinolone resistance genes. qnrS contributes to the protection of DNA gyrase and topoisomerase IV from quinolones. oqxA and oqxB encode efflux pump proteins [23, 42]. These inhibitory mechanisms are mainly reported in Enterobacteriaceae [42]. The low detection of these genes in this study indicated that some quinolone resistance genes have partially spread among human intestinal bacteria, although bacterial species harboring these quinolone resistance genes in the human large intestine have not been identified.

Although antibiotic resistance genes were individually analyzed in this study, other studies have analyzed and reported the presence of multi-antibiotic resistance genes in mobile elements in human large intestinal bacteria [12, 21, 26, 43]. It has been shown that macrolide resistance genes (ermB and ermF) are often linked to tetracycline resistance genes (tetM, tetQ, and tetX). mefA and tetO often exist in the same transposon. In this study, although both ermB and tetM were detected in only 9.4% of subjects, both ermF and tetQ were detected in 96.9% of subjects. Furthermore, ermF and tetX and/or tetQ were simultaneously detected in 53.1% of subjects. Both mefE/E and tetO were detected in more than 90% of subjects. These results enable us to speculate that multi-antibiotic resistance genes are likely present and widespread among large intestinal bacteria of young adult Japanese.

In this study, the presence and spread of some antibiotic resistance genes, particularly against tetracyclines and macrolides, in human large intestinal bacteria were identified. However, details regarding antibiotic-resistant bacteria colonized in the large intestine remain uncertain. Further studies, including culture-based studies, are needed to understand the number and species of antibiotic-resistant bacteria colonizing the human large intestine and to analyze the structure of antibiotic resistance genes.

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