Bioscience of Microbiota, Food and Health Vol. 36 (4), 151–154, 2017

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

Yuji OHASHI[1]* and Tomohiko FUJISAWA[1]

[1]Laboratory of Food Hygiene, Department of Food Science and Technology, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan

Received February 7, 2017; Accepted July 14, 2017; Published online in J-STAGE August 3, 2017

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 (bla_{TEM}), 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*, bla_{TEM}, 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

*Corresponding author. Yuji Ohashi, Laboratory of Food Hygiene, Department of Food Science and Technology, Nippon Veterinary and Life Science University, 1-7-1 Kyonan-cho, Musashino, Tokyo 180-8602, Japan. Tel. +81-422-31-4151, Fax +81-422-33-2094, E-mail: ohashi@nvlu.ac.jp

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*, *ereA*, *ereB*, *mphA*, *mefA/E*, and *msrA/B*), chloramphenicol (*cat*), β-lactam antibiotics (bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{OXA}), fluoroquinolones (*qnrA*, *qnrB*, *qnrS*, *qnrD*, *qepA*, *oqxA*, *oqxB*, and *aac(6')-Ib-cr*), ampicillin (*ampC*), methicillin (*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 10^5 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 10^5 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 *tetQ* and *tetW* were most commonly detected in fecal bacterial genomes. In this study, *ermB*, *mefA/E*, *tetW*, and *tetQ* 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 bla_{TEM} (62.5%) that encodes β-lactamase, which inactivates

Table 1. Detection of antibiotic resistance genes with specific PCR in the feces of Japanese individuals

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)
	ereA	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	bla_{SHV}	7 (21.9)
	$bla_{CTX\text{-}M}$	2 (6.3)
	bla_{TEM}	20 (62.5)
	bla_{OXA}	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*, bla_{TEM}, 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 (bla_{TEM}), 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 CTn*7853* 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 Tn*4351* and Tn*4400* [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 Tn*5405* 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 bla_{TEM}, bla_{SHV} 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 bla_{TEM} and bla_{SHV} 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 *mefA/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.

REFERENCES

1. Cummings JH, Macfarlane GT. 1997. Role of intestinal bacteria in nutrient metabolism. Clin Nutr 16: 3–11. [CrossRef]
2. Mackie RI, Sghir A, Gaskins HR. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. Am J Clin Nutr 69 Suppl: 1035S–1045S. [Medline]
3. Dibner JJ, Richards JD. 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 84: 634–643. [Medline] [CrossRef]
4. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG Jr. 2002. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci USA 99: 6434–6439.

[Medline] [CrossRef]
5. Goossens H, Ferech M, Vander Stichele R, Elseviers M, ESAC Project Group. 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 365: 579–587. [Medline] [CrossRef]
6. Hu Y, Yang X, Lu N, Zhu B. 2014. The abundance of antibiotic resistance genes in human guts has correlation to the consumption of antibiotics in animal. Gut Microbes 5: 245–249. [Medline] [CrossRef]
7. van Schaik W. 2015. The human gut resistome. Philos Trans R Soc Lond B Biol Sci 370: 20140087. [Medline] [CrossRef]
8. von Wintersdorff CJ, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, Savelkoul PH, Wolffs PF. 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7: 173. [Medline] [CrossRef]
9. Card RM, Warburton PJ, MacLaren N, Mullany P, Allan E, Anjum MF. 2014. Application of microarray and functional-based screening methods for the detection of antimicrobial resistance genes in the microbiomes of healthy humans. PLoS One 9: e86428. [Medline] [CrossRef]
10. Sommer MOA, Dantas G, Church GM. 2009. Functional characterization of the antibiotic resistance reservoir in the human microflora. Science 325: 1128–1131. [Medline] [CrossRef]
11. Francino MP. 2016. Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. Front Microbiol 6: 1543. [Medline] [CrossRef]
12. Ghosh TS, Gupta SS, Nair GB, Mande SS. 2013. *In silico* analysis of antibiotic resistance genes in the gut microflora of individuals from diverse geographies and age-groups. PLoS One 8: e83823. [Medline] [CrossRef]
13. Hu Y, Yang X, Qin J, Lu N, Cheng G, Wu N, Pan Y, Li J, Zhu L, Wang X, Meng Z, Zhao F, Liu D, Ma J, Qin N, Xiang C, Xiao Y, Li L, Yang H, Wang J, Yang R, Gao GF, Wang J, Zhu B. 2013. Metagenome-wide analysis of antibiotic resistance genes in a large cohort of human gut microbiota. Nat Commun 4: 2151. [Medline] [CrossRef]
14. Godon JJ, Zumstein E, Dabert P, Habouzit F, Moletta R. 1997. Molecular microbial diversity of an anaerobic digestor as determined by small-subunit rDNA sequence analysis. Appl Environ Microbiol 63: 2802–2813. [Medline]
15. Miele A, Bandera M, Goldstein BP. 1995. Use of primers selective for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in VanA isolates. Antimicrob Agents Chemother 39: 1772–1778. [Medline] [CrossRef]
16. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother 40: 2562–2566. [Medline]
17. Aminov RI, Garrigues-Jeanjean N, Mackie RI. 2001. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. Appl Environ Microbiol 67: 22–32. [Medline] [CrossRef]
18. Lanz R, Kuhnert P, Boerlin P. 2003. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet Microbiol 91: 73–84. [Medline] [CrossRef]
19. Hummel AS, Hertel C, Holzapfel WH, Franz CMAP. 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. Appl Environ Microbiol 73: 730–739. [Medline] [CrossRef]
20. Klare I, Konstabel C, Werner G, Huys G, Vankerckhoven V, Kahlmeter G, Hildebrandt B, Müller-Bertling S, Witte W, Goossens H. 2007. Antimicrobial susceptibilities of *Lactobacillus, Pediococcus* and *Lactococcus* human isolates and cultures intended for probiotic or nutritional use. J Antimicrob Chemother 59: 900–912. [Medline] [CrossRef]
21. Chen J, Yu Z, Michel FC Jr, Wittum T, Morrison M. 2007. Development and application of real-time PCR assays for quantification of erm genes conferring resistance to macrolides-lincosamides-streptogramin B in livestock manure and manure management systems. Appl Environ Microbiol 73: 4407–4416. [Medline] [CrossRef]
22. Böckelmann U, Dörries HH, Ayuso-Gabella MN, Salgot de Marçay M, Tandoi V, Levantesi C, Masciopinto C, Van Houtte E, Szewzyk U, Wintgens T, Grohmann E. 2009. Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Appl Environ Microbiol 75: 154–163. [Medline] [CrossRef]
23. Osińska A, Harnisz M, Korzeniewska E. 2016. Prevalence of plasmid-mediated multidrug resistance determinants in fluoroquinolone-resistant bacteria isolated from sewage and surface water. Environ Sci Pollut Res Int 23: 10818–10831. [Medline] [CrossRef]
24. Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. 2007. Sensitive quantitative detection of commensal bacteria by rRNA-targeted reverse transcription-PCR. Appl Environ Microbiol 73: 32–39. [Medline] [CrossRef]
25. Seville LA, Patterson AJ, Scott KP, Mullany P, Quail MA, Parkhill J, Ready D, Wilson M, Spratt D, Roberts AP. 2009. Distribution of tetracycline and erythromycin resistance genes among human oral and fecal metagenomic DNA. Microb Drug Resist 15: 159–166. [Medline] [CrossRef]
26. Roberts MC. 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 245: 195–203. [Medline] [CrossRef]
27. Bartha NA, Sóki J, Urbán E, Nagy E. 2011. Investigation of the prevalence of *tetQ, tetX* and *tetX1* genes in *Bacteroides* strains with elevated tigecycline minimum inhibitory concentrations. Int J Antimicrob Agents 38: 522–525. [Medline] [CrossRef]
28. Clark NC, Olsvik O, Swenson JM, Spiegel CA, Tenover FC. 1999. Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aadA*) in *Enterococcus faecalis*. Antimicrob Agents Chemother 43: 157–160. [Medline]
29. Shoemaker NB, Vlamakis H, Hayes K, Salyers AA. 2001. Evidence for extensive resistance gene transfer among *Bacteroides* spp. and among *Bacteroides* and other genera in the human colon. Appl Environ Microbiol 67: 561–568. [Medline] [CrossRef]
30. Roberts MC. 2004. Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. Mol Biotechnol 28: 47–62. [Medline] [CrossRef]
31. Klaassen CH, Mouton JW. 2005. Molecular detection of the macrolide efflux gene: to discriminate or not to discriminate between *mef*(A) and *mef*(E). Antimicrob Agents Chemother 49: 1271–1278. [Medline] [CrossRef]
32. Aminov RI, Mackie RI. 2007. Evolution and ecology of antibiotic resistance genes. FEMS Microbiol Lett 271: 147–161. [Medline] [CrossRef]
33. Scott KP, Melville CM, Barbosa TM, Flint HJ. 2000. Occurrence of the new tetracycline resistance gene *tet*(W) in bacteria from the human gut. Antimicrob Agents Chemother 44: 775–777. [Medline] [CrossRef]
34. Flórez AB, Ammor MS, Alvarez-Martín P, Margolles A, Mayo B. 2006. Molecular analysis of tet(W) gene-mediated tetracycline resistance in dominant intestinal Bifidobacterium species from healthy humans. Appl Environ Microbiol 72: 7377–7379. [Medline] [CrossRef]
35. Aires J, Doucet-Populaire F, Butel MJ. 2007. Tetracycline resistance mediated by *tet*(W), *tet*(M), and *tet*(O) genes of *Bifidobacterium* isolates from humans. Appl Environ Microbiol 73: 2751–2754. [Medline] [CrossRef]
36. Ammor MS, Flórez AB, Alvarez-Martín P, Margolles A, Mayo B. 2008. Analysis of tetracycline resistance *tet*(W) genes and their flanking sequences in intestinal *Bifidobacterium* species. J Antimicrob Chemother 62: 688–693. [Medline] [CrossRef]
37. Derbise A, Aubert S, El Solh N. 1997. Mapping the regions carrying the three contiguous antibiotic resistance genes *aadE*, *sat4*, and *aphA-3* in the genomes of staphylococci. Antimicrob Agents Chemother 41: 1024–1032. [Medline]
38. Werner G, Hildebrandt B, Witte W. 2001. Aminoglycoside-streptothricin resistance gene cluster *aadE-sat4-aphA-3* disseminated among multiresistant isolates of *Enterococcus faecium*. Antimicrob Agents Chemother 45: 3267–3269. [Medline] [CrossRef]
39. Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. 2008. Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 14 Suppl 1: 144–153. [Medline] [CrossRef]
40. Sköld O. 2000. Sulfonamide resistance: mechanisms and trends. Drug Resist Updat 3: 155–160. [Medline] [CrossRef]
41. Wang N, Yang X, Jiao S, Zhang J, Ye B, Gao S. 2014. Sulfonamide-resistant bacteria and their resistance genes in soils fertilized with manures from Jiangsu Province, Southeastern China. PLoS One 9: e112626. [Medline] [CrossRef]
42. Poirel L, Cattoir V, Nordmann P. 2012. Plasmid-mediated quinolone resistance; interactions between human, animal, and environmental ecologies. Front Microbiol 3: 24. [Medline] [CrossRef]
43. Zonenschain D, Rebecchi A, Morelli L. 2009. Erythromycin- and tetracycline-resistant lactobacilli in Italian fermented dry sausages. J Appl Microbiol 107: 1559–1568. [Medline] [CrossRef]