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NOTE

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Prevalence of 16S rRNA methylases in Gram-negative bacteria derived from companion animals and livestock in Japan

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ABSTRACT. The emergence and spread of aminoglycoside-resistant bacteria are a public health concern. The acquisition of the genes encoding 16S rRNA methylases, such as *armA*, *rmtA*, and *rmtB*, confers high-level resistance to aminoglycosides. However, the prevalence has not been well investigated in Japanese veterinary fields. To determine the prevalence of 16S rRNA methylases in animals, we detected 16S rRNA methylases genes in Gram-negative bacteria from animals. Here, we report the isolation of *rmtB* and *armA* from two of the 446 *Escherichia coli* (0.5%) and one of the 103 *Klebsiella* spp. isolates (1.0%) from companion animals, respectively. However, none of the isolations were observed from 2445 *E. coli* isolates derived from livestock in Japan. The prevalence of 16S rRNA methylases in animals, especially in companion animals, should be carefully monitored in Japanese veterinary fields to avoid the spreading of the genes.

KEY WORDS: aminoglycoside resistance, companion animals, livestock, 16S rRNA methylases

The spread of antibiotic-resistant bacteria and resistance genes in clinical settings and veterinary fields is a global public health concern [23]. Therefore, the WHO recommends the monitoring of antibiotic resistance in each country [25]. In response to this recommendation, the trend in antibiotic resistance in Japanese livestock has been continually monitored since 1999, and this system is known as the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) [16].

On the other hand, the prevalence of antibiotic-resistant bacteria and resistance genes in companion animals has not been monitored in Japan, and several local and intermittent studies have been reported [7, 18]. In 2016, the government of Japan proposed the "National Action Plan on Antimicrobial Resistance 2016–2020" to address these antimicrobial problems. The importance of antimicrobial surveillance in veterinary fields is also documented from the view of One-Health approach in that plan.

Aminoglycosides are used for severe bacterial infectious diseases in both clinical settings and veterinary fields [13], and emergence of aminoglycoside-resistant bacteria is problematic. In Japan, the amounts of aminoglycosides usage were lower than those of some antibiotics in livestock and companion animals [17]. In aminoglycosides, streptomycin is relatively used in pigs and streptomycin resistance in *E. coli* are highly isolated from pigs [16, 17]. Most aminoglycosides bind to the decoding aminoacyl-tRNA recognition site of the 16S rRNA that is part of the bacterial 30S ribosome, and subsequently interfere with bacterial growth through inhibition of protein synthesis [15]. Bacteria acquire resistance against aminoglycosides through several mechanisms. Aminoglycoside-modifying enzymes [aminoglycoside acetyltransferase (AAC), aminoglycoside phosphotransferase (APH), and aminoglycoside nucleotidyltranferases (ANT)], which inactivate specific aminoglycosides, are the most prevalent mechanisms [21]. In addition, 16S rRNA methylases has been reported as a novel aminoglycoside resistance mechanism [24]. Differently from aminoglycoside-modifying enzymes, 16S rRNA methylases can confer high-level aminoglycoside resistance by modifying specific nucleotides in the aminoglycoside binding site of 16S rRNA [24].

In general, 16S rRNA methylases genes are located in transferable plasmids [24]. In addition, 16S rRNA methylases-harboring

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plasmids frequently contain other classes of antibiotic resistance genes such as β-lactamase genes [24]. Therefore, these observations warn the increase of multi-drug resistant bacteria by an acquisition of 16S rRNA methylase-harboring plasmid. Although 16S rRNA methylases possessing Gram-negative bacteria has been found in livestock and companion animals in foreign countries [5, 24, 27], the isolation and prevalence have not been reported in Japanese animals. In this study, we investigated the prevalence of 16S rRNA methylase genes in Gram-negative bacteria isolated from companion animals and livestock in Japan.

A total of 212 and 234 *Escherichia coli* isolated from feces samples of dogs that visited 13 veterinary hospitals (all these hospitals are located in Ebetsu city, Japan) in 2005 and 2015–2016, respectively. A total of 1,029 and 1,418 *E. coli* isolates derived from livestock (cattle, pigs, and chicken feces) were collected by the JVARM in 2004–2005 and 2013–2014, respectively [16]. In addition, a total of 103 *Klebsiella* spp. [9], 60 *Enterobacter* spp. [8], and 81 *Acinetobacter* spp. isolates, obtained from clinical specimens collected from dogs and cats between 2003 and 2015, were used for this study. Bacterial identification was conducted using matrix-assisted laser desorption/ionization—time of light mass spectrometry (MALDI-TOF MS) with the Bruker MALDI Biotyper system (Bruker Daltonics, Bremen, Germany) [3].

To screen aminoglycoside-resistant isolates, minimum inhibitory concentrations (MICs) of gentamicin (Sigma-Aldrich, St. Louis, MO, U.S.A.) were determined using the agar dilution method according to Clinical Laboratory Standards Institute (CLSI) guidelines [2, 24]. *E. coli* ATCC25922, *Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC29212, and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains. In gentamicin-resistant isolates, MICs of several types of aminoglycosides [amikacin, neomycin, and apramycin (all obtained from Sigma-Aldrich)] were determined to screen putative 16S rRNA methylase positive isolates according to a previous study [24]. Interpretations for neomycin and apramycin resistance were defined according to a previous report [10], because of the no definition in CLSI guidelines. MIC of arbekacin (Sigma-Aldrich), which is not modified by most aminoglycoside-modifying enzymes, was also determined in the putative 16S rRNA methylase positive isolates [4]. The breakpoint for arbekacin was similar to that for amikacin as defined by CLSI guidelines (≥32 μg/m*l*) [2].

Some *E. coli* isolates derived from canine feces were resistant to gentamicin (MIC ≥16 μg/m*l*; 15.6 and 29.5% in 2005 and 2015–2016, respectively; Table 1). In contrast, *E. coli* isolates derived from livestock feces were rarely resistant to gentamicin (1.9 and 1.0% in 2004–2005 and 2013–2014, respectively; Table 1). Gentamicin-resistant *Klebsiella*, *Enterobacter*, and *Acinetobacter* spp. isolates derived from companion animals in 2003–2015 were 31.1, 26.2, and 6.2% of the total, respectively (Table 1). From the MIC values of aminoglycosides, three putative 16S rRNA methylases-positive isolates were found in two *E. coli* isolates (RGU-60 and RGU-78) and one *Klebsiella pneumoniae* isolate (KL39) (Table 2). In contrast, none of the putative 16S rRNA methylase positive isolates were found in *Enterobacter* or *Acinetobacter* spp. derived from companion animals.

Three of the putative 16S rRNA methylases-positive isolates were screened for 16S rRNA methylases genes (i.e., *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, and *npmA*) using PCR and DNA sequencing [4, 6, 29]. The *rmtB* gene was found in two *E. coli* isolates from dogs in 2005 (0.9% of total isolates in 2005 and 0.5% of *E. coli* isolates from canine feces), and *armA* gene was found in one *K. pneumoniae* isolate (1.0%) derived from canine feces in 2015 (Tables 1 and 2).

MICs of streptomycin, ampicillin, and tetracycline (all obtained from Sigma-Aldrich) were determined as described above. Each isolate was evaluated for the presence of genes for aminoglycoside-modifying enzymes (*aadA1*, *aac(3)*, *aadB*, *aphA1*, *aphA2*, *strA*, *strB*, and *aac(6')-Ib-cr*) [14, 19], β-lactamases (bla_{IEM}, bla_{SHV}, $bla_{\text{CMY-2}}$, $bla_{\text{ctx-M}}$, and bla_{DHA}) [12, 20, 28], and tetracycline resistance (*tetA, tetB, tetC, tetD, tetE,* and *tetG*) [11] using PCR.

Isolates RGU-60 and KL39 were resistant to neomycin and possessed the aminoglycoside phosphotransferase gene *aphA1*, which is related to neomycin (fradiomycin) and kanamycin resistance (Table 2). All three 16S rRNA methylases-positive isolates were resistant to streptomycin and possessed the aminoglycoside phosphotransferase genes, *strA* and *strB*, which are related to streptomycin resistance. All three 16S rRNA methylases positive isolates were resistant to ampicillin and possessed the bla_{TEM} gene (Table 2). In addition, isolate KL 39 possessed the β-lactamase genes bla_{SHV} and bla_{DHA}, and isolates RGU-60 and KL39 were resistant to tetracycline and possessed the *tetA* gene.

Transferability of 16S rRNA methylases genes in *E. coli* was determined using previously described broth-mating methods, with slight modifications [22]. Briefly, the recipients were rifampicin-resistant *E. coli* K12 DH5α strain and mating was conducted at 37°C. Transconjugants were selected on Mueller-Hinton agar supplemented with 50 μg/m*l* rifampicin (Sigma-Aldrich) and 4 μg/m*l* gentamicin. Broth-mating methods were repeated three times. Some transconjugants were randomly selected and characterized by MIC determination of tested antibiotics and possession of antibiotic resistance genes.

The *rmtB* genes were transferred from isolates RGU-60 and RGU-78 to the recipient strain using the broth mating method, with transfer frequencies of 1.7×10^{-5} and 2.4×10^{-3}, respectively (Table 2). All transconjugants were resistant to gentamicin, amikacin, arbekacin, streptomycin, and ampicillin (Table 2) and possessed the *rmtB*, *strA*, *strB*, and bla_{TEM} genes. Some transconjugants from an isolate, RGU-60, as donor were susceptible to neomycin, apramycin, and tetracycline. In these transconjugants, some resistance genes (*aphA1* and *tetA*) were not transferred together with *rmtB* genes.

In this study, the prevalence of gentamicin resistance in *E. coli* isolates from Japanese livestock was found to be low (<2.0%), and no 16S rRNA methylases genes were found in these isolates. In China, amikacin-resistant *E. coli* and 16S rRNA methylases genes (*rmtB*, *armA*, and *rmtE*) were observed in 19.7 and 18.5% of isolates derived from livestock, respectively [27]. In general, usage of veterinary antibiotics appears to contribute to the appearance of antibiotic resistance in *E. coli* isolates from healthy livestock [1]. In addition, an increase in prevalence of 16S rRNA methylases genes is attributed to use of amikacin and/or arbekacin [24]. These antibiotics are used as growth promoters in livestock in China [27] but not in Japan. Therefore, these observations may explain the low prevalence of gentamicin-resistant *E. coli* in livestock in Japan.

Our results showed the higher prevalence of gentamicin-resistant Gram-negative bacteria in companion animals than in

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Table 1. Prevalence of gentamicin-resistant bacteria and 16S rRNA methylases genes in Japanese animals

Origin	Bacterial species	Year	n	Gentamicin resistance[a)]	16S rRNA methylases
Canine fecal samples	*Escherichia coli*	2005	212	33 (15.6%)	*rmtB* 2 (0.9%)
	E. coli	2015–2016	234	69 (29.5%)	0
Cattle, Pig, Chicken fecal samples	*E. coli*	2004–2005	1,029	20 (1.9%)	0
	E. coli	2013–2014	1,418	14 (1.0%)	0
Canine, Feline clinical specimens	*Klebsiella* spp.	2003–2015	103	32 (31.1%)	*armA* 1 (1.0%)
	Enterobacter spp.	2003–2015	65	17 (26.2%)	0
	Acinetobacter spp.	2003–2015	81	5 (6.2%)	0

a) Gentamicin resistance indicates a minimum inhibitory concentration ≥16 μg/ml.

Table 2. Characterization of 16S rRNA methylases-positive isolates from dogs and their transconjugants

	Strain name	Bacterial species	Year	MIC (μg/ml)[a)]								Antibiotic resistance genes			
				GM (16)[b)]	AMK (64)[b)]	NEO (32)[c)]	APR (64)[c)]	ABK (32)[d)]	SM (64)[c)]	ABPC (32)[b)]	TET (16)[b)]	16S rRNA methylases	Aminoglycoside-modifying enzyme	Beta-lactamase genes	Tetracycline resistance gene
16S rRNA methylase-positive isolates	RGU-60	*Escherichia coli*	2005	**>256**	**>256**	**>256**	16	**>128**	**>128**	**>128**	**64**	*rmtB*	*aphA1, strA, strB*	bla_{TEM}	*tetA*
	RGU-78	*E. coli*	2005	**>256**	**>256**	8	16	**>128**	**>128**	**>128**	1	*rmtB*	*strA, strB*	bla_{TEM}	
	KL39	*Klebsiella pneumoniae*	2015	**>256**	**>256**	**256**	8	**>128**	**64**	**>128**	**512**	*armA*	*aphA1, strA, strB*	bla_{TEM}, bla_{SHV}, bla_{DHA}	*tetA*
Recipient	DH5α	*E. coli*		0.25	0.5	2	1	1	0.25	2	2				
Transconjugants	TC-RGU-60–1			**>256**	**>256**	1	1	**>128**	**>128**	**>128**	1	*rmtB*	*strA, strB*	bla_{TEM}	
	TC-RGU-60–2			**>256**	**>256**	**>256**	16	**>128**	**>128**	**>128**	**64**	*rmtB*	*aphA1, strA, strB*	bla_{TEM}	*tetA*
	TC-RGU-60–3			**>256**	**>256**	**>256**	16	**>128**	**>128**	**>128**	2	*rmtB*	*aphA1, strA, strB*	bla_{TEM}	
	TC-RGU-78–1			**>256**	**>256**	1	1	**>128**	**>128**	**>128**	1	*rmtB*	*strA, strB*		

a) Values in parentheses indicate breakpoints. Bold type indicates resistance to individual antibiotics. b) Clinical Laboratory Standards Institute (CLSI) breakpoints [2]. c) Previously reported breakpoints [10]. d) Reference from CLSI breakpoints for amikacin. ABK; arbekacin, ABPC; ampicillin, AMK; amikacin, APR; apramycin, GM; gentamicin, MIC; minimum inhibitory concentration, NEO; neomycin, SM; streptomycin, TET; tetracycline.

livestock. Although the total amount of aminoglycosides usage in companion animals were not higher than those of the other antibiotics in companion animals, the amount of gentamicin usage were more than half in aminoglycosides [17]. Gentamicin are frequently used for companion animals mainly as external medicine (Personal communication). External usage of antibiotics would affect the antibiotic resistance in Enterobacteriaceae, although the direct verification has not been reported. The high rates of gentamicin resistance in companion animals would be related to the usage of gentamicin in companion animals.

16S rRNA methylases-positive strains were resistant to not only aminoglycosides but also to other classes of antibiotics, and *rmtB* genes were easily transferred to other *E. coli* strains. In addition, some resistance genes (*strA, strB*, and bla_{TEM}) were invariably transferred with 16S rRNA methylases genes. In general, 16S rRNA methylases genes are present in plasmids with other antibiotic resistance genes [24]. Although more detailed analyses are needed to characterize the 16S rRNA methylases-harboring plasmid found in this study, these plasmids contribute to multi-drug resistance.

Two types of 16S rRNA methylases genes were detected in companion animals, which are in close contact with humans. It indicates the possibility of the transmission of 16S rRNA methylase gene possessed bacteria and/or the harboring plasmids between human and companion animals, at relatively easy. According to the previous study, the sporadic spread of a specific *K. pneumoniae* lineage that possessed *rmtB* has been reported from companion animals, and this lineage (ST37) is also isolated form clinical setting in China [26]. This observation may support the possibility. On the contrast, the prevalence of 16S rRNA methylase genes in companion animals in this study was lower compared with the neighbor country, China, corresponding with the low prevalence of 16S rRNA methylase genes in human clinical settings in Japan [24]. Thus, current risk of the transmission of 16S rRNA methylase gene between human and companion animals should be estimated low in Japan. However, it should be required the continuous monitoring from the view of multidrug resistance of the isolated 16S rRNA methylase positive bacteria and the co-transferable aspect with other antimicrobial resistance.

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REFERENCES

1. Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T. and Tamura, Y. 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the feces of food-producing animals in Japan. *Jpn. J. Infect. Dis.* **58**: 369–372. [Medline]
2. Clinical and Laboratory Standards Institute. 2013.Performance Standards for Antimicrobial Disk Dilution Susceptibility Tests for Bacteria Isolated from Animals, Document VET01-A4, 4th ed., Wayne.
3. Dierig, A., Frei, R. and Egli, A. 2015. The fast route to microbe identification: matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). *Pediatr. Infect. Dis. J.* **34**: 97–99. [Medline] [CrossRef]
4. Doi, Y. and Arakawa, Y. 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. *Clin. Infect. Dis.* **45**: 88–94. [Medline] [CrossRef]
5. Doi, Y., Wachino, J. I. and Arakawa, Y. 2016. Aminoglycoside resistance: The emergence of acquired 16S Ribosomal RNA methyltransferases. *Infect. Dis. Clin. North Am.* **30**: 523–537. [Medline] [CrossRef]
6. Green, K. D., Chen, W. and Garneau-Tsodikova, S. 2011. Effects of altering aminoglycoside structures on bacterial resistance enzyme activities. *Antimicrob. Agents Chemother.* **55**: 3207–3213. [Medline] [CrossRef]
7. Harada, K., Niina, A., Nakai, Y., Kataoka, Y. and Takahashi, T. 2012. Prevalence of antimicrobial resistance in relation to virulence genes and phylogenetic origins among urogenital *Escherichia coli* isolates from dogs and cats in Japan. *Am. J. Vet. Res.* **73**: 409–417. [Medline] [CrossRef]
8. Harada, K., Shimizu, T., Mukai, Y., Kuwajima, K., Sato, T., Kajino, A., Usui, M., Tamura, Y., Kimura, Y., Miyamoto, T., Tsuyuki, Y., Ohki, A. and Kataoka, Y. 2017. Phenotypic and molecular characterization of antimicrobial resistance in *Enterobacter* spp. isolates from companion animals in Japan. *PLoS One* **12**: e0174178. [Medline] [CrossRef]
9. Harada, K., Shimizu, T., Mukai, Y., Kuwajima, K., Sato, T., Usui, M., Tamura, Y., Kimura, Y., Miyamoto, T., Tsuyuki, Y., Ohki, A. and Kataoka, Y. 2016. Phenotypic and molecular characterization of antimicrobial reesistance in *Klebsiella* spp. isolates from companion animals in Japan: clonal dissemination of multidrug-resistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*. *Front. Microbiol.* **7**: 1021. [Medline] [CrossRef]
10. Hu, Y., Liu, L., Zhang, X., Feng, Y. and Zong, Z. 2017. In vitro activity of neomycin, streptomycin, paromomycin and apramycin against carbapenem-resistant Enterobacteriaceae clinical strains. *Front. Microbiol.* **8**: 2275. [Medline] [CrossRef]
11. Jun, L. J., Jeong, J. B., Huh, M.D., Chung, J.K., Choi, D.L., Lee, C.H. and Jeong, H. D. 2004. Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in *Edwardsiella tarda* isolated from fish farms in Korea. *Aquaculture* **240**: 89–100. [CrossRef]
12. Kojima, A., Ishii, Y., Ishihara, K., Esaki, H., Asai, T., Oda, C., Tamura, Y., Takahashi, T. and Yamaguchi, K. 2005. Extended-spectrum-beta-lactamase-producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese veterinary antimicrobial resistance monitoring program. *Antimicrob. Agents Chemother.* **49**: 3533–3537. [Medline] [CrossRef]
13. Kotra, L. P., Haddad, J. and Mobashery, S. 2000. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob. Agents Chemother.* **44**: 3249–3256. [Medline] [CrossRef]
14. Kozak, G. K., Boerlin, P., Janecko, N., Reid-Smith, R. J. and Jardine, C. 2009. Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. *Appl. Environ. Microbiol.* **75**: 559–566. [Medline] [CrossRef]
15. Magnet, S. and Blanchard, J. S. 2005. Molecular insights into aminoglycoside action and resistance. *Chem. Rev.* **105**: 477–498. [Medline] [CrossRef]
16. National Veterinary Assay Laboratory Ministry of Agriculture Forestry and Fisheries. 2012. Report on the Japanese veterinary antimicrobial resistance monitoring system 2008 to 2011. http://www.maff.go.jp/nval/tyosa_kenkyu/taiseiki/pdf/jvarm2008_2011.pdf [accessed on April 24, 2019].
17. National Veterinary Assay Laboratory Ministry of Agriculture Forestry and Fisheries. 2017. Sales amounts and sales volumes (active substance) of antibiotics, synthetic antibacterials, anthellmintics and antiprotozoals. http://www.maff.go.jp/nval/iyakutou/hanbaidaka/pdf/h29hanbaikoukin.pdf

[accessed on April 24, 2019].
18. Okubo, T., Sato, T., Yokota, S., Usui, M. and Tamura, Y. 2014. Comparison of broad-spectrum cephalosporin-resistant *Escherichia coli* isolated from dogs and humans in Hokkaido, Japan. *J. Infect. Chemother.* **20**: 243–249. [Medline] [CrossRef]
19. Park, C. H., Robicsek, A., Jacoby, G. A., Sahm, D. and Hooper, D. C. 2006. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother.* **50**: 3953–3955. [Medline] [CrossRef]
20. Pérez-Pérez, F. J. and Hanson, N. D. 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J. Clin. Microbiol.* **40**: 2153–2162. [Medline] [CrossRef]
21. Ramirez, M. S. and Tolmasky, M. E. 2010. Aminoglycoside modifying enzymes. *Drug Resist. Updat.* **13**: 151–171. [Medline] [CrossRef]
22. Usui, M., Hiki, M., Murakami, K., Ozawa, M., Nagai, H. and Asai, T. 2012. Evaluation of transferability of R-plasmid in bacteriocin-producing donors to bacteriocin-resistant recipients. *Jpn. J. Infect. Dis.* **65**: 252–255. [Medline] [CrossRef]
23. Ventola, C. L. 2015. The antibiotic resistance crisis: part 1: causes and threats. *P&T* **40**: 277–283. [Medline]
24. Wachino, J. and Arakawa, Y. 2012. Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: an update. *Drug Resist. Updat.* **15**: 133–148. [Medline] [CrossRef]
25. World Health Organization. Public health importance of antimicrobial resistance. http://www.who.int/antimicrobial-resistance/en/ [accessed on April 21, 2019].
26. Xia, J., Fang, L. X., Cheng, K., Xu, G. H., Wang, X. R., Liao, X. P., Liu, Y. H. and Sun, J. 2017. Clonal spread of 16S rRNA methyltransferase-producing *Klebsiella pneumoniae* ST37 with high prevalence of ESBLs from companion animals in China. *Front. Microbiol.* **8**: 529. [Medline] [CrossRef]
27. Xia, J., Sun, J., Cheng, K., Li, L., Fang, L. X., Zou, M. T., Liao, X. P. and Liu, Y. H. 2016. Persistent spread of the *rmtB* 16S rRNA methyltransferase gene among *Escherichia coli* isolates from diseased food-producing animals in China. *Vet. Microbiol.* **188**: 41–46. [Medline] [CrossRef]
28. Xu, L., Ensor, V., Gossain, S., Nye, K. and Hawkey, P. 2005. Rapid and simple detection of bla_{CTX-M} genes by multiplex PCR assay. *J. Med. Microbiol.* **54**: 1183–1187. [Medline] [CrossRef]
29. Yan, J. J., Wu, J. J., Ko, W. C., Tsai, S. H., Chuang, C. L., Wu, H. M., Lu, Y. J. and Li, J. D. 2004. Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. *J. Antimicrob. Chemother.* **54**: 1007–1012. [Medline] [CrossRef]