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Detection and Characterization of Aparmycin-Resistant *Escherichia coli* from Humans in Korea

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To investigate apramycin resistance in humans in Korea, a total of 138 human *Escherichia coli* strains confirmed as gentamicin-resistant were collected from Korean Culture Collection Antimicrobial-Resistant Microbes. Apramycin resistance (minimum inhibitory concentrations $\geq$1,024 μg/ml) was observed in 16 (11.6%) of the 138 gentamicin-resistant *E. coli* (GREC) strains. Among the seven different kinds of aminoglycoside resistance genes tested, only four kinds were detected in the apramycin-resistant GREC strains: *aac (3)-II*, *aac (3)-III*, *aac (3)-IV*, and *armA*. The *aac (3)-IV* gene was found in all apramycin-resistant GREC strains, whereas *aac(3)-II*, *aac(3)-III*, and *armA* genes were detected in 8 (50.0%), 6 (37.5%), and 1 (6.3%) GREC strains resistant to apramycin, respectively. Of 16 apramycin-resistant GREC strains, transfer of apramycin resistance was observed in seven (43.8%), and co-transfer of resistance to other antimicrobials along with apramycin resistance was also found in four strains (25.0%) by broth mating. The results of this study suggest that more prudential use of apramycin in animals is needed.

Introduction

Escherichia coli are commensal and pathogenic bacteria in the intestinal flora in humans and animals. Apramycin, a veterinary aminoglycoside antibiotic, has been introduced in livestock around the world since late 1970s. Apramycin resistance is conferred by the aminoglycoside-modifying enzyme (AME) 3-N-aminoglycoside acetyltransferase type-IV [aac (3)-IV],[2] which also confers resistance to tobramycin and gentamicin that are used to treat serious infections in humans. Although apramycin has not been used for human medicine, resistance to this antibiotic has been detected among human isolates of *Klebsiella pneumoniae*,[12,17] *E. coli*,[12,17,20] and Enterobacteriaceae.[3,7,16,17,19] In addition, it was suggested that exchange of apramycin resistance via conjugative plasmid could occur between humans and animals.[3,9]

In Korea, apramycin has been used for treatment or prevention of enteric diseases in animals since 1983. This antibiotic was also authorized as feed additives (www.nvrqs.go.kr/Ex_Notice/Law/View.asp). In a recent study from Korea,[4] a relatively high level of apramycin resistance in commensal and pathogenic *E. coli* strains isolated from pigs was documented. Although a higher level of apramycin-resistant Enterobacteriaceae isolates from humans has been reported from countries that have used apramycin in animals, little attention has been paid to resistance to this antibiotic in humans in Korea. *E. coli* was reported to be one of the important pathogens in urinary and blood infections in Korean hospital.[13] The aim of this study was, therefore, to investigate the prevalence of apramycin resistance in *E. coli* isolates from humans in Korea. Characterization of the apramycin-resistant *E. coli* isolates was also performed.

Materials and Methods

Bacterial collection

A total of 138 gentamicin-resistant *E. coli* (GREC) isolates from humans were provided from Korean Culture Collection Antimicrobial-Resistant Microbes. Of the 138 isolates, 102 were isolated from 5 hospitals during 1999–2003 (35 isolated before 1999, 23 in 1999, 26 in 2001, 7 in 2002, and 11 in 2003). Information on the rest of the strains was not available. Of 138 isolates, 35 were recovered from samples of urine ($n = 30$), pus ($n = 3$), blood ($n = 1$), and bile ($n = 1$), but there was no information on the origin of the samples for the rest of the isolates.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) for apramycin, gentamicin, and tobramycin resistance in GREC were determined by an agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[5] Antimicrobials were obtained from Sigma Chemical

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Co. (St Louis. Mo). GREC isolates that presented apramycin resistance and transconjugants were examined for susceptibility using agar disk diffusion method described by Bauer *et al.*[1] for the following 14 antimicrobials: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cephalothin (30 μg), cefoxitin (30 μg), cefotaxime (30 μg), cefepime (30 μg), amikacin (30 μg), neomycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), nalidixic acid (30 μg), and chloramphenicol (30 μg). Cartridges of antimicrobial-containing discs were obtained from Becton Dickinson (Sparks, MD; BBL, Sensi-Disk). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as quality control strains. Inhibition zone diameters were interpreted in accordance with the guidelines of the CLSI.[5] Breakpoints for gentamicin and tobramycin (≥8 μg/ml), and apramycin (≥16 μg/ml) were used as described by CLSI,[5] and the Danish Integrated Antimicrobial Resistance Monitoring and Research Program,[6] respectively.

Aminoglycoside resistance gene profile

The AME contents of all apramycin-resistant GREC were determined by PCR using primer sets for seven different genes: *aac (3)-I*, *aac (3)-II*, *aac (3)-III*, *aac (3)-IV*, *ant (2″)-I*, *armA*, and *aac (6)-Ib*, based on previously described methods.[10,18]

Transferability

Transferability of apramycin resistance was investigated by broth mating method using *E. coli* J53 (sodium azide^r) as the recipient. Overnight cultures of 0.05 ml of donor and 0.45 ml of recipient were each added to 4.5 ml of fresh tryptic soy broth (Becton Dickinson) and the mixtures were incubated at 37°C with gentle shaking for 16–18 h. Transconjugants were selected on MacConkey agar plate containing apramycin (16 μg/ml) and sodium azide (150 μg/ml).

Results

Of 138 GREC strains tested, 16 (11.6%) showed resistance to apramycin (MICs ≥1,024 μg/ml). All apramycin-resistant GREC isolates were also confirmed to be resistant to tobramycin and gentamicin in this study. They all had resistance to four or more antimicrobials such as beta-lactams, aminoglycosides, (fluoro) quinolone, phenicols, tetracyclines, and folate pathway inhibitors. Of the 16 apramycin-resistant GREC, 10 (62.5%) strains showed multiple drug resistance to ≥10 antimicrobials, including gentamicin and apramycin (Table 1).

Of the seven different types of aminoglycoside resistance genes tested, four kinds were detected in apramycin-resistant GREC: *aac (3)-IV*, *aac (3)-II*, *aac (3)-III*, and *armA*. The *aac (3)-IV* gene was detected in all apramycin-resistant GREC, whereas the other genes, *aac (3)-II*, *aac (3)-III*, and *armA*, were detected in 8 (50.0%), 6 (37.5%), and 1 (6.3%) strains, respectively. Except for four isolates, all apramycin-resistant GREC carried two or three different AME genes in each isolate (Table 1).

By broth mating, transferability of apramycin resistance was observed in 7 (43.8%) of 16 apramycin-resistant strains. Co-transfer of resistance to other antimicrobials along with apramycin resistance was also found in 4 (25.0%) strains (Table 1).

Discussion

This is the first detection of apramycin resistance in human isolates in Korea. Apramycin resistance in human clinical isolates such as *E. coli* and *K. pneumoniae* has also been reported from other countries, including the United Kingdom,[9,12] Belgium,[3] Spain,[8,17] and China.[20] Although apramycin has been used for the prevention of digestive disease in food animals since 1983, this antibiotic has never been used in humans in Korea. Nevertheless, in this study, apramycin resistance was detected in human *E. coli* isolates recovered before 1999. Higher prevalence (10.9%) of *aac (3)-IV*, apramycin resistance gene, was observed in countries that had used the antibiotic in animals such as Belgium, England, France, and Germany, compared to the prevalence (0.7%) in countries that had never used apramycin.[15] Our result is similar to those for apramycin use in other countries. The *aac (3)-IV* gene was detected in all apramycin-resistant strains in this study. This gene confers apramycin resistance and also encodes cross resistance to other aminoglycosides such as gentamicin, tobramycin, and netilmicin.[3] All apramycin-resistant strains also showed resistance to gentamicin and tobramycin. Although we could not determine whether the gentamicin and tobramycin resistance was attributed to this gene or not, we assume that gentamicin and tobramycin resistance might be conferred by the *aac (3)-IV*, considering the fact that four strains that had not carried acetyltransferase genes other than the *aac (3)-IV* also showed resistance to these two antibiotics in this study.

Various species of human Enterobacteriaceae isolates containing the *armA* gene have also been reported from several European countries[8] and Korea.[14] One isolate carrying the *armA* gene showed a high level of resistance to gentamicin (>256) and tobramycin (>256) in this study. Unlike other modifying enzymes that vary in their substrate ranges, the acquired methylases confer high-level resistance to most of clinically important aminoglycosides.[8] The isolate also showed resistance against many antimicrobial agents such as ampicillin, cephalothin, amikacin, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, nalidixic acid, neomycin, and tetracycline. As previously documented,[8] our result also suggests the importance of coordinated surveillance of human and animal isolates.

Apramycin is used exclusively in veterinary medicine. Nevertheless, apramycin resistance has been observed in human isolates. The most likely explanation for the emergence of apramycin resistance in human isolates of Enterobacteriaceae, in particular *E. coli*, is that mobility of the *aac3-IV* gene on plasmids may have contributed to the selection and dissemination of these strains in a human environment.[12,20] In the present study, apramycin resistance was transferred in seven (43.8%) isolates, and resistance to other antimicrobials along with apramycin resistance was also co-transferred in four stains. Although infection or colonization in man with such organisms may be transient, the genes encoding apramycin resistance may be transferred by conjugation to other strains of the Enterobacteriaceae found in the normal human intestinal flora.

The occurrence of apramycin resistance in strains with multiple antimicrobial resistances is of major concern to both veterinary and human, because multiple resistant isolates may lead to inefficacy of a valuable antibiotic in treating serious infections. In this study, all apramycin-resistant *E. coli* were also resistant to 4 or more antimicrobials, including

Table 1. Characterization of Apramycin-Resistant *Escherichia coli* Strains Isolated From Humans

Isolates	Year	Origin	Sample	MICs (μg/ml)			Antimicrobial resistance pattern	Frequency of transfer	Transferred resistance	Resistance gene
				TM	GM	APR				
7-CCARM-1010	Before 1999	Hospital (Y)	Urine	128	128	>1,024	AM,CF,FOX,SM,GM,N,CIP,NA,SXT,CM,TE	1.7×10^{-3}	APR	*aac(3)-IV, aac(3)-II*
12-CCARM-1016	Before 1999	Hospital (Y)	Urine	128	256	>1,024	AM,CF,FOX,SM,GM,N,CIP,NA,SXT,CM,TE	1.3×10^{-1}	APR	*aac(3)-IV, aac(3)-II*
15-CCARM-1023	Before 1999	Hospital (Y)	Urine	256	32	>1,024	AM,SM,CIP,NA	3.9×10^{-2}	APR	*aac(3)-IV*
16-CCARM-1025	Before 1999	Hospital (Y)	Pus	128	8	>1,024	AM, CF,SM,CIP	1.2×10^{-2}	APR,AM	*aac(3)-IV*
43-CCARM-1170	1999	Hospital (C)	Unknown	128	4	>1,024	CF,SM,CIP,NA	NT	APR	*aac(3)-IV, aac(3)-III*
56-CCARM-1265	2001	Hospital (K)	Unknown	128	64	>1,024	N,CIP,TE	1.1×10^{-1}	APR,AM,N,TE	*aac(3)-IV*
59-CCARM-1268	2001	Hospital (K)	Unknown	128	64	>1,024	AM,CF,FOX,SM,N,CIP,NA, SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-II, aac(3)-III*
62-CCARM-1274	2001	Hospital (K)	Unknown	64	128	>1,024	AM,CF,FOX,SM,N,CIP,NA, SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-II, aac(3)-III*
74-CCARM-1417	2002	Hospital (K)	Unknown	64	128	>1,024	AM,CF,FOX,SM,N,CIP,NA, SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-II, aac(3)-III*
76-CCARM-1423	2002	Hospital (K)	Unknown	32	4	1,024	AM,CF,FOX,SM,GM,CIP,NA,SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-III*
77-CCARM-1424	2002	Hospital (K)	Unknown	64	8	>1,024	AM,CF,FOX,SM,GM,CIP,NA,SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-III*
134-CCARM-15335	2003	KONAR	Unknown	>256	>256	>1,024	AM,CF,AN,N,CIP,NA,SXT, CM,TE	NT	–	*aac(3)-IV, armA*
141-CCARM-15367	2003	KONAR	Unknown	128	32	1,024	AM,CF,N,CIP,NA,SXT,CM,TE	6.9×10^{-2}	APR,AM,N,CM	*aac(3)-IV, aac(3)-II*
83-CCARM-1460	Unknown	Unknown	Unknown	128	256	1,024	AM,GM,N,CIP,NA,SXT,CM	1.4×10^{-2}	APR,N,SXT,CM	*aac(3)-IV, aac(3)-II*
111-CCARM-1517	Unknown	Unknown	Unknown	64	8	>1,024	AM,CF,CIP,NA,SXT,CM,TE	NT	–	*aac(3)-IV*
119-CCARM-1539	Unknown	Unknown	Unknown	128	256	>1,024	AM,CF,FOX,SM,N,CIP,NA, SXT,CM,TE	NT	–	*aac(3)-IV, aac(3)-II*

MICs, minimal inhibitory concentrations; TM, tobramycin; GM, gentamicin; APR, apramycin; AM, ampicillin; CF, cephalothin; FOX, cefoxitin; SM, streptomycin; GM, gentamicin; N, neomycin; CIP, ciprofloxacin; NA, Nalidixic acid; SXT, trimethoprim/sulfamethoxazole; CM, chloramphenicol; TE, tetracycline; –, not found; NT, not transferred.

tobramycin and gentamicin. Similar result was reported from Denmark.[11] All apramycin/gentamicin-resistant strains isolated from pigs in Denmark showed multiple drug resistance. This finding might be one of the reasons for the persistence of the apramycin/gentamicin-resistant isolates in humans and animals.

The emergence of the *aac (3)-IV* gene in bacteria might be the selection pressure of gentamicin and tobramycin in humans. Another possible explanation could be related with the use of apramycin and gentamicin in animals. However, the lack of epidemiological data on the isolates in this study made it difficult for us to determine whether the resistance in humans was acquired individually by selective pressure or by transmission from animals or animal products. However, our result indicates that veterinary antimicrobials, especially those that can share cross-resistance with antimicrobials for human use, should be used more cautiously in animals.

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Disclosure Statement

The authors have no competing interests to disclose.

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