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Original Article

Reduced rates of antimicrobial resistance in *Staphylococcus intermedius* group and *Escherichia coli* isolated from diseased companion animals in an animal hospital after restriction of antimicrobial use☆

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ABSTRACT

The 2016 National Action Plan aims for reduction in antimicrobial resistance (AMR) to tetracyclines, third-generation cephalosporins, and fluoroquinolones in *Escherichia coli* isolates from livestock: to lower the tetracycline resistance of *E. coli* to 33% or less; to maintain the third-generation cephalosporin resistance of *E. coli* at the same level as in the other G7 countries as of 2020; and to maintain the fluoroquinolone resistance of *E. coli* at the same level as in the other G7 countries as of 2020. A relatively unexplored facet of reducing AMR is the impact of minimizing transmission of AMR strains by companion animals. In this study we compared AMR rates in *Staphylococcus intermedius* group (SIG) and *E. coli* isolated from diseased companion animals in an animal hospital before and after restriction of antimicrobial use. Our study spanned a 4.5-year period from 2014 to June 2018 during which antimicrobial use was restricted in 2016. During this period, abundance of methicillin-resistant SIG isolates from the hospital dropped from 41.5% to 9.3%, and that of extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates dropped from 29.5% to 9.5%. Tests for antimicrobial susceptibility revealed significantly reduced rates of AMR to enrofloxacin and levofloxacin in SIG isolates, and to cefazolin in *E. coli* isolates after antimicrobial use was restricted. Our observations suggest that restriction of antimicrobial use, especially that of third-generation cephalosporins and fluoroquinolones, is an effective method for reducing AMR rates. These findings will be relevant in guiding antimicrobial restriction approaches in other animal hospitals and clinics.

1. Introduction

Transmission of bacterial pathogens between animals and humans is a risk inherent to humanity's continued close proximity to companion animals. Many Japanese people keep companion animals, such as dogs and cats, in their homes, and some medical institutes and nursing homes are beginning to introduce animal-assisted therapy for mental health care (including cognitive disorders) of patients and the elderly. Furthermore, advances in veterinary medical technology have extended the lives of animals, especially household pets. Indeed, the “White Paper on Household Animals 2016” [1] reported that the average life span of Japanese household dogs in 2014 was 13.7 years. According to the “One Health” concept of comprehensive health control of humans, contact animals, and their environments [2], maintenance of the health of both pets and their owners will benefit from studies on the transmission of bacterial pathogens with or without antimicrobial resistance (AMR) between humans and animals.

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AMR rates have been increasing in companion animals. The Veterinary Infection Control Association (VICA) Sepsis Working Group recently reported isolation of bacteria from companion animals by blood culture in Japan in periods 2010–2013 and 2014–2016 [3]. *Escherichia coli* and *Staphylococcus intermedius* group (SIG) bacteria were most prevalent in both periods, a result confirmed by independent urine sample testing [4]. In the blood culture results, the percentages of AMR strains increased between 2010–2013 and 2014–2016, from 22.7% to 28.0% for extended-spectrum β-lactamase (ESBL) *E. coli,* and from 44.4% to 69.6% for methicillin-resistant staphylococci (MRS) SIG.

While studies on AMR in livestock and strategies to reduce their rates are well established, relatively little is known about AMR in companion animals. The goals of the National Action Plan on Antimicrobial Resistance for 2020 proposed by the Japanese Prime Minister, Mr. Shinzo Abe, at the 2016 G7 ISE-SHIMA SUMMIT consists of three aims [5]: to reduce human antimicrobial use; to reduce AMR rates in bacterial isolates from humans; and to reduce AMR rates in bacterial isolates from livestock. The Action Plan aims to reduce *E. coli* AMR rates in livestock isolates: to lower the tetracycline resistance of *E. coli* to 33% or less; to maintain the third-generation cephalosporin resistance of *E. coli* at the same level as in the other G7 countries as of 2020; and to maintain the fluoroquinolone resistance of *E. coli* at the same level as in the other G7 countries as of 2020. Meanwhile, little is known about changes in AMR rates in SIG and *E. coli* isolated from animal hospitals and clinics in Japan. To address this knowledge gap, we compared changes in AMR rates among SIG and *E. coli* strains from Kurita Animal Hospital before and after the hospital's restriction of antimicrobial use. Our findings indicate that restriction of antimicrobial use significantly reduces AMR rates in a variety of scenarios.

2. Materials and methods

2.1. Veterinary clinical setting and selection of antimicrobials

Three veterinary doctors examined and treated diseased companion animals at the Kurita Animal Hospital, which has 2 consulting rooms for visiting animals, and 46 treating rooms for hospitalization, including 6 intensive care units and 7 isolation units for contagious diseases. Severe infectious diseases including sepsis were suspected when either the veterinarians or pet owners observed reduced activity, appetite loss, chill, or breathing abnormalities such as abnormally rapid breathing [6]. Specimens were derived from either sterile (such as blood or joint fluid), or non-sterile samples (such as ear discharge or exposed pus) and were taken immediately from the sites indicating significant signs by the veterinary clinicians [6,7]. Veterinary practitioners submitted specimens together with request sheets describing companion animal information to the Sanritsu Zelkova Veterinary Laboratory to identify causal bacterial pathogens since 2015.

To decrease antimicrobial use, the Hospital introduced strict antimicrobial selection criteria to treat diseased dogs and cats from 2016 onwards (Table 1), targeting mainly third-generation cephalosporins and fluoroquinolones [8]. Of third-generation cephalosporins, cefovecin, a long-acting drug for two weeks by single dosing, was firstly restricted. Antimicrobials were initially selected based on Gram-staining results until antimicrobial susceptibility testing (AST) results were obtained from the Veterinary Laboratory. During the first visit of animals with non-life-threatening infections, one of the reagents in Table 1 was selected for exclusive use as necessary. Where AST results were known, a suitable antimicrobial was chosen preferentially from the first-line drugs in Table 1. Drug selection was in accordance with instructions, and antimicrobials contraindicated as the first-line uses (fluoroquinolones and third-generation cephalosporins) were used only when cultured bacteria were susceptible and first-line drugs were ineffective.

Antimicrobial use was strictly limited in the case of some types of infection. Antimicrobial use was restricted for skin diseases; pets with secondary infections suspected to be caused by self-mutilation due to severe itching were not treated with systemic antimicrobials on the first visit. Some dogs with atopic dermatitis were successfully treated without antimicrobials by combining local antiseptic application with oral administration of an anti-itching drug for pyotraumatic dermatitis [9]. Cats with feline cystitis were not treated with antimicrobials without the positive urine culture result unless there were overt signs of infection such as pyuria. For non-life-threatening infections, symptoms and signs were evaluated every 4–5 days after starting treatment; drugs were discontinued immediately upon disappearance of symptoms and signs.

2.2. Estimation of antimicrobial use

We estimated antimicrobial use before and after antimicrobial restriction from 2014 through to the end of June in 2018 by subtracting the stocked titers (mg) from the purchased titers (mg) according to class: penicillins, cephalosporins including cefovecin, aminoglycosides, macrolides, lincosamides, tetracyclines, peptides, carbapenems or penems, fluoroquinolones, thiamphenicols, and others. We also correlated average antimicrobial use per animal with the total amount, for each antimicrobial class, by calculating the numbers of animals examined and treated by veterinarians at the Hospital during selected periods.

2.3. Bacterial species identification and AST results

We performed Gram-staining and selective culturing for aerobic and anaerobic bacteria at the Veterinary Laboratory to identify the causative bacterial species using MicroScan WalkAway Plus System (Beckman Coulter, Inc., Tokyo, Japan) for automated identification [10]. We assessed antimicrobial susceptibility by AST using the broth microdilution method (MicroScan WalkAway Plus System) according to CLSI guidelines [11,12]. For example, identification of ESBL-production in *E. coli* was based on at least a 3-fold reduction of minimum inhibitory concentrations (MICs) of either cefotaxime or ceftazidime upon addition of the β-lactamase inhibitor clavulanic acid. Detection of MRS was based on $\geq$4 μg/mL oxacillin MIC in *S. aureus* and *S. lugdunensis,* and $\geq$0.5 μg/mL oxacillin MIC in SIG and coagulase-negative staphylococci other than *S. lugdunensis*. Antimicrobial susceptibilities of enrofloxacin were determined using the disk diffusion method in accordance with the guidelines outlined in Performance Standard CLSI VET01-S2 [3,4,13]. We were unable to distinguish between *S. pseudintermedius*, *S. intermedius*, and *S. delfini* because the three species have the same biochemical properties [14] and we were unable to conduct genetic analyses (e.g., staphylococcal chromosomal cassette *mec* typing, genotyping contributing to ESBL-production, multilocus sequence typing, and others) because no strains were collected for storage at the Veterinary Laboratory.

2.4. Evaluation of changes in AMR rates

Kurita Animal Hospital started to send the Veterinary Laboratory clinical specimens from the pets for bacterial species identification and AST results since 2015. To evaluate changes in AMR rates at the Hospital over the study period (from 2015 to June 2018) we extracted data from the Veterinary Laboratory database on SIG and *E. coli* isolated from companion animals and compared changes

Table 1
How to select antimicrobials for treatment of diseased dogs and cats in Kurita Animal Hospital.

Antimicrobial selection	Antimicrobial characteristics	For diseased dogs	For diseased cats
First selected drugs Can be used for empiric therapy.	Narrow spectrum drugs Limited risk of AMR for human health	Chloramphenicol, macrolides, penicillins (except for antipseudomonal penicillin), clavulanic acid-amoxicillin, lincosamides, sulfamethoxazole-trimethoprim, tetracyclines, 1st-generation cephalosporins	Macrolides, penicillins (except for antipseudomonal penicillin), clavulanic acid-amoxicillin, **lincosamides, tetracyclines,** 1st-generation cephalosporins
Second selected drugs Should be used according to susceptibility test results.	Broad spectrum drugs Some risk of AMR for human health	Fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides (as veterinary drugs)	**Chloramphenicol, fluoroquinolones, sulfamethoxazole-trimethoprim,** 3rd-generation cephalosporins, aminoglycosides (as veterinary drugs)
Third selected drugs	Broad spectrum or irreplaceable drugs	Fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides (as human drugs)	Fluoroquinolones, 3rd-generation cephalosporins, aminoglycosides (as human drugs)
Should be used according to susceptibility test results when first and second choice drugs are not appropriate.	High risk of AMR for human health	penems, 2nd-generation cephalosporins, antipseudomonal penicillins, tazobactam-piperacillin, fosfomycin, metronidazole	penems, 2nd-generation cephalosporins, antipseudomonal penicillins, tazobactam-piperacillin, fosfomycin, **metronidazole**
Last selected drugs Should be used only when life-threatening infection is expected.	Critically important drugs for veterinary and human medicines	Carbapenems, glycopeptides	Carbapenems, glycopeptides

AMR, antimicrobial resistance. The antimicrobials to be used with cation in cats are shown in bold letters.

in AMR rates at the Hospital with those in AMR rates at the Veterinary Laboratory. We also assessed changes in AMR rates to the following antimicrobials in SIG and *E. coli* strains from the Kurita Animal Hospital during the same period: ampicillin, sulbactam-ampicillin, clavulanic acid-amoxicillin, gentamicin, minocycline, erythromycin, clindamycin, cefazolin, cefpodoxime, cefmetazole, enrofloxacin, levofloxacin, sulfamethoxazole-trimethoprim, teicoplanin, vancomycin, imipenem, and meropenem.

2.5. Approval of animal ethics committee

The Ethics Committee of the Sanritsu Zelkova Veterinary Laboratory approved this study design (approval number SZ20181018) to maintain privacy of the affected companion animals.

2.6. Statistical analysis

We used Fisher's exact probability test (two-sided) to analyze significant differences in the rates of AMR (including MRS and ESBL-production) in SIG and *E. coli* isolated from Kurita Animal Hospital before (in 2015) and after (in 2016, 2017, and 2018) restriction of antimicrobial use. A p value of <0.05 was considered to indicate statistically significance.

3. Results

3.1. Estimation of antimicrobial use

Veterinarians at the Hospital examined and treated 12,328 animals in 2014, 13,509 in 2015, 13,923 in 2016, 14,385 in 2017, and 7395 to June 2018. Changes in the estimated average amounts of antimicrobial use per animal from 2014 to June 2018 according to antimicrobial class are shown in Table 2. We observed approximately a one-third reduction (the almost same level in 2014) of antimicrobial use from 294.3 mg/animal in 2015 to 204.5 mg/animal in 2017 and 203.8 mg/animal in 2018, while there was an increase of the use from 199.7 mg/animal in 2014 to that in 2015.

We confirmed the reduced use of third-generation cephalosporins in 2017 and 2018 and fluoroquinolones in 2016, 2017, and 2018. As compared with the amounts of cefovecin in 2014 (3.4 mg/animal) and 2015 (1.7 mg/animal), there were the decreased amounts in 2016 (0.4 mg/animal), 2017 (0.5 mg/animal), and 2018 (0.2 mg/animal). Furthermore, use of three classes of 2nd-generation cephalosporins, peptides, and thiamphenicols was entirely eliminated after restriction of antimicrobial use was imposed. There was no use of carbapenems/penems and fosfomycin in 2018, with decreased use of tetracyclines in 2018. On the other hand, we found similar use of penicillins, 1st-generation cephalosporins, aminoglycosides, and lincosamides in 2018, with increased use of macrolides, as compared with those in 2015.

3.2. Evaluation of changes in AMR rates

Distributions in clinical sources of companion animal-origin staphylococcal and *E. coli* isolates from the Hospital are shown in Table 3. SIG isolates were mainly from skin-derived specimen, open pus, and ear/nose-origin specimen from 2015 to June 2018. On the other hand, *E. coli* strains were mainly from urogenital tracts-derived specimen and open pus during the same period.

MRS and ESBL production among isolates from the Hospital dropped significantly after restriction of antimicrobial use. AMR rates of SIG and *E. coli* strains isolated from the pets at the Hospital as compared with those at the Veterinary Laboratory are shown in Table 4. The percentage of SIG isolates that were MRS in the Hospital dropped from 41.5% in 2015 to 30.8% in 2016, 27.6% in 2017, and 9.3% in 2018; similarly, the percentage of ESBL-producing *E. coli* isolated from the Hospital dropped from 29.5% in 2015 to 15.6% in 2016, 23.3% in 2017, and 9.5% in 2018. On the other hand, there were no overall changes in the rates of MRS (54.7%–60.1%) and ESBL-production (34.1%–38.5%) in isolates at the Veterinary Laboratory during the same period.

In addition, variations in AMR rates to various antimicrobials in the SIG and *E. coli* isolates from the diseased animals from the Hospital are shown in Table 5. AST revealed significantly reduced rates of AMR to enrofloxacin and levofloxacin in SIG isolates and to cefazolin in *E. coli* isolates after antimicrobial restriction from 2016 to June 2018. Furthermore, from 2017 through 2018 there were significant reductions of AMR to sulbactam-ampicillin in SIG isolates and to gentamicin in *E. coli* isolates. In 2018, AST showed significant reductions in AMR to ampicillin, gentamicin, erythromycin, and clindamycin in SIG and to ampicillin, minocycline (AMR 0%), enrofloxacin (0%), and levofloxacin (0%) in *E. coli*. We found no emergence of either teicoplanin/vancomycin-resistant SIG or imipenem/meropenem-resistant *E. coli* isolates throughout.

Table 2
Changes in estimated average amounts of antimicrobial use per animal during selected periods according to the classes in Kurita Animal Hospital.

Antimicrobial class	2014	2015	2016	2017	2018 [January to June]
Penicillins (%)	120.1 (60.1)	179.7 (61.1)	174.5 (61.1)	147.9 (72.3)	125.1 (61.4)
Total use of cephalosporins (%)	59.9 (30.0)	91.8 (31.2)	90.9 (31.8)	39.0 (19.1)	63.9 (31.4)
1st-generation cephalosporins (%)	46.4 (23.2)	84.1 (28.6)	83.0 (29.1)	36.4 (17.8)	63.7 (31.3)
2nd-generation cephalosporins (%)	0 (0)	0.4 (0.1)	0 (0)	0 (0)	0 (0)
3rd-generation cephalosporins (%)	13.5 (6.8)	7.3 (2.5)	7.9 (2.8)	2.6 (1.3)	0.2 (0.1)
Aminoglycosides (%)	1.3 (0.7)	0.6 (0.2)	0.7 (0.2)	0.8 (0.4)	0.4 (0.2)
Macrolides (%)	2.0 (1.0)	1.1 (0.4)	9.0 (3.2)	6.3 (3.1)	5.9 (2.9)
Lincosamides (%)	6.7 (3.4)	9.3 (3.2)	3.5 (1.2)	4.2 (2.1)	7.4 (3.6)
Tetracyclines (%)	4.9 (2.5)	3.9 (1.3)	4.8 (1.7)	3.0 (1.5)	1.0 (0.5)
Peptides (%)	0 (0)	0.4 (0.1)	0 (0)	0 (0)	0 (0)
Carbapenems and penems (%)	1.2 (0.6)	2.2 (0.7)	0 (0)	1.3 (0.6)	0 (0)
Fluoroquinolones (%)	1.6 (0.8)	2.3 (0.8)	0.5 (0.2)	0.3 (0.1)	0.1 (0.0)
Thiamphenicols (%)	0 (0)	1.1 (0.4)	0 (0)	0 (0)	0 (0)
Other antimicrobials (fosfomycin) (%)	2.0 (1.0)	1.9 (0.6)	1.8 (0.6)	1.7 (0.8)	0 (0)
Total amount (%)	199.7 (100)	294.3 (100)	285.7 (100)	204.5 (100)	203.8 (100)

We estimated average antimicrobial use per animal (mg/animal) during limited periods by taking the stocked titers off the purchased titers according to the class.
Kurita Animal Hospital has introduced the restriction of antimicrobial use into the veterinary clinical settings since 2016.

Table 3
Distributions in clinical sources of companion animal-origin staphylococcal and *Escherichia coli* isolates from Kurita Animal Hospital.

	2015	2016	2017	2018 [January to June]
Total number of SIG isolates	106	120	123	54
Skin-derived specimen (%)	39 (36.8)	48 (40.0)	50 (40.7)	18 (33.3)
Open pus (%)	39 (36.8)	32 (26.7)	30 (24.4)	17 (31.5)
Ear/nose-origin specimen (%)	23 (21.7)	21 (17.5)	17 (13.8)	10 (18.5)
Eye-origin specimen (%)	2 (1.9)	9 (7.5)	7 (5.7)	1 (1.9)
Urogenital tracts-derived specimen (%)	1 (0.9)	7 (5.8)	8 (6.5)	8 (14.8)
Mouth/throat-origin specimen (%)	1 (0.9)	2 (1.7)	8 (6.5)	0 (0)
Blood (%)	1 (0.9)	0 (0)	0 (0)	0 (0)
Other non-sterile specimen (%)	0 (0)	1 (0.8)	3 (2.4)	0 (0)
Total number of *E. coli* isolates	61	77	86	42
Urogenital tracts-derived specimen (%)	9 (14.8)	23 (29.9)	27 (31.4)	14 (33.3)
Open pus (%)	33 (54.1)	16 (20.8)	24 (27.9)	15 (35.7)
Skin-derived specimen (%)	1 (1.6)	7 (9.1)	11 (12.8)	4 (9.5)
Ear/nose-origin specimen (%)	7 (11.5)	6 (7.8)	3 (3.5)	3 (7.1)
Mouth/throat-origin specimen (%)	1 (1.6)	6 (7.8)	6 (7.0)	2 (4.8)
Eye-origin specimen (%)	4 (6.6)	3 (3.9)	3 (3.5)	0 (0)
Uterus-derived specimen (%)	1 (1.6)	9 (11.7)	4 (4.7)	0 (0)
Blood (%)	0 (0)	2 (2.6)	3 (3.5)	1 (2.4)
Other sterile specimen (%)	1 (1.6)	0 (0)	1 (1.2)	0 (0)
Other non-sterile specimen (%)	4 (6.6)	5 (6.5)	4 (4.7)	3 (7.1)

SIG, Staphylococcus intermedius group. Kurita Animal Hospital has introduced the restriction of antimicrobial use into the veterinary clinical settings since 2016.

Table 4
Changes in antimicrobial resistance rates among companion animal-origin staphylococcal and *Escherichia coli* isolates from an animal hospital as compared with those at Sanritsu Zelkova Veterinary Laboratory.

	2015	2016	2017	2018 [January to June]
Kurita Animal Hospital				
Methicillin-resistant SIG isolates (%)	44 (41.5)	37 (30.8)	34 (27.6)*	5 (9.3)††
Methicillin-susceptible SIG isolates (%)	62 (58.5)	83 (69.2)	89 (72.4)	49 (90.7)
Total number (%)	106 (100)	120 (100)	123 (100)	54 (100)
ESBL-producing *E. coli* isolates (%)	18 (29.5)	12 (15.6)	20 (23.3)	4 (9.5)†
ESBL-non-producing *E. coli* isolates (%)	43 (70.5)	65 (84.4)	66 (76.7)	38 (90.5)
Total number (%)	61 (100)	77 (100)	86 (100)	42 (100)
Sanritsu Zelkova Veterinary Laboratory				
Methicillin-resistant SIG isolates (%)	1364 (60.1)	1478 (56.5)	1268 (56.4)	538 (54.7)
Methicillin-susceptible SIG isolates (%)	904 (39.9)	1136 (43.5)	979 (43.6)	446 (45.3)
Total number (%)	2268 (100)	2614 (100)	2247 (100)	984 (100)
ESBL-producing *E. coli* isolates (%)	791 (37.6)	856 (34.1)	986 (36.1)	527 (38.5)
ESBL-non-producing *E. coli* isolates (%)	1310 (62.4)	1655 (65.9)	1743 (63.9)	841 (61.5)
Total number (%)	2101 (100)	2511 (100)	2729 (100)	1368 (100)

SIG, Staphylococcus intermedius group; ESBL, extended-spectrum β-lactamase; *, p value of <0.05 in 2015 vs. 2017; †, p value of <0.05 in 2015 vs. 2018; ††, p value of <0.01 in 2015 vs. 2018.
We used Fisher's exact probability test (two-sided) to analyze differences in the antimicrobial resistance of each bacterial species before and after the restriction of antimicrobial use.
Kurita Animal Hospital has introduced the restriction of antimicrobial use into the veterinary clinical settings since 2016.
Using database at Sanritsu Zelkova Veterinary Laboratory, we evaluated antimicrobial resistance of each species isolated from other areas.

Table 5
Changes in resistance rates to various antimicrobials in companion animal-origin staphylococcal and *Escherichia coli* isolates from Kurita Animal Hospital.

	2015	2016	2017	2018 [January to June]
Total number of SIG isolates	106	120	123	54
Ampicillin (%)	92 (86.8)	92 (76.7)	94 (76.4)	36 (66.7)##
Sulbactam-ampicillin (%)	44 (41.5)	37 (30.8)	34 (27.6)†	5 (9.3)##
Gentamicin (%)	64 (60.4)	64 (53.3)	61 (49.6)	22 (40.7)#
Minocycline (%)	4 (3.8)	4 (3.3)	11 (8.9)	1 (1.9)
Erythromycin (%)	62 (58.5)	59 (49.2)	66 (53.7)	15 (27.8)##
Clindamycin (%)	60 (56.6)	63 (52.5)	70 (56.9)	21 (38.9)#
Enrofloxacin (%)	63 (59.4)	54 (45.0)*	48 (39.0)††	12 (22.2)##
Levofloxacin (%)	65 (61.3)	54 (45.0)*	51 (41.5)††	12 (22.2)##
Sulfamethoxazole-trimethoprim (%)	45 (42.5)	42 (35.0)	44 (35.8)	16 (29.6)
Teicoplanin (%)	0 (0)	0 (0)	0 (0)	0 (0)
Vancomycin (%)	0 (0)	0 (0)	0 (0)	0 (0)
Total number of *E. coli* isolates	61	77	86	42
Ampicillin (%)	32 (52.5)	35 (45.5)	42 (48.8)	11 (26.2)##
Clavulanic acid-amoxicillin (%)	12 (19.7)	9 (11.7)	9 (10.5)	4 (9.5)
Gentamicin (%)	10 (16.4)	8 (10.4)	3 (3.5)††	1 (2.4)#
Minocycline (%)	8 (13.1)	4 (5.2)	7 (8.1)	0 (0)#
Cefazolin (%)	33 (54.1)	16 (20.8)**	25 (29.1)††	9 (21.4)##
Cefpodoxime (%)	18 (29.5)	12 (15.6)	20 (23.3)	4 (9.5)
Cefmetazole (%)	0 (0)	1 (1.3)	0 (0)	1 (2.4)
Enrofloxacin (%)	11 (18.0)	9 (11.7)	17 (19.8)	0 (0)##
Levofloxacin (%)	11 (18.0)	9 (11.7)	6 (7.0)	0 (0)##
Sulfamethoxazole-trimethoprim (%)	5 (8.2)	14 (18.2)	9 (10.5)	2 (4.8)
Imipenem (%)	0 (0)	0 (0)	0 (0)	0 (0)
Meropenem (%)	0 (0)	0 (0)	0 (0)	0 (0)

SIG, Staphylococcus intermedius group; *, p value of <0.05 in 2015 vs. 2016; **, p value of <0.01 in 2015 vs. 2016; †, p value of <0.05 in 2015 vs. 2017; ††, p value of <0.01 in 2015 vs. 2017.
#, p value of <0.05 in 2015 vs. 2018; ##, p value of <0.01 in 2015 vs. 2018.
We used Fisher's exact probability test (two-sided) to analyze differences in the antimicrobial resistance of each bacterial species before and after the restriction of antimicrobial use.
Kurita Animal Hospital has introduced the restriction of antimicrobial use into the veterinary clinical settings since 2016.

4. Discussion

Here we reported that restriction of antimicrobial use leads to significant reductions in AMR rates in SIG and *E. coli* strains isolated from diseased companion animals in a Japanese animal hospital. Our results are consistent across strains and antibiotics and highlight the efficacy of such an approach in tackling the growing problem of AMR. By reducing AMR in companion animals, the risk of spreading AMR microbes to humans is mitigated, leaving antimicrobials available exclusively for urgent cases. To the best of our knowledge, this is the first such study of companion animal AMR, although our work is limited by being conducted in only one institute.

Our results suggest that antimicrobial use should be minimized for treatment of companion animals, in line with the Danish Small Animal Veterinary Association's published guidelines [15]. These guidelines recommend minimal antimicrobial use, including minimization of use of carbapenem-class and anti-MRS-class antibiotics in order to prevent the emergence of carbapenem-resistant bacteria, multidrug-resistant *Pseudomonas aeruginosa* isolates, and vancomycin-resistant bacteria in veterinary clinical settings. This recommendation is compatible with our established selection of antimicrobials to treat diseased dogs and cats (Table 1).

We found a relationship between the restriction of antimicrobial use and AMR during this study period. There were fluctuations of the total amounts of antimicrobial use per animal year by year (from 2014 to 2018) before and after the restriction. Cefovecin of third-generation cephalosporins was firstly restricted with the limited use of fluoroquinolones. We could confirm the reduced amounts of cefovecin and fluoroquinolones in 2016, 2017, and 2018 after the restriction and could observe those of third-generation cephalosporins in 2017 and 2018, although change in the total amounts was limited between 2015 and 2016. The decreased AMR rates to some antimicrobials were shown to be started in 2016 (Table 5). Finally, the abundance of MRS in SIG isolates dropped to 9.3%, and that of ESBL-producing *E. coli* isolates dropped to 9.5% (Table 4). In addition, AST indicated the reduced rates of AMR to enrofloxacin and levofloxacin in SIG isolates, and to cefazolin in *E. coli* isolates after the restriction.

Our results indicate that non-antimicrobial treatment of exposed wounds and skin diseases is effective and beneficial. SIG isolates were shown to be mainly from skin-derived specimen (33.3%–40.7%) obtained at the Kurita Animal Hospital during the study period. The Hospital demonstrated successful pyotraumatic dermatitis treatment without antimicrobial use with local procedures employing antiseptic solution and oral administration of an anti-itching drug [9]. Indeed, some European countries have legal restrictions regarding use of certain MRS-associated antimicrobials such as mupirocin in veterinary clinical settings [16]. Saputra et al. [17] recently described AMR in coagulase-positive staphylococci isolated from companion animals in Australia: detection of methicillin-resistant *S. pseudintermedius* was associated with infections of sites such as surgical areas or skin and soft tissue; isolates from dogs with surgical site infections were three-times more likely to be MRS if the dogs had received prior antimicrobial treatment. Therefore, future veterinary treatment will benefit from developing non-antimicrobial strategies to treat the most common infections associated with methicillin-resistant *S. pseudintermedius* such as surgical sites or traumatic wounds, skin diseases such as pyoderma, and otitis externa [16].

Gandolfi-Decristophoris et al. [18] reported ESBL-producing *Enterobacteriaceae* among healthy cats and dogs living in nursing homes and households. Those pets that had received an antimicrobial therapy in the three months prior to the study had a higher risk of being carriers of ESBL-producing *Enterobacteriaceae*. Strikingly, another study [19] indicated a strong association of ESBL-producing *Enterobacteriaceae* in household cats fed with raw pet food, since the pet food was often contaminated

with ESBL-producing bacteria. Thus, veterinarians will need to consider the risk factors such as feeding conditions in addition to history of antimicrobial use when examining and treating diseased animals.

In conclusion, our observations suggest that restriction of antimicrobial use, especially that of third-generation cephalosporins and fluoroquinolones, is a promising method for effective reduction of AMR rates. Further studies are needed to confirm this, but our results suggest that in the future, the VICA's published guidelines limiting the use of antimicrobials in other animal hospitals and clinics, will contribute to the ongoing battle against AMR.

Conflicts of interest

All authors (G.K., Y.T., Y.M., and T.T.) and the VICA AMR Working Group have no conflicts of interest to declare in relation to this article.

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