Jpn. J. Infect. Dis., 72, 94–98, 2019

Original Article

Species Identification of β-Hemolytic Streptococci from Diseased Companion Animals and Their Antimicrobial Resistance Data in Japan (2017)

Yasuto Fukushima[1], Yuzo Tsuyuki[1,2], Mieko Goto[1], Haruno Yoshida[1], and Takashi Takahashi[1]*

[1]*Laboratory of Infectious Diseases, Graduate School of Infection Control Sciences & Kitasato Institute for Life Sciences, Kitasato University, Tokyo 108-8641; and*
[2]*Division of Clinical Laboratory, Sanritsu Zelkova Veterinary Laboratory, Kanagawa 213-0032, Japan*

SUMMARY: This study aimed to identify the species and assess the antimicrobial resistance (AMR) of β-hemolytic streptococci isolated from companion animals in Japan. Strains were isolated from clinical specimens of 131 companion animals that exhibited symptoms in April–May 2017. We identified strains by 16S rRNA sequencing and assessed their antimicrobial susceptibility using the broth microdilution method. AMR genes *erm*(A)-*erm*(B)-*mef*(A) and *tet*(M)-*tet*(O)-*tet*(K)-*tet*(L)-*tet*(S) in all isolates were amplified by PCR. 16S rRNA sequencing identified β-hemolytic streptococcal species as *Streptococcus canis* (*n* = 117, 89.3%), *S. agalactiae* (*n* = 7), *S. dysgalactiae* subsp. *equisimilis* (*n* = 5), *S. dysgalactiae* subsp. *dysgalactiae* (*n* = 1), and *S. equi* subsp. *zooepidemicus* (*n* = 1). Overall AMR rates were 39.7% for minocycline, 19.8% for erythromycin, and 17.6% for clindamycin, with a minimum inhibitory concentration (MIC_{90}) of > 4, > 2, and > 1 μg/mL, respectively. AMR genotyping showed the presence of single or mixed types: *erm*(B)-*mef*(A) and *tet*(M)-*tet*(O)-*tet*(L)-*tet*(S). There was a significant relationship between tetracycline-resistance genotypes and open pus/skin-derived specimens. These observations identify some unique features of β-hemolytic streptococcal isolates from companion animals in Japan, such as the dominant isolation of *S. canis* and resistance to tetracycline, macrolide, and lincosamide antibiotics, in terms of species identification and AMR properties.

INTRODUCTION

Many individuals in Japan keep companion animals in their homes, including dogs and cats. Additionally, medical institutes and nursing homes are starting to introduce animal-assisted therapy as a mental health service for hospital patients and elderly individuals. As a result, animals and humans are living increasingly closely. Furthermore, remarkable advances in veterinary medical technology have helped to extend the lives of animals, especially household pets. According to the "White Paper on Household Animals 2016" (1), the average life span of household dogs in Japan in 2014 was 13.7 years. Therefore, based on the "One Health" concept, a comprehensive control of the health of humans, contact animals, and their related environments (2), bacterial pathogens with the potential for transmission between humans and animals need to be investigated in order to maintain the health of both pets and their owners.

Streptococcus canis, belonging to the β-hemolytic Lancefield carbohydrate antigen group G streptococci, has been associated with multiple documented pyogenic syndromes in cats by an institutional boarding facility (3). This species also appears to have been the zoonotic source of human infections in several case studies (4–6). *S. canis*-related invasive zoonotic infections may be underdiagnosed, as species-level identification has not been performed for the same.

The β-hemolytic *S. agalactiae* carbohydrate group B proliferates in the milk of cows and can result in intramammary infections. In addition, this pathogen has been previously isolated from several invasive infections, including sepsis/bacteremia with unknown foci, infectious endocarditis, septic arthritis, meningitis, and others among human neonatal or elderly subjects (7,8). Therefore, human and veterinary clinicians should be aware of possible infectious diseases resulting from this zoonotic bacterium.

S. dysgalactiae subsp. *equisimilis* isolates having β-hemolytic activity and groups G /C/A antigens are increasingly recovered from severe invasive infections, such as streptococcal toxic shock syndrome, necrotizing fasciitis, meningitis, infectious endocarditis, sepsis, septic arthritis, osteomyelitis, and others in human patients worldwide (9). These *S. dysgalactiae* subsp. *equisimilis* strains are zoonotic and are carried in both humans and animals, including house pets and horses (10,11).

For identification of β-hemolytic streptococci, the Lancefield carbohydrate grouping is used in veterinary clinical settings. Although it only included one institute, Kimura et al. (12) reported the prevalence and antimicrobial susceptibility of *Streptococcus* spp. isolated from infected dogs and cats in a veterinary hospital between 2006 and 2013. Of the 96 strains tested, 79 (82.3%) were identified as group G streptococci. However, few studies have analyzed all β-hemolytic

Received June 4, 2018. Accepted October 9, 2018.
J-STAGE Advance Publication October 31, 2018.
DOI: 10.7883/yoken.JJID.2018.231
*Corresponding author: Mailing address: Laboratory of Infectious Diseases, Graduate School of Infection Control Sciences & Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan. Tel: +81-3-5791-6428, Fax: +81-3-5791-6441, E-mail: taka2si@lisci.kitasato-u.ac.jp

streptococcal isolates from diseased companion animals in Japan at the species-level with respect to their antimicrobial resistance (AMR) phenotypes or genotypes.

We aimed to determine the species and subspecies-level identification and AMR properties of β-hemolytic streptococcal isolates recovered from diseased dogs and cats nationwide in Japan in 2017.

MATERIALS AND METHODS

Collection of bacterial isolates and background of companion animals: Clinical specimens, submitted by veterinary practitioners, along with request sheets including the information of companion animals nationwide, were immediately sent to the Sanritsu Zelkova Veterinary Laboratory to examine them for causative bacterial agents. These specimens were taken from diseased companion animals, which visited either clinics or hospitals during the study period of April to May 2017, with significant symptoms or signs found either by pet owners or the veterinary clinicians (13). Specimens were derived from either sterile (blood, joint fluid, etc.) or non-sterile samples (ear discharge, open pus, etc.). Each specimen was inoculated on sheep blood agar plates and incubated in 5% CO_2 at 35°C for 24 h. Gray-white colonies with β-hemolytic activity were subjected to latex agglutination testing with group-specific antisera for Lancefield carbohydrate antigen classification (Seroiden Strepto Kit, Eiken Chemical Co., Ltd., Tokyo, Japan). All β-hemolytic streptococcal isolates (one isolate per companion animal) were stored between −70°C and −80°C until processed. The companion animal information (species, sex, age, clinical specimen, date collected, and Japanese prefecture where the practitioners worked) was taken from the request sheets. Storedt β-hemolytic streptococcal isolates, along with the companion animal information, were sent to the Laboratory of Infectious Diseases (Graduate School of Infection Control Sciences & Kitasato Institute for Life Sciences) for further genotypic and phenotypic analyses.

Determination of species identification: DNA was extracted from the β-hemolytic streptococcal isolates by suspending in TE buffer and boiling at 97°C for 10 min as previously described (14). We identified the β-hemolytic streptococcal strains at the species-level based on 16S rRNA sequencing data (13,15) (Table 1). The isolates identified were unambiguously identified, with ≥ 98.7% similarity to the 16S rRNA sequence of the corresponding strain.

Confirmation of species-level identification accuracy: We evaluated the accuracy of the species-level identification by 16S rRNA sequencing by including PCR amplification of a *S. canis cfg* (co-hemolysin CAMP-factor) (16,17), *S. agalactiae dltS* (histidine kinase membrane sensor protein) (18), *S. dysgalactiae* subsp. *equisimilis emm* (virulence factor M protein) (19), *S. equi sodA* (superoxide dismutase A), and its *seeI* (exotoxin) (20). Table 1 shows the oligonucleotide primers used for these genes and their PCR amplicon size. All *emm* genotyping was based on the Centers for Disease Control and Prevention database <http://www2a.cdc.gov/ncidod/biotech/strepblast.asp>.

Antimicrobial susceptibility testing and determination of resistance genes: The minimum inhibitory concentrations (MICs) of various antimicrobial agents, including penicillin G, ampicillin, cefepime, cefotaxime, ceftriaxone, cefozopran, meropenem, minocycline (MIN), erythromycin (ERY), azithromycin (AZM), clindamycin (CLI), levofloxacin (LVX), vancomycin, and chloramphenicol (CHL), were determined using the broth microdilution method (MICroFAST Panel Type 7J for *Streptococcus* spp., Beckman Coulter Inc., Tokyo, Japan), according to the Clinical and Laboratory Standards Institute guidelines for β-hemolytic streptococci (21). The quality of the susceptibility testing was controlled using two American Type Culture Collection (ATCC) strains (ATCC29212 and ATCC4961). The AMR rate and MIC values, defined as the lowest antibiotic concentration at which 50% (MIC_{50}) and 90% (MIC_{90}) of each strain was inhibited, were calculated for each β-hemolytic streptococcal isolate against each antibiotic.

The presence of macrolide/lincosamide (ML)-class resistance genes, *erm*(A), *erm*(B), and *mef*(A), in addition

Table 1. Oligonucleotide primers for targeted genes and their PCR amplicons' size

Targeted gene (specific species)	Primer	Direction	Sequence (5′→3′)	Expected amplicon size (bp)	Reference
16S rRNA (universal)	27F[1)]	Forward	AGAGTTTGATCMTGGCTCAG	1,497	[13,15]
	1485R[1)]	Reverse	TACGGTTACCTTGTTACGAC		
cfg (*S. canis*)	camp-canis-I	Forward	CAATTAACTAATAAGGTAGAACAG	238	[16,17]
	camp-canis-II	Reverse	CTCTCTCAAAACGGGTG		
dltS (*S. agalactiae*)	dlts-F	Forward	CTGTAAGTCTTTATCTTTCTCG	199	[18]
	dlts-R	Reverse	TCCATTCGCTTAGTCTCC		
emm (*S. dysgalactiae* subsp. *equisimilis*)	emm1[1)]	Forward	TATTSGCTTAGAAAATTAA	Variable and 180 bp used for genotyping	[19][2)]
	emm2	Reverse	GCAAGTTCTTCAGCTTGTTT		
sodA (*S. equi* subsp. *equi* & *S. equi* subsp. *zooepidemicus*)	sodA-F	Forward	CAGCATTCCTGCTGACATTCGTCAGG	235	[20]
	sodA-R	Reverse	CTGACCAGCCTTATTCACAACCAGCC		
seeI (*S. equi* subsp. *equi*)	seeI-F	Forward	GAAGGTCCGCCATTTTCAGGTAGTTTG	520	[20]
	seeI-R	Reverse	GCATACTCTCTCTGTCACCATGTCCTG		

[1)]: The same primers are used for both PCR amplification and sequencing.
[2)]: PCR protocol for *emm* genotyping including the primer sequences was indicated at the following URL: https://www.cdc.gov/streplab/protocol-emm-type.html.

to tetracycline (TET)-class resistance genes, *tet*(M), *tet*(O), *tet*(K), *tet*(L), and *tet*(S), in all β-hemolytic streptococcal isolates was confirmed by PCR as previously described (22,23). We also confirmed the sequences of these resistance genes from several isolates positive by PCR. The relationships between AMR genotypes and their phenotypes, identified bacterial species or companion animal background, were evaluated.

Approval of animal ethics committee: The Ethics Committee of the Sanritsu Zelkova Veterinary Laboratory approved this study design (approval number SZ20170316) to maintain privacy of the affected companion animals.

Statistical analysis: We used the Fisher's exact probability test (two-sided) to analyze the significance of the relationships between AMR genes and the background of the companion animals. A p value of < 0.05 indicated statistical significance.

RESULTS

β-hemolytic streptococcal isolates and companion animal demographics: Out of 1,947 clinical specimens obtained from dogs and cats, 131 β-hemolytic streptococcal isolates, (isolation rate 6.7%), including groups G ($n = 119$), B ($n = 7$), C ($n = 4$), and A ($n = 1$), were collected from 19 prefectures from April–May of 2017. The most common prefectures sampled were Chiba ($n = 43$), Tokyo ($n = 28$), Aichi ($n = 12$), Kanagawa ($n = 10$), Ibaraki ($n = 8$), Saitama ($n = 7$), Okayama ($n = 4$), and Nara ($n = 4$). Isolates were obtained from ear/nose-origin ($n = 40$), open pus ($n = 36$), urogenital tracts-derived ($n = 21$), eye-origin ($n = 15$), skin-derived ($n = 10$), uterine ($n = 1$), and other ($n = 8$) specimens from dogs ($n = 113$) and cats ($n = 18$). The companion animal demographics were as follows: mean age, 10.3 years; age range, 1–19 years; sex, 80 males and 51 females.

AMR: Species identification by 16S rRNA sequencing revealed the 131 β-hemolytic streptococcal isolates consisted of *S. canis* ($n = 117$, 89.3%), *S. agalactiae* ($n = 7$, 5.3%), *S. dysgalactiae* subsp. *equisimilis* ($n = 5$, 3.8%), *S. dysgalactiae* subsp. *dysgalactiae* ($n = 1$, 0.8%), and *S. equi* subsp. *zooepidemicus* ($n = 1$, 0.8%). Group G isolates included *S. canis* ($n = 117$) and *S. dysgalactiae* subsp. *equisimilis* ($n = 2$), and group C consisted of *S. dysgalactiae* subsp. *equisimilis* ($n = 2$), *S. dysgalactiae* subsp. *dysgalactiae* ($n = 1$), and *S. equi* subsp. *zooepidemicus* ($n = 1$). Groups B and A included only one species each, *S. agalactiae* ($n = 7$) and *S. dysgalactiae* subsp. *equisimilis* ($n = 1$), respectively.

Genetic assessment of the accuracy of identification: All *S. canis* isolates contained the *cfg* gene, and all *S. agalactiae* possessed the *dltS* gene. Three *emm* subtypes were detected in the *S. dysgalactiae* subsp. *equisimilis* isolates: *stC9431.0* ($n = 3$), *stG245.0* ($n = 1$), and *stG485.0* ($n = 1$). However, we did not detect *emm* in the *S. dysgalactiae* subsp. *dysgalactiae* strain. One *S. equi* subsp. *zooepidemicus* isolate had the *sodA* gene without possession of *seeI*.

Antimicrobial susceptibility and AMR genotypes: The antimicrobial sensitivities of the collected β-hemolytic streptococcal strains to the oral and parenteral antibiotics are shown in Table 2. The overall AMR rates were 39.7% ($n = 52$) for MIN, 19.8% ($n = 26$) for ERY, and 17.6% ($n = 23$) for CLI, with an MIC_{90} of > 4, > 2, and > 1 μg/mL, respectively. We also found β-hemolytic streptococcal isolates resistant to AZM ($n = 26$), LVX ($n = 5$ in *S. canis* and *S. agalactiae*), and CHL ($n = 1$ in *S. agalactiae*). No strains were resistant to all β-lactams. All MIN-resistant strains were either *S. canis* ($n = 48$, 41.0%) or *S. agalactiae* ($n = 4$), ERY/AZM-resistant isolates were *S. canis* ($n = 22$, 18.8%), *S. agalactiae* ($n = 3$), and *S. dysgalactiae* subsp. *equisimilis* ($n = 1$), and CLI-resistant strains were *S. canis* ($n = 20$, 17.1%) and *S. agalactiae* ($n = 3$). All resistant streptococcal strains were isolated from non-sterile samples and were obtained in 14 (73.7%) prefectures of Japan.

Our study included TET-resistance genotypes alone

Table 2. Antimicrobial activity of oral and parenteral antibiotics against 130 β-hemolytic streptococcal isolates from diseased companion animals in Japan

Antibiotic	*S. canis* ($n = 117$)			*S. agalactiae* ($n = 7$)			*S. dysgalactiae* ($n = 6$)		
	MIC range (μg/mL)	MIC_{50}	MIC_{90}	MIC range (μg/mL)	MIC_{50}	MIC_{90}	MIC range (μg/mL)	MIC_{50}	MIC_{90}
Penicillin G	≦0.03	≦0.03	≦0.03	≦0.03	≦0.03	≦0.03	≦0.03	≦0.03	≦0.03
Ampicillin	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06	≦0.06
Cefepime	≦0.5	≦0.5	≦0.5	≦0.5	≦0.5	≦0.5	≦0.5	≦0.5	≦0.5
Cefotaxime	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.06	≦0.06	≦0.06
Ceftriaxone	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12
Cefozopran	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12
Meropenem	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12	≦0.12
Minocycline[1)]	≦0.5–> 4	≦0.5	> 4	≦0.5–> 4	> 4	> 4	≦0.5–4	≦0.5	4
Erythromycin[1)]	≦0.12> 2	≦0.12	> 2	≦0.12–> 2	≦0.12	> 2	≦0.12–> 2	≦0.12	> 2
Azythromycin[1)]	≦0.25–> 4	≦0.25	> 4	≦0.25–> 4	≦0.25	> 4	≦0.25–> 4	≦0.25	> 4
Clindamycin[1)]	≦0.12–> 1	≦0.12	> 1	≦0.12–> 1	≦0.12	> 1	≦0.12	≦0.12	≦0.12
Levofloxacin[1)]	≦0.25–> 8	0.5	1	≦0.25–> 8	0.5	> 8	≦0.25–0.5	0.5	0.5
Vancomycin	≦0.12–0.5	0.25	0.5	≦0.12–0.5	0.5	0.5	≦0.25	≦0.25	≦0.25
Chloramphenicol[1)]	≦4–8	≦4	≦4	≦4–> 16	≦4	> 16	≦4	≦4	≦4

MIC, minimum inhibitory concentration. One strain of *S. equi* subsp. *zooepidemicus* was excluded from this table.
[1)]: The overall resistance rates to minocycline, erythromycin, azythromycin, clindamycin, levofloxacin, and chloramphenicol were 40% ($n = 52$), 20% ($n = 26$), 20% ($n = 26$), 17.7% ($n = 23$), 3.8% ($n = 5$), and 0.8% ($n = 1$), respectively.

(*n* = 40, 30.5%), ML-resistance genotypes alone (*n* = 3, 2.3%), and mixed TET+ML-resistance genotypes (*n* = 25, 19.1%). The TET-resistance genes detected alone were *tet*(M) (*n* = 16), *tet*(O) (*n* = 21), and *tet*(S) (*n* = 3). The ML-resistance genes alone were *erm*(B) (*n* = 2) and *mef*(A) (*n* = 1). The mixed resistance genes were *erm*(B)+*tet*(O) (*n* = 14), *erm*(B)+*tet*(M) (*n* = 3), *erm*(B)+*tet*(L) (*n* = 3), *mef*(A)+*tet*(O) (*n* = 2), *mef*(A)+*tet*(M) (*n* = 1), *erm*(B)+*mef*(A)+*tet*(O) (*n* = 1), and *erm*(B)+*tet*(M)+*tet*(O) (*n* = 1). We did not detect *erm*(A) or *tet*(K) in the β-hemolytic streptococcal isolates. *S. canis* strains included 60 (51.3%) isolates with these AMR genes, whereas *S. agalactiae* had 6 (85.7%) and *S. dysgalactiae* subsp. *equisimilis* had 2 (40.0%). There was a significant relationship between overall AMR genotypes ($p = 0.029$) and TET-resistance genotypes alone ($p = 0.029$) in open pus/skin-derived specimens. All β-hemolytic streptococcal strains with AMR genes were isolated from non-sterile samples and were obtained from 15 (78.9%) Japanese prefectures.

DISCUSSION

Of the 131 β-hemolytic streptococcal strains, we obtained five species identifications, *S. canis*, *S. agalactiae*, *S. dysgalactiae* subsp. *equisimilis*, *S. dysgalactiae* subsp. *dysgalactiae*, and *S. equi* subsp. *zooepidemicus*. To the best of our knowledge, this is the first large-scale nationwide monitoring of β-hemolytic streptococcal isolates from diseased companion animals in Japan. A previous study of streptococcal isolates from 393 dogs in the USA (24) identified the major species/subspecies as *S. canis* (*n* = 88, 22.4%), *S. dysgalactiae* subsp. *equisimilis* (*n* = 13, 3.3%), and *S. equi* subsp. *zooepidemicus* (*n* = 4, 1.0%), which were comparable with our observations.

In 2015, we compared the species/subspecies identification methods of 16S rRNA sequencing with the results obtained by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and an automated biochemical identification method (Vitek 2 system with the Gram positive ID Card) using 72 group G streptococcal strains (13). There were discrepancies between the 16S rRNA sequencing results and the identification data from both MALDI-TOF MS and the automated biochemical assay. 16S rRNA sequencing identified 68 strains as *S. canis*, whereas MALDI-TOF MS identified 18 (26.5%) as *S. canis*, and the automated biochemical method identified 37 (54.4%) as *S. canis*. On the other hand, we observed a good correlation rate (89.3%) of species/subspecies identification in the 131 β-hemolytic streptococcal strains by the manual identification assay (Rapid ID 32 STREP API v4.0, SYSMEX bioMérieux Co., Ltd., Tokyo, Japan). This method identified 94.0% of the 117 *S. canis* strains confirmed by sequencing (preliminary results). The sensitivity, specificity, and positive and negative predictive values of this assay to identify *S. canis* compared to 16S rRNA sequencing were 94.0%, 92.9%, 99.1%, and 65.0%, respectively (data not shown). Moreover, the blood-origin *S. canis* isolate from the human case of bacteremia was identified by a manual identification assay (99.9% probability) (17). As such, this kit seems to be useful in identifying β-hemolytic streptococcal strains, such as S. canis in veterinary clinical situations, although further research is needed to evaluate its performance capacity compared to other kits.

Three *emm* genotypes including *stC9431.0*, *stG245.0*, and *stG485.0* were detected in the animal-derived *S. dysgalactiae* subsp. *equisimilis* isolates in this investigation. Two *emm* types (*stC9431.0* and *stG6792.3*) with *stC1929.1* (original type from a skin wound of a house cat) were observed in the 2015 *S. dysgalactiae* subsp. *equisimilis* strain (13). The *stC9431.0* was recovered from sterile human specimens in the USA (25), and *stG6792.3*, *stG245.0*, and *stG485.0* are the major isolate types found in human patients in Japan (14). These results seem to involve the zoonotic profiles of *S. dysgalactiae* subsp. *equisimilis*, although both studies had small numbers of isolates. A large-scale study to examine the animal-origin of *S. dysgalactiae* subsp. *equisimilis* strains is needed to clarify the zoonotic features.

A previous investigation reported AMR rates of 27% for TET and 10.8% for ML in *S. canis* strains (*n* = 37) from dogs collected in Denmark (2000–2005) (26). In 2015, we conducted antimicrobial susceptibility testing (MICroFAST Panel Type 5J) using group G streptococcal strains (13). Overall, the AMR rate against TET was 20.8% (*n* = 15) and 5.6% (*n* = 4) to ERY/clarithromycin. There were four (5.6%) and two (2.8%) group G streptococcal isolates resistant to CLI and LVX, respectively. All resistant strains were *S. canis*. On the other hand, we found that the overall AMR rates were 39.7% (41.0% of *S. canis*) for MIN, 19.8% (18.8% of *S. canis*) for ERY/AZM, and 17.6% (17.1% of *S. canis*) for CLI in 2017. The changes in AMR rates among *S. canis* isolates from 2015 and 2017 are summarized in Table 3. Therefore, TET and ML resistance rates seem to have increased rapidly among β-hemolytic streptococcal strains, especially *S. canis* in Japan. Furthermore, AMR genotyping indicated single or mixed types *erm*(B)-*mef*(A) and *tet*(M)-*tet*(O)-*tet*(L)-*tet*(S) in our investigation. Pinho et al. (27) also demonstrated either *tet*(M) or *tet*(O) as the sole or mixed determinants *tet*(M)+*tet*(L), *tet*(L)+*tet*(S), and *erm*(B)+*tet*(O) in TET-resistant *S. canis* isolates (*n* = 23; AMR rate, 27%). Additionally, the Japanese Veterinary Antimicrobial Resistance Monitoring System reported that TET-class antibiotics constituted 46.2% of the overall antimicrobials (converted weight in tons to bulk powder) used for animals (28). Thus, AMR to TET and/or ML-classes should be considered when these different antimicrobials are not effective in veterinary clinical practices.

This study had two limitations. First, most of the clinical samples were non-sterile (ear/nose, open pus,

Table 3. Changes in AMR rates among *S. canis* isolates from 2015 and 2017

Antimicrobial class	Resistance rate (%) in 2015 (total No. = 68)	Resistance rate (%) in 2017 (total No. = 117)	*P* value by the Fisher's exact probability test (two-sided)
Tetracycline	22.1 (*n* = 15)	41.0 (*n* = 48)	0.01
Macrolide	5.9 (*n* = 4)	18.8 (*n* = 22)	0.016
Lincosamide	5.9 (*n* = 4)	17.1 (*n* = 20)	0.039

urogenital tract, eye, and skin samples). Future studies using sterile samples from severely diseased companion animals should be performed to discard invasive infections (streptococcal toxic shock syndrome, necrotizing fasciitis, etc.). Second, our study contained limited demographics in terms of the companion animals studied, with regards to animal species, sex, age, clinical specimens, bacterial isolation dates, and the prefectures in which the veterinarians worked. More detailed information, including underlying medical conditions, diagnosis of infectious diseases, therapeutic approaches (surgical procedure, supportive treatment, and treatment with antimicrobials), and outcomes (survival/death and related sequelae) should be collected from veterinary doctors in future studies.

In conclusion, our results show the dominant isolation of *S. canis* and AMR to TET and/or ML-classes of β-hemolytic streptococcal isolates from diseased companion animals in Japan (April–May 2017). These unique characteristics could be of use to Japanese veterinary practitioners when examining and treating companion animals with clinical symptoms or signs of streptococcal infections. In future, these strains should be monitored throughout the country sequentially and additional β-hemolytic streptococcal isolates need to be characterized.

Acknowledgments The authors wish to thank Drs. Yoshiteru Murata (Murata Animal Hospital, Chiba, Japan), Goro Kurita (Kurita Animal Hospital, Ibaraki, Japan), and Daisuke Taniyama (Tokyo Saiseikai Central Hospital, Tokyo, Japan) for their helpful assistances and suggestions.

Conflict of interest None to declare.

REFERENCES

1. Anicom. White Paper on Household Animals 2016. Available at <https://www.anicom-page.com/hakusho/book/pdf/book_201612.pdf>. Accessed May 11, 2018. Japanese.
2. Centers for Disease Control and Prevention. One Health. Available at <https://www.cdc.gov/onehealth/index.html>. Accessed May 11, 2018.
3. Morrow BL, McNatt R, Joyce L, et al. Highly pathogenic β-hemolytic streptococcal infections in cats from an institutionalized boarding facility and a multi-species comparison. J Feline Med Surg. 2016;18:318-27.
4. Amsallem M, Iung B, Bouleti C, et al. First reported human case of native mitral infective endocarditis caused by *Streptococcus canis*. Can J Cardiol. 2014;30:1462:e1-2.
5. Cheong BM, Lim AY. Sharing a microbe with man's best friend: A case of canine streptococcal infection in a diabetic patient. Med J Malaysia. 2015;70:318-9.
6. Lacave G, Coutard A, Troché G, et al. Endocarditis caused by *Streptococcus canis*: an emerging zoonosis? Infection. 2016;44:111-4.
7. Takahashi T, Ubukata K, Watanabe H. Invasive infection caused by *Streptococcus dysgalactiae* subsp. *equisimilis*: characteristics of strains and clinical features. J Infect Chemother. 2011;17:1-10.
8. Shibayama A, Yoshizaki T, Tamaki M, et al. Pyogenic sternoclavicular arthritis caused by *Streptococcus agalactiae* in an elderly adult with diabetes mellitus. J Am Geriatr Soc. 2016;64:1376-7.
9. Brandt CM, Spellerberg B. Human infections due to *Streptococcus dysgalactiae* subspecies *equisimilis*. Clin Infect Dis. 2009;49:766-72.
10. Schrieber L, Towers R, Muscatello G, et al. Transmission of *Streptococcus dysgalactiae* subsp. *equisimilis* between child and dog in an Aboriginal Australian community. Zoonoses Public Health. 2014;61:145-8.
11. Acke E, Midwinter AC, Lawrence K, et al. Prevalence of *Streptococcus dysgalactiae* subsp. *equisimilis* and *S. equi* subsp. *zooepidemicus* in a sample of healthy dogs, cats and horses. N Z Vet J. 2015;63:265-71.
12. Kimura Y, Shimada E, Miyamoto T, et al. Prevalence and antimicrobial drug susceptibility of enterococci and streptococci in dogs and cats. J Jpn Vet Med Assoc. 2014;67:499-505. Japanese.
13. Tsuyuki Y, Kurita G, Murata Y, et al. Identification of group G streptococcal isolates from companion animals in Japan and their antimicrobial resistance patterns. Jpn J Infect Dis. 2017;70:394-8.
14. Kim S, Byun JH, Park H, et al. Molecular epidemiological features and antibiotic susceptibility patterns of *Streptococcus dysgalactiae* subsp. *equisimilis* isolates from Korea and Japan. Ann Lab Med. 2018;38:212-9.
15. Kakuta R, Yano H, Hidaka H, et al. Severe acute otitis media caused by mucoid *Streptococcus pyogenes* in a previously healthy adult. Tohoku J Exp Med. 2014;232:301-4.
16. Hassan AA, Khan IU, Abdulmawjood A, et al. Development of PCR assays for detection of *Streptococcus canis*. FEMS Microbiol Lett. 2003;219:209-14.
17. Taniyama D, Abe Y, Sakai T, et al. Human case of bacteremia caused by *Streptococcus canis* sequence type 9 harboring the *scm* gene. IDCases. 2017;7:48-52.
18. Murayama SY, Seki C, Sakata H, et al. Capsular type and antibiotic resistance in *Streptococcus agalactiae* isolates from patients, ranging from newborns to the elderly, with invasive infections. Antimicrob Agents Chemother. 2009;53:2650-3.
19. Takahashi T, Sunaoshi K, Sunakawa K, et al. Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections. Clin Microbiol Infect. 2010;16:1097-103.
20. Alber J, El-Sayed A, Lämmler C, et al. Multiplex polymerase chain reaction for identification and differentiation of *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi*. J Vet Med B Infect Dis Vet Public Health. 2004;51:455-8.
21. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 22nd informational supplement. Document M100-S22. Wayne, PA: CLSI; 2012.
22. Malhotra-Kumar S, Lammens C, Piessens J, et al. Multiplex PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. Antimicrob Agents Chemother. 2005;49:4798-800.
23. Haenni M, Saras E, Bertin S, et al. Diversity and mobility of integrative and conjugative elements in bovine isolates of *Streptococcus agalactiae*, *S. dysgalactiae* subsp. *dysgalactiae*, and *S. uberis*. Appl Environ Microbiol. 2010;76:7957-65.
24. Lamm CG, Ferguson AC, Lehenbauer TW, et al. Streptococcal infection in dogs: a retrospective study of 393 cases. Vet Pathol. 2010;47:387-95.
25. Ahmad Y, Gertz RE Jr, Li Z, et al. Genetic relationships deduced from *emm* and multilocus sequence typing of invasive *Streptococcus dysgalactiae* subsp. *equisimilis* and *S. canis* recovered from isolates collected in the United States. J Clin Microbiol. 2009;47:2046-54.
26. Pedersen K, Pedersen K, Jensen H, et al. Occurrence of antimicrobial resistance in bacteria from diagnostic samples from dogs. J Antimicrob Chemother. 2007;60:775-81.
27. Pinho MD, Matos SC, Pomba C, et al. Multilocus sequence analysis of *Streptococcus canis* confirms the zoonotic origin of human infections and reveals genetic exchange with *Streptococcus dysgalactiae* subsp. *equisimilis*. J Clin Microbiol. 2013;51:1099-109.
28. The AMR One Health Surveillance Committee. Nippon AMR One Health Report (NAOR) 2017. Tokyo: Tuberculosis and Infectious Diseases Control Division, Health Service Bureau, Ministry of Health, Labour and Welfare; 2017.