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Note

Bacteria isolated from companion animals in Japan (2014–2016) by blood culture

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ABSTRACT

We aimed to identify microorganisms isolated by blood culture (BC) from companion animals and to determine antimicrobial resistance of these isolates during 2014–2016 at veterinary laboratory, in comparison with those during 2010–2013, in Japan. Clinical data (animal species, visiting animals/hospitalized animals, and others except for disease type and clinical course including history of antimicrobial agent use) on ill animals at veterinary clinics or hospitals were obtained. We retrospectively analyzed animal-origin BC results extracted from the database in 2014–2016 and those obtained in 2010–2013. BC-positive samples were from most of dogs ($n = 174$ in 2014–2016 and $n = 86$ in 2010–2013). *Escherichia coli* ($n = 50$, 25.1%) and *Staphylococcus intermedius* group (SIG) bacteria ($n = 23$, 11.6%) were most prevalent in 2014–2016, while the percentages of *E. coli* ($n = 22$, 25.3%) and SIG ($n = 9$, 10.3%) in 2010–2013 were similar to those in 2014–2016. Percentages of extended-spectrum β-lactamase (ESBL)-producing *E. coli* and methicillin-resistant staphylococci (MRS) rate of SIG bacteria isolated in 2014–2016 were 28.0% and 69.6% (vs. 22.7% and 44.4% in 2010–2013), respectively. Fourteen ESBL-producing *E. coli* in 2014–2016 were isolated from 7 visiting animals and 7 hospitalized ones, whereas the sixteen MRS of SIG were from 7 visiting animals and 9 hospitalized ones. Our observations support the prevalent microorganisms isolated by BC and their antimicrobial resistance patterns for two study periods.

1. Text

The Clinical and Laboratory Standards Institute (CLSI) [1,2] recommends using blood culture (BC) in clinical practice to detect invasive microorganisms. Although clinicians often perform BC on samples from patients, veterinarians rarely do this for ill companion animals (dogs and cats), because it is very difficult to collect an adequate blood volume (5–10 mL) for one culture bottle from such animals. To establish BC procedures for diseased animals, a Versa TREK system (Kohjin Bio Co., Ltd., Saitama, Japan) modified for culture bottles was introduced in Japan in 2010 to isolate aerobic and anaerobic bacteria from a smaller volume (0.1–1 mL) of blood.

Lee et al. [3] examined how many sets of BC bottles were needed to detect invasive microorganisms in human adult patients. The results of this study indicated that two sets from each sample obtained from patients during the first 24 h of disease onset was sufficient to detect bacteria in approximately 90% of these cases. Thus, multiple sets of BCs should be performed to detect blood-origin bacteria in clinical practice.

Many individuals in Japan keep animals in their homes. In addition, some hospitals and nursing homes have introduced animal-assisted therapy for mental health care of patients and elderly individuals. In addition, remarkable advances in veterinary medical technology have resulted in extending the lives of animals, especially household pets. According to the “White Paper on

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Household Animals 2016" [4], the average life span of household dogs was 13.7 years in Japan in 2014. Therefore, based on the "One Health" concept [5], BC-positive bacteria and ones that can be transmitted between humans and animals should be investigated to maintain the health of both pets and their owners.

It is important to determine the species and antimicrobial susceptibility of bacteria isolated by BC to diagnose and treat invasive infection in diseased companion animals. However, there have been few nation-wide studies based on the use of BC in veterinary practice in Japan. The purpose of this study was to determine differences in the species and antimicrobial resistance (AMR) of bacteria isolated by BC from animals in 2014–2016, in comparison with those isolated in 2010–2013, because there were requests for two sets of BC since 2014.

Pets were brought to either veterinary clinics or hospitals because of poor activity, appetite loss, chills, or abnormal respiration (i.e., tachypnea), which are suggestive of either sepsis or severe infection. To examine the causative bacterial agents in the blood specimens submitted by veterinary practitioners, the specimens were sent to the Sanritsu Zelkova Veterinary Laboratory and were immediately tested using the Versa TREK system (one set or more). Blood specimens were obtained from animals that showed significant signs and visited either veterinary clinics or hospitals nationwide from January 1, 2014 to December 31, 2016, and from January 1, 2010 to December 31, 2013. Clinical data (i.e., animal species, visiting animals/hospitalized animals, gender, age, collection date, and Japanese prefecture in which the practitioners worked except for disease type and clinical course including history of antimicrobial agent use) on these animals were also obtained. One set of BC bottles was defined to be both aerobic bottle and anaerobic one applied. The one set or more for BC from the same animal were counted as one request by the corresponding veterinarian.

When BCs were positive, we performed Gram-staining and the selective subcultures for aerobic and anaerobic bacteria, using the supernatants from positive cultures to identify the bacterial species. MicroScan WalkAway Plus System (Beckman Coulter, Inc., Tokyo, Japan) was applied as the automated identification system [6]. BCs were maintained for a maximum of 7 days, until bacteria were detected or samples were determined negative. Antimicrobial susceptibility testing was then performed using the broth microdilution method (MicroScan WalkAway Plus System) according to the CLSI guidelines [7,8]. For example, determination of extended-spectrum β-lactamase (ESBL)-producing in *E. coli* was based on the either 3-fold or more reduction of minimum inhibitory concentration (MIC) by clavulanic acid with either cefotaxime or ceftazidime as compared with that of cefotaxime or ceftazidime alone. Determination of methicillin-resistant staphylococci (MRS) was based on the ≥4 μg/mL of MIC by oxacillin in *S. aureus* and *S. lugdunensis* and the ≥0.5 μg/mL of MIC by oxacillin in *S. intermedius* group (SIG) and coagulase-negative staphylococci except for *S. lugdunensis*. Antimicrobial susceptibilities of enrofloxacin and orbifloxacin were determined using the disk diffusion method in accordance with the guidelines outlined in CLSI VET01-S2 [9,10]. The data on isolated blood-origin microorganisms and their antimicrobial susceptibility were entered into a database at the study institution. SIG consisting of *S. pseudintermedius*, *S. intermedius*, and *S. delfini*, was not able to be specifically distinguished because the three species exhibit the same biochemical properties [11]. Concordance of species identification of the staphylococci using automated identification, matrix-assisted laser desorption ionization-time of flight mass spectrometry, and different fragment of thermonuclease gene amplified [12] was confirmed with the isolates from the conventional sterile site (blood, joint fluid, ascites, and intestinal lymphnode) collected in 2017, because the isolates collected in 2014–2016 were not stored (see the Supplementary table & figure). We retrospectively compared the results from animals in 2014–2016 to those obtained in 2010–2013.

For the protection of the animals studied, the Ethics Committee of the Sanritsu Zelkova Veterinary Laboratory examined and approved the protocol before the study began.

Table 1
Speciation of microorganisms isolated from companion animals in Japan by blood culture.

Species identified	Number (%) in 2010–2013	Number (%) in 2014–2016
Enterobacteriaceae	33 (37.9)	79 (39.7)
Escherichia coli	22 (25.3)	50[a] [b] (25.1)
Serratia marcescens	4	9
Klebsiella pneumoniae	3	13[a] [b]
Klebsiella spp.		1
Enterobacter aerogenes	2	1
E. cloacae		1
Proteus mirabilis	1	1
Morganella morganii	1	
Kluyvera cryocrescens		1
Providencia rettgeri		1
Salmonella serogroup O4		1[a]
***Staphylococcus* spp.**	16 (18.4)	37 (18.6)
S. intermedius group	9 (10.3)	23[a] [b] (11.6)
S. aureus	3	4[a]
S. schleiferi	1	6[a]
Coagulase-negative staphylococci	2	3
S. epidermidis	1	1
***Enterococcus* spp.**	10 (11.5)	9 (4.5)
E. faecium	8	4[b]
E. faecalis	2	4[a]
Enterococcus spp.		1
***Streptococcus* spp.**	5 (5.7)	15 (7.5)
Group G streptococci	5	5[a]
Group C streptococci		2[a]
S. bovis		1
α-streptococci		3
γ-streptococci		3
Other *Streptococcus* spp.		1
Anaerobes	8 (9.2)	37 (18.6)
Bacteroides fragilis group	2	3[a]
B. vulgatus		2
Bacteroides spp.		2
Peptostreptococcus spp.	2	3
Clostridium perfringens	1	5[b]
Clostridium spp.	1	2
Fusobacterium spp.	1	3
Prevotella spp.	1	2
Actinomyces spp.		1
Propionibacterium acnes		12[b]
Lactobacillus spp.		1[b]
Eubacterium spp.		1
Non-fermentative microorganisms	5 (5.8)	9 (4.5)
Pseudomonas aeruginosa	3	7[a]
Acinetobacter spp.	1	1
Alcaligenes spp.	1	1
Others	10 (11.5)	13 (6.5)
Corynebacterium bovis		1
Corynebacterium spp.	6	5[b]
Pasteurella multocida	3	1
Pasteurella spp.		1
Campylobacter spp.		1
Agrobacterium spp.		2[b]
Aeromonas hydrophila		1
Bacillus spp.	1	1[b]
Total	87 (100)	199 (100)
Rate of bacterial isolation by blood culture (%)	22	21.1
Percentage of 2 sets of the culture bottles	0	4.6

The remaining set of the culture bottles was only one set applied.
The blood culture-positive samples were from most of dogs (n = 86 in 2010–2013 and n = 174 in 2014–2016).
[a] Of 2 sets of the culture bottles, both sets were positive.
[b] Of 2 sets of the culture bottles, one set alone was positive.

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