***In vitro* Activity of 24 Antimicrobial Agents against *Staphylococcus* and *Streptococcus* Isolated from Diseased Animals in Japan**

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ABSTRACT. A total of 88 *Staphylococcus* and 61 *Streptococcus* isolates from diseased animals throughout Japan were examined in 2000 for the minimum inhibitory concentrations of 24 different antimicrobials by the agar dilution method standardized by the Japanese Society of Chemotherapy. The resistance rates to aminobenzylpenicillin (36.4%) and benzylpenicillin (35.2%) were high in *Staphylococcus* isolates, and those to oxytetracycline (45.9%) and kanamycin (21.3%) were high in *Streptococcus* isolates. Two isolates resistant to oxacillin harbored the *mecA* gene. One was *Staphylococcus epidermidis* derived from a pig with arthritis, and the other *Staphylococcus cohnii* from a head of cattle with mastitis.

KEY WORDS: antimicrobial susceptibility, *Staphylococcus* spp., *Streptococcus* spp.

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Staphylococci and streptococci are prevalent among apparently healthy animals, and sometimes become causative agents of the inflammatory diseases such as mastitis, arthritis, pneumonia, and meningitis [1, 2, 7]. The health management is generally practiced for preventing diseases, and the major treatment of diseases is antimicrobial therapy with efficient antimicrobial agents. The antimicrobial use for therapy of bacterial infection leads to developing antimicrobial resistant bacteria. Particularly, emerging antimicrobial resistance of zoonotic organisms in domestic animals becomes potential public health threats throughout the world. In the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) system, several bacterial pathogens in diseased animals and some food-borne organisms [3, 4] and commensal organisms [9] in healthy domestic animals were monitored for antimicrobial susceptibility. In the present study, we describe the antimicrobial susceptibilities of *Staphylococcus* and *Streptococcus*, targeted bacterial species for the monitoring, isolated from diseased animals.

A total of 88 *Staphylococcus* and 61 *Streptococcus* isolates from clinical specimen of several origins were kindly supplied by Livestock Hygiene Service Centers around Japan in 2000 on the request for an isolate per case. Bacterial species and origins of isolates tested are shown in Table 1. All the isolates were stored in 10% skim milk at –80°C until use. The minimum inhibitory concentrations (MICs) of 24 different antimicrobials listed in Tables 2 and 3 were determined by the agar dilution method standardized by the Japanese Society of Chemotherapy [10]. Mueller Hinton Agar (MHA, Difco) was used for MIC determination for

staphylococci and MHA with 5% sheep blood for streptococci. The MIC of oxacillin (MIPIC) was determined in the respective agar containing 2% NaCl incubated at 35°C for 24 hr [11]. The resistance break point was determined on the basis of the MIC population of the isolates.

In staphylococci, the resistance rates to aminobenzylpenicillin (ABPC, 36.4%), benzylpenicillin (PCG, 35.2%), oxytetracycline (OTC, 17.0%), dihydrostreptomycin (DSM, 12.5%), and erythromycin (EM, 10.2%) were relatively high (Table 2). Those to the remaining drugs were lower than 10%. All isolates were susceptible to cephalosporins, chloramphenicol, and quinolone antibiotics except one isolate which was resistant to some quinolone antibiotics. As for streptococci, the resistance rates to OTC (45.9%) and kanamycin (KM, 21.3%) were high (Table 3). Four isolates were resistant to EM, two were to PCG, and the other two was to either ABPC or gentamicin (GM). All isolates were susceptible to cephalosporins and quinolone antibiotics. The resistance rates to PCG and ABPC were high in staphylococci, and so were to OTC or KM in streptococci.

In regard to animal species, the resistance rates to PCG and ABPC (30.4 and 31.9%, respectively) were high in *Staphylococcus* isolates from cattle, though OTC resistances were rare (4.3%). Yoshimura *et al.* [15] showed a similar trend in *Staphylococcus aureus* isolates from cattle. The multidrug-resistant (MDR) isolates accounted for 32.8% (21/61) and 25.6% (2/8) in *Staphylococcus aureus* and coagulase-negative staphylococci, respectively (Table 4). Eleven of the 23 MDR isolates were resistant to ABPC in the combination with OTC, EM, and/or some aminoglycoside antibiotics (DSM, KM, and GM). Besides, ABPC resistances were rare (4.3%) in *Streptococcus* isolates from cattle. The majority of *Staphylococcus* and *Streptococcus* isolates were derived from bovine mastitis (61/69 and 28/29, respectively). Generally, for mastitis caused by staphy-

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Table 1. Bacterial species used in this study and their origins

Genus	Species	Cattle	Swine	Poultry	Horse	Canine	Total
<i>Staphylococcus</i>	<i>S. aureus</i>	61	3	2			66
	<i>S. hyicus</i>	1	6	2			9
	<i>S. epidermidis</i>	1	1				2
	<i>S. xylosus</i>	2					2
	<i>S. lugdunensis</i>	1			1		2
	<i>S. chromogenes</i>	1	1				2
	<i>S. simulans</i>		1				1
	<i>S. haemolyticus</i>	1					1
	<i>S. cohnii</i>	1					1
	<i>S. sciuri</i>			1			1
	<i>S. delphini</i>					1	1
Subtotal		69	12	5	1	1	88
<i>Streptococcus</i>	<i>S. suis</i>	1	21				22
	<i>S. dysgalactiae</i>	8	3				11
	<i>S. agalactiae</i>	8	1				9
	<i>S. uberis</i>	7					7
	<i>S. bovis</i>	6		1			7
	<i>S. porcinus</i>		2				2
	<i>S. acidominimus</i>	1					1
	<i>S. mitis</i>	1					1
	<i>S. sp.</i>		1				1
Subtotal		32	28	1	0	0	61

Table 2. Antimicrobial susceptibilities of staphylococci isolates of animal origin

Antimicrobial agents	MIC for staphylococci			Resistance Breakpoint (mg/l)	No. of resistance (%)			
	range (mg/l)	50%	90%		Cattle(n=69)	Swine(n=12)	Others(n=7)	Total(n=88)
Benzylpenicillin	≤0.05–6.25	≤0.05	1.56	≥0.39	21(30.4)	7(58.3)	3(42.9)	31(35.2)
Aminobenzylpenicillin	≤0.05–6.25	0.10	1.56	≥0.39	22(31.9)	7(58.3)	3(42.9)	32(36.4)
Nafcillin	≤0.05–1.56	0.20	0.20					
Oxacillin	≤0.05–50	0.39	0.39	≥3.13	1(1.4)	1(8.3)	0(0)	2(2.3)
Cloxacillin	0.1–3.13	0.39	0.39					
Cefazolin	0.1–1.56	0.39	0.39					
Cefuroxime	0.1–6.25	0.78	1.56					
Ceftiofur	0.1–6.25	0.78	1.56					
Cefquinome	≤0.05–1.56	0.39	0.78					
Dihydrostreptomycin	0.78–100<	3.13	>100	≥25	6(8.7)	4(33.3)	1(14.3)	11(12.5)
Gentamycin	≤0.05–100<	0.20	0.39	≥3.13	4(5.8)	0(0)	0(0)	4(4.5)
Kanamycin	0.2–100<	0.78	1.56	≥12.5	4(5.8)	3(25.0)	0(0)	7(8.0)
Erythromycin	0.2–100<	0.39	0.39	≥3.13	2(2.9)	6(50.0)	1(14.3)	9(10.2)
Oxytetracycline	0.2–100<	0.39	50	≥6.25	3(4.3)	8(66.7)	4(57.1)	15(17.0)
Chloramphenicol	1.56–12.5	6.25	6.25					
Nalidixic acid	12.5–100<	50	50					
Oxolinic acid	0.78–100	1.56	6.25	≥25	0(0)	1(8.3)	0(0)	1(1.1)
Benofloxacin	0.2–100	0.39	0.78	≥6.25	0(0)	1(8.3)	0(0)	1(1.1)
Difloxacin	0.2–100	0.20	0.39	≥6.25	0(0)	1(8.3)	0(0)	1(1.1)
Danofloxacin	0.1–100	0.20	0.39	≥3.13	0(0)	1(8.3)	0(0)	1(1.1)
Enrofloxacin	≤0.05–100	0.10	0.20	≥6.25	0(0)	1(8.3)	0(0)	1(1.1)
Orbifloxacin	0.2–50	0.39	0.78	≥6.25	0(0)	1(8.3)	0(0)	1(1.1)
Ofloxacin	0.2–25	0.39	0.39	≥3.13	0(0)	1(8.3)	0(0)	1(1.1)
Sulphadimethoxine	0.78–100<	50	>100					
Some of antimicrobials					25(36.2)	10(83.3)	4(57.1)	39(44.3)

lococci or streptococci, penicillin or tetracycline was administered therapeutically. These results suggest that the veterinarians have to pay attention to multidrug-resistant bacteria in choosing the therapeutic antimicrobials, espe-

cially in mastitis due to staphylococci.

In porcine isolates, *Staphylococci* resistant to PCG (58.3%), ABPC (58.3%), DSM (33.3%), KM (25.0%), EM (50.0%) or OTC (66.7%) were highly prevalent. All porcine

Table 3. Antimicrobial susceptibilities of streptococci isolates of animal origin

Antimicrobial agents	MIC for streptococci			Resistance Breakpoint (mg/l)	No. of resistance (%)			
	range (mg/l)	50%	90%		Cattle(n=32)	Swine(n=28)	Others(n=1)	Total(n=61)
Benzylpenicillin	≤0.05–1.56	≤0.05	0.1	≥0.39	1(3.2)	1(3.6)	0(0)	2(3.3)
Aminobenzylpenicillin	≤0.05–0.78	≤0.05	0.1	≥0.39	1(3.2)	0(0)	0(0)	1(1.6)
Nafcilline	≤0.05–1.56	≤0.05	0.2					
Oxacilline	≤0.05–1.56	0.39	0.39					
Cloxacilline	≤0.05–6.25	0.78	1.56					
Cefazolin	≤0.05–0.78	0.1	0.2					
Cefuroxime	≤0.05–0.78	≤0.05	0.1					
Ceftiofur	≤0.05–3.13	0.1	0.39					
Cefquinome	≤0.05–0.39	≤0.05	0.1					
Dihydrostreptomycin	1.56–100<	25	>100					
Gentamycin	≤0.05–100	1.56	6.25	≥50	1(3.2)	1(3.6)	0(0)	1(1.6)
Kanamycin	0.78–100<	12.5	100	≥50	12(37.5)	1(3.6)	0(0)	13(21.3)
Erythromycin	≤0.05–100<	0.1	6.25	≥50	0(0)	4(14.3)	0(0)	4(6.6)
Oxytetracycline	≤0.05–25	1.56	25	≥3.13	8(25.0)	20(71.4)	0(0)	28(45.9)
Chloramphenicol	0.39–12.5	1.56	3.13					
Nalidixic acid	50–100<	>100	>100					
Oxolinic acid	1.56–100<	50	100					
Benofloxacin	0.39–6.25	1.56	1.56					
Difloxacin	0.2–6.25	1.56	3.13					
Danofloxacin	0.1–6.25	0.78	0.78					
Enrofloxacin	≤0.05–1.56	0.39	0.78					
Orbifloxacin	0.39–3.13	1.56	3.13					
Ofloxacin	0.39–3.13	0.78	1.56					
Sulphadimethoxine	25–100<	>100	>100					
Some of antimicrobials					16(50.0)	25(89.3)	0(0)	41 (67.2)

Table 4. Antimicrobial resistance types of staphylococci isolates from diseased animals

Antimicrobial resistance types*	<i>S. aureus.</i>			Coagulase-negative staphylococci		
	Cattle	Swine	others	Cattle	Swine	others
DSM	1	0	0	0	0	0
OTC	1	0	0	0	0	1
PCG-ABPC	12	0	0	0	0	0
ABPC-EM	1	0	0	0	0	0
EM-OTC	0	1	0	1	1	0
DSM-KM-EM-OTC	0	0	0	0	1	0
PCG-ABPC-OTC	0	0	1	0	2	0
PCG-ABPC-DSM	4	1	0	0	1	0
PCG-ABPC-GM-KM	4	0	0	0	0	0
PCG-ABPC-OTC-EM	0	1	0	0	0	1
PCG-ABPC-DSM-OTC	0	0	1	0	0	0
PCG-ABPC-OTC-DSM-KM-EM	0	0	0	0	1	0
PCG-ABPC-MPIPC-DSM-OTC	0	0	0	1	0	0
PCG-ABPC-MPIPC-KM-OTC-OA-EM-FQs	0	0	0	0	1	0
Susceptible	38	0	0	6	2	3

* Abbreviations: PCG, benzylpenicillin; ABPC, aminobenzylpenicillin; MPIPC, oxacilline; DSM, dihydrostreptomycin; GM, gentamycin; KM, kanamycin; EM, erythromycin; OTC, oxytetracycline; OA, oxolinic acid; FQs, benofloxacin, danofloxacin, difloxacin, enrofloxacin, ofloxacin and orbifloxacin.

isolates were MDR except two susceptible isolates (Table 4). On the contrary, *Streptococcus* isolates resistant to OTC (71.4%) or EM (14.3%) were prevalent, whereas PCG-, ABPC-, DSM-, or KM-resistant isolates were rare in pigs. Previous investigations showed that tetracycline (86.9%), KM (71.4%), and streptomycin (29.5%) resistance were highly prevalent among 689 *Streptococcus suis* isolates from pigs in 1987 through 1996 [6]. Though the break point

of DSM could not be defined in this study, most of the isolates showed high MIC value of DSM (≥25 µg/ml). The present results were similar to the previous ones expect for KM resistance. Furthermore, high prevalence of OTC resistance in staphylococci and streptococci may be associated with the frequent use of tetracycline antibiotics in pig industry (13).

Finally, two isolates were resistant to MPIPC (25 and 50

$\mu\text{g/ml}$). One of them derived from a pig with arthritis was *Staphylococcus epidermidis*, and the other from cattle with mastitis was *Staphylococcus cohnii*. The presence of the *mecA* gene was examined by the PCR method described previously [8]. As the isolates were positive for PCR detecting *mecA* (data not shown), both the isolates were identified as methicillin-resistant coagulase-negative staphylococci (MRCNS). These MRCNS isolates were susceptible to vancomycin (MIC 0.78 or 1.56 $\mu\text{g/ml}$). The MRCNS isolate from a pig was resistant to PC, ABPC, KM, EM, OTC, OA and all the fluoroquinolones tested. The pig had been treated with ABPC, OTC, danofloxacin, and tylosin. Though the MRCNS isolate may be associated with the antimicrobial treatment, *S. epidermidis* is the most common MRCNS among human isolates (5). Besides, the MRCNS isolate from cattle was resistant to PC, ABPC, DSM, and OTC, and the animal had been treated with cefazolin. As the isolate was susceptible to cephalosporins, the antimicrobial treatment did not play any role in selection pressure. In any case, though the origin of *mecA* is not clear now, the staphylococci harboring *mecA* was prevalent among not only horses [2, 12, 14] and chickens [8], but also pigs and cattle in Japan.

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REFERENCES

- Asai, T., Niwa, K., Jinnouti, T., Mori, M. and Suzuki, G. 1996. *J. Jpn. Vet. Med. Assoc.* **49**: 666–668.
- Anzai, T., Kamada, M., Kaneramaru, T., Sugita, S., Shimizu, A. and Higuchi, T. 1996. *J. Equine Sci.* **7**: 7–11.
- Esaki, H., Morioka, A., Ishihara, K., Kojima, A., Shiroki, S., Tamura, Y. and Takahashi, T. 2004. *J. Antimicrob. Chemother.* **53**: 266–270.
- Ishihara, K., Kira, T., Ogikubo, K., Morioka, A., Kojima, A., Kijima-Tanaka, M., Takahashi, T. and Tamura, Y. 2004. *Int. J. Antimicro. Agents* **24**: 63–69.
- Ito, T., Kuwahara, K., Hisata, K., Okuma, K., Cui, L. and Hiramatsu, K. 2004. *J. Jpn. Assoc. Infect. Dis.* **78**: 459–469.
- Kataoka, Y., Yoshida, T. and Sawada, T. 2000. *J. Vet. Med. Sci.* **62**: 1053–1057.
- Kataoka, Y., Sugimoto, C., Nakazawa, M., Morozumi, T. and Kashiwazaki, M. 1993. *J. Vet. Med. Sci.* **55**: 623–626.
- Kawano, J., Shimizu, A., Saitoh, Y., Yagi, M., Saito, T. and Okamoto, R. 1996. *J. Clin. Microbiol.* **34**: 2072–2077.
- Kijima-Tanaka, M., Ishihara, K., Morioka, A., Kojima, A., Ohzono, T., Ogikubo, K., Takahashi, T. and Tamura, Y. 2003. *J. Antimicrob. Chemother.* **51**: 447–451.
- Mitsuhashi, S., Goto, S., Jo, K., Kawata, T., Kozaki, N., Nishino, T., Ogawa, N. and Tanami, H. 1981. *Chemotherapy (Jpn.)* **29**: 76–79.
- National Committee for Clinical Laboratory Standards. 2002. 2nd ed, Approved standard. NCCLS document M31-A2. National Committee for Clinical Laboratory Standards, Villanova.
- Shimizu, A., Kawano, J., Yamamoto, C., Kakutani, O., Anzai, T. and Kamada, M. 1997. *J. Vet. Med. Sci.* **59**: 935–937.
- Tamura, Y. 2003. Trends in Antimicrobial agents for Veterinary Use and Control Measures of Antimicrobial Resistance. *J. Jpn. Vet. Med. Assoc.* **56**: 685–691.
- Yasuda, R., Kawano, J., Onda, H., Takagi, M., Shimizu, A. and Anzai, T. 2000. *Am. J. Vet. Res.* **61**: 1451–1455.
- Yoshimura, H., Ishimaru, M., and Kojima, A. 2002. *J. Vet. Med. B Infect. Dis. Vet. Public Health* **49**: 457–460.