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Isolation of Bacteria from Clinical Specimens in Veterinary Medical Teaching Hospital and Trend of Antimicrobial Susceptibility

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Abstract : Bacteria that are resistant to several different groups of antibiotics have increased during the past few years. The importance of surveillance of antimicrobial resistance is now widely recognized. Unfortunately, this development has not been documented continuously in veterinary medicine in Korea. Therefore, the clinical prevalence and trend of antimicrobial susceptibility of aerobic isolates were investigated in this study. Total 121 isolates of aerobic bacteria were isolated from clinical specimens of dogs and cats at Veterinary Medical Teaching Hospital of Seoul National University from May 2001 to October 2002. Among them, the most common isolated species was *Staphylococcus* spp. (48 isolates), followed by *E.coli* (26 isolates), *Enterococcus* spp. (21 isolates), *Klebsiella pneumoniae* (9 isolates), *Streptococcus* spp. (6 isolates), *Enterobacter cloacae* (3 isolates), *Pseudomonas aeruginosa* (3 isolates), *Corynebacterium xerosis* (2 isolates), *Chryseomonas* spp. (2 isolates), and *Providencia stuartii* (1 isolate). The susceptibility of isolates to antibiotics was determined by the disk diffusion method. Gram-positive bacterial isolates were showed high susceptibilities to amikacin, amoxacillin/clavulanate, ceftazidime, and oxacillin, while Gram-negative bacterial isolates were showed high susceptibilities to amikacin and ceftazidime. *Staphylococcus* spp. were showed high susceptibilities to amikacin, amoxicillin/clavulanate, ceftazidime, cephalothin, and oxacillin. *Streptococcus* spp. and *E.coli* were showed high susceptibilities to amikacin and ceftazidime. Of the 48 staphylococci, seven Methicillin Resistant staphylococci were observed (14.6%), distributed among *S. auricularis* (1), *S. hemolyticus* (2), *S. sciuri* (1), *S. saprophyticus* (1), *S. warneri* (2) isolates. One strain of *E.coli* and one strain of *Corynebacterium xerosis* were resistant to all antibiotics tested. And, resistance trends between the year 2000 (from July 1999 to September 2000) and 2002 (from May 2001 to October 2002) were compared. Resistance to antibiotics was increased in both Gram-positive and Gram-negative bacterial isolates ($p < 0.05$). The resistance rates of *Staphylococcus* spp., *E.coli* and *Klebsiella pneumoniae* to all antibiotics tested were also increased ($p < 0.05$). This study investigated increasing resistance between the year 2000 and 2002 in Veterinary Medical Teaching Hospital of Seoul National University. Surveillance resistance is helpful to alert to veterinarian and select of appropriate therapy. Antimicrobial susceptibility surveillance of isolates should urgently be continued in veterinary medicine.

Key words **:** antimicrobial susceptibility, aerobic bacteria, dog, cat

Introduction

Antibiotics are essential in the therapy of animal diseases, especially in the bacterial infections. Many veterinarians have overdosed and misused antibiotics without antimicrobial susceptibility test. It may contribute to the emergence of resistant strains of bacteria[2]. In addition, inappropriate antibiotic using has also contributed to the emergence of multi-drug resistant bacteria[14]. Antimicrobial resistance may be transferred between bacteria by plasmids, tranposons, or insertion-sequence mechanisms[16]. Transferable plasmids may possess genes encoding resistance to antibiotics. Thus, a single transfer can result in the acquisition of several antimicrobial resistance determinants[8]. The emergence of bacterial resistance to antibiotics during therapy is always a problem of great concern in clinical medicine.

Most of all animal patients who came to Veterinary medical Teaching Hospital of Seoul National University already have been received antibiotics therapy several times without the susceptibility test. However, the detailed information of antibiotic therapy prior to treatment in this hospital could not be obtained. In practice, many veterinarians tend to rely on clinical experience and use antibiotics with which they are familiar, or often broad-spectrum antibiotics, which are cheaper and have fewer side effects. However, the effectiveness of antibiotics may be decreased, due to the development of resistance. This is the reason that antimicrobial susceptibility test is more emphasized. Many factors influence antibiotic susceptibilities, and the continuous testing has been needed to monitor the susceptibility trend[9].

In this study, we report aerobic bacteria isolated from animal patients in Veterinary Medical Teaching Hospital of Seoul National University as well as susceptibility pattern. And, this study shows the comparison of aerobic bacterial antibiotics resistant rates between the year 2000 (from July 1999 to September 2000) and 2002 (from May 2001 to October 2002).

The purpose of this study is to investigate changes in susceptibility of aerobic bacterial isolates to antibiotics.

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Materials and methods

Bacterial isolates

Aerobic bacteria were isolated from clinical specimens including dogs and cats at Veterinary Medical Teaching Hospital of Seoul National University from May 2001 to October 2002. Clinical specimens in culturing aerobic bacteria were swabs of purulent material, pus, draining tracts, lower respiratory tract, nasal discharge, peritonial aspirates, ear, eye, urine, postoperative infection site, and urogenital system. Tracheal washing and nasal discharge were collected from the dog with chronic respiratory tract infection.

Clinical specimens were collected by the swab method using Amies transport medium (BBL Culture swab plus®: BBL Microbiology System, MD, USA). Liquid specimen which was collected by the needle method was plated directly on the agar. Urine specimens were collected by cystocentesis which was sterile[7,12].

Conditions of aerobic culture

Media for initial processing were blood agar containing w/v pr v/v and MacConkey agar. Swabs were inoculated onto blood agar and onto MacConkey agar (KOMED, Korea). The plates were incubated at 37°C for 24 hrs aerobically. Characteristics of colony on blood agar or on MacConkey agar were recorded. Selecting after predominant colony on blood agar was selected, Gram stain was used to classified the bacterial colonies according to the stain pattern, Gram staining, catalase test, oxidase test, and analysis with GNI and GPI card by VITEK system (bio Merieux Vitek, Hazelwood, MO, USA). Isolates were stored at -70°C in 15% glycerol broth until used.

Antimicrobial susceptibility test

Briefly, a colony was selected from the blood agar (KOMED, Korea), and then transferred to the Muller-Hinton broth. This broth was incubated at 37°C until it exceeded the turbidity of a 0.5 McFarland standard. The turbidity was adjusted with sterile saline to a density of a 0.5 McFarland standard (approx. 1×10^5 organism per ml). A sterile swab was dipped into the adjusted suspension, and inoculated the Muller-Hinton agar (KOMED, Korea) by streaking the swab over the entire agar surface. This procedure was repeated 5 times, as rotating the plate. The disks were placed on the surface of the agar with Disk Sensi (Becton Dickinson Microbiology System, MD, USA.[1] Antibiotics used were amikacin (30 μg), ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (10 μg), neomycin (30 μg), penicillin G (10 unit), amoxacillin/clavulanic acid (20/10 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), cephalothin (30 μg), ceftazidime (30 μg), clindamycin (2 μg), trimethoprim-sulfa (23.75/1.25 μg), and oxacillin (1 μg). The plate was inverted and placed it in an incubator at 37°C aerobically. After 24 hrs, the diameters of the zones of complete inhibition were measured.

Statistical analysis

The comparison of resistant rates between the year 2000 (from July 1999 to September 2001) and 2002 (from May to October 2002) was analyzed by χ^2 test with 95% reliability.

Data of the year 2000 were obtained from the dissertation for the degree of master by Hee Yoo, which was published in Seoul National University on Feb. 2000.

Results

Aerobic bacteria isolated from clinical specimens

One hundred twenty one isolates were obtained from 118 dogs and 3 cats. These were collected from skin, eye, ear, urine, postoperative infection site, genital system, tracheal washing, wound, and nasal discharge (Table 1).

As shown in table 2, the most common isolated species was *Staphylococcus* spp. (48 isolates), followed by *E.coli* (26 isolates), *Enterococcus* spp. (21 isolates), *Klebsiella pneumo-*

Table 1. Isolated aerobic bacteria by the sampling sites

Aerobic bacteria	skin	eye	urine	ear	post operative	genital system	tracheal washing	wound	nasal discharge
Staphylococcus spp.	26	10	4	3	1	1	2	1	
E.coli	4	5	3	1	3	4	2	1	3
Enterococcus spp.	10	4	1	4	1			1	
Klebsiella pneumoniae		1	4	1	1		1		1
Streptococcus spp.	3	1		1	1				
Pseudomonas aeruginosa		1	1					1	
Enterobacter cloacae	1		1			1			
Chryseomonas spp.	1			1					
Corynebacterium xerosis	2								
Providencia stuartii				1					
Total	47	22	14	12	7	6	5	4	4

Table 2. Aerobic bacteria from clinical specimens

Organism		No. of Isolates
Staphylococcus spp.		48
	S. simulans	15
	S. epidermidis	9
	S. haemolyticus	8
	S. warneri	8
	S. auricularis	5
	S. sciuri	2
	S. saprophytica	1
Streptococcus spp.		6
	S. agalactiae	5
	S. uberis	1
Enterococcus spp.		21
	E. faecalis	11
	E. gallinarum	7
	E. faecium	3
E. coli		26
Klebsiella pneumoniae		9
Enterobacter cloacae		3
Pseudomonas aeruginosa		3
Corynebacterium xerosis		2
Chryseomonas spp.		2
	C. indologenes	1
	C. luteola	1
Providencia stuartii		1

niae (9 isolates), *Streptococcus* spp. (6 isolates), *Enterobacter cloacae* (3 isolates), *Pseudomonas aeruginosa* (3 isolates), *Corynebacterium xerosis* (2 isolates), *Chryseomonas* spp. (2 isolates), and *Providencia stuartii* (1 isolate). Among staphylococci, the most common isolated species *S. simulans* (15 isolates), followed by *S. epidermidis* (9 isolates), *S. hemolyticus* (8 isolates), *S. warneri* (8 isolates) and so forth. And, *Klebsiella pneumoniae* and *Staphylococcus* spp. were common isolates in urine samples.

Antimicrobial susceptibility of aerobic bacterial isolates

Gram-positive bacterial isolates susceptibility patterns are shown in Table 3. Gram-positive bacterial isolates were highly susceptible to amikacin (81.4%), amoxacillin/clavulanate (74.7%), ceftazidime (74.7%), and oxacillin (72.0%). This result indicated that amikacin, amoxacillin-clavulanate, ceftazidime, and oxacillin were relatively effective against Gram-positive bacterial isolates.

Gram-negative bacterial isolates were highly susceptible to amikacin (77.1%) and ceftazidime (82.9%) (Table 4). This data also indicated that amikacin and ceftazidime were relatively effective against Gram-negative bacterial isolates.

However, susceptibility patterns of individual isolated species were variable (Tables 3 and 4). *Staphylococcus* spp. showed

Table 3. Antimicrobial susceptibility of the isolates to antibiotics

	Staphylococcus spp. (n=48)			*Streptococcus* spp. (n=6)			*Enterococcus* spp. (n=21)			Total (n=75)		
	R	I	S	R	I	S	R	I	S	R	I	S
Amikacin	0	0	48	1	0	5	12	1	8	13	1	61
	(0.0)	(0.0)	(100.0)	(16.7)	(0.0)	(83.3)	(57.1)	(4.8)	(38.1)	(17.3)	(1.3)	(81.3)
Amoxacillin/	8	0	40	2	1	3	7	1	13	17	2	56
clavulanate	(16.7)	(0.0)	(83.3)	(33.3)	(16.7)	(50.0)	(33.3)	(4.8)	(61.9)	(22.7)	(2.7)	(74.7)
ampicillin	32	0	16	3	1	2	8	0	13	43	1	31
	(66.7)	(0.0)	(33.3)	(50.0)	(16.7)	(33.3)	(38.1)	(0.0)	(61.9)	(57.3)	(1.3)	(41.3)
Ceftazidime	7	1	40	1	0	5	9	1	11	17	2	56
	(14.6)	(2.1)	(83.3)	(16.7)	(0.0)	(83.3)	(42.9)	(4.8)	(52.4)	(22.7)	(2.7)	(74.7)
Cephalothin	8	0	40	3	0	3	16	1	4	27	1	47
	(16.7)	(0.0)	(83.3)	(50.0)	(0.0)	(50.0)	(76.2)	(4.8)	(19.0)	(36.0)	(1.3)	(62.7)
Chloramphenicol	15	1	32	3	0	3	9	1	11	27	2	46
	(31.3)	(2.1)	(66.7)	(50.0)	(0.0)	(50.0)	(42.9)	(4.8)	(52.4)	(36.0)	(2.7)	(61.3)
Ciprofloxacin	16	0	32	4	0	2	10	1	10	30	1	44
	(33.3)	(0.0)	(66.7)	(66.7)	(0.0)	(33.3)	(47.6)	(4.8)	(47.6)	(40.0)	(1.3)	(58.7)
Erythromycin	28	0	20	5	0	1	15	2	4	48	2	25
	(58.3)	(0.0)	(41.7)	(83.3)	(0.0)	(16.7)	(71.4)	(9.5)	(19.0)	(64.0)	(2.7)	(33.3)
Gemtamicin	13	4	31	2	2	2	15	0	6	30	6	39
	(27.1)	(8.3)	(64.6)	(33.3)	(33.3)	(33.3)	(71.4)	(0.0)	(28.6)	(40.0)	(8.0)	(52.0)
Neomycin	6	10	32	1	2	3	12	3	6	19	15	41
	(12.5)	(20.8)	(66.7)	(16.7)	(33.3)	(50.0)	(57.1)	(14.3)	(28.6)	(25.3)	(20.0)	(54.7)
Norfloxacin	15	2	31	4	0	2	11	3	7	30	5	40
	(31.3)	(4.2)	(64.6)	(66.7)	(0.0)	(33.3)	(52.4)	(14.3)	(33.3)	(40.0)	(6.7)	(53.3)
Penicillin G	36	0	12	4	0	2	10	0	11	50	0	25
	(75.0)	(0.0)	(25.0)	(66.7)	(0.0)	(33.3)	(47.6)	(0.0)	(52.4)	(66.7)	(0.0)	(33.3)
Oxacillin	7	0	41	2	0	4	12	0	9	21	0	54
	(14.6)	(0.0)	(85.4)	(33.3)	(0.0)	(66.7)	(57.1)	(0.0)	(42.9)	(28.0)	(0.0)	(72.0)
Timethoprim-	28	0	20	4	0	2	12	0	9	44	0	31
sulfa	(58.3)	(0.0)	(41.7)	(66.7)	(0.0)	(33.3)	(57.1)	(0.0)	(42.9)	(58.7)	(0.0)	(41.3)
clindamycin	28	2	18	4	0	2	17	0	4	49	2	24
	(58.3)	(4.2)	(37.5)	(66.7)	(0.0)	(33.3)	(81.0)	(0.0)	(19.0)	(65.3)	(2.7)	(32.0)

( ) : %, R : resistant, I : intermediate, S : susceptible

Table 4. Antimicrobial susceptibility of the isolates to antibiotics

	E.coli (n=26)			*Klebsiella pneumoniae* (n=9)			Total (n=35)		
	R	I	S	R	I	S	R	I	S
Amikacin	3	0	23	5	0	4	8	0	27
	(11.5)	(0.0)	(88.5)	(55.6)	(0.0)	(44.4)	(22.9)	(0.0)	(77.1)
Amoxacillin/clavulanate	9	3	14	5	1	3	14	4	17
	(34.6)	(11.5)	(53.8)	(55.6)	(11.1)	(33.3)	(40.0)	(11.4)	(48.6)
Ampicillin	20	0	6	9	0	0	29	0	6
	(76.9)	(0.0)	(23.1)	(100.0)	(0.0)	(0.0)	(82.9)	(0.0)	(17.1)
Ceftazidime	1	2	23	3	0	6	4	2	29
	(3.8)	(7.7)	(88.5)	(33.3)	(0.0)	(66.7)	(11.4)	(5.7)	(82.9)
Cephalothin	13	3	10	7	0	2	20	3	12
	(50.0)	(11.5)	(38.5)	(77.8)	(0.0)	(22.2)	(57.1)	(8.6)	(34.3)
Chloramphenicol	11	0	15	5	0	4	16	0	19
	(42.3)	(0.0)	(57.7)	(55.6)	(0.0)	(44.4)	(45.7)	(0.0)	(54.3)
Ciprofloxacin	14	1	11	7	0	2	21	1	13
	(53.8)	(3.8)	(42.3)	(77.8)	(0.0)	(22.2)	(60.0)	(2.9)	(37.1)
Erythromycin	21	0	5	8	0	1	29	0	6
	(80.8)	(0.0)	(19.2)	(88.9)	(0.0)	(11.1)	(82.9)	(0.0)	(17.1)
Gemtamicin	14	1	11	6	1	2	20	2	13
	(53.8)	(3.8)	(42.3)	(66.7)	(11.1)	(22.2)	(57.1)	(5.7)	(37.1)
Neomycin	10	3	13	4	2	3	14	5	16
	(38.5)	(11.5)	(50.0)	(44.4)	(22.2)	(33.3)	(40.0)	(14.3)	(45.7)
Norfloxacin	14	0	12	7	0	2	21	0	14
	(53.8)	(0.0)	(46.2)	(77.8)	(0.0)	(22.2)	(60.0)	(0.0)	(40.0)
Penicillin G	21	0	5	9	0	0	30	0	5
	(80.8)	(0.0)	(19.2)	(100.0)	(0.0)	(0.0)	(85.7)	(0.0)	(14.3)
Oxacillin	17	0	9	8	0	1	25	0	10
	(65.4)	(0.0)	(34.6)	(88.9)	(0.0)	(11.1)	(71.4)	(0.0)	(28.6)
Timethoprim-sulfa	17	0	9	6	0	3	23	0	12
	(65.4)	(0.0)	(34.6)	(66.7)	(0.0)	(33.3)	(65.7)	(0.0)	(34.3)
Clindamycin	19	0	7	8	0	1	27	0	8
	(73.1)	(0.0)	(26.9)	(88.9)	(0.0)	(11.1)	(77.1)	(0.0)	(22.9)

( ) : %, R : resistant, I : intermediate, S : susceptible

high susceptibilities to amikacin (100%), amoxacillin/clavulanate (83.3%), ceftazidime (83.3%), cephalothin (83.3%), and oxacillin (85.4%). *Streptococcus* spp. showed high susceptibilities to amkacin (83.3%) and ceftazidime (83.3%). *E.coli* showed high susceptibilities to amkacin (88.5%) and ceftazidime (88.5%). However, the susceptibility rates of *Enterococcus* spp. and *Klebsiella pneumoniae* to tested antibiotics were less than 70%.

Moreover, 69 (57%) in total 121 isolates were resistant to 5 or more antibiotics (Table 5). Among them, 26 isolates (54%) in staphylococci, 4 isolates (67%) in *Streptococcus* spp., 19 isolates (73%) in *E.coli*, 8 isolates (89%) in *Klebsiella pneumoniae*, 3 isolates (100%) in *Pseudomonas aeruginosa*, and 1 isolate (50%) in *Corynebacterium xerosis* showed multi-drug resistance (> 5 antibiotics).

And, 7 isolates in staphylococci were resistant to oxacillin, distributed among *S. auricularis* (1), *S. hemolyticus* (2), *S. sciuri* (1), *S. saprophyticus* (1), *S. warneri* (2) isolates. Six isolates of these Methicillin Resistant staphylococci showed multi-drug resistance (> 10 antibiotics) (Table 6). Additionally, one strain of *E.coli* and one strain of *Corynebacterium xerosis* were resistant to all tested antibiotics.

The comparison of resistance rates between the year 2000 and 2002

The susceptibility trend of tested antibiotics to Gram positive bacterial isolates was variable(Fig 1). The resistant rates of Gram positive bacterial isolates to amoxacillin/clavulanate, cephalothin, ciprofloxacin, oxacillin, trimethoprim-sulfa, and clindamycin were increased, while the resistant rates of penicillin G (19.5%) and ampicillin (23.3%) were decreased. The resistant rates of all tested antibiotics against Gram-positive isolates were increased (12.7%).

In Gram-negative bacterial isolates, the efficacy of all tested

Table 5. Distribution and number of multi-drug resistant isolates

	No. of resistant antibiotics										
	15	14	13	12	11	10	9	8	7	6	5
Staphylococcus spp. (n=48)	0	0	2	3	1	2	1	5	1	4	7
Streptococcus spp. (n=6)	0	1	1	0	0	0	1	0	0	1	0
Enterococcus spp. (n=21)	0	2	3	1	0	0	1	0	0	0	1
E.coli (n=26)	1	1	1	2	3	2	7	0	1	1	0
Klebsiella pneumoniae (n=9)	0	2	3	1	0	0	1	0	0	0	1
Pseudomonas aeruginosa (n=3)	0	0	0	1	0	2	0	0	0	0	0
Corynebacterium xerosis (n=2)	1	0	0	0	0	0	0	0	0	0	0

Table 6. Multi-drug resistant isolates (> 10 antibiotics)

	AN	AMC	AM	CAZ	CF	C	CIP	E	GM	N	NOR	P	OX	TxS	CC	note*
Staphylococcus spp.																
S.hemolyticus		R	R	R	R		R	R	R	R	R	R	R	R	R	13
S.hemolyticus		R	R	R		R	R	R			R	R	R	R	R	11
S.hemolyticus			R			R	R	R	R	R	R	R		R	R	10
S.saprophytica		R	R	R	R	R	R	R	R		R	R	R	R	R	13
S.sciuri		R	R	R	R	R		R	R	R		R	R	R	R	12
S.warneri		R	R			R	R	R	R	R	R	R	R	R	R	12
S.warneri		R	R		R	R	R	R	R		R	R	R	R	R	12
S.warneri			R	R	R		R	R	R		R	R		R	R	10
Streptococcus spp.																
S.agalactiae	R	R	R		R	R	R	R	R	R	R	R	R	R	R	14
S.agalactiae		R	R	R	R	R	R	R	R		R	R	R	R	R	13
Enterococcus spp.																
E.faecalis	R	R	R		R	R	R	R	R	R	R	R	R	R	R	14
E.faecalis	R			R	R	R	R	R	R	R	R		R		R	11
E.faecalis	R			R	R	R	R	R	R	R	R		R		R	11
E.faecium	R	R	R		R		R	R	R		R	R	R	R	R	12
E.galinarum		R	R	R	R		R		R	R	R	R	R	R	R	12
E.galinarum	R				R	R	R	R	R	R	R			R	R	10
E.coli																
	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15
	R	R	R		R	R	R	R	R	R	R	R	R	R	R	14
		R	R		R	R	R	R	R	R	R	R	R	R	R	13
		R	R		R	R	R	R	R		R	R	R	R	R	12
		R	R		R		R	R	R	R	R	R	R	R	R	12
			R		R	R	R	R	R	R	R	R		R	R	11
		R	R		R		R	R	R		R	R	R	R	R	11
		R	R		R	R		R	R	R		R	R	R	R	11
			R		R		R	R	R	R	R	R		R	R	10
			R		R		R	R	R		R	R	R	R	R	10
Klebsiella pneumoniae																
	R	R	R		R	R	R	R	R	R	R	R	R	R	R	14
	R	R	R		R	R	R	R	R	R	R	R	R	R	R	14
	R	R	R	R	R		R	R	R	R	R	R	R		R	13
	R	R	R	R	R		R	R	R	R	R	R	R	R		13
		R	R	R	R	R	R	R	R		R	R	R	R	R	13
	R		R		R	R	R	R	R		R	R	R	R	R	12
Pseudomonas aeruginosa																
		R	R		R	R	R	R	R		R	R	R	R	R	12
		R	R		R	R		R		R		R	R	R	R	10
		R	R		R	R		R		R		R	R	R	R	10
Corynebacterium xerosis																
	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	15

* No. of resistant antibiotics ; AN:amikacin, AMC:amoxacillin/clavulanate, AM:ampicillin, CAZ:ceftazidime, CF:cephalothin, CIP:ciprofloxacin, E:erythromycin, GM:gentamicin, N:neomycin, NOR:norfloxacin, P:penicillinG, OX:oxacillin, TxS:trimethoprim-sulfa, CC:clindamycin

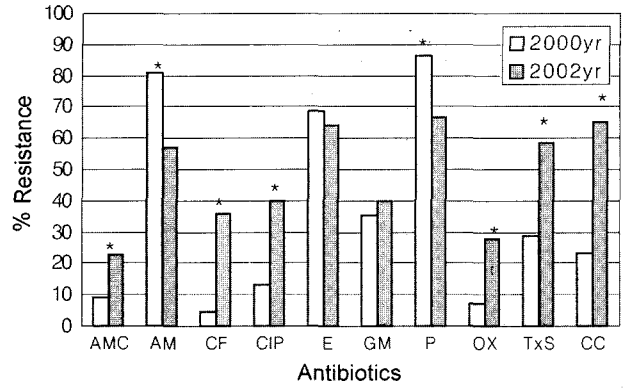

Fig 1. A comparison of G(+) bacterial resistance trends between the year 2000 and 2002.

*change of resistant degree with statistically significance
2000 yr: July 1999 to Sep 2000, 2002 yr: May 2001 to Sep 2002
AMC: amoxacillin/clavulanate, AM: ampicillin, CF: cephalothin, CIP: ciprofloxacin, E: erythromycin, GM: gentamicin, P: peni-cillinG, OX: oxacillin, TxS: trimethoprim-sulfa, CC: clindamycin

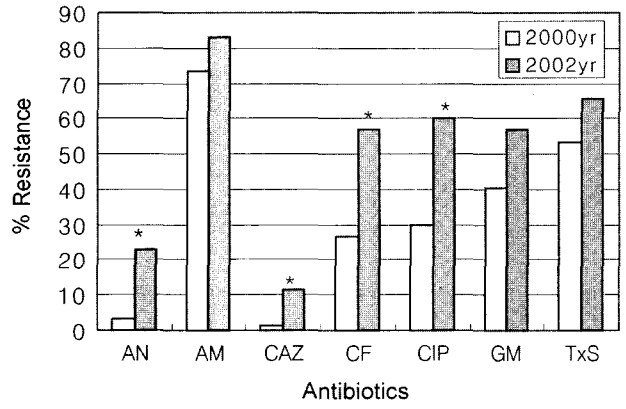

Fig 2. A comparison of G(-) bacterial resistance trends between the year 2000 and 2002.

*change of resistant degree with statistically significance
2000 yr: July 1999 to Sep 2000, 2002 yr: May 2001 to Sep 2002
AN: amikacin, AM: ampicillin, CAZ: ceftazidime, CF: cephalothin, CIP: ciprofloxacin, GM: gentamicin, TxS: trimethoprim-sulfa

antibiotics was deteriorated (18.4% increase in resistance) (Fig 2). The resistant rates of Gram negative bacterial isolates to amikacin, ceftazidime, cephalothin, and ciprofloxacin were increased.

The effectiveness of penicillin G to *Staphylococcus* spp. was somewhat returned (19% decrease in resistance) (Fig 3). However, the resistant rate of *Staphylococcus* spp. for all tested antibiotics was increased (2.0%). The resistant rate of *E.coli* to all tested antibiotics was also increased (6.6%) (Fig 4). Finally, the resistant rate of *Klebsiella pneumoniae* to all tested antibiotics was also increased (20.0%), despite no change in resistant rates to individual antibiotics ($p < 0.05$) (Table 7).

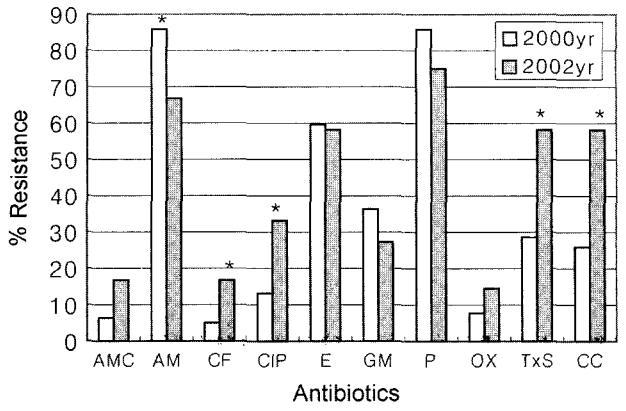

Fig 3. A comparison of *Staphylococcus* spp. resistance trends between the year 2000 and 2002.

*change of resistant degree with statistically significance
2000 yr: July 1999 to Sep 2000, 2002 yr: May 2001 to Sep 2002
AMC: amoxacillin/clavulanate, AM: ampicillin, CF: cephalothin, CIP: ciprofloxacin, E: erythromycin, GM: gentamicin, P: penicillinG, OX: oxacillin, TxS: trimethoprim-sulfa, CC: clindamycin

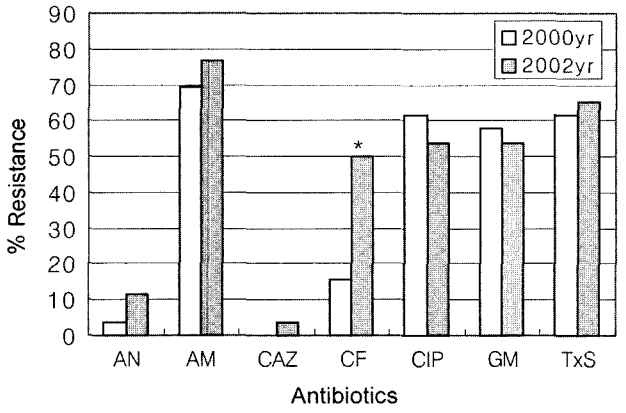

Fig 4. A comparison of *E.coli* resistance trends between the year 2000 and 2002.

*change of resistant degree with statistically significance
2000 yr: July 1999 to Sep 2000, 2002 yr: May 2001 to Sep 2002
AN: amikacin, AM: ampicillin, CAZ: ceftazidime, CF: cephalothin, CIP: ciprofloxacin, GM: gentamicin, TxS: trimethoprim-sulfa

Disccusion

When choosing appropriate antibiotics to treat diseases, periodic examination of bacteriological culture should be always performed. Clinician must judge whether certain bacteria were causing the progression of the diseases and must also consider the characteristics of those bacteria. Undoubtedly, selection of antibiotics should be carried out through susceptibility test, because the prevalence and degree of antimicrobial resistance in the veterinary medicine are increasing worldwide[18].

Staphylococcus simulans and *Staphylococcus epidermidis* were the most prevalent among staphylococci species in this

Table 7. A comparison of resistance trends by isolated species between the year 2000 and 2002.

	Klebsiella pneumoniae		
	2000 (n=8)	2002 (n=9)	note*
Amikacin	1	5	
Amipicilin	8	9	
Ceftazidime	1	3	
Cephalothin	3	7	
Ciprofloxacin	3	7	
Gentamicin	4	6	
Trimethoprim-sulfa	7	6	
Total	27	43	20.0%

*change of resistant degree with statistically significance
2000 yr : July 1999 to Sep 2000, 2002 yr : May 2001 to Sep 2002

study. Those coagulase-negative staphylococci (CNS) are the group of opportunistic pathogens since they are found as normal inhabitants of the skin and mucous membranes. For this reason, CNS are often assuming that they are contaminating clinical samples but are not involved in the primary infection[3]. However, there is evidence that these bacteria may be responsible for primary infections[11]. In dogs, those strains may be considered as potential pathogens, as they have been isolated from deep pyodermas[13].

Seven isolates of 48 staphylococci isolated in this study expressed methicillin resistance, which were all CNS. Additionally, 6 isolates of them were resistant to 10 or more antibiotics as shown in table 8. CNS strains have become a serious problem as they express methicillin resistance, which leads to significant limitation in therapy[3].

In the present study, roughly 87% of aerobic isolates are resistant to at least one antibiotic agent, and 57% of them were resistant to 5 or more antibiotics. More seriously, one strain of *E.coli* and one strain of *Corynebacterium xerosis* were resistant to all antibiotics tested, which represent the emergence of multi-drug resistant strains in infections. If veterinarians choose antibiotics for treatment without previous susceptibility testing in this case, they may be in danger of excessive and rotational using of antibiotics. Multi-drug resistance in isolates was a great problem. In some cases, multi-drug resistant strains of hospital origin have become so serious, just as in the preantibiotic era[6]. Patients with infections due to resistant organisms are likely to require more and longer hospital stays[5]. Antimicrobial resistance has resulted in prolonged and more serious illness, the use of more expensive and often more toxic drugs and drug combination, and increased fatality rates. Additionally, multi-drug resistant bacteria from companion animal can be transmitted to human, and it has become more serious in veterinary medicine and in human medicine. Thus, the occurrence and spread of multi-drug resistant strains need to be monitored, as infections caused by such strains can be difficult to treat.

Likewise, surveillance data from this study indicated that resistance to antibiotics was increasing in both Gram-positive and Gram-negative bacteria ($p < 0.05$). Interestingly, the effectiveness of penicillin G and ampicillin to Gram-positive bacteria was somewhat returned. It may be resulted in less use of them. The resistance rates of *Staphylococcus* spp., *E.coli*, and *Klebsiella pneumoniae* to all tested antibiotics were increased ($p < 0.05$). It may be mainly due to excessive and inappropriate use of antibiotics. These results showed that antibiotic resistance in veterinary medicine is progressive and imposes limitations on the choice of antibiotics for therapy in many different infections. Overdose of antibiotics and poor compliance with infection-control measures have been identified as the major reasons for increasing trends in antimicrobial resistance. Additionally, antibiotics used in veterinary hospitals are too often unnecessarily broad-spectrum drugs. Veterinarians should use broad-spectrum antibiotics for the shortest duration possible; if antibiotic therapy is begun for coverage of a possible infection of unknown type, such agents should be changed to those with the narrowest spectrum of activity based on microbiological results of culture and susceptibility[14]. Though the correlation of *in vitro* susceptibility test with the *in vivo* results of treatment is less than 100%, the result of antimicrobial susceptibility test may contribute to the most effective treatment of the patient[4,5,17]. Susceptible, intermediate, and resistant categories are based on levels of antibiotics achieved in the serum with normal kidney and liver function. Drugs concentration in urine may be effective for urinary tract infection even when the categorical interpretation is resistant. Conversely, drugs that do not penetrate well to a poorly vascularized area may not be effective even though the result of sensitivity test is susceptible. Aside from susceptibility patterns, other factors influencing the choice of antibiotics include their pharmacokinetics, effect on the normal flora, and ability to penetrate into sites of infection. *In vitro* resistance to antibiotics does not mean clinical treatment failure.

If the effectiveness of antibiotics is to be preserved, it is essential not only to control their appropriate use but also learn which doses and duration of therapy, will minimize the resistant bacteria[10]. However, inappropriate use of antibiotics is only part of the problem. In addition to appropriate antibiotics, infection control must be part of controlling resistance on hospitals. Hospitals have considerable potential for the spread of antimicrobial resistance. Poor compliance with infection-control measures is largely responsible for the dissemination of resistant isolates. Using of antibiotics with caution and controlling of infection are the best strategy for preventing the emergence and spread of nosocomial multi-drug resistant pathogens.

Surveillance of antimicrobial resistance is considered to be necessary for selection of appropriate therapy, and for monitoring changes in resistance rates in veterinary medicine. And, monitoring of susceptibility may be helpful to alert veterinarians and attempt to reverse resistance.

This study emphasized the need for the isolation of anaerobic bacteria as pathogens and for surveillance resistance, in order to select appropriate antibiotics. And, antimicrobial

susceptibility surveillance of isolates should urgently be continued in veterinary medicine.

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대학 동물병원 임상 검체로부터 분리된 호기성 세균과 항생제 감수성 양상

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요 약 : 항생제 저항성의 증가가 우려되고 있는 현재, 수의 임상에서는 지속적인 항생제 내성의 조사가 이루어지지 않고 있었다. 이에 본 논문에서는 대학 동물병원에 내원한 증례의 임상검체들로부터 분리한 호기성 세균과 그들의 항생제 감수성 양상을 조사하고자 하였다. 2001년 5월부터 2002년 10월까지 서울대학교 수의과대학 부속동물병원에 내원한 개, 고양이에서 채취한 임상 검체로부터 총 121주의 호기성 세균이 분리되었는데, 가장 많이 분리된 세균은 *Staphylococcus spp.* (48주)였으며 이어서 *E.coli* (26주), *Enterococcus spp.* (21주) 등의 순으로 빈도수가 높게 분리되었다. 항생제 감수성 검사 결과, 그람 양성균은 amikacin, amoxacillin/clavulanate, ceftazidime, oxacillin에 높은 감수성을 나타내었고, 그람 음성균은 amikacin과 ceftazidime에 높은 감수성을 나타내었다. 이 중, *Staphylococcus spp.*는 amikacin, amoxacillin/clavulanate, ceftazidime, oxacillin, cephalothin에 높은 감수성을 나타내었고, *Streptococcus spp.*와 *E.coli* 는 amikacin과 ceftazidime에 높은 감수성을 나타내었다. *Enterococcus spp.*와 *Klebsiella pneumoniae*는 70% 이상의 감수성을 나타내는 항생제가 없었다. 이 외에도 7주의 Methicillin Resistant *staphylococci*가 분리되었으며, 실험에 사용한 모든 항생제에 저항성을 가지는 균주가 *E.oli*와 *Corynebacterium xerosis*에서 각각 1주씩 분리되었다. 2000년 (1999년 7월-2000년 9월)과 2002년 (2001년 5월-2002년 10월)의 항생제 저항성을 비교했을 때, 그람 양성균과 그람 음성균의 전체 항생제에 대한 저항성은 증가했음을 확인할 수 있었는데 특히 *Staphylococcus spp.*와 *E.coli, Klebsiella pneumoniae*의 전체 항생제에 대한 저항성이 유의성 있게 증가하였음을 확인할 수 있었다($p<70.05$). 이상과 같이 동물 병원 임상 검체에서 원인균의 분리, 동정은 항생제 선택에 도움을 줄 수 있는 중요한 자료를 제공하며, 항생제 저항성의 증가를 확인함으로써 이를 치료에 이용할 수 있게 되었다. 따라서 수의 임상에서 항생제 저항성의 변화 양상에 대한 조사는 계속해서 이루어져야 되리라 사료된다.

주요어 : 항생제 감수성, 호기성 세균, 개, 고양이