

Antimicrobial Susceptibility of Coagulase-Negative Staphylococci Isolated from Bovine Mastitis Between 2003 and 2008 in Korea

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A total of 1,444 coagulase-negative staphylococci (CNS) isolates from bovine mastitic milk samples collected during 2003–2008 in Korea were identified to the species level. Of 14 species identified, *S. simulans*, *S. haemolyticus*, and *S. sciuri* accounted for over 60% of the isolates. All the CNS isolates were tested for susceptibility to eight antimicrobials commonly used in dairy cattle. With a few exceptions, similar resistance patterns were observed among the CNS species: penicillin and ampicillin showed the lowest activity, whereas amikacin, cephalothin, and gentamicin were highly effective. About 39% (557/1,444) of the CNS isolates were pan-susceptible, whereas 12% (175/1,444) showed resistance to four or more antimicrobials tested.

Keywords: Coagulase-negative staphylococci, antimicrobial resistance, mastitis

Coagulase-negative staphylococci (CNS) in dairy production are often considered minor pathogens, causing only subclinical or mild clinical mastitis as opportunistic bacteria [21]. Recently, however, the importance of CNS as mastitis-causing agents has increased because they are the most frequently isolated organisms from bovine mastitis in several countries [3, 15, 18] including Korea [12]. In addition, multi-antimicrobial resistance was often seen in CNS [10, 21], and they may also function as a reservoir for the transfer of antimicrobial resistance genes to *S. aureus* [1].

Staphylococci other than *S. aureus* are known to comprise 39 characterized species and more than 10 CNS species of these have been isolated from clinical samples of mastitis [16]. CNS species may differ in antimicrobial susceptibility, virulence factors, host response to infection,

and transmissibility, and thus species identification is needed to select appropriate antimicrobial therapy and to develop species-specific management practices [18]. However, researchers have generally regarded CNS, which were associated with bovine mastitis, as one homogenous group. Only recently have species-specific differences in CNS isolated from bovine mastitis been investigated [20].

Identification of CNS to the species level is needed to assess their antimicrobial susceptibility and to develop effective control strategies for CNS-associated mastitis. Therefore, the purpose of this study was to identify and determine the susceptibility of different CNS species isolated from bovine mastitis in Korea.

Sample collection and bacterial examination of the milk samples were conducted as described previously [13]. Briefly, a total of 20,386 quarter milk samples were collected from lactating cows on 552 dairy herds in Korea during January 2003 to June 2008. Milk samples with somatic cell counts (SCC) of greater than 200,000 cells/ml were subjected to bacteriological examination using standard laboratory techniques [14] in the Mastitis Diagnostic Laboratory, National Veterinary Research and Quarantine Service. All organisms presumptively identified as staphylococci by colony morphology, hemolytic patterns, and Gram staining were examined for coagulase production using the tube coagulase method and rabbit plasma. Coagulase-negative staphylococci were identified to the species level using the VITEK system (bioMérieux, Hazelwood, MO, U.S.A.) as described by the manufacturer.

In vitro antimicrobial susceptibility test was conducted by the disc diffusion method and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute [4]. Commercially available antimicrobial sensitivity discs (BBL; Becton-Dickinson, Cockeysville, MD, U.S.A.) were used: amikacin (30 µg/disc), gentamicin (10 µg/disc), kanamycin (30 µg/disc), ampicillin (10 µg/disc), cephalothin (30 µg/disc), tetracycline (30 µg/disc), erythromycin (15 µg/

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Table 1. Species of coagulase-negative staphylococci isolated from milk samples (SCC >200,000 cells/ml) submitted to the Mastitis Diagnostic Laboratory of National Veterinary Research and Quarantine Service, 2003–2008.

Bacterial spp.	Number of isolated bacteria (%)						
	2003 (n=402)	2004 (n=286)	2005 (n=245)	2006 (n=273)	2007 (n=107)	2008 (n=131)	Total (n=1,444)
<i>S. simulans</i>	126 (31.3)	102 (35.6)	65 (26.5)	46 (16.8)	18 (16.8)	32 (24.4)	389 (26.9)
<i>S. haemolyticus</i>	83 (20.6)	71 (24.8)	53 (21.6)	34 (12.4)	24 (22.4)	10 (7.6)	275 (19.0)
<i>S. sciuri</i>	124 (30.8)	19 (6.6)	38 (15.5)	11(3.8)	7 (6.5)	11 (8.4)	210 (14.5)
<i>S. auricularis</i>	2 (0.5)	3 (1.0)	11 (4.5)	66 (24.1)	26 (24.3)	54 (41.2)	162 (11.2)
<i>S. xylosus</i>	18 (4.5)	41 (14.3)	35 (14.3)	16 (5.8)	7 (6.5)	9 (6.9)	126 (8.7)
<i>S. warneri</i>	14 (3.5)	10 (3.5)	7 (2.9)	14 (5.1)	8 (7.5)	4 (3.1)	57 (3.9)
<i>S. epidermidis</i>	10 (2.5)	13 (4.5)	12 (4.9)	10 (3.6)	2 (1.9)	1 (0.8)	48 (3.3)
<i>S. hominis</i>	6 (1.5)	6 (2.1)	6 (2.4)	10 (3.6)	4 (3.7)	4 (3.1)	36 (2.4)
<i>S. saprophyticus</i>	7 (1.7)	14 (4.9)	2 (0.8)	6 (2.1)	4 (3.7)	0 (0.0)	33 (2.2)
Others ^a	12 (3.0)	7 (2.4)	16 (6.5)	60 (21.9)	7 (6.5)	6 (4.6)	108 (7.4)

^aIncluded 38 CNS isolates identified to the species level (17 *S. cohnii*, 10 *S. hyicus*, 6 *S. capitis*, 4 *S. lentus*, and 1 *S. intermedium*) and 70 unidentified CNS isolates.

disc), and penicillin (10 unit/disc). *S. aureus* ATCC 25923 was used as the quality control reference organism.

Of the 1,444 isolates identified as CNS, 70 (4.8%) could not be identified to the species level, and the remaining 1,374 were identified to 14 species of *Staphylococcus* in this study (Table 1). The predominant species were *S. simulans*, followed by *S. haemolyticus*, *S. sciuri*, *S. auricularis*, *S. xylosus*, *S. warneri*, *S. epidermidis*, *S. hominis*, and *S. saprophyticus*. A small number of *S. cohnii* (1.1%, 17/1,444), *S. hyicus* (0.6%, 10/1,444), *S. capitis* (0.4%, 6/1,444), *S. lentus* (0.2%, 4/1,444), and *S. intermedium* (0.06%, 1/1,444) were also isolated. Although variation exists between

herds and countries, *S. simulans* and *S. chromogenes* were reported to be the most common species in bovine mastitis [16]. Our finding that *S. simulans* was the most predominant species is in agreement with those reported from Norway [23], Finland [22], and Brazil [10]. However, *S. chromogenes* was not identified in this study.

The *in vitro* susceptibilities of the 1,444 CNS isolates to the eight antimicrobial agents are presented in Table 2. Generally, similar patterns of resistance were observed among the species of CNS, with a few exceptions: unlike other species that showed moderate susceptibility to penicillin, over 90% of *S. xylosus* isolates showed penicillin

Table 2. Antibacterial susceptibility of coagulase-negative staphylococci isolated from milk samples (SCC>200,000 cells/ml) submitted to the Mastitis Diagnostic Laboratory of National Veterinary Research and Quarantine Service, 2003–2008.

Species	Organisms Total	Number of susceptible isolates (%)							
		P ^a	AM	CF	AN	GM	TE	K	E
<i>S. simulans</i>	389	249 (64.0)	256 (65.8)	385 (98.9)	378 (97.1)	365 (93.8)	342 (87.9)	333 (85.6)	291 (74.8)
<i>S. haemolyticus</i>	275	142 (51.6)	145 (52.7)	260 (94.5)	255 (92.7)	239 (86.9)	249 (90.5)	203 (73.8)	187 (68.0)
<i>S. sciuri</i>	210	72 (34.2)	84 (40.0)	178 (84.7)	191 (90.5)	172 (81.9)	139 (66.1)	145 (69.0)	102 (48.5)
<i>S. auricularis</i>	162	71 (43.8)	116 (71.6)	133 (82.0)	146 (90.1)	148 (91.3)	123 (75.9)	114 (70.3)	119 (73.4)
<i>S. xylosus</i>	126	19 (8.7)	43 (34.1)	121 (96.0)	125 (99.2)	122 (96.8)	89 (70.6)	122 (96.8)	87 (69.0)
<i>S. warneri</i>	57	22 (38.5)	24 (42.1)	56 (98.2)	56 (98.2)	47 (82.4)	44 (77.1)	42 (73.6)	42 (73.6)
<i>S. epidermidis</i>	48	25 (52.0)	27 (56.2)	47 (97.9)	45 (93.7)	40 (83.3)	32 (66.6)	32 (66.6)	32 (66.6)
<i>S. hominis</i>	36	18 (50.0)	25 (69.4)	33 (91.6)	35 (97.2)	31 (86.1)	30 (83.3)	25 (69.4)	28 (77.7)
<i>S. saprophyticus</i>	33	17 (51.5)	17 (51.5)	27 (81.8)	29 (87.8)	26 (78.7)	23 (69.6)	20 (60.6)	13 (39.3)
<i>S. cohnii</i>	17	10 (58.8)	11 (64.7)	16 (94.1)	17 (100.0)	17 (100.0)	15 (88.2)	10 (58.8)	8 (47.0)
<i>S. hyicus</i>	10	3 (30.0)	5 (50.0)	10 (100.0)	7 (70.0)	8 (80.0)	9 (90.0)	7 (70.0)	8 (80.0)
<i>S. capitis</i>	6	4 (66.6)	2 (33.3)	6 (100.0)	5 (83.3)	5 (83.3)	5 (83.3)	3 (50.0)	4 (66.6)
<i>S. lentus</i>	4	0 (0.0)	0 (0.0)	3 (75.0)	4 (100.0)	4 (100.0)	2 (50.0)	4 (100.0)	2 (50.0)
<i>S. intermedium</i>	1	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)
UNID ^b	70	29 (41.4)	37 (52.8)	61 (87.1)	63 (90.0)	57 (81.4)	55 (78.5)	53 (75.7)	43 (61.4)
Total	1,444	681(47.1)	793 (55.0)	1,337 (92.5)	1,357 (93.9)	1,282 (88.7)	1,158 (80.1)	1,114 (77.1)	967 (66.9)

^aP: penicillin; AM: ampicillin; CF: cephalothin; AN: amikacin; GM: gentamicin; TE: tetracycline; K: kanamycin; E: erythromycin.

^bUNID: unidentified CNS.

Table 3. The numbers of CNS isolates resistant to the given number of antimicrobials and the resistance patterns most frequently observed among the CNS isolates.

Antimicrobials	Numbers of		Most frequently observed pattern of resistance	
	Resistant isolates (%)	Resistance patterns ^b	Types	Number of isolates/ Total number of resistant isolates (%)
0	557 (38.5)	0	-	-
1	200 (13.8)	7	P ^a	86/200 (43.0)
2	370(25.6)	16	P-AM	293/370 (79.1)
3	142 (9.8)	14	P-AM-TE	60/142 (42.2)
4	82 (5.6)	13	P-AM-GM-K	37/82 (45.1)
5	44 (3.0)	12	P-AM-GM-TE-K	12/44 (27.2)
6	26 (1.8)	9	P-AM-GM-TE-K-E	13/26 (50.0)
7	6 (0.4)	3	P-AM-AN ^a -GM-TE-K-E	3/6 (50.0)
8	17 (1.1)	1	P-AM-AN-GM-TE-K-E-CF	17/17 (100.0)
Total	1,444	75		521/887

^aP: penicillin; AM: ampicillin; CF: cephalothin; AN: amikacin; GM: gentamicin; TE: tetracycline; K: kanamycin; E: erythromycin.

^bPatterns of combined resistance against the same number of different kinds of antimicrobials.

resistance. Moreover, none of the *S. lentus* isolates showed susceptibility to both penicillin and ampicillin. Among the 1,444 CNS isolates tested, the highest rate of susceptibility was generally observed for amikacin (93.9%), followed by cephalothin (92.5%), gentamicin (88.7%), tetracycline (80.1%), and kanamycin (77.1%). The rates of susceptibility to these antimicrobials are similar to those of previous reports from Korea [7, 8]. Although a direct comparison between the studies is difficult because different methods have been used, the susceptibilities of CNS in this study are mostly lower than those reported from other countries such as Finland [15], Argentina [6], Germany [9], and Sweden [2]. The percentage of the erythromycin-resistant CNS (33.1%) in this study is also much higher than those reported from Finland (5.4%) [15], Argentina (5.7%) [6], Germany (7.4%) [9], and Sweden (3.6%) [2].

Overall, penicillin and ampicillin showed the lowest activities against CNS in this study, which is in agreement with those of previous reports from Korea [8] and other countries [6, 11, 19]. The most common resistance mechanism in staphylococci is known to be β -lactamase production, which results in resistance to penicillin G and aminopenicillins [21]. However, the percentage of penicillin resistance (52.9%) observed in this study is much higher than those of previous studies from Korea [7, 8] and most other countries such as Argentina (27.6%) [6], Switzerland (31%) [5], Finland (32%) [15], and the U.S. (22.1%) [17].

About 39% (557/1,444) of the CNS isolates were pan-susceptible, and 13.8% (200/1,444) were resistant against penicillin only. Simultaneous resistance to four or more antimicrobials was observed in 12% (175/1,444) of the isolates. The multidrug resistance observed in this study is much lower than that reported from Brazil [10], where almost all CNS isolates from bovine mastitis showed resistance to two or more antimicrobial agents. Seventy-

five different resistance patterns were identified among the 887 strains resistant to at least one antimicrobial agent in this study. Besides penicillin–ampicillin resistance, the most frequently observed pattern of multiple resistances was the penicillin–ampicillin–gentamicin–kanamycin combination found in 37 of 887 (4.2%) resistant strains (Table 3).

The present study evaluated the antimicrobial susceptibilities of CNS isolates from bovine mastitic milk samples collected for about 6 years throughout Korea. This is the first report of the distribution and the antimicrobial resistance of CNS species on such a large scale in Korea. In this study, we observed relatively higher resistance of CNS against most antimicrobials tested, particularly against penicillin and ampicillin, compared with most of the previous studies from other countries. These results support the fact that the antimicrobial resistance problem is comparatively more serious in Korea.

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