Antibiogram of *Staphylococcus intermedius* from Animals, Staff and the Environment in Animal Hospitals in Korea and Their Transmission of *mecA* Gene

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**Introduction**

*Staphylococcus intermedius* is the most frequently isolated coagulase-positive staphylococci from both healthy and diseased dogs (1). *S. intermedius* is a common cause of otitis externa, pyoderma and wound infections in companion animals (2). Studies have shown this species to be increasingly resistant to the antibiotic methicillin and vancomycin which have been used in the last stages of infection. Although *S. intermedius* infections are rare in humans, there are several case reports in which the infections have been fatal (3). This may signify the organism to be zoonotic. As veterinarians and staff in animal hospitals are frequently exposed to dogs, they may have a high risk of infection. In animal hospitals are frequently exposed to dogs, infections have been fatal (3). This may signify the organism to be zoonotic. As veterinarians and staff in animal hospitals are frequently exposed to dogs, they may have a high risk of *S. intermedius* infections and may also play the role of host. The purpose of this study was to isolate the strains from various sources at animal hospitals in Korea, examine their susceptibility to antibiotics and determine the presence of horizontal transmission of *S. intermedius* by comparing the *S. intermedius* strains with the *mecA* gene from human, animals and the environment in animal hospitals.

**Materials and Methods**

A total of 529 samples were obtained where 271 are from 54 animals, 170 samples from 77 hospital staff and 88 from the hospital environment of five animal hospitals (4 teaching hospitals and 1 private referral hospital). The animal samples (n=271) were taken from the anus (n=52), horizontal ear canal (n=83), nasal mucosa (n=61), skin (n=60), wound infection area (n=8), and urine specimen (n=7). Human samples were taken from hand (palm and the skin between fingers) and nasal cavity. All samples were taken using BBL Culture Swab (Becton, Dickson and Company) or Meat/Turkey Carcass Sampling Kit (Nasco, Canada). Two hospitals were tested twice each with an interval of 3 months and 19 months, respectively. *S. intermedius* was isolated using various biochemical tests and confirmed by Vitek 2 GPI card (bioMerieux, Lyon, France) and PCR with *S. intermedius*-specific primers. *S. intermedius* was also tested for antibiotic susceptibility to 16 different antibiotics from 11 classes by disk diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) guideline: ampicillin (10 µg), amoxicillin-clavulanic acid (30µg), amikacin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), cephaptolin (30 µg), ciprofloxacin (5 µg), ceftaxime (30 µg), erythromycin (15 µg), nitrofurantoin (300 µg), gentamicin (10 µg), imipenem (10 µg), penicillin (10 units), trimethorim-sulfamethoxazole (23.75 µg, 1.25 µg), tetracycline (30 µg), vancomycin (30 µg) (BD BBL, Sparks, MD, USA). In addition, the minimal inhibitory concentrations (MICs) to oxacillin (Sigma-Aldrich, St. Louise, MO, USA), vancomycin (Sigma-Aldrich) and orbifloxacin (Riedel-deHaen, Seelze, Germany) were determined by using a microdilution test according to the guidelines of the CLSI. Isolates with vancomycin MIC ≥ 16 mg/L and orbifloxacin MIC ≥ 8 mg/L were classified as being vancomycin and orbifloxacin resistant. The isolates with oxacillin MIC ≥ 4 mg/L were classified as being methicillin-resistant (MRSI). Methicillin/oxacillin resistance strains of *S. intermedius* were determined by PCR with primer sets targeting the *mecA* gene; *meca1*- 5’-AAAAATCGATGGTAAAGGGTGCC-3’ and *meca2*- 5’-AGTTTCGAGTGATCGGATCG-3’ (4), and Primer-1- 5’-CGTGTTACAGTACGGGTG-3’ and Primer-2- 5’-GAATGATGGCAACTAAGTTC-3’ (5).

For the classification of the methicillin-resistant *Staphylococcal cassette chromosome mec* (SCCmec) group of MRSI, the new multiplex PCR method developed by Boye et al. (6) was used, and the relation between these *mecA* gene strains from human, animals and the environment was determined further using the pulsed field gel electrophoresis (PFGE) (7).

**Results and Discussion**

Out of 529 samples, a total of 119 (22.5%) *S. intermedius* (animals, 73 (61.3%); hospital staffs, 34 (28.6%); and hospital environment, 12 (10.1%) were isolated.
The following antimicrobial agents showed over 90% susceptibility by disk diffusion test, cephalothin (CF) 92.4%; amoxicillin-clavulanic acid (AmC) 94.1%; vancomycin (Va) 98.3%; imipenem (IPM) 99.2%; nitrofurantoin (F/M300) 99.2%; amikacin (AN) 100% whereas penicillin (P) and ampicillin (AM) showed a resistant rate of 97.5% and 98.3% respectively. Multi-drug resistance to at least 3 antimicrobial classes was shown in 70 (58.8%) of 119 strains (animals, 58.9% (43/73); veterinary staffs, 58.8% (20/34); and hospital environment 58.3% (7/12)). The MIC tests showed that 49 (41.2%) were resistant to oxacillin and 70 (58.8%) were susceptible, and 42 (35.3%) were resistant, 5 samples (4.2%) were intermediate, and 72 were (60.5%) susceptible to orbifloxacin. All samples were susceptible to vancomycin (100%) (Fig. 1). Out of 49 oxacillin-resistant strains 11 strains carried mecA gene, classifying MRSI according to the CLSI guidelines.

*S. intermedius* strains which were obtained from five animals (24 samples) with skin disease or a history of skin disease did not carry the mecA gene. Among the 39 mecA gene strains, only 20 (51.3%); animals, 10; veterinary staffs, 6; and environment, 4 could be classified by multiplex PCR for SCCmec typing. The most common SCCmec type was V (19 of 20), followed by type IV (1 of 20) (Figure 2). The PFGE result showed an animal, the veterinary staffs in charge of that animal of a hospital where the animal was hospitalized had the same PFGE patterns, suggesting possible transmission of MRSI among them. PFGE results of other MRSI isolates also showed the same pattern between an animal and a veterinary staff. (Fig. 3).

The high antibiotic resistant rate of *S. intermedius* isolates to penicillin and ampicillin might be the result to the frequently use of penicillin G (54 kg, 2004; 87 kg, 2005) and ampicillin (57 kg, 2004; 61 kg, 2005) in small animal hospitals in the past few years (8). A total of 39 isolates (32.8%) possessed the mecA gene and among them only 20 isolates could be classified. The different source and bacteria species of the samples and the regional differences may be the reason for the low classification rate. It is not clear how the MRSI strains are transmitted but the results of this study show that a horizontal transmission of MRSI or the mecA gene from hospital staff, animal and the environment may be present. To prevent transmission and to avoid the outbreak of disease caused by *S. intermedius* and MRSI, prudent use of antibiotics and strict infection control practice in animal hospitals should be stimulated. In addition, continuous monitoring and molecular epidemiological studies should be followed.

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References


Fig. 1 Result of antimicrobial susceptibility test of *S. intermedius* isolates to oxacillin, vancomycin, and orbifloxacin determined by broth microdilution method (MIC)

Fig. 2 SCC mecA typing of MRSI isolates from animals, veterinary staffs, and the environments of animal hospitals using multiplex PCR (6) *V*, Group IV; *IV*, Group V

Fig. 3 PFGE of MRSI isolates from animals, veterinary staffs, and the environment of animal hospitals

1. Veterinary staff 1 (hand); 2. Animal (skin); 3. Animal (horizontal ear canal); 4. Animal (pad); 5. Animal (wound region); 6. Environment (door knob); 7. Veterinary staff 2 (hand); 7. Animal (anus); 9. Veterinary staff (nasal mucosa) 2-5, from single dog