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Antimicrobial Susceptibility and Distribution of Multidrug-Resistant Organisms Isolated from Environmental Surfaces and Hands of Healthcare Workers in a Small Animal Hospital

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Abstract

Various multidrug-resistant organisms (MDROs) are present in animal hospitals. To investigate the bacteria that are capable of causing healthcare-associated infections (HAI) in a small animal referral hospital, as well as their drug resistance, samples were collected from 14 hospital environment surfaces and the hands of 5 healthcare workers (HCWs); bacteria were then isolated, identified, and tested for antimicrobial resistance. Thirty-four bacterial strains were isolated, namely staphylococci (35%), *Bacillus* spp. (32%), *Acinetobacter nosocomialis* (12%), enterococci (12%), *Pseudomonas aeruginosa* (3%), *Paenibacillus thermophilus* (3%), and *Pantoea calida* (3%). Among the 12 staphylococcal isolates, 8 possessed *mecA* gene; 9, methicillin-resistant staphylococci (MRS). All 3 enterococcal isolates were multidrug-resistant (MDR) and 1 possessed *vanA* gene, showing resistance to vancomycin. Among the clinically important bacteria, 35% were MDR, and only 1 strain, *Enterococcus feacalis,* was susceptible to all antimicrobials. Here, MDROs that cause opportunistic infections were found in an animal hospital and on the hands of HCWs. The present study was the first to find vancomycin-resistant enterococci on the hands of HCW in a small animal hospital. Therefore, in addition to reducing HAI, infection monitoring and management in small animal hospitals are needed to reduce the spread of MDROs from a One Health perspective.

Key Words: Healthcare-associated infection (HAI), Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), Vancomycin-resistant enterococci (VRE)

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Introduction

In both human and veterinary medicine, control of healthcare-associated infections (HAIs) in hospitals is very important. If patients admitted and hospitalized for treatment acquire new infections from hospitals, morbidity and mortality increase and the duration of hospitalization is prolonged, which can cause secondary problems such as financial loss. Multidrug-resistant organisms (MDROs) are especially important in HAIs because they may contaminate variable fomites or environmental surfaces in animal hospitals[12]. Thus, awareness of this problem is increasing in veterinary medicine[33]. In veterinary medicine, major MDROs linked to HAIs include methicillin-resistant staphylococci (MRS), enterococci, *Escherichia coli*, *Salmonella* spp., *Acinetobacter* spp., and *Pseudomonas* spp.[33], which are also may present on the epidermis or mucosal surface of hosts.

Patients may acquire HAIs from direct contact with the hands of healthcare workers (HCWs) from contaminated hospital environments[13]. The hands of HCW are a potential reservoir for resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA); there have been reports of both the environment and HCWs in animal hospitals being contaminated by bacteria[12,21]. Because these results indicate the possibility of resistant bacteria being transmitted between humans and animals to cause infections in animal patients and HCWs that lead to zoonotic diseases, infection monitoring and control are needed in animal hospitals. Pets are known to play a crucial role in transmitting resistant bacteria to humans and other animals[3,8,39]. Once study showed that nosocomial infections occurred in 82% of 38 veterinary teaching hospitals, and zoonotic infections occurred in 50% of these hospitals[2]. Therefore, infection control in animal hospitals should be approached not only from the HAI but also from the One Health perspective, which requires inclusion of more broad-based infectious agents than those found in human hospitals. In human medicine, infectious agents such as bacteria, viruses, and fungi are under stringent monitoring to maintain the hygiene of hospital environments, but such effort is still lacking in animal hospitals[40].

In human medicine, fomites (e.g., stethoscopes, thermometers, electronic equipments, gowns), environmental surfaces, and the hands of HCW are known to be potential vectors of bacteria that cause HAIs, and HAI prevention guidelines continue to be updated[14,22,35]. In veterinary medicine, there have not been as many studies as in human medicine on what types of bacteria that are transmitted in the environment and by HCWs and what resistances these bacteria may have[12,15,17]. Therefore, the objective of the present study was to investigate environmental and hands of HCWs contamination to identify potential sources of bacterial transmission in a small animal hospital, as well as to identify the bacterial strains and their antimicrobial resistance.

Materials and methods

Sampling: Sampling of isolates from 5 veterinary HCWs and the environment was conducted on November 2014 at a referral animal hospital in the Republic of Korea. Surface swab specimens were collected from the hospital environment, including chairs in the waiting room, the treatment tabletop, the doctor's office, the surgical preparation room, cages, a computed tomography (CT), keyboards of computers after patient contact (two each), and the swabs were inoculated onto blood agar plates. The tips of all fingers and thumbs of HCWs were imprinted on a hand plate (Easy Checker, Komed, Korea) with 5% sheep's blood agar (BD, Sparks, MD) to identify search for bacteria. Overall, 20 HCW's hand samples and 14 environmental samples were collected. HCWs who were subjects in the present study had not visited a hospital or received any treatment in the past 2 weeks, whereas environmental sampling was conducted

at 2 pm, when diagnosis and treatment activities were being performed. All environments were sterilized at 9 am, and diagnosis and treatment began at 10 am. A total of 34 isolates of diverse bacterial spp. identified from these sources were included in this study.

Bacterial identification by 16S rDNA sequence analysis: To identify bacteria obtained from samples, we performed a polymerase chain reaction (PCR) of the 16S rDNA gene and sequenced the PCR products. PCR amplification and sequencing of the 16S rDNA were performed as previously described[27]. The universal eubacterial primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') were used. The 16S rRNA gene sequences from each bacterial isolate were compared with reference sequences using BLAST searches in the GenBank and EzTaxon public databases.

Antimicrobial susceptibility testing: Among the 34 bacterial isolates identified, 20 clinically important bacterial isolates, including *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp., and *Pseudomonas* spp. were tested for in vitro susceptibility to various antibiotics by the broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2014). The antimicrobial agents tested in this study were penicillin, ampicillin, tetracycline, ciprofloxacin, imipenem, meropenem, colistin, cefotaxime, cefazolin, amikacin, vancomycin, teicoplanin, azithromycin, linezolid, oxacillin, clindamycin, trimethoprim-sulfamethoxazole, and gentamicin. *Streptococcus pneumoniae* ATCC 49619, *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 25922 were used as quality control strains.

Detection of antibiotic resistance determinant genes: PCR was conducted to detect a *mecA* gene in staphylococcal isolates when resistance to oxacillin was shown; a multiplex PCR was conducted to detect *van* genes (*vanA*, *vanB*, and *vanC*) in enterococcal isolates when resistance to vancomycin was shown. All PCR testing was performed using primer pairs and amplification conditions described previously [25,34].

Statistical analysis: Prism 6 Version 6.01 (GraphPad) was used for the statistical analysis. For comparison of data between groups, a Fisher's exact test was performed. For all comparisons, a value of $P < 0.05$ was considered significant.

Results

A total of 34 bacterial isolates were identified from 7 environmental surfaces within an animal hospital and the hands of 5 HCWs, with 28 gram-positive isolates (82.4%) and 6 gram-negative isolates (17.6%) (Table 1). Strains isolated included the following, in order of frequency: staphylococci (35.3%), *Bacillus* spp. (32.4%), *Acinetobacter nosocomialis* (11.8%), enterococci (11.8%), *Pseudomonas aeruginosa* (2.9%), *Paenibacillus thermophilus* (2.9%), and *Pantoea calida* (2.9%). Among the staphylococcal strains, the highest numbers of strains isolated were *S. pseudintermedius* (6 isolations), followed by *S. aureus* and *S. haemolyticus* with 2 isolates each, and *S. epidermidis* and *S. warneri* with one isolate each. Among the *Bacillus* spp., 5 isolates of *B. cereus*, 3 of *B. methylotrophicus*, 1 of *B. siamensis*, 1 of *B. subtilis*, and 1 of *B. sonorensis* were isolated. Among the enterococci, 1 isolates of *E. faecalis* and 3 of *E. faecium* were isolated.

Of the environmental surfaces that were sampled, bacteria were not isolated from the samples from chairs in the waiting room and the treatment table. The highest amount of bacteria was isolated from a computer keyboard and a doctor's office. In contrast, bacteria were isolates from all samples from the hands of HCWs. Among the bacteria isolated, the antimicrobial susceptibility testing was performed in the clinically important bacteria (CIB)[33] (Table 2). Among the environmental surfaces from which

Table 1. Identification of bacterial isolates from environmental surfaces and hands of healthcare worker by 16S rDNA sequence analysis

	Bacterial species	Number of isolates[a]
Surface		
Chairs in a waiting room	–	–
Treatment table	–	–
Keyboard in a doctor's office	*Acinetobacter nosocomialis*	6
	Bacillus cereus	> 100
	Staphylococcus epidermidis	> 50
A doctor's office	*Bacillus methylotrophicus*	3
	Bacillus siamensis	> 50
	Staphylococcus haemolyticus	2
	Staphylococcus warneri	> 100
Cage	*Bacillus methylotrophicus*	1
	Pseudomonas aeruginosa	1
Computed tomography	*Enterococcus faecalis*	1
	Staphylococcus pseudintermedius	> 50
Surgical preparation room	*Paenibacillus thermophilus*	1
Hands		
Healthcare worker 1	*Acinetobacter nosocomialis*	12
	Bacillus methylotrophicus	20
	Bacillus subtilis subsp. Inaquosorum	3
	Enterococcus faecium	9
	Staphylococcus pseudintermedius	21
Healthcare worker 2	*Bacillus cereus*	5
	Staphylococcus aureus	> 50
Healthcare worker 3	*Acinetobacter nosocomialis*	10
	Bacillus cereus	1
	Bacillus sonorensis	5
Healthcare worker 4	*Pantoea calida*	2
	Staphylococcus haemolyticus	8
Healthcare worker 5	*Acinetobacter nosocomialis*	> 100
	Enterococcus faecium	15
	Staphylococcus pseudintermedius	> 300

[a]Number of isolates. > 50, 51–100; > 100, 101–150; > 300, 301–350.

bacteria were isolated, CIB were not found only in the surgical preparation room, whereas CIB were isolated from all hands of HCWs. From the computer keyboard and CT, two or more CIBs were isolated. The percentage of CIBs isolated from hands and the environment were not significantly different ($P = 0.1627$).

Antimicrobial susceptibility testing, performed on CIB only, showed only one *E. faecalis* was susceptible to all antimicrobial agents tested. *E. faecium*, *S. epidermidis, S. haemolyticus,* and *S. pseudintermedius* showed MDR. Neither *A. nosocomialis* nor *Pseudomonas aeruginosa* showed MDR, and the percentage of MDRO among all CIB was 35% (7/20). Among all staphylococci, 81.8% (9/11) were methicillin-resistant, of which, 44.4% (4/9) were MDR. A total of 2 *S. aureus* isolates were MRSA, whereas a total of 3 of 6

Table 2. Results of antimicrobial susceptibility testing of clinically important bacteria isolated from environmental surfaces and hands of healthcare workers

Bacterial species	Source	Genes[a]	MDR[b]	Antimicrobial susceptibility[c]/Interpretive breakpoints (μg/ml)									
				Tet	Cipro	Imi	Mero	Coli	Cefo	Cefa	Ami		
Pseudomonas aeruginosa	Environment		–	32 ND	0.12 S	0.5 S	0.25 S	2 S	> 64 ND	> 64 ND	8 S		
Acinetobacter nosocomialis	Environment		–	0.12 S	0.25 S	< 0.06 S	0.12 S	0.5 S	1 S	> 64 ND	1 S		
A. nosocomialis	Hand		–	0.25 S	0.25 S	< 0.06 S	0.25 S	0.5 S	2 S	> 64 ND	2 S		
A. nosocomialis	Hand		–	0.25 S	0.5 S	0.25 S	2 S	64 R	16 I	> 64 ND	2 S		
A. nosocomialis	Hand		–	0.25 S	0.12 S	< 0.06 S	0.12 S	0.5 S	2 S	> 64 ND	2 S		
				Tet	Cipro	Van	Tei	Amp	Azi	Lin			
Enterococcus faecalis	Environment		–	< 0.06 S	1 S	1 S	0.25 S	1 S	4 ND	1 S			
E. faecium	Hand		MDR	64 R	64 R	0.5 S	0.25 S	64 R	16 ND	1 S			
E. faecium	Hand		MDR	> 64 R	> 64 R	0.5 S	< 0.12 S	> 64 R	> 32 ND	1 S			
E. faecium	Hand	*vanA* (+)	MDR	64 R	> 64 R	> 64 R	64 R	> 64 R	8 ND	1 S			
				Tet	Cipro	Van	Azi	Lin	Oxa	Pen	Clin	SXT	Gen
Staphylococcus aureus	Hand	*mecA* (+)	–	0.12 S	0.25 S	0.5 S	1 S	1 S	32 R	64 R	0.12 S	0.06/1.18 S	0.5 S
S. aureus	Hand	*mecA* (+)	–	0.12 S	0.25 S	1 S	2 S	1 S	32 R	64 R	0.12 S	0.06/1.18 S	0.5 S
S. epidermidis	Environment	*mecA* (+)	MDR	< 0.06 S	4 R	2 S	8 R	0.5 S	32 R	8 R	2 I	0.12/2.37 S	64 R
S. haemolyticus	Environment	*mecA* (+)	MDR	32 R	16 R	1 S	> 32 R	0.5 S	> 64 R	> 64 R	> 64 R	1/19 S	> 64 R
S. haemolyticus	Hand	*mecA* (+)	–	< 0.06 S	0.25 S	0.25 S	0.5 S	0.25 S	16 R	2 R	0.12 S	0.12/2.37 S	32 R
S. pseudintermedius	Environment		–	16 R	1 S	0.5 S	0.5 S	0.5 S	0.12 S	8 R	0.12 S	0.5/9.5 S	8 I
S. pseudintermedius	Hand		–	32 R	32 R	0.5 S	4 I	0.5 S	0.25 S	< 0.06 S	2 I	1/19 S	2 S
S. pseudintermedius	Hand	*mecA* (−)	MDR	8 I	0.12 S	1 S	8 R	0.25 S	1 R	8 R	1 I	0.5/9.5 S	16 R
S. pseudintermedius	Hand	*mecA* (+)	–	< 0.06 S	16 R	0.5 S	0.5 S	0.5 S	32 R	16 R	0.12 S	0.5/9.5 S	8 I
S. pseudintermedius	Hand	*mecA* (+)	–	< 0.06 S	16 R	0.5 S	1 S	0.5 S	64 R	64 R	0.12 S	0.5/9.5 S	8 I
S. pseudintermedius	Hand	*mecA* (+)	MDR	16 R	16 R	0.5 S	> 32 R	1 S	> 64 R	> 64 R	> 64 R	0.5/9.5 S	> 64 R

[a]Genes, antibiotic resistance determinant genes.
[b]Multidrug resistance (MDR), resistant to three or more antimicrobial classes.
[c]Antimicrobial susceptibility; Ami = amikacin, Amp = ampicillin, Azi = azithromycin, Cefa = cefazolin, Cefo = cefotaxime, Cipro = ciprofloxacin, Clin = clindamycin, Coli = colistin, Gen = gentamicin, I = intermediate, Imi = imipenem, Lin = linezolid, Mero = meropenem, Oxa = oxacillin, Pen = penicillin, R = resistant, S = susceptible, Tei = teicoplanin, Tet = tetracycline, SXT = trimethoprim-sulfamethoxazole, Van = vancomycin.

(50%) *S. pseudintermedius* strains isolated were methicillin-resistant *S. pseudintermedius* (MRSP). Among MRS, one *S. pseudintermedius* did not possess *mecA* gene, whereas 72.7% (8/11) of staphylococci contained *mecA*. Moreover, among the 4 strains of staphylococci that were MDR, 3 were *mecA* (+). Among all staphylococci, none was resistant to trimethoprim-sulfamethoxazole, vancomycin, and linezolid. Among all MRS, strains that were MDR were all resistant to azithromycin, oxacillin, penicillin, clindamycin, and gentamicin.

The percentage of enterococci that were MDR was 75% (3/4), and all of these showed resistance to tetracycline, ciprofloxacin, and ampicillin. One *E. faecium* resistant to vancomycin contained *vanA* gene. All enterococcal isolates showed no resistance to linezolid. Among the *A. nosocomialis* strains, 1 showed resistance to colistin. Moreover, the percentage of resistant strains of CIB isolated from hands and the environment did not show a significant difference ($P = 1.0$).

Discussion

This work identifies environmental surfaces and hands of HCWs as the bacterial contamination sources in a small animal hospital and MDROs were also observed. These results suggest that patients may be difficult to treat when HAI is caused by MDROs, so it is necessary to thoroughly disinfect the hospital environmental surfaces and hand hygiene of HCWs and MDROs can be transmitted to HCWs as well.

In this study, bacteria were not isolated from chairs in the waiting room and the treatment table. This outcome was likely because the chairs in the waiting room and treatment table were cleaned and sterilized frequently. The treatment table, specifically, is sterilized between each patient. In contrast, 2 strains of CIB each (*A. nosocomialis* and *S. epidermidis*) were cultured from a computer keyboard and CT equipment, which are cleaned less often, yet have frequent contact with patients and/or HCWs. Places that have frequent contact yet are not cleaned often, such as computer keyboards or door handles, have been reported to serve as a source of bacterial contamination in human hospitals[7,31]. Typical sources of HAIs in human hospitals are employees who have contact with patients, visitors, patient care equipment, medical devices, and the hospital environment[18]. There are similar results from animal hospitals[1,12,15]. Sampling from all surfaces without sink in a veterinary teaching hospital, including cages, floor of the examining room, door handle, keyboard, telephone, and scale, resulted in isolation of enterococci and staphylococci[15]. In the present study, major pathogens such as MRS, enterococci, *A. nosocomialis,* and *P. aeruginosa* were isolated from the environment. Moreover, CIB were isolated from the all hands of HCWs. CIB were isolated ($P = 0.1627$) and resistance genes were identified ($P = 1.0$) from hands and the environment, with no significant difference between the two sources. In addition, because the most of the bacteria isolated from the keyboard and the doctor's office where pet histories were taken from owners, it is necessary to recommend hand hygiene not only for HCWs but also for the owners.

One of the major mechanisms by which staphylococci become resistant to methicillin is by acquiring the *mecA* gene. MRS possess an additional penicillin binding protein (PBP2a) that is encoded by *mecA* or *mecC*, and this protein confers resistance to beta-lactam antibiotic through cross-linking in the process of cell wall synthesis[26]. The reason why MRS are a subject of infection control is that the *mecA* gene can be passed to other bacteria, making them resistant to antibiotics with a beta-lactam ring as the base (e.g., penicillins, cephalosporins, and carbapenems)[38]. Resistance to other classes of antibiotics, including fluoroquinolones, lincosamides, macrolides, tetracyclines, and trimethoprim-sulfonamides, has also been observed[9,24]. In the present study, there were a total of 11 strains of staphylococci, of which 9 were MRS and 8 were *mecA* (+).

Although MRS showed some differences in their levels of resistance, they did show resistance to antibiotics other than vancomyin, linezolid, and trimethoprim-sulfamethoxazole; thus, the findings were similar to those of previous studies. Moreover, MRSA found in the present study were not MDR, and of a total of 3 strains of MRSP, 1 isolation was MDR with resistance to 7 antimicrobial agents tested.

MRSA is a commensal organism that accounts for 1–1.5% in humans[28]. MRSA is known to be present in dogs at a lower prevalence (< 1%)[4]. In humans, MRSA is a well-known MDR nosocomial pathogen, and the prevalence of MRSA colonization among workers in small animal practice has been reported to be 4.4%[16], which makes it an emerging pathogen in animal hospitals. Moreover, the incidence of MRSA carriage in hospitalized dogs was 8.9%, whereas the incidence in HCWs in animal hospitals was 17.9%, which was higher than that of the general population[21]. MRSA contamination has been reported in animal hospital environments[15,17], and MRSA in outbreaks in veterinary teaching hospitals were suspected to have been transmitted from humans to animals, as HAI has also been reported[32]. There have been reports of not only MRSA infection in animals, but cases of humans being infected from pets that are MRSA carriers[23]; thus, it is a pathogen that can cause HAIs, while at the same time causing disease in human. In the present study, MRSA was not found in the hospital environment, but it was found on the hands of HCWs. The number of HCWs who were the subjects of the present study was low, which limited the significant results obtained from comparisons. However, because MRSA was found, methods that can reduce MRSA colonization are clearly needed.

S. pseudintermedius is the most common bacteria found on the skin of dogs, and it is a common cause of both HAIs and skin infections in dogs and cats[36]. The first case of human *S. pseudintermedius* infection was reported in 2006[37]. Moreover, because another report indicated that MRSP is capable of colonizing both dogs and humans[5,29], it is monitored in animal hospitals. In a Japanese animal hospital, the same strain of MRSP was detected in both dogs and veterinary staff[29], and people who work in an environment with small animals or owners of dogs infected with MRSP tend to have greater MRSP colonization rates than those who do not[11,29]. In the present study, *S. pseudintermedius* was found in both the environment and on hands, and among the 6 strains isolated, 3 were *mecA* (+) and 2 were MDR. Unlike in human hospitals, MRSP is a major causative agent of HAI in animal hospitals; as such, a suitable infection control system to prevent MSRP is needed.

In the present study, enterococci had virulence traits and were MDR. Moreover, they can cause infectious diseases that are difficult to treat[30]; therefore, they are a subject of monitoring in infection control systems in animal hospitals. Because enterococci are members of the gastrointestinal flora of cats and dogs and are excreted in feces, HAI can occur because of fecal contamination in hospital environments[19]. Moreover, there is a report indicating that enterococci isolated from 10 animal hospital surfaces were *E. faecium* (35.4%), *E. faecalis* (33.2%), *E. hirae* (28.3%), and *E. gallinarum* (2.5%), and 53% of *E faecium* were MDR, showing resistance to enrofloxacin, ampicillin, and doxycycline, which are all commonly used in veterinary medicine[20]. In the present study, three *E. faecium* and one of *E. faecalis* were isolated, with 100% of *E. faecium* being MDR. *E. faecium* exhibited resistance to ciprofloxacin, ampicillin, and tetracycline. In another study of animal hospital environments and hands of HCWs, enterococci were isolated, and 3 years of observations showed a gradual increase in antimicrobial resistance in *E. faecium*. However, vancomycin-resistant enterococci (VRE) were not isolated[15], and, to the best of our knowledge, the present study is the first in which VRE have been isolated from hands of HCWs in a small animal hospital. Because VRE exhibit MDR, the

choice of therapeutic agents for severe infections is limited, and resistance genes (*vanA*) transmitted to other bacterial species, especially staphylococci, is a problem[10]. Moreover, enterococci are durable and can survive at high temperature and through chlorine and alcohol-based disinfection[6]; thus, they pose a threat in medical settings. Therefore, according to the results in the present study, VRE can be spread through the hands of HCWs, and to prevent such transmission, it is necessary to manage infections by focusing on hand hygiene.

Because the present study had a small sample size, statistical evaluations of the prevalence of bacteria, results of antimicrobial susceptibility testing, and number of resistant strains were limited. However, in the present study, we examined representative resistance genes inducing MRS and VRE. In the future, the number of animal hospitals being studied should be increased for a larger sample size, and studies should be conducted with additional fomites in animal hospitals.

Conclusion

In reality, it is impossible for a hospital to eliminate bacteria. However, even if the carriage rate is low, the presence of resistant or highly virulent bacterial strains in a hospital environment can pose a major threat to both patients and HCWs. Therefore, infection control in animal hospitals is very important from a One Health perspective of protecting both animals and humans, and a systematic infection control protocol that is appropriate for animal hospitals is needed to enhance hospital hygiene.

Conflicts of Interest

These is no conflict of interest.

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