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Original Article

한국 3차 병원 중환자실의 환경 배양검사결과: 의료 환경 안전성과 의료관련 감염 개선에 대한 고찰

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Environmental Culture of Bacteria at the Intensive Care Unit of a Tertiary Hospital in Korea: A Consideration for Improving Medical Environmental Safety and Healthcare-associated Infection

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Background: Intensive care unit (ICU) infections cause major health and financial problems worldwide. Inanimate surfaces and environmental contamination can play a role in the cross-infection of pathogens and associated patient infection. Here, we aimed to identify the pathogens that are present in the ICUs.

Methods: This study analyzed bacterial cultures on 160 environmental samples from the ICU at a tertiary hospital in Incheon.

Results: From cultures of 160 samples, 407 bacteria of 38 species were isolated; of these, 109 (26.8%) were gram-negative and 298 (73.2%) were gram-positive. The common isolation sites were keyboards (38 strains), bed linen sheets (average head, waist, and foot seats) (36 strains), bedside rails (33 strains), and curtains (27 strains). The common bacteria isolated were coagulase-negative staphylococci (CNS) (222 strains, 54.5%), *Acinetobacter baumannii* (48 strains, 11.8%), *Pseudomonas aeruginosa* (33 strains, 8.1%), and *Enterococcus faecium* (24 strains, 5.9%). A total of 60 multidrug-resistant strains were isolated. There were multidrug-resistant *Acinetobacter baumannii* (MRAB) (n=32), multidrug-resistant *Pseudomonas aeruginosa* (MRPA) (n=2), vancomycin-resistant *Enterococcus* (VRE) (n=20), and carbapenem-resistant *Enterobacteriaceae* (CRE) (n=6).

Conclusion: It was confirmed that large numbers of multidrug-resistant bacteria, such as VRE and CRE, colonized the environment in the ICU of this tertiary hospital. Taken together, the findings of this study will inform consideration of new intervention plans for in-hospital medical infection control programs in the future, especially in critical care units.

Key Words: Bacteria, Multidrug resistance, Intensive care units, Cross infection, Infection control

Introduction

Healthcare-associated infections (HAIs) include a broad range of infections. In the past, this term was only used for hospital or nosocomial infections, but currently, it includes medical services associated with infections

and healthcare-associated infections, even if the patient is not in a medical facility [1]. HAI has long been a key issue affecting health care quality, thereby leading to increased mortality rates and medical costs, especially when multidrug-resistant bacteria infect inpatients in an intensive care unit (ICU) [2,3].

The extensive use of antibiotics in the ICUs limits the choice of the next treatment, a situation which can lead to the emergence of multidrug-resistant bacteria. Patients in the ICUs have a high risk of infection for several reasons, including underlying illnesses, immunosuppression, and various medical treatments, such as the use of mechanical ventilation, central venous catheters, and urinary tract catheters. Several studies have reported that the medical infection rates in ICUs are 5-10 times higher than those in general hospital wards [4-7].

Such infections are known to be caused by microorganisms that are present on the hands of healthcare professionals or the beds of patients; in addition, these may also be caused by frequent contact with medical devices and healthcare professional work stations involving equipment, such as telephones, keyboards, and medical records. Gram-negative and gram-positive bacteria are known to be viable for several months under dry surface conditions, and their viability increases under moderate humidity and low temperature conditions. The rate of cross-contamination of microorganisms from one surface to another is affected by several factors, including the type of microorganism, medical device usage, humidity, initial microbial distribution, hand hygiene rules, practical application of infection control by nurses, the number of infected patients in the hospital, the structure of the ICU (private or shared rooms), and in-hospital antibiotic management programs [8-12].

In an ICU, various treatments are administered to patients, and many close contacts occur in a day. In addition, medical equipment used to monitor the condition of patients and extend life, is distributed in the environment around the ICU beds. Hence, more elaborate disinfection management is required for areas where healthcare workers (HCWs) are in frequent contact with patients and equipment. Accordingly, confirmation of the microbe distribution in various medical environments may play a crucial role in managing infections in medical institutions and informing the design of new interventions.

In this study, we isolated and cultured bacteria from the hospital environment, specifically the ICU. We also aimed to determine the status of cross-contamination of antibiotic-resistant bacteria in the medical environment, by investigating the distribution of medically related infectious disease pathogens, such as carbapenem-resistant *Enterobacteriaceae* (CRE), multidrug-resistant *Pseudomonas aeruginosa* (MRPA), multidrug-resistant *Acinetobacter baumannii* (MRAB), vancomycin-resistant *Staphylococcus aureus* (VRSA), and vancomycin-resistant *Enterococcus* (VRE) [13].

Materials and Methods

1. Object

The ICU of the Gil Medical Center (GMC), a tertiary hospital in Incheon, was categorized into two zones, and one bed was designated for study in each zone. Zone A and zone B were in the same ICU, separated by different columns and different pathways. Twenty sites per bed were selected, and environmental samples were collected from these sites every week from 17th October, 2019 through 5th November, 2019 (Fig. 1).

2. Environmental harvesting and bacterial separation culture

The environmental surface area was wiped using a sterile phosphate-buffered saline (PBS) moist gauze; the gauze was then placed in a sterile bag and moved to the laboratory at 4°C. The samples in the wet gauze were dispensed and plated in 100 μL of Blood agar (Asan Pharmaceutical, Korea), Tryptic Soy Agar (BBL, Fisher Scientific, USA), MacConkey agar (BD BBL, Fisher Scientific, USA), CHROMagar™ mSuperCARBA™ (CHROMagar company, France), CHROMagar™ VRE (CHROMagar company, France) and CHROMagar™ Staph aureus (CHROMagar company, France), and cul-

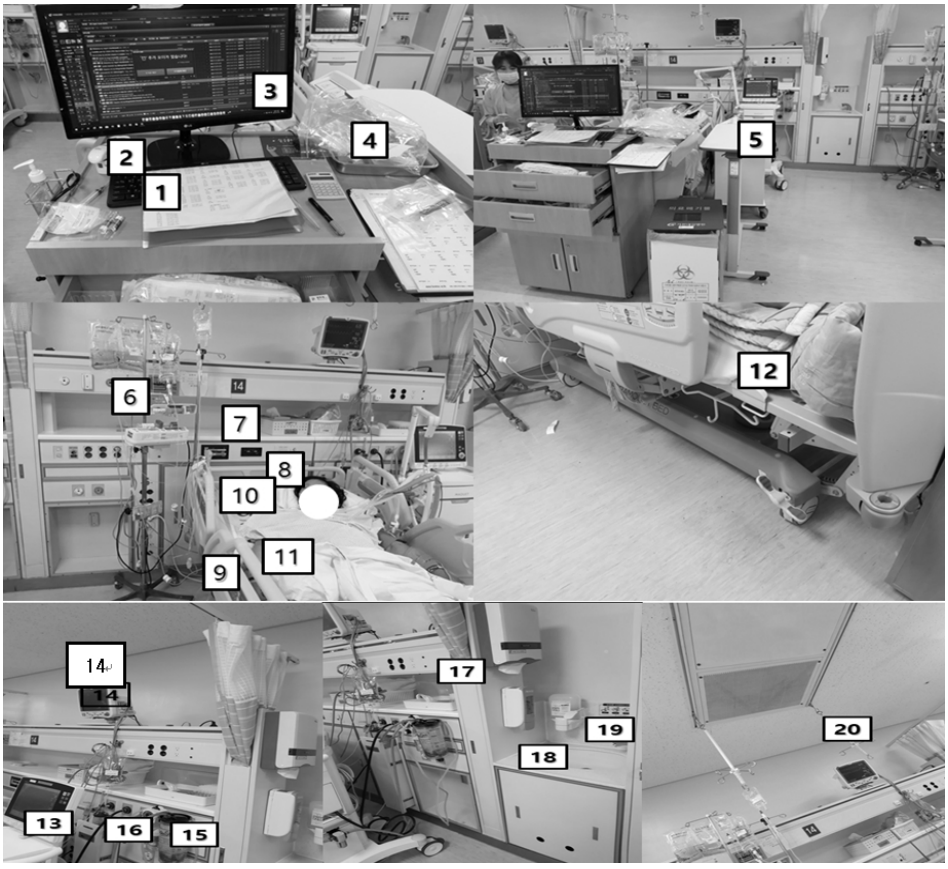

Fig. 1. Sites of environmental culture. (1) Keyboard of computer, (2) mouse of computer, (3) monitor of a computer at bedside, (4) patient trays, (5) bed removable table, (6) infusion pump modulator, (7) bedside shelves, (8) bedside rails, (9) bed modulator (remote controller), (10) bed linen (head), (11) bed linen (body), (12) bed linen (leg), (13) mechanical ventilator, (14) patient monitoring modulator, (15) suction bottle, (16) ventilator and other infusion line area, (17) patient curtain, (18) washstands, (19) water tap, (20) infusion pump holder.

tured at 37°C for 24 h. All colonies grown in the media were isolated on TSA medium, and the bacteria were identified using a mass spectrometer (MALDI-TOF, Bruker). If the score was ≥2.0, the result was judged valid. In the database, calculates an arbitrary unit score value between 0 and 3 reflecting the similarity between the sample and the reference spectrum, and displays the top 10 matching database records. Standard Bruker interpretative criteria were applied. Briefly, scores of ≥2.0 were accepted for species assignment and scores of ≥1.7 but <2.0 for identification to the genus level. Scores below 1.7 were considered unreliable.

3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was performed using the broth microdilution method with customized Sensititre KRCDC2F, KORN (Trek Diagnostic Systems, OH, USA) and MIC-Strip Vancomycin (MERLIN Diagnostika, Germany), in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [14]. The following antimicrobial agents were used for testing: ampicillin, azithromycin, cefoxitin, ceftazidime, ceftriaxone, cefotaxime, imipenem, gentamicin, amikacin, streptomycin, tetracycline, nalidixic acid, ciprofloxacin, trimethoprim/sulfamethoxazole, chloramphenicol, colistin, meropenem, ertapenem, imipenem, doripenem, and vancomycin.

The designation of resistant pathogens is as follows. MRPA and MRAB refer to *P. aeruginosa* and *A. baumannii*, respectively, which are resistant to all three classes of antimicrobial agents, carbapenem, aminoglycoside, and fluoroquinolone. CRE refers to Enterobacteriaceae, which is resistant to carbapenem antibiotics. VRSA and VRE refer to vancomycin-resistant *S. aureus* and enterococci, respectively. The application of antibiotic resistance criteria is based on the infectious disease diagnostic test guidelines [13] of the KDCA (Korea Disease Control and Prevention Agency).

Table 1. Results of bacterial culture of intensive care unit environment by site (A and B zones)*

A zone	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	Total (%)
Enterococcus faecalis	1							1		1	2	2					1				8 (3.6)
Enterococcus faecium	3					1		2		2		2				1	3			1	15 (6.7)
Staphylococcus aureus	1			1						1	1				1	1	1				7 (3.1)
CNS	9	9	4	8	1	5		10	3	8	11	11	5	3	4	4	9	2	1	5	112 (49.7)
Paenibacillus spp.										2			1			1					4 (1.8)
Bacillus spp.			1							1	1	1			1				1	3	9 (4)
Corynebacterium striatum												2									2 (0.9)
Bifidobacterium infantis	2									1											3 (1.3)
Micrococcus luteus	1															1		1			3 (1.3)
Streptococcus mitis	1																				1 (0.4)
Corynebacterium aurimucosum						1															1 (0.4)
Acinetobacter baumannii	2	1	1	1		1		1	1	2	1	3	2		2	2	2	3		2	27 (12)
Acinetobacter bereziniae																		1	1		2 (0.9)
Enterobacter aerogenes								1				2			1						4 (1.8)
Pseudomonas aeruginosa								1	1	1	2	1						4	3		13 (5.8)
Klebsiella pneumoniae		1									2							2			5 (2.2)
Stenotrophomonas maltophilia															1				1		2 (0.9)
Enterobacter cloacae																		1			1 (0.4)
Cupriavidus pauculus																		2	1		3 (1.3)
Neisseria subflava	1																				1 (0.4)
Pseudomonas putida																			1		1 (0.4)
Pantoea calida																				1	1 (0.4)
Total isolated	21	11	6	10	1	8	0	16	5	19	20	24	8	3	10	10	16	16	9	12	225
%	9.3	4.9	2.7	4.4	0.4	3.6		7.1	2.2	8.4	8.9	10.7	3.6	1.3	4.4	4.4	7.1	7.1	4	5.3	100

B zone	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20	Total (%)
Enterococcus faecalis																	1				1 (0.5)
Enterococcus faecium	1					2		1			1	2					1	1			9 (4.9)
Staphylococcus aureus	1											1			1		1				4 (2.2)
CNS	12	7	2	4	1	7	2	8	13	10	8	12	2	2	6	4	4	4	1	7	110 (60.4)
Paenibacillus spp.								1													1 (0.5)
Bacillus spp.	1				1										2						4 (22)
Stenotrophomonas maltophilia												1									1 (0.5)
Micrococcus luteus										1								1			2 (1.1)
Enterococcus arium		1																			1 (0.5)
Acinetobacter baumannii	2	1		1		1	1	2		1	2	1	1	1	1	1	2	1		2	21 (11.5)
Acinetobacter bereziniae																		1	1		2 (1.1)
Pseudomonas aeruginosa		1		1	2		1	2	1	2	1	1	1	1	2	2		2			20 (11)
Klebsiella pneumoniae																		1	1		2 (1.1)
Neisseria subflava					1																1 (0.5)
Acinetobacter nosocomialis															1						1 (0.5)
Paenibacillus urinalis												1					1				2 (1.1)
Total	17	10	2	6	5	10	4	17	5	14	12	19	4	4	13	7	10	11	3	9	182
%	9.3	5.5	1.1	3.3	2.7	5.5	2.2	9.3	2.7	7.7	6.6	10.4	2.2	2.2	7.1	38	5.5	6	1.6	4.9	100

*The numbers in the table indicate numbers of strains that are isolated at selected site.

Abbreviations: CNS, coagulase-negative staphylococci; *Spp*, species; A, B1, keyboard of computer; A, B2, mouse of computer; A, B3, monitor of computer at bedside; A, B4, patient trays; A, B5, bed removable table; A, B6, infusion pump modulator; A, B7, bedside shelves; A, B8, bedside rails; A, B9, bed modulator (remote controller); A, B10, bed linen (head); A, B11, bed linen (body); A, B12, bed linen (leg); A, B13, mechanical ventilator; A, B14, patient monitoring modulator; A, B15, suction bottle; A, B16, ventilator and other infusion line area; A, B17, patient curtain; A, B18, washstands; A, B19, water tap; A, B20, infusion pump holder.

Results

From 17th October to 5th November, 2019, a total of four bacterial tests were conducted on the surrounding environment of patients admitted to the ICU every week. Table 1 shows the bacterial separation results from each of the 20 sites in zones A and B. In this study, 38 species of 407 bacteria strains were isolated from the samples obtained from the ICU environment. Of these, 109 (26.8%) were gram-negative and 298 (73.2%) were gram-positive. The common species were coagulase-negative Staphylococcus (222 strains, 54.5%); *A. baumannii* (48 strains, 11.8%); *P. aeruginosa* (33 strains, 8.1%); *E. faecium* (24 strains, 5.9%); and *Bacillus* species (13 strains, 3.2%), in order of distribution (Table 2). Among the 20 sites, the highest number of bacteria was separated from the keyboard (38 strains), followed by bed linen sheets (average of the head, waist, and foot seats) (36 strains), bedside rails (33 strains), washbasin (27 strains), curtains (26 strains), suction bottles (23 strains), and mouse and intravenous fluid bag holder (21 strains) (Table 3). Total 225 bacteria of 33 species were isolated from the samples collected from zone A; by species, coagulase-negative Staphylococcus (CNS) (112 strains, 49.8%), *A. baumannii* (27 strains, 12%), *E. faecium* (15 strains, 6.7%), and *P. aeruginosa* (13 strains, 5.8%) were the predominant isolates in this zone. A total of 182 bacteria from 26 species were isolated from the samples collected from zone B. CNS (110 strains, 60.4%); *A. baumannii* (21 strains, 11.5%); and *P. aeruginosa* (20 strains, 11%) were the

Table 2. Classification of a total of 407 identified bacterial strains by species

	A zone (N=225)	B zone (N=182)	Total (N=407)	Rate (%)
Staphylococcus aureus	7	4	11	2.7
CNS	112	110	222	54.5
Streptococcus mitis	1	0	1	0.2
Enterococcus faecalis	8	1	9	2.2
Enterococcus faecium	15	9	24	5.9
Enterococcus arium	0	1	1	0.2
Paenibacillus spp.	2	1	3	0.7
Corynebacterium aurimucosum	1	0	1	0.2
Corynebacterium striatum	2	0	2	0.5
Bacillus spp.	9	4	13	3.2
Bifidobacterium infantis	3	0	3	0.7
Micrococcus luteus	3	2	5	1.2
Acinetobacter baumannii	27	21	48	11.8
Acinetobacter bereziniae	2	2	4	1.0
Acinetobacter nosocomialis	0	1	1	0.2
Cupriavidus pauculus	3	0	3	0.7
Enterobacter aerogenes	4	0	4	1.0
Enterobacter cloacae	1	0	1	0.2
Neisseria subflava	1	1	2	0.5
Klebsiella pneumonia	5	2	7	1.7
Stenotrophomonas maltophilia	2	1	3	0.7
Pseudomonas putida	1	0	1	0.2
Pantoea calida	1	0	1	0.2
Pseudomonas aeruginosa	13	20	33	8.1
Paenibacillus urinalis	2	2	4	1.0

Abbreviations: N, number; CNS, coagulase-negative staphylococci; *Spp*, species.

Table 3. Relative distributions of bacterial isolates by site of cultures

Site	A zone (N=225)	B zone (N=182)	Total (N=407)	Rate (%)
S1	21	17	38	9.3
S2	11	10	21	5.2
S3	6	2	8	2.0
S4	10	6	16	3.9
S5	1	5	6	1.5
S6	8	10	18	4.4
S7	0	4	4	1.0
S8	16	17	33	8.1
S9	5	5	10	2.5
S10	19	14	33	8.1
S11	20	12	32	7.9
S12	24	19	43	10.6
S13	8	4	12	2.9
S14	3	4	7	1.7
S15	10	13	23	5.7
S16	10	7	17	4.2
S17	16	10	26	6.4
S18	16	11	27	6.6
S19	9	3	12	2.9
S20	12	9	21	5.2

Abbreviations: S1, keyboard of computer; S2, mouse of computer; S3, monitor of the computer on the bedside; S4, patient trays; S5, bed removable table; S6, infusion pump modulator; S7, bedside shelves; S8, bedside rails; S9, bed modulator (remote controller); S10, bed linen (head); S11, bed linen (body); S12, bed linen (leg); S13, mechanical ventilator; S14, patient monitoring modulator; S15, suction bottle; S16, ventilator and other infusion line area; S17, patient curtain; S18, washstands; S19, water tap; S20, infusion pump holder.

most commonly isolated species in this zone.

For all the strains isolated, the target strain for the pathogen causing medically infectious diseases was selected, and antibiotic and resistance gene (for carbapenemase-producing *Enterobacteriaceae* (CPE) and VRE confirmation) tests were performed to confirm the presence or absence of resistant bacteria. Sixty strains of infectious antibiotic-resistant bacteria were isolated in this study: 32 strains of MRAB, 2 strains of MRPA, 20 strains of VRE, 6 strains of CRE, and no VRSA. All VREs were positive for the Van A resistance gene. Of the six CRE strains (*Klebsiella pneumoniae*, four strains; *Enterobacter aerogenes*, one strain; and *Enterobacter cloacae*, one strain), the strain carrying the resistance gene (CPE) was *Klebsiella pneumoniae* (four strains). Using sequence analysis, all of the KPC-2 genotypes were confirmed (data not shown).

Table 4 shows the antibiotic-resistant strains identified in zones A and B. In zone A, 31 antibiotic-resistant bacteria were isolated: CRE (4 strains, CPE 2 strains); MRAB (11 strains); MRPA (2 strains); and VRE (14 strains). In zone B, 29 resistant bacteria were isolated: CRE (2 strains, CPE 2 strains); MRAB (21 strains); and VRE (6 strains).

From the samples of Zone A, two CREs were isolated from the bedside rail and washstand; six MRABs were isolated from the keyboard, bed sheet, mechanical ventilator, curtain, washstand, and infusion holder; two MRPAs were isolated from the bed sheet and washstand; and VRE was isolated from seven sites: keyboard, infusion pump remote control, bedside rail, bed sheet, vent line, curtain, and infusion holder (Table 4).

Table 4. frequency of isolation of multidrug-resistant bacteria by cultures sites (A, B zone) : The number of times cultured during 4 repeated culture tests is indicated by a + mark

Pathogens	CRE (CPE)		MRAB		MRPA		VRE	
Site	A zone	B zone	A zone	B zone	A zone	B zone	A zone	B zone
S1			++	++			++	+
S2				+				
S3								
S4				+				
S5								
S6				+			+	+
S7				+				
S8	+			++			++	
S9								
S10				+			++	
S11			+	++	+			+
S12				+			++	++
S13			++	+				
S14				+				
S15				+				
S16				+			+	
S17			++	++			+++	
S18	+++	+	++	+	+			+
S19		+						
S20			++	++			+	
Total (A 31/B 29)	4 (2)	2 (2)	11	21	2	0	14	6

Abbreviations: S1, keyboard of computer; S2, mouse of computer; S3, monitor of the computer on the bedside; S4, patient trays; S5, bed removable table; S6, infusion pump modulator; S7, bedside shelves; S8, bedside rails; S9, bed modulator (remote controller); S10, bed linen (head); S11, bed linen (body); S12, bed linen (leg); S13, mechanical ventilator; S14, patient monitoring modulator; S15, suction bottle; S16, ventilator and other infusion line area; S17, patient curtain; S18, washstands; S19, water tap; S20, infusion pump holder; CRE, carbapenem-resistant *Enterobacteriaceae*; CPE, carbapenemase-producing *Enterobacteriaceae*; MRAB, multidrug-resistant *Acinetobacter baumannii*; MRPA, multidrug-resistant *Pseudomonas aeruginosa*; VRE, vancomycin-resistant *Enterococcus*.

Among the drug-resistant pathogens, MRAB was the most commonly isolated, with a proportion of 20%, followed by VRE 12.5%, CRE 6.3%, and MRPA 1.3%. The most common site from which resistant bacteria were isolated was the washstands in both zones A and B. In the case of bed linen on which the patient was lying, VRE, MRAB, and MRPA were detected in zone A, VRE, and MRAB in zone B, and MRAB in the curtains in zone B (Table 4). There was no outbreak of bacterial infection during the study period in GMC.

Discussion

Various microorganisms exist in the hospital environment. These microorganisms are known to cause infection by several routes, including air transmission (pulmonary tuberculosis, chickenpox, and measles), droplet transmission (including influenza, Middle East respiratory syndrome virus, and coronavirus disease 2019), or contact transmission (multidrug resistant bacteria, such as VRE and CRE) [1]. In-hospital transmission of pathogens usually occurs through the hands of HCWs, who are in direct contact with infected patients; however, it may also occur through the contaminated hospital environment or the hands of HCWs exposed to equipment [15]. Our study demonstrated that there are many bacteria, especially MDR-bacteria, in a real ICU environment; hence, it is necessary to perform routine surveillance cultures or to have environmental infection control programs.

A previous study reported that the surface of a medical device that was in frequent use in an ICU was re-contaminated within 4 h after standard disinfection [16]. Environmental contamination in the ICU can occur not only through equipment that is directly used for patient care (for example, stethoscopes, ultrasound equipment, infusion pumps, and the surface of mechanical ventilators), but also through medical record equipment (for example, medical charts, computer keyboards, mice, and monitoring devices) [8,16].

In one study undertaken from 2010 to 2012, cultures were performed on the samples collected from the hands of ICU HCWs—nurse, doctor, environmental cleaner, guide, and nursing assistant—and the ICU environment: bed, bed linen, nurse station, Ambu bag, patient table, oxygen mask, ventilator, telephone, patient, and medical record file. Approximately 1.4% and 16.5% *A. baumannii*, 5.9% and 8.1% *S. aureus*, 20.9% and 18.7% *S. epidermis*, and 1% and 1.3% *Enterococcus* species were isolated from the HCWs and ICU environment, respectively [17]. In addition, MRAB (94%, 54.5%), MRSA (59.6%, 67.3%), and VRE (0%, 25%) were isolated from HCWs and environmental specimens, respectively. Ventilators, oxygen masks, and bed linen were the most commonly contaminated sites in this study [17]. These findings are similar to those reported in the present study.

Microorganisms that can cause HAI include not only the microbes from the environment of a medical institution or HCWs, but also those residing in the bodies of patients. In general, human skin carries bacteria at a concentration of 4×10^4-1×10^6 colony-forming units per 1 cm^2, and 10^6 skin cells are eliminated from normal skin every day. As the eliminated skin cells contain living microorganisms, environmental contamination by microorganisms is common in areas around the patient, such as patient gowns and bed linen [18]. Our study led to the detection of drug-resistant bacteria, such as MRAB, MRPA, and VRE, in patient bed linen.

There are reports that 40%-60% of HAIs occurring in the ICU are caused by the normal flora of patients, and 20%-40% by cross-infection from the hands of HCWs [19,20]. In one cohort study, cultures from the gloves and gowns of HCWs were prepared after treating a patient infected with *A. baumannii*. About one-third of the gowns and gloves of HCWs were positive for *A. baumannii*, and about 80% of the ward environments of infected patients were contaminated with *A. baumannii*. The independent risk factors for HAI were as follows: HCWs contaminated with multidrug-resistant (MDR) bacteria (OR=4.2, 95% CI=2.7.6.5), HCWs stay in the room of infected patients for more than 5 min (OR=2.0, 95% CI=1.2.3.4), physical examination (OR=1.7, 95% CI=1.1.2.8), and ventilator contact (OR=1.8, 95% CI=1.1.2.8) [21]. Resistant bacteria present in the patient's surrounding environment may be a risk factor for increased medical-related

infections caused by multidrug-resistant bacteria. Our data demonstrated that there are many MDR bacteria in the ICU environment that pose a high risk of HAI.

The viability of pathogens on environmental surfaces is related to the unique characteristics of microorganisms, such as species, presence or absence of biofilm formation, and distribution concentration, and environmental factors, such as UV irradiation, temperature, humidity, concentration of organic substances, and object surfaces [22-24]. In studies on the viability of bacteria, viruses, and fungi in the medical setting, coagulase-negative staphylococci could survive for 8-21 days on clothing and towels, and *P. aeruginosa* was reported to survive for up to 24 h on clothing and towels [25]. In our study, bacteria were found in linen and bed sheets, a similar situation.

Resistance to disinfectants may be produced by the unique characteristics of a microorganism. For example, the wax layer of the bacterial cell wall and the extracellular membrane of gram-negative bacteria inhibit the invasion of disinfectants into cells; it is well known that the higher the concentration of gram-positive and gram-negative bacteria, fungi, and viruses, the longer their survival times on environmental surfaces [26]. Biofilm is a three-dimensional structure of microorganisms, formed in a polymeric matrix secreted by microorganisms. More than 90% of a biofilm is composed of exopolymeric substances. Pathogens can form biofilms in the epithelial cells, bones, teeth, and blood vessel linings of hosts, as well as medical and dental devices, such as catheters, various implants, and artificial organs [27,28]. The humidity in the medical environment of the ICU makes it easier to form biofilms, and these are not easy to remove, because biofilms are more than 1,000 times more resistant to disinfectants than bacteria in a planktonic form [29]. *P. aeruginosa* biofilms survive even after 5 min of exposure to 2,000 ppm peracetic acid, on the surface of endoscopes [30]. In our study, 11.8% of coagulase-negative staphylococci were isolated, and *P. aeruginosa*, which are multidrug resistant and biofilm-forming, were also identified in both zones A and B. As mentioned above, among the bacteria that cause HAIs, MDR bacteria are more easily transmitted between patients than the bacteria with high sensitivity to antibiotics. This is because the selective pressure from broad-spectrum antibiotics commonly used in hospitalized patients inhibits the normal flora in patients, making it easier for them to carry MDR bacteria [1]. Moreover, the microorganisms themselves undergo structural changes, such as forming biofilms, to lower the effects of disinfecting, and the surviving pathogens have sufficient potential to cause cross-contamination among infected patients, HCWs, and hospital environments.

This study has several strengths. First, it is most similar to real-world data, in that the environment of the ICU of a tertiary hospital in use was analyzed, and the samples were serially tracked at more than 20 clinical sites. Second, it was confirmed that the positivity rate of MDR bacteria was high, by analyzing the frequency of the identified bacterial strains and checking the proportion of resistant bacteria. These findings can inform infection control policies and infection control interventions in the ICU.

This study also has some limitations. First, it was not possible to demonstrate a direct relationship between cultures from the environment and patient identification in the ICU. Even when strains are identified in the environment, not all are related to patient infection, but they can be considered sufficiently related when considering the frequent procedures and treatments in the ICU. In previous studies, when HCWs treated the patients infected with *A. baumannii*, the gown and gloves were contaminated with a probability of about a third, and about 80% of the hospitalization wards of *A. baumannii*-infected patients had the bacteria isolated from the environment [17,21]. Identification of the bacteria in environmental culture, especially MDR strains, may be important in the ICU situation. Second, there is a lack of information on whether identifiable bacteria occur more frequently in the ICU than in the general hospital wards situations. However, real-world data in the ICU were reflected. All cultures were measured serially, and many findings matched the culture results from those of previous studies [17,18]; however, we believe this problem can be overcome by further detailed studies. Third, it was not possible to confirm the comparison before and after infection interven-

tion, by checking only the total bacteria isolated from each site. Since this study aimed to search for strains in the ICU in everyday situations without intervention, we thought that performing these comparisons would affect the results. Based on the proportion and type of environmentally cultivated strains identified in this study, it is necessary to study additional infection intervention methods.

Although the number of topics in infection control is very large, the overall focus is mainly on patients, pathogens, and medical staff, while interest in the environment is relatively less. The standard preventive guideline for avoiding HAI is to perform random microbial tests on the samples obtained from the air, water, and environmental surfaces of medical institutions. It is stated that this process should be implemented for the evaluation of environmental conditions.

In conclusion, this study confirmed, based on the results of random bacteriological tests in the ICU of a tertiary hospital, that MDR bacteria, such as VRE and CRE, were distributed in different patterns. This finding suggests that even if an outbreak does not occur, microbial testing of the environment may be necessary. Taken together, the findings of this study will help in designing a new intervention plan for in-hospital HAI management in the future.

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