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The hospital environment as an ecological driver of multidrug-resistant organisms: evidence from intensive isolation and operating rooms

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Abstract

Purpose: Multidrug-resistant organisms (MDROs) present a significant challenge for hospital infection control, with environmental surfaces acting as key reservoirs. This study sought to identify MDRO species, evaluate their biofilm-forming capacity, and quantify microbial bioburden in intensive care isolation and operating rooms. **Methods:** Environmental sampling was conducted in 16 rooms. Air and surface samples, including floors, walls, and bedside monitors, were collected using a microbiological air sampler and sterile swabs. Samples were cultured to determine total bioburden and detect MDROs, with bacterial identification performed using VITEK 2. Antimicrobial resistance was assessed using the Kirby-Bauer disc diffusion test, and bioburden levels were compared to national standards. **Results:** Forty-nine MDRO isolates were identified: 3 methicillin-resistant *Staphylococcus aureus* (MRSA, 6.12%), 19 methicillin-resistant coagulase-negative staphylococci (MRCONS, 38.78%), 24 carbapenem-resistant *Acinetobacter* spp. (CRA, 48.98%), and 3 extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (6.12%). Mean microbial bioburden remained within acceptable limits for air (57.44 CFU/m³), walls (70.63 CFU/100 cm²), and bedside monitors (22.63 CFU/100 cm²), but exceeded thresholds on floors (753.75 CFU/100 cm²). Biofilm-forming capacity varied: MRCONS (26.32% strong biofilm), CRA (8.33% strong biofilm), and ESBL-producing Enterobacterales (66.67% weak biofilm). **Conclusion:** MDROs were detected in all sampled rooms, with CRA as the predominant species. Floors exhibited microbial loads above acceptable standards, highlighting the necessity for improved cleaning protocols. Enhanced environmental infection control strategies are essential to reduce MDRO transmission in healthcare settings.

Keywords: biofilm; environmental surveillance; healthcare-associated infection; microbial contamination; multidrug-resistant organisms

INTRODUCTION

Healthcare-associated infections (HAIs) are defined as infections acquired by patients who were free of infection at the time of hospital admission. The burden of HAIs is disproportionately higher in developing countries compared to high-income nations, with the risk estimated to be 2–20 times greater and incidence rates up to six times higher [1]. Data from the Centers for Disease Control and Prevention (CDC) and the

National Nosocomial Infection Surveillance (NNIS) program indicated that, between 1992 and 1997, HAIs were the fourth leading cause of death in the United States, accounting for at least 20,000 deaths annually [2].

In Southeast Asia, the prevalence of HAIs is 21.6%, with Indonesia reporting the highest prevalence (30.4%) and Singapore the lowest (8.4%) [3]. The most frequently reported pathogens include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter* spp., Enterobacterales such as *Klebsiella* spp., Escherichia coli, and *Candida* spp. [4]. A substantial proportion of these organisms are multidrug-resistant organisms (MDROs), posing significant challenges for hospital infection control programs.

Environmental contamination represents a critical route of MDRO transmission. High-touch surfaces in patient care areas, such as bed controls, call buttons, and bedside tray tables, are recognized as important reservoirs for MDRO persistence [5,6]. Pathogens such as vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and extended-pectrum β-lactamase (ESBL)-producing Enterobacterales can survive on surfaces for extended periods, facilitating transmission to patients, visitors, and healthcare personnel [7].

A Comprehensive evaluation of MDRO presence, biofilm-forming capacity, and microbial bioburden in critical hospital environments—particularly intensive care isolation units and operating rooms—provides valuable insights into environmental transmission dynamics. However, data on the environmental burden of MDROs and their biofilm-forming ability in Indonesian healthcare facilities remain scarce, underscoring the need for local evidence to inform targeted infection prevention and control strategies.

METHODS

Study design and setting

This study employed an observational, descriptive-analytic cohort design. It was conducted in August 2024 in the intensive isolation room and operating room at the Academic Hospital of Universitas Gadjah Mada, Yogyakarta, Indonesia.

The study was carried out in a hospital setting, focusing on isolation and operating rooms. Inclusion criteria were: (1) rooms used to treat patients requiring airborne or contact isolation in intensive care, and (2) rooms equipped with a pressure control system.
Exclusion criteria were: (1) rooms that are undergoing construction or renovation, and (2) rooms not used for patient care. Of 46 intensive care rooms and eight operating rooms, 16 met the eligibility criteria, comprising eight isolation intensive care rooms and eight operating rooms.

Data collection

Environmental sampling

Environmental samples were collected by trained research staff from air, floors, walls, and bedside monitors under normal room conditions, i.e., not immediately after routine or terminal cleaning. Air sampling was performed using the MAS-100 Microbial Air Monitoring System by aspirating 100 mL of air onto blood agar and chromogenic media. Surface sampling was conducted using pre-moistened sterile swabs, applied to a 10 × 10 cm area on each site (floor, wall, and bedside monitor). All samples were processed using standard microbiological procedures.

Sample processing and culture methods

Specimens were inoculated onto blood agar plates to quantify total bioburden and onto selective chromogenic media (Staphylococcus, Acinetobacter, and ESBL agar) to identify MDROs. Bacterial identification was confirmed using the VITEK 2 system. Antimicrobial susceptibility was determined using the Kirby–Bauer disc diffusion method, with cefoxitin as a confirmatory test for MRSA and meropenem for carbapenem-resistant organisms (CROs). Bioburden results were compared against national standards: ≤180 CFU/m³ for operating room air, ≤200 CFU/m³ for intensive care unit air, and 0–5 CFU/cm² or ≤500 CFU/100 cm² for surfaces.

Bacterial identification and antibiotic resistance testing

Colony morphology on chromogenic media was used for presumptive identification: *Staphylococcus* spp. produced green colonies on Staphylococcus agar; *Acinetobacter* spp. produced red colonies on Acinetobacter agar; ESBL-producing *Klebsiella*, *Enterobacter*, and *Citrobacter* spp. produced metallic blue colonies, while ESBL-producing *E. coli* produced dark pink to reddish colonies. Antibiotic susceptibility was assessed according to CLSI standards. Bacterial suspensions (10^8 CFU/mL, adjusted to McFarland standard) were spread on Mueller–Hinton agar, and antibiotic discs were applied. Plates were incubated at 37 °C for 18–24 hours, and inhibition zones were measured to classify isolates as sensitive, intermediate, or resistant.

Biofilm formation test

Biofilm-forming ability was evaluated using the microtiter plate assay. Colonies were inoculated into 10

mL tryptic soy broth (TSB) and incubated at 37 °C for 24 hours. Cultures were diluted 1:100 in fresh medium, and 200 µL was added to 96-well polystyrene microplates, including positive and negative controls. After 24 hours of incubation, wells were washed three times with PBS to remove non-adherent cells, stained with 0.1% crystal violet, washed with distilled water, and air-dried. Bound dye was solubilized with 5% acid isopropanol, and optical density (OD) was measured at 595 nm using an Enzyme-Linked Immunosorbent Assay (ELISA) reader. Biofilm formation was classified according to the Stepanović criteria [8].

Microbiological media and quality control

The following media were used: blood agar, Mueller–Hinton agar, and chromogenic media for Staphylococcus, Acinetobacter, and ESBL-producing Enterobacterales. All media were freshly prepared under aseptic conditions at the Microbiology Laboratory, Faculty of Medicine, Universitas Gadjah Mada (UGM). Media quality was ensured by incubating prepared plates and saline controls at 37 °C for 18–24 hours before use to confirm sterility.

Quality control of bacterial identification

Quality control for MDRO identification was performed using two clinical isolates of each target organism (CRAB, ESBL-producing *K. pneumoniae*, and ESBL-producing *E. coli*) that had been previously confirmed by VITEK 2. Each isolate was cultured on chromogenic media to verify standard colony phenotypes: MRSA-green colonies on chromogenic Staphylococcus agar with clustered cocci on Gram staining, catalase positive, coagulase positive, and cefoxitin resistance; MRCONS similar microscopic and biochemical features but coagulase negative; CRAB-round red colonies on chromogenic Acinetobacter agar; ESBL K. pneumoniae-dark turquoise colonies; ESBL *E. coli*-light purple colonies. When colony phenotypes were doubtful, VITEK 2 was used for confirmation.

Data analysis

The main outcomes of interest were the baseline prevalence rates of MDROs detected in the hospital environment, including air, walls, floors, and patient bedside monitors, representing high-touch surfaces. Data were analyzed to calculate microbial bioburden levels and the percentage of MDRO contamination across different surfaces and air samples.

RESULTS

Specimen collection, consisting of environmental swabs and air samples, was conducted on August 19, 2024, in eight airborne ICU rooms and eight operating rooms. Specimens were collected during the operating room (OR) and ICU activity. Measurements included room area, lighting level (illuminance), air humidity, air pressure, and air bioburden counts. The average room area in the airborne ICU room was 39.44 m² (standard minimal value: 16 m²), and OK was 49.2 m² (standards recommend a minimum of 42 m² for a general OR and 50 m² for primary specialty OR).

The mean lighting level (illuminance) in operating rooms was 581.25 lux (standard value: 300-500 lux), while in the ICU it was 251.38 lux (standard value: 250 lux). The mean temperature in operating rooms was 19.41 °C (standard value: 22-27 ^{0}C), while in ICUs it was 22.75 °C (standard value: 22-23 ^{0}C). Average air humidity was 65.08% (standard value: 40-60%) in operating rooms and 57.84% (standard value: 40-60%) in ICUs.

Air pressure measurements showed +5.25 Pa in operating rooms (standard value: minimum +2.5 Pascal (Pa)) and -2.65 Pa in airborne ICUs (standard value: minimum -2.5 Pascal (Pa)) (Table 1). This finding shows that the room size, lighting level (illuminance), air humidity, and air pressure significantly exceeded the minimum requirements set by the standards for ICU and OK, but not in operating rooms, where average air humidity was lower.

Table 1. The description of the conditions of the airborne isolation intensive care unit (ICU) and operating room (OK) in surveillance MDRO Academic Hospital UGM 2024

Loc	Average room size (m^2)	Average lighting (lux)	Average temperature (^{0}Celsius)	Average humidity (%)	Average air pressure (Pa)
OK	49.20	581.25	19.41	65.07	5.25
ICU	39.43	251.38	22.75	57.83	-2.65

Note: Loc (Location), OK (Operating Room), ICU(Airborne Isolation Intensive Room), Pa(Pascal), CFU(Colony Forming Unit)

A total of 49 MDRO isolates were recovered in this study, consisting of 24 CRA (48.98%), 19 MRCoNS (38.78%), 3 MRSA (6.12%), and 3 Extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) (6.12%) (Table 2). Isolates obtained from operating rooms accounted for 30/49 (61.22%) of the total, while 19/49 (38.78%) were recovered from ICU rooms. In operating rooms, MRCoNS predominated (17/30; 56.67%), followedby CRA (11/30; 36.67%). By contrast, isolates from ICU rooms were dominated by CRA 13/19(68.42%) (Table 2).

Table 2. MDRO isolates according to the room type (operating room and isolation intensive room) in surveillance at the MDRO Academic Hospital UGM 2024

Bacterial isolate	OK	ICU	Sub total
	n (%)	n (%)	n (%)
MRSA	0 (0)	3 (15.79)	3 (6.12)
MRCoNS	17 (56.67)	2 (10.53)	19 (38.78)
CRA	11 (36.67)	13 (68.42)	24 (48.98)
ESBL-E	2 (6.67)	1 (5.26)	3 (6.12)
Total	30 (100)	19 (100)	49 (100)

Notes: OK (Operating Room), ICU (ICU Airborne), MRSA (Methicillin-resistant *Staphylococcus aureus*), MRCoNS (Methicillin-resistant coagulase-negative staphylococci), ESBL-E (Extended-spectrum beta-lactamase-producing *Enterobacterales*), CRA (Carbapenem-resistant *Acinetobacter* spp.)

Table 3. The microbial bioburden from the air, the floor, the wall, and the patients' bedside monitor in blood agar in surveillance, MDRO, Academic Hospital, UGM 2024

Location	Microbial Bioburden			
	Air (CFU/m³)	Floor (CFU/100cm²)	Monitor (CFU/100 cm²)	Wall (CFU/100 cm²)
OK	27.375	320.375	13.875	132.375
ICU	87.5	1187.125	31.375	8.875
OK + ICU	57.438	753.75	22.625	70.625

Notes: OK(Operating Room), ICU(Airborne Isolation Intensive Room), CFU(Colony Forming Unit), Standard value for microbial bioburden from hospital surface (0-5 CFU/cm² or 0-500 CFU/100 cm²), standard value from air sampling for OR (below 180 CFU/m³) and for ICU (below 200 CFU/m³)

The microbial bioburden of the air, the floor, the wall, and the patient's bedside monitor was measured from blood agar after 24 hours of incubation. The measured air microbial loads in both OR and ICU were within acceptable limits, with values 87.5 CFU/m³ (standard value: below 180 CFU/m³) for OR and 27.375 CFU/m³ (standard value: below 200 CFU/m³) for ICU (Table 3). However, the bacteria bioburden from the floor exceeded the recommended threshold. The microbial bioburden in OK is 320 CFU/100 cm², while in ICU it is 1187 CFU/m² (Standard value: 0-5 CFU/cm² or 0-500 CFU/100 cm²).

Multidrug-Resistant Organism (MDRO) isolates were recovered from air, floor, bedside monitors, and the walls of OR and ICU rooms. Of the 49 MDRO isolates, 26 (53.06%) originated from floor surfaces, 11 (22.45%) from air, 10 (20.41%) from bedside monitors, and 2 (4.08%) from walls. Among the 11 isolates yielded from air specimens, 8 isolates were carbapenem-resistant *Acinetobacter* spp. (CRA) and 3 were MRCoNS. From floor samples, 14 CRA isolates, 8 MRCoNS isolates, 3 ESBL-E isolates, and 1 MRSA isolate were obtained. The patients' bedside monitor samples yielded 7 isolates, including 2 MRSA and 1 CRA. The wall yielded 1 MRCoNS isolate and 1 CRA isolate (Table 4).

The biofilm test was done by calculating the Cutoff value of optical density (ODc) and classifying the bacteria into four categories: Non-biofilm producer (ODs ≤ ODc), weak biofilm producer (ODc < ODs ≤ (2 x ODc)), moderate biofilm producer ((2 x ODc) < ODs ≤ (4 x ODc)), and strong biofilm producer (ODs > (4 x ODc)) (Table 5). The biofilm formation test was conducted on 49 bacterial isolates isolated from 16 locations. Of the 49 isolates, 21/49 (42.86%) were non-biofilm-forming, 16/49 (32.65%) were weakly biofilm-forming, 7/49 (14.29%) were strongly biofilm-forming, and 5/49 (10.20%) were moderately biofilm-forming. Among MRSA isolates (n = 3), all were non-biofilm-forming. Of the 19 MRCoNS isolates, 7 (36.84%) were non-biofilm-forming, 4 (21.05%) were weak biofilm-forming, 3 (15.79%) were moderate biofilm-forming, and 5 (26.32%) were strong biofilm-forming. Among the 24 CRA isolates, 10 (41.67%) were non-biofilm-forming, 10 (41.67%) were weak biofilm-forming, while 2 isolates each (8.33%) exhibited moderate and vigorous biofilm formation. Of the 3 ESBL-producing Enterobacterales (ESBL-E) isolates, 2 (66.67%) were weak biofilm-forming, and 1 (33.33%) was non-biofilm-forming (Table 5).

Table 4. The number of multidrug-resistant organisms (MDRO) isolated from the air, the floor, the wall, and patients' bedside monitors using chromogenic Staphylococcus agar, Acinetobacter chromogenic agar, and ESBL chromogenic agar in surveillance at the MDRO Academic Hospital UGM 2024

MDRO Isolate	Air (11 isolates)		Floor (26 isolates)		Monitor (10 isolates)		Wall (2 isolates)		Total (49 isolates)
	OK	ICU	OK	ICU	OK	ICU	OK	ICU	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
MRSA	0	0	0	1 (9.09)	0	2 (66.67)	0	0	3 (6.12)
MRCONS	3 (50)	0	7 (46.67)	1 (9.09)	6 (85.71)	1 (33.33)	1 (50)	0	19 (38.78)
CRA	3 (50)	5 (100)	6 (40)	8 (72.73)	1 (14.29)	0	1 (50)	0	24 (48.98)
ESBL-E	0	0	2 (13.33)	1 (9.09)	0	0	0	0	3 (6.12)
Total	6(100)	5(100)	15(100)	11(100)	7(100)	3(100)	2(100)	0	49(100)

Notes: OK (Operating Room), ICU (Airborne Isolation Intensive Room), CFU (Colony Forming Unit), MRSA (Methicillin Resistant *Staphylococcus aureus*), MRCoNS (Methicillin-resistant coagulase-negative staphylococci), ESBL-E (Extended-spectrum beta-lactamase producing Enterobacterales), CRA (Carbapenem-resistant *Acinetobacter* spp.)

Table 5. The biofilm formation capacity of MDRO isolates (MRSA, MRCoNS, CRA, and ESBL-E) based on the location of specimen collection-(OK and ICU)

Location	Isolate	Non-biofilm forming	Weak biofilm forming	Moderate biofilm forming	Strong biofilm forming	Sub total
		n(%)	n(%)	n(%)	n(%)	n(%)
OK	**MRSA**	0	0	0	0	0
	MRCONS	6 (35.29)	3 (17.65)	3 (17.65)	5 (29.41)	17 (100)
	CRA	6 (54.55)	4 (36.36)	0	1 (9.09)	11 (100)
	ESBL-E	1 (50)	1 (50)	0	0	2 (100)
	Sub Total	13 (43.33)	8 (26.67)	3 (10)	6 (20)	30 (100)
ICU	**MRSA**	3 (100)	0	0	0	3 (100)
	MRCONS	1 (50)	1 (50)	0	0	2 (100)
	CRA	4 (30.77)	6 (46.15)	2 (15.38)	1 (7.69)	13 (100)
	ESBL-E	0	1 (100)	0	0	1 (100)
	Sub Total	8 (42.11)	8 (42.11)	2 (10.53)	1 (5.26)	19 (100)
OK and ICU	**MRSA**	3 (100)	0	0	0	3(100)
	MRCONS	7 (36.84)	4 (21.05)	3 (15.79)	5 (26.32)	19 (100)
	CRA	10 (41.67)	10 (4167)	2 (8.33)	2 (8.33)	24 (100)
	ESBL-E	1 (33.33)	2 (66.67)	0	0	3 (100)
	Sub Total	**21 (42.86)**	**16 (32.65)**	**5 (10.20)**	**7 (14.29)**	**49(100)**

Notes: OK (Operating Room), ICU (Airborne Isolation Intensive Room), CFU (Colony Forming Unit), MRSA (Methicillin Resistant *Staphylococcus aureus*), MRCoNS (Methicillin-resistant coagulase-negative staphylococci), ESBL-E (Extended-spectrum beta-lactamase producing Enterobacterales), CRA (Carbapenem-resistant *Acinetobacter* spp.)

DISCUSSION

The condition of Airborne Isolation Intensive Care Units (ICUs) and Operating Rooms (ORs) is critical for preventing the transmission of multidrug-resistant organisms (MDROs) and maintaining safe environments for surgical procedures. Key factors influencing the efficacy of these environments include room layout, temperature, humidity, lighting, air pressure, and bacterial burden, particularly in healthcare settings in Indonesia.

Room layout is essential in controlling air exchange rates and microbial contamination. In hospitals, particularly in operating rooms, sufficient space enables adequate ventilation and more effortless mobility for healthcare workers, thereby improving overall air quality [9]. Design specifications for ICU and OR environments generally advocate for minimum room sizes to facilitate adequate ventilation and the use of antimicrobial surfaces. The pressurization of these rooms is critical; maintaining negative pressure in isolation rooms is necessary to prevent air contaminants from escaping into adjacent areas [10]. Temperatures in operating and isolation rooms typically range between 20–26°C, with specific guidelines recommending cooler conditions in surgical settings (18-23°C) for the comfort of surgical staff and to minimize infections [11,12]. Evidence suggests that rooms maintained at appropriate temperatures can significantly reduce airborne microbial colonization.

Fluctuating temperatures and excessive humidity, with optimal relative humidity maintained between 30% and 60%, can facilitate bacterial proliferation, underscoring the need for stringent environmental controls to prevent infection [13,14]. Lighting also influences microbial survival; well-illuminated environments are associated with reduced microbial activity due to photoinactivation of bacteria and fungi [12]. Studies conducted in Indonesia have shown that HEPA filters can reduce airborne fungal and bacterial contaminants by up to 70% [13]. Routine environmental monitoring often identifies temporal and activity-related fluctuations in bacterial counts, underscoring the need for continuous surveillance to inform cleaning and disinfection protocols [15,16].

The Centers for Disease Control and Prevention (CDC) recommends that environmental sampling be conducted in specific, targeted scenarios, such as outbreak investigations, research, monitoring hazardous environmental conditions, and quality assurance activities [17]. Routine microbiological monitoring of air and surfaces in ORs and ICUs should be performed at least semiannually [13]. When enhanced cleaning protocols are implemented, before-and-after validation sampling is necessary to assess effectiveness [18]. More frequent monitoring of cleaning processes, such as weekly or monthly, can be performed using non-microbiological methods, such as fluorescent markers, to verify thoroughness [17]. In the event of a suspected outbreak, high-frequency (daily) investigative sampling of high-touch surfaces, air, and equipment is warrant-

ed, as contamination may be intermittent. Notably, one study demonstrated that sampling only on "Day 1" of an investigation missed 35% of contamination [19].

The prevalence and characteristics of bacterial isolates in operating rooms (ORs) and isolation intensive care units (ICUs) are critical for managing the risk of hospital-acquired infections. The types and frequencies of bacterial isolates can differ significantly between these two environments due to variations in their design, purpose, and patient demographics. Studies indicate that multiple bacterial strains are present in both ICUs and ORs, necessitating vigilant monitoring and infection control. In ICUs, Pseudomonas aeruginosa is the predominant isolate, representing up to 32.3% of all isolates, while methicillin-resistant Staphylococcus aureus (MRSA) is detected in approximately 6.8% of samples [20]. Additional research has identified *Acinetobacter baumannii, Klebsiella spp.*, and *Escherichia coli as* significant pathogens due to their potential for antibiotic resistance [21,22].

Studies assessing bacterial loads on various surfaces in ORs and ICUs have found that environmental samples from air, floors, and bedside areas exhibit differing levels of contamination. One study reported that mean aerobic bacterial loads in the indoor air of surgical wards exceeded standard limits, indicating a heightened risk of airborne bacterial infection. Furthermore, this study highlighted a correlation between airborne bacterial counts and the incidence of surgical site infections (SSIs), reinforcing the importance of ongoing air quality monitoring [23].

Microbial assessments frequently utilize blood agar to culture fastidious organisms, while chromogenic agars enable the specific isolation of targeted pathogens, including *Staphylococcus aureus* and gram-negative bacteria. Research has shown that blood agar effectively recovers a broad spectrum of bacteria from environmental samples, supporting the growth of both aerobic and anaerobic species [24]. Chromogenic media are also essential for differentiating pathogenic strains; for instance, chromogenic MRSA agar allows for rapid identification of MRSA [25].

The biofilm-forming ability of multidrug-resistant organism (MDRO) isolates, including methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCoNS), carbapenem-resistant *Acinetobacter baumannii* (CRA), and Enterobacterales with extended-spectrum beta-lactamases (ESBL), varies considerably based on the site of specimen collection. Recognizing these differences is essential for effective infection control in healthcare settings, especially in ICUs and surgical environments.

Evidence indicates that environmental factors significantly influence the biofilm formation capacity of these MDROs. Isolates obtained from high-contact surfaces, such as patient bed rails and medical devices, produce more biofilm than those from less frequently touched areas. Ribeiro et al. observed that biofilm-forming bacteria, including MRSA and CRA, exhibited higher resistance when cultured from ICU surfaces than from peripheral locations, suggesting that environmental pressures may contribute to the accumulation of resistant strains [26].

From a public health perspective, this study highlights important implications for environmental infection prevention and control in hospital settings. The elevated microbial bioburden observed on floor surfaces indicates that standard cleaning protocols may be inadequate for effectively controlling environmental contamination, particularly in high-risk areas. Floors may serve as reservoirs for pathogen persistence and indirect transmission.

Furthermore, the identification of biofilm-forming multidrug-resistant organisms (MDROs) underscores the need for cleaning strategies that use disinfectants with demonstrated biofilm-disruptive properties, particularly in critical care and operating room settings. Relying solely on quantitative microbial counts may not adequately capture MDRO-related risks; therefore, incorporating qualitative surveillance for specific MDROs could enhance infection prevention policies. The detection of MDROs in air samples underscores the importance of regularly maintaining HVAC and HEPA filtration systems and implementing policies to control staff movement, patient flow, and equipment transfer between contaminated and sterile areas. Collectively, these results indicate the need for improved environmental surveillance and targeted interventions to mitigate the risk of healthcare-associated infections, thereby promoting patient safety and public health.

This study is subject to several limitations concerning its scope, design, and methodology. First, as a single-center study conducted at a single hospital, the findings may not be generalizable to other healthcare settings. Second, the cross-sectional design, with environmental sampling performed on a single day, does not account for temporal variations in microbial load that may arise at different times or under varying patient activity levels.

Additionally, environmental sampling was not conducted immediately after routine cleaning, limiting the ability to assess the effectiveness of the current cleaning protocol. The sampling was confined to air, floors, walls, and bedside monitors, so contamination on other high-touch surfaces, such as bed rails, bed

controls, door handles, and keyboards, was not evaluated. Finally, although this study identifies potential environmental reservoirs of multidrug-resistant organisms, it does not establish a direct link between environmental contamination and healthcare-associated infections in patients.

Future research should employ longitudinal or before-and-after study designs to assess changes in microbial bioburden and the prevalence of multidrug-resistant organisms (MDROs) following targeted interventions, such as enhanced cleaning protocols or the use of disinfectants with established antibiofilm efficacy. Additionally, environmental sampling should be expanded to include other high-touch surfaces, such as bed rails, bed controls, door handles, keyboards, and non-critical medical equipment.

Incorporating molecular techniques, such as molecular typing or whole-genome sequencing, is also recommended to evaluate potential clonal relationships between environmental and clinical isolates, thereby providing a more comprehensive understanding of the role of environmental contamination in healthcare-associated infections. A multidisciplinary approach involving infection control teams, hospital management, and public health authorities could further enhance the evidence base for environmentally focused infection prevention policies.

CONCLUSION

Routine environmental monitoring, strict adherence to infection control standards, and targeted interventions to disrupt biofilm formation are essential to mitigate the risk of MDRO persistence and transmission. Strengthening hospital infection prevention policies through evidence-based environmental surveillance will be critical to safeguarding patient safety, particularly in high-risk clinical settings.

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Authors' contribution
D.F.W: Conceptualization, Methodology, Writing – original draft, Investigation, Funding acquisition; E.W.D: Formal analysis, Investigation, Validation; G.S.P: Data curation, Project administration; A.R: Writing – review & editing, Software, Visualization; T.N: Supervision, Resources.

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Data availability
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement
This study was reviewed and approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, with ethical clearance number KE-0787-05-2024.

Conflicts of interest
The author(s) declare(s) no conflict of interest regarding the publication of this article.

Use of artificial intelligence (AI)
Nothing to declare.

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