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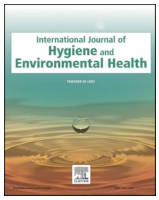

Environmental contamination with highly resistant microorganisms after relocating to a new hospital building with 100% single-occupancy rooms: A prospective observational before-and-after study with a three-year follow-up

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ABSTRACT

Introduction: Inanimate surfaces within hospitals can be a source of transmission for highly resistant microorganisms (HRMO). While many hospitals are transitioning to single-occupancy rooms, the effect of single-occupancy rooms on environmental contamination is still unknown. We aimed to determine differences in environmental contamination with HRMO between an old hospital building with mainly multiple-occupancy rooms and a new hospital building with 100% single-occupancy rooms, and the environmental contamination in the new hospital building during three years after relocating.

Methods: Environmental samples were taken twice in the old hospital, and fifteen times over a three-year period in the new hospital. Replicate Organism Direct Agar Contact-plates (RODACs) were used to determine colony forming units (CFU). Cotton swabs premoistened with PBS were used to determine presence of methicillin-resistant *Staphylococcus aureus,* carbapenemase-producing *Pseudomonas aeruginosa,* highly resistant Enterobacterales, carbapenem-resistant *Acinetobacter baumannii,* and vancomycin-resistant *Enterococcus faecium*. All identified isolates were subjected to whole genome sequencing (WGS) using Illumina technology.

Results: In total, 4993 hospital sites were sampled, 724 in the old and 4269 in the new hospital. CFU counts fluctuated during the follow-up period in the new hospital building, with lower CFU counts observed two- and three years after relocating, which was during the COVID-19 pandemic. The CFU counts in the new building were equal to or surpassed the CFU counts in the old hospital building. In the old hospital building, 24 (3.3%) sample sites were positive for 49 HRMO isolates, compared to five (0.1%) sample sites for seven HRMO isolates in the new building ($P < 0.001$). In the old hospital, 89.8% of HRMO were identified from the sink plug. In the new hospital, 71.4% of HRMO were identified from the shower drain, and no HRMO were found in sinks.

Discussion: Our results indicate that relocating to a new hospital building with 100% single-occupancy rooms significantly decreases HRMO in the environment. Given that environmental contamination is an important source for healthcare associated infections, this finding should be taken into account when considering hospital designs for renovations or the construction of hospitals.

1. Introduction

Inanimate surfaces in hospitals, especially in patient rooms and bathrooms, can be a reservoir for pathogenic and possibly highly resistant microorganisms (HRMO) (Weber et al., 2013). From these environmental reservoirs, microorganisms can be transmitted to patients. Depending on the species, microorganisms are able to survive in the

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List of abbreviations

Aztreonam broth	Tryptic soy broth with aztreonam 75 mg/L
CFU	Colony forming units
CPE	Carbapenemase-producing Enterobacterales
CP-PA	Carbapenemase-producing *Pseudomonas aeruginosa*
CR-AB	Carbapenem-resistant *Acinetobacter baumannii*
Erasmus MC	Erasmus MC University Medical Center, Rotterdam, the Netherlands
ESBL	Extended-spectrum β-lactamase
ESBL-E	Extended-spectrum β-lactamase-producing Enterobacterales
FCW	Facility care worker
HAI	Healthcare-associated infections
HRMO	Highly resistant microorganisms
ICU	Intensive Care Unit
IPC	Infection prevention and control
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
PBS	Phosphate buffered saline
RODAC	Replicate Organism Direct Agar Contact-plates
TSA	Trypticase Soy Agar
TSB	Tryptic Soy Broth
Vancomycin broth	Tryptic soy broth with vancomycin 50 mg/L
VIM-PA	VIM-positive *Pseudomonas aeruginosa*
VRE	Vancomycin-resistant *Enterococcus faecium*
WGS	Whole genome sequencing

environment for long periods of time, ranging from a few hours up to several months or even years (Kramer et al., 2006; Suleyman et al., 2018). Environmental contamination of patient rooms can therefore be a prolonged source of pathogens. A review of 1561 published outbreaks has identified that the hospital environment was the source in almost one fifth of the studied outbreaks (Gastmeier et al., 2006). Furthermore, various studies have shown that when the previous room occupant was colonized or infected with an HRMO, subsequent patients had an increased risk for acquisition of that microorganism (Mitchell et al., 2015; Wu et al., 2019). This illustrates that transmission via the environment also occurs in non-outbreak settings. Additionally, Chen et al. showed transmission from the environment to patients and vice versa for methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and *Clostridioides difficile* (Chen et al., 2019). These findings all highlight the importance of achieving a microbiologically safe hospital environment for patients. Cleaning is a key component for this, but hospital design, disinfection practices, and surface composition should be taken into account as well.

New hospital designs nowadays frequently consist of either mainly or only single-occupancy rooms. Research indicates that single-occupancy rooms are an important infection prevention and control (IPC) measure, and are part of aiming for a healing environment in general (Schreuder et al., 2016; Stiller et al., 2016). Transitioning from multiple-occupancy rooms to single-occupancy rooms eliminates the risk of acquiring a microorganism from infected or colonized roommates (*i.e.* via direct or indirect contact), but not from prior room occupants (*i.e.* indirect contact). Currently, literature about the effect of single-occupancy rooms on environmental contamination is lacking.

On May 18, 2018, the Erasmus MC University Medical Center, Rotterdam, the Netherlands (Erasmus MC), relocated from an old hospital building with mainly multiple-occupancy rooms and shared bathrooms to a newly constructed hospital building with 100% single-occupancy rooms with private bathrooms. This provided a unique opportunity to study differences in environmental contamination between multiple- and single-occupancy rooms. We aimed to determine differences in environmental contamination between multiple-occupancy rooms and single-occupancy rooms in a non-outbreak setting, by determining the overall number of colony forming units (CFU) and the presence of HRMO on different locations in patient rooms and bathrooms. Second, we aimed to determine changes in environmental contamination of the newly constructed hospital over a three-year follow up-period. Third, we aimed to determine if there was persistent contamination of surfaces over time by using whole genome sequencing (WGS), and to identify clusters.

2. Methods

2.1. Study design

This prospective observational before-and-after study was performed in the Erasmus MC, a university hospital in Rotterdam, the Netherlands. Environmental sampling was performed between April 2018, and May 2021. The relocation to the new hospital building took place during the study period, at May 18, 2018. Samples were taken at two moments in the old hospital building; two weeks and one week before relocating (Fig. 1). In the new hospital building, samples were taken at 15 different moments; two weeks, one week and one day before relocating patients, and one day, one week, two weeks, one, three, six, nine, 12, 15, 18, 24, and 36 months after relocating patients (Fig. 1).

2.2. Study setting

2.2.1. Old hospital building

The old hospital building of the Erasmus MC opened in 1961, consisted of 1200 beds, and had mainly two- and four-patient rooms and shared bathrooms. Exceptions were the adult Intensive Care Unit (ICU), which consisted of only single-occupancy rooms; the isolation ward, which consisted of single-occupancy rooms with anterooms and private bathrooms, and three hematology wards, which consisted of mainly single-occupancy rooms with anterooms and private bathrooms. Additionally, hematology ward I had one three-patient room, hematology ward II had two two-patient rooms, and hematology ward III had two two-patient and two three-patient rooms, all with attached bathrooms. Two of the hematology wards were located at another location in Rotterdam; the Erasmus MC Cancer Institute, location Daniel den Hoed. The Cancer Institute also relocated to the new hospital building on May 18, 2018.

In the old hospital building, 10 two-person rooms, 15 four-person rooms, four isolation rooms with anteroom, three hematology rooms with anteroom, 10 ICU rooms, of which two with anteroom, and nine bathrooms were sampled. Two hematology rooms were located at the Cancer Institute. Of the sampled bathrooms, one belonged to a hematology room and one to an isolation room. In Supplementary file 1, the medical specialty corresponding to the sampled patient rooms and bathrooms is described.

2.2.2. New hospital building

The new hospital building consisted of 503 single-occupancy rooms with private bathrooms, 22 isolation rooms with anterooms and private bathrooms, and 56 single-occupancy adult ICU rooms. While isolation rooms in the old hospital building where located at one ward, isolation rooms in the new building were located at multiple wards in the hospital

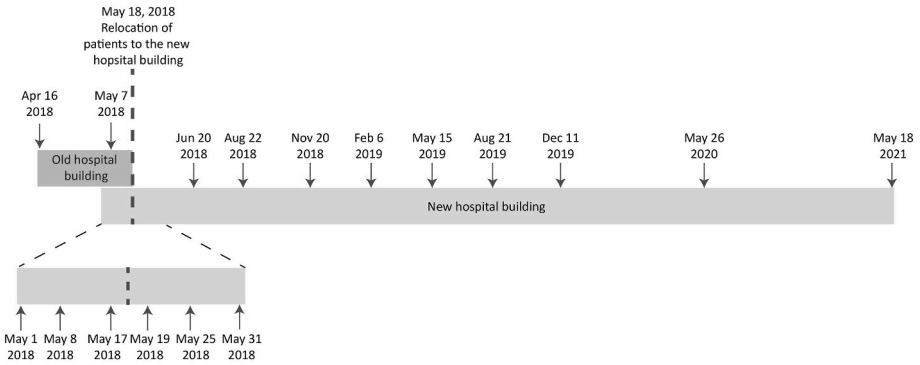

Fig. 1. Timeline of the study. Arrows indicate the sampling moments in the old and the new hospital building.

building.

In the new hospital building, 30 single-occupancy rooms, of which three hematology and four isolation rooms, all with anterooms; 10 ICU rooms, of which two with anteroom; and 10 bathrooms were sampled. Bathrooms sampled in the new building belonged to eight included single-occupancy rooms, one included hematology room, and one included isolation room (Supplementary file 1). Rooms were selected before the start of sampling and the same rooms were sampled during each sampling moment, unless it was not possible to enter the room (*e.g.* patient was in a clinically unstable condition or was admitted with an indication for isolation in a normal patient room). In these circumstances, a nearby patient room was sampled.

2.3. Sample sites

Sample sites in patient rooms were the nightstand, table, wall, sink, and the top and bottom of the sink plug (Supplementary file 2). When multiple nightstands or tables were present in a patient room, all were sampled. In four-person rooms, two locations on the wall were sampled. Sample sites in bathrooms were the toilet seat, shower chair, shower drain, door handle on the inside of the bathroom, the sink, and the top and bottom of the sink plug (Supplementary file 2). Sink plugs were installed in 2013 in six wards, including the ICU, as an IPC measure, to prevent splashing of water from the sink drain. In the old building, sink plugs were not present in 31 sinks. When not present, the top of the sink drain was sampled, which was considered the same sample site as the bottom of the sink plug for analyses. In the new hospital building, a sink plug was present in all sinks, with the exception of one sampled bathroom sink, where the top of the sink drain was sampled. In rooms with an anteroom (*e.g.* hematology and isolation rooms), the sink was located in the anteroom instead of in the patient room. Furthermore, in both the old and the new hospital building, two ICU rooms had a sink in the anteroom and a sink in the patient rooms. For these rooms, both the sink and sink plug in the anteroom, as well as the sink and sink plug in the patient room were sampled.

2.4. Sampling methods

To determine the total number of CFUs, Replicate Organism Direct Agar Contact-plates (RODAC) with Trypticase Soy Agar (TSA) with Lecithin and Polysorbate 80 (Bruker Daltonics, Bremen, Germany) were used. Of all sample sites, one RODAC per sampling moment was taken, with the exception of the bottom of the sink plug. Since it was not feasible to sample the bottom of the sink plug with a RODAC, CFU counts were not determined for this location. The RODACs were pressed firmly on surfaces for about 10 s, according to standard practice. For the door handle and the top of the sink plug, the RODAC was carefully rotated over the surface, to ensure that the whole RODAC came in contact with the surface. Sterile cotton swabs (BSN medical, Almere, the Netherlands) were used to determine the presence of MRSA, vancomycin-resistant *Enterococcus faecium* (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacterales (ESBL-E), carbapenemase-producing Enterobacterales (CPE), carbapenemase-producing *Pseudomonas aeruginosa* (CP-PA), and carbapenem-resistant *Acinetobacter baumannii* (CR-AB). For each sampling site, two swabs were pre-wetted with phosphate buffered saline (PBS) before sampling a standardized surface of 100 cm^2 (Supplementary file 2). During sampling, swabs were rotated and moved in multiple directions as predefined in our sampling protocol (Supplementary file 2). Due to the specific shapes of door handles, shower drains and the top and bottom of the sink plug, no standardized surface of 100 cm^2 was sampled. Instead, the complete surfaces were sampled, while the swab was rotated and moved in multiple directions according to our protocol. Directly after sampling, in random order, one swab was placed in a tryptic soy broth (TSB) with aztreonam 75 mg/L (aztreonam broth) and one swab in TSB with vancomycin 50 mg/L (vancomycin broth).

2.5. Microbiological methods

RODACs were incubated twice overnight at 35 °C, after which CFUs were counted. When more than 100 colonies were counted, this was reported as >100 CFU. Both the vancomycin and the aztreonam broth were incubated for 24 h at 35 °C.

On the incubated aztreonam broth, a *vanA, vanB, mecA/mecC* PCR was performed using established procedures. When the *vanA/B* PCR was positive, a *Brilliance*™ VRE (Oxoid, Basingstoke, UK), was inoculated and incubated twice overnight at 35 °C. All suspected *Enterococcus* spp. colonies were identified to species level using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany) running the MBT Compass Library, Revision E; MBT 7854 MSP Library and MBT Compass Library, Revision F MBT 8468 MSP Library. For *E. faecium* isolates, an additional *vanA* and *vanB* PCR was performed. When the *mecA/mecC* PCR was positive, a TSA plate with 5% sheep blood (blood agar [Becton Dickinson, New Jersey, USA]) and a BBL™ CHROMagar ™ MRSA II* (Becton Dickinson, New Jersey, USA) were inoculated and incubated twice overnight at 35 °C. All morphologically suspected *S. aureus* isolates were identified using MALDI-TOF. A cefoxitin disk diffusion was performed on a Mueller Hinton agar (Becton Dickinson, New Jersey, USA). A growth inhibition zone of <22 mm was considered resistant and confirmatory for MRSA.

From the incubated vancomycin broth, a CHROMID® CARBA SMART Agar (bioMérieux, Marcy-l'Etoile, France), and an ESBL plate (Oxoid, Basingstoke, UK) were inoculated and incubated twice overnight at 35 °C. All morphologically different colonies were identified to species level using MALDI-TOF. For *P. aeruginosa, A. baumannii*, and Enterobacterales isolates growing on the CARB side of the CHROMID® CARBA SMART agar, a PCR was performed to detect bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ genes using established procedures. For isolates growing on the OXA side, an OXA-48-like PCR was performed. When the PCR was negative, a CIM test was performed for *P. aeruginosa*

and Enterobacterales, and an antimicrobial susceptibility test with VITEK®2 (bioMérieux) for *A. baumannii*. Colonies growing on the ESBL plate were identified to species level using MALDI-TOF. Antimicrobial susceptibility was determined with VITEK®2, and a combination disk-diffusion method (ESBL + AmpC Screen Kit; Rosco Diagnostica, Taastrup, Denmark) was performed to phenotypically confirm the presence of an ESBL. A CIM test was performed when the presence of a carbapenemase was suspected as well.

Antibiotic susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (European Committee on Antimicrobial Susceptibility Testing: Clinical breakpoints, 2017). All identified MRSA, VRE, ESBL-E, CPE, CP-PA and CR-AB isolates were stored at −80 °C.

2.5.1. Whole genome sequencing

WGS was performed for all identified isolates. DNA was extracted using the MagNA pure 96 platform (Roche Applied Science, Mannheim, Germany) and shipped to Novogene (HongKong, China) for sequencing. Genomic DNA was fragmented by shearing to a size of ~350 bp. Libraries were prepared using the NEBNext® DNA Library Prep kit (New England Biolabs, Ipswich, MA, USA) and subjected to 150 bp paired-end sequencing generating >100 × coverage using Illumina. Incidental, samples were sequenced using the in-house platform. Library preparation was conducted with the Illumina DNA Prep (Illumina, San Diego, CA, United States). Sequencing was conducted using the iSeq 100 System (Illumina) generating 150 bp paired-end reads. *De novo* genomic assemblies were generated using CLC Genomics Workbench v21 (Qiagen, Hilden, Germany). Presence of antibiotic resistance genes was analyzed using the web-based interface of the Comprehensive Antimicrobial Resistance Database (CARD - https://card.mcmaster.ca/accessed on July 4.2022). The analysis was restricted to include perfect and strict hits (Alcock et al., 2020; Jia et al., 2017). Plasmid replicon types were detected using the online Plasmidfinder software v2.1 (https://cge.food.dtu.dk/services/PlasmidFinder accessed on November 16, 2022/) with default settings (Carattoli et al., 2014) Identification confirmation was performed using the Type strain genome server (TYGS) (https://tygs.dsmz.de)/(Meier-Kolthoff and Göker, 2019). For *Enterobacter* spp. and *P. aeruginosa*, conventional Multi Locus Sequence Types (MLST) and core-genome MLST (cgMLST) or whole-genome MLST analysis (wgMLST) was performed using the available schemes available in BioNumerics (Applied Maths, St-Martens-Latem, Belgium) and for *K. pneumoniae* and *E. faecium* using the schemes available in SeqSphere (Ridom, Munster, Germany). For *Citrobacter freundii* an *ad hoc* wgMLST scheme was created in SeqSphere using the cgMLST Target Definer v1.5 with the genomic sequence of the Type strain (ATCC 8090, accession nr. CP049015.1) as seed genome and 24 NCBI Refseq genomes as penetration query genomes. Genomes improperly assigned to *C. freundii* and plasmid based genes were excluded. The resulting scheme consisted of 3162 core genes and 1142 accessory genes. The sequence data for this study has been deposited in BioProject ID: PRJNA904531.

2.5.2. Cleaning protocol

In both hospital buildings, the same external company was hired for environmental cleaning of hospital surfaces. Both in the old and new hospital building, rooms were cleaned daily with microfiber cloths dampened with water, unless disinfection was indicated. Sinks were part of this daily cleaning routine and the protocol for sink cleaning remained unchanged during the study period. To ensure quality, internal and external audits were performed regularly. After a patient in the old building was discharged, the nightstand and bed were removed to be cleaned, but no additional cleaning measures were taken besides daily cleaning. In the new hospital building, the whole room was cleaned before a new patient could be admitted to the room. Additionally, cleaning staff received extra training after relocating. Also, in the new building, facility care workers (FCW) were introduced. Several cleaning tasks were transferred from the cleaning staff to the FCW. In general, when no disinfection was indicated, the cleaning staff was responsible for the cleaning of the built in furniture, where the FCW was responsible for the cleaning of the other equipment and furniture in the room.

2.6. Statistical analyses

The different patient rooms were categorized in 1) general patient rooms (*i.e.* two- and four-person rooms in the old hospital building, and single-occupancy rooms on general wards in the new hospital building), 2) ICU rooms, 3) rooms with an anteroom (*i.e.* isolation rooms and hematology rooms), and 4) bathrooms. CFU counts per RODAC were converted into CFU counts per square cm (cm^2), by dividing the CFU counts by the surface of the RODAC. CFU counts per cm^2 were presented as medians. Differences between the sample moments in the old hospital building and between the two hospital buildings were analyzed using the Mann-Whitney-U test, differences within the new hospital building were analyzed with the Wilcoxon Signed-Ranks Test. Presence of HRMO was defined as yes/no, and presented with numbers and percentages, and analyzed with chi-squared analyses. $P < 0.05$ was considered statistically significant. IBM Statistical Package for the Social Sciences Solutions (SPSS) version 25 (IBM Corp., Armonk, New York, USA) was used for all analyses.

3. Results

3.1. Colony forming units over time

In total, 4993 sample sites were sampled, 724 in the old building and 4269 in the new building. RODACs were taken from 4211 out of 4993 (84.3%), 673 out of 724 (93.0%) sample sites in the old hospital building, and 3536 out of 4269 (82.8%) in the new hospital building. For nine (0.2%) sample sites the RODAC went missing in the laboratory, and the other 773 (15.5%) sample sites were bottom of sink plugs, where no RODACs were taken according to our sampling protocol. The highest median number of CFUs per cm^2 was identified from the shower drain (3.95 CFUs per cm^2), and the lowest from the wall (0.04 CFUs per cm^2).

The observed CFU counts per cm^2 at both sampling moments in the old hospital building are presented in Supplementary file 3. The CFU counts determined one month before relocating to the new hospital building were used as the reference for the old hospital building (Table 1). Before relocating patients to the new hospital building, we observed significantly lower CFU counts ($P < 0.05$, Table 1) for almost all locations in single-occupancy rooms and bathrooms compared to the old hospital building, but not for ICU rooms and for rooms with an anteroom (Table 1). After relocating patients, we observed an overall build-up in CFU counts during the first three months to a median of 0.47 CFU per cm^2, and fluctuating CFU counts after this moment (Fig. 2). The CFU counts in the new building were equal to or surpassed the median number of CFU counts in the old building within nine months for single-occupancy rooms, within 18 months for ICU rooms, within one month for rooms with anteroom, and within three months for bathrooms (Table 1). For the single-occupancy rooms, we observed significantly lower CFU counts ($P < 0.05$, Table 1) six months after relocating for all locations, while we observed significantly higher CFU counts ($P < 0.05$, Table 1) nine months after relocating. For the bathrooms, we noticed significantly lower CFU counts up to one month after relocation (Table 1). For the ICU rooms, the sink did not reach the same median number of CFU counts as in the old building, and we observed significantly lower CFU counts for the sink throughout the three year follow-up period (Table 1). At the two sampling moments during the COVID-19 pandemic (May 2020 and May 2021), we observed significantly lower CFU counts ($P < 0.05$, Table 1) in single-occupancy rooms, but not in other room types (Fig. 2, Table 1).

Table 1
The median CFU count per cm^2 determined in the new hospital building compared to the median CFU count per cm^2 determined in the old hospital building one month before relocating.

Room type	Sample site	Old hospital building	Two weeks before	One week before	One day before	One day after	One week after	Two weeks after	One month after	Three months after	Six months after [a]	Nine months after	12 months after	15 months after	18 months after	24 months after	36 months after
Single-occupancy room [b]	Overall	0.74	0.08 ↓89%	0.08 ↓89%	0.12 ↓84%	0.20 ↓73%	0.12 ↓84%	0.16 ↓78%	0.23 ↓69%	0.51 ↓31%	0.08 ↓89%	1.13 ↑52%	0.55 ↓26%	0.31 ↓58%	0.63 ↓15%	0.16 ↓78%	0.12 ↓84%
	Nightstand	1.46	0.20 ↓86%	0.23 ↓84%	0.39 ↓73%	0.66 ↓55%	0.59 ↓60%	1.05 ↓28%	0.86 ↓41%	1.37 ↓6%	0.41 ↓72%	3.40 ↑133%	1.09 ↓25%	0.94 ↓36%	2.19 ↑50%	0.70 ↓52%	0.43 ↓71%
	Table	1.29	0.04 ↓97%	0.12 ↓91%	0.10 ↓92%	0.20 ↓84%	0.39 ↓70%	0.51 ↓60%	0.66 ↓49%	0.78 ↓40%	0.47 ↓64%	3.13 ↑143%	0.78 ↓40%	0.31 ↓76%	2.19 ↑70%	0.27 ↓79%	0.39 ↓70%
	Wall	0.12	0.00 ↓100%	0.00 ↓100%	0.00 ↓100%	0.00 ↓100%	0.04 ↓67%	0.00 ↓100%	0.00 ↓100%	0.04 ↓67%	0.04 ↓67%	0.47 ↑292%	0.12 0%	0.00 ↓100%	0.08 ↓33%	0.04 ↓67%	0.00 ↓100%
	Sink	0.31	0.12 ↓61%	0.04 ↓87%	0.12 ↓61%	0.20 ↓35%	0.08 ↓74%	0.08 ↓74%	0.27 ↓13%	0.39 ↑26%	0.04 ↓87%	0.59 ↑90%	0.31 0%	0.59 ↑90%	0.27 ↓13%	0.16 ↓48%	0.23 ↓26%
	Top of sink plug	0.39	0.00 ↓100%	0.04 ↓90%	0.12 ↓69%	0.20 ↓49%	0.39 0%	0.27 ↓31%	0.12 ↓69%	0.63 ↑62%	0.00 ↓100%	0.23 ↓41%	0.63 ↑62%	0.59 ↑51%	0.63 ↑62%	0.08 ↓79%	0.12 ↓69%
ICU room	Overall	0.25	0.04 ↓84%	0.12 ↓62%	0.18 ↓28%	0.18 ↓28%	0.20 ↓20%	0.08 ↓68%	0.08 ↓68%	0.12 ↓62%	0.21 ↓16%	0.08 ↓68%	0.14 ↓44%	0.12 ↓62%	0.33 ↑32%	0.10 ↓60%	0.08 ↓68%
	Nightstand	0.25	-	-	0.57 ↑128%	0.55 ↑120%	0.59 ↑136%	0.08 ↓68%	0.12 ↓52%	0.23 ↓8%	0.59 ↑136%	0.47 ↑88%	0.23 ↓8%	0.35 ↑40%	0.63 ↑152%	0.55 ↑120%	0.53 ↑112%
	Wall	0.06	0.00 ↓100%	0.04 ↓33%	0.00 ↓100%	0.00 ↓100%	0.04 ↓33%	0.00 ↓100%	0.02 ↓66%	0.02 ↓66%	0.04 ↓33%	0.04 ↓33%	0.00 ↓100%	0.06 0%	0.27 ↑350%	0.10 ↑67%	0.04 ↓33%
	Sink	1.56	0.18 ↓88%	0.20 ↓87%	0.33 ↓79%	0.45 ↓71%	0.20 ↓87%	0.21 ↓87%	0.21 ↓87%	0.47 ↓70%	0.29 ↓81%	0.14 ↓91%	0.20 ↓87%	0.16 ↓90%	0.23 ↓85%	0.06 ↓96%	0.12 ↓92%
	Top of sink plug	0.41	0.02 ↓95%	0.10 ↓76%	0.14 ↓66%	0.06 ↓85%	0.31 ↓24%	0.16 ↓61%	0.08 ↓80%	0.68 ↑66%	0.47 ↑15%	0.00 ↓100%	0.16 ↓61%	0.08 ↓80%	0.39 ↓5%	0.00 ↓100%	0.04 ↓90%
Room with anteroom	Overall	0.23	0.20 ↓13%	0.12 ↓48%	0.47 ↑104%	0.04 ↓83%	0.12 ↓48%	0.12 ↓48%	0.23 0%	0.16 ↓30%	0.16 ↓30%	0.18 ↓22%	0.16 ↓30%	0.23 0%	0.39 ↑70%	0.16 ↓30%	0.20 ↓13%
	Nightstand	0.51	0.47 ↓8%	0.27 ↓47%	0.35 ↓15%	0.63 ↑24%	0.39 ↓24%	1.56 ↑206%	1.17 ↑129%	1.56 ↑206%	0.59 ↑16%	1.29 ↑153%	0.27 ↓47%	0.39 ↓24%	1.17 ↑129%	1.88 ↑269%	1.84 ↑261%
	Table	0.35	0.20 ↓43%	0.20 ↓43%	0.47 ↑34%	1.37 ↑291%	0.31 ↓11%	0.59 ↑69%	0.27 ↓23%	0.35 0%	2.73 ↑680%	1.48 ↑323%	0.55 ↑57%	1.25 ↑257%	2.46 ↑603%	0.51 ↑46%	0.55 ↑57%
	Wall	0.04	0.12 ↑200%	0.04 0%	0.00 ↓100%	0.00 ↓100%	0.00 ↓100%	0.00 ↓100%	0.00 ↓100%	0.04 0%	0.00 ↓100%	0.16 ↑300%	0.00 ↓100%	0.04 0%	0.08 ↑100%	0.00 ↓100%	0.12 ↑200%
Bathroom	Sink	0.23	0.16 ↓30%	0.12 ↓48%	0.47 ↑104%	0.00 ↓100%	0.04 ↓83%	0.08 ↓65%	0.16 ↓30%	0.04 ↓83%	0.04 ↓83%	0.04 ↓83%	0.08 ↓65%	0.23 0%	0.12 ↓48%	0.08 ↓65%	0.04 ↓83%
	Top of sink plug	0.20	0.20 0%	0.08 ↓60%	0.04 ↓80%	0.04 ↓80%	0.20 0%	0.08 ↓60%	0.27 ↑35%	0.35 ↑75%	0.08 ↓60%	0.08 ↓60%	0.04 ↓80%	0.08 ↓60%	0.39 ↑95%	0.08 ↓60%	0.08 ↓60%
	Overall	1.76	0.08 ↓95%	0.08 ↓95%	0.10 ↓94%	0.39 ↓78%	0.51 ↓71%	0.55 ↓69%	1.02 ↓42%	1.76 0%	1.13 ↓36%	3.81 ↑116%	1.52 ↓14%	3.24 ↑84%	3.40 ↑104%	1.64 ↓7%	1.31 ↓26%
	Toilet seat	1.56	0.10 ↓94%	0.16 ↓90%	0.12 ↓26%	0.27 ↓83%	0.98 ↓37%	1.15 ↓62%	1.04 ↓33%	1.52 ↓3%	2.58 ↑65%	3.05 ↑96%	1.09 ↓30%	2.25 ↑44%	1.66 ↑6%	2.13 ↑37%	1.27 ↓19%
	Shower chair	2.11	0.04 ↓98%	0.12 ↓94%	0.59 ↓72%	3.52 ↑67%	1.17 ↓45%	1.89 ↓10%	2.30 ↑9%	2.56 ↑21%	0.59 ↓72%	3.95 ↑87%	1.07 ↓49%	3.95 ↑87%	3.40 ↑61%	1.97 ↓7%	3.14 ↑49%
	Shower drain	3.95	0.57 ↓86%	0.06 ↓98%	0.12 ↓97%	3.13 ↓21%	0.68 ↓83%	1.35 ↓66%	3.95 0%	3.95 0%	3.95 0%	3.95 0%	3.95 0%	3.95 0%	3.95 0%	3.95 0%	3.95 0%
	Door handle	2.07	0.08 ↓96%	0.10 ↓95%	0.08 ↓96%	0.20 ↓90%	0.25 ↓88%	0.57 ↓72%	0.25 ↓88%	0.86 ↓58%	0.45 ↓78%	2.77 ↑34%	1.33 ↓36%	0.82 ↓60%	1.66 ↓20%	0.47 ↓77%	0.20 ↓90%
	Sink	0.94	0.06 ↓94%	0.14 ↓85%	0.04 ↓96%	0.31 ↓67%	0.20 ↓79%	0.10 ↓89%	0.66 ↓30%	1.17 ↑24%	0.57 ↓39%	3.40 ↑262%	1.04 ↑11%	2.25 ↑139%	2.27 ↑141%	0.80 ↓15%	0.61 ↓35%
	Top of sink plug	1.76	0.04 ↓98%	0.04 ↓98%	0.00 ↓100%	0.47 ↓73%	0.27 ↓85%	0.04 ↓98%	0.37 ↓79%	1.56 ↓11%	0.41 ↓77%	3.67 ↑109%	0.66 ↓62%	2.64 ↑50%	0.51 ↓71%	1.68 ↓5%	0.82 ↓53%

Arrows and percentages indicate the change in CFU counts compared to the old hospital. For example, an overall CFU/cm^2 count of 0.08 in single-occupancy rooms two weeks before relocating is a decrease of 89% compared to a CFU count/cm^2 of 0.74 in the old building. This is a significant reduction, indicated by the color of the cell. Green cells indicate a significant decrease in CFU counts, red cells indicate a significant increase in CFU counts. Light green/light red $0.05<P<0.01$, Green/Red $0.01\leq P<0.001$, Dark green/dark red $P\leq 0.001$. Before; before relocating patients, After; after relocating patients. Abbreviation: CFU colony forming units. [a] The RODACs for the single-occupancy rooms and bathrooms were accidentally incubated for 24 hours instead of 48 hours [b] Single-occupancy rooms were compared with the combined median CFU counts per cm^2 of two- and four patient rooms in the old hospital building.

3.2. *Presence of highly resistant microorganisms in the environment*

In the old building, 49 HRMO isolates were identified from 24 of the 724 (3.3%) sampled sites (Table 2). Thirty-seven out of 49 (75.5%) isolates were identified from patient rooms, not the ante-room or bathroom, and 44 out of 49 (89.8%) isolates were identified on the top or bottom of the sink plug (Table 2). In the new building, seven HRMO isolates were identified from five of the 4269 (0.1%) sampled sites, a significant decrease compared to the old building ($P < 0.001$) (Table 2). All seven isolates were identified in the patient bathroom, five (71.4%) were identified from the shower drain (Table 2). In the new building, no HRMO were identified from the top or bottom of sink plugs (Table 2).

In the old hospital building, 16 ESBL-E isolates were identified on 15 sample sites (eight *Enterobacter* spp., five *Citrobacter* spp., three *Klebsiella* spp.), 24 CP-PA isolates on 13 sample sites, and nine CPE isolates on five sample sites (four *C. freundii* isolates on three locations and five *Enterobacter* spp. isolates on three locations) (Table 2). In the new building, we identified three VRE isolates on three sample sites, three CPE isolates on one location (*E. hormaechei*) and one ESBL-E isolate on one sample site (*K. pneumoniae*) (Table 2). The three VRE positive locations were all identified in the same bathroom, one week after relocating. In both hospital buildings, no MRSA and CR-AB were detected.

WGS was performed on all strains. Unfortunately, due to human error, we were unable to link the results of the WGS of isolates identified in the old hospital building to the locations where the isolates were found. Details of the analysis of the isolates were shown in Supplementary file 4. Most noteworthy, in CP-PA isolates a bla_{VIM-2} gene was detected, whereas in carbapenem-resistant *C. freundii* it involved a bla_{KPC-2} gene and in carbapenem-resistant *Enterobacter* spp. a bla_{OXA-48} gene was detected. AmpC type beta-lactamase genes (*e.g.* bla_{CMY} and bla_{DHA}) were most often found in *C. freundii* (6 out of 8 isolates). In this relatively small collection of isolates, seven isolates (two *C. freundii* and five *E. asburiae*) contained an *mcr-9* variant gene, but this involved several clonally related isolates. Upon clone correction this involved 3 strains. Three *mcr*-9 positive isolates had an minimum inhibitory concentration (MIC) of 0.5 μg/mL, and four strains had an MIC of 8 μg/mL, as measured by Vitek 2. No other *mcr* genes were detected. In isolates that were considered to be genetically closely related, variation in the

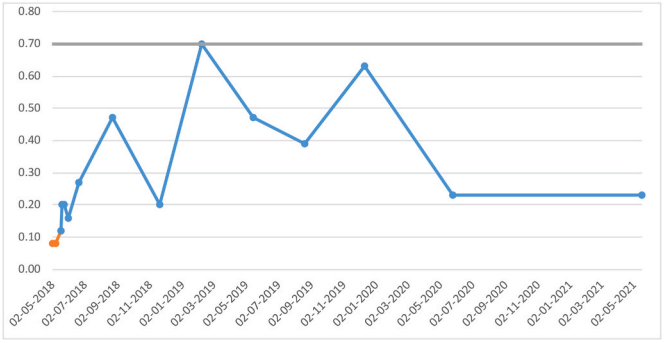

Fig. 2. Overall median CFU count per cm^2 determined over time in the new hospital building and the CFU count per cm^2 determined in the old hospital building one month before relocating as a reference. Orange line; CFU count in the new hospital building before relocating patients. Blue line; CFU count in the new hospital building after relocating patients. Grey line; CFU count observed one month before relocating patients in the old building, as reference value. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

presence of AMR genes was detected.

4. Discussion

The relocation to the new hospital building with 100% single-occupancy rooms with private bathrooms resulted in a significant reduction of environmental contamination with HRMO during the three-year follow-up period. We observed lower CFU counts up to three months after relocating, with fluctuating CFU counts after that moment. Two- and three years after relocating, during the COVID-19 pandemic, CFU counts in single-occupancy rooms were significantly lower compared to the multiple-occupancy rooms in the old hospital building.

Our findings should be considered in the broader context of the relocation. Besides the transition to 100% single-occupancy rooms, the introduction of a final cleaning after discharge of a patient in the new building might be associated with the reduction in environmental contamination with HRMO. Such a final cleaning is, however, more feasible in a single-occupancy room compared to a multiple-occupancy room. A second explanation for the higher number of HRMO identified in the old building is the number of VIM-positive *Pseudomonas aeruginosa* (VIM-PA) that was identified. The presence of VIM-PA in the old

Table 2
Number of sample sites positive for highly resistant microorganisms, and the number of resistant isolates detected on the sites during both sampling moments in the old hospital building and all sampling moments in the new hospital building.

Old hospital building							New hospital building						
Room type	Sample site	Positive sample sites (%)	ESBL-E	CPE	CP-PA	VRE	Room type	Sample site	Positive sample sites (%)	ESBL-E	CPE	CP-PA	VRE
Two- and four patient room	Nightstand (n = 149)	–	–	–	–	–	Single-occupancy room	Nightstand (n = 315)	–	–	–	–	–
	Table (n = 79)	–	–	–	–	–		Table (n = 324)	–	–	–	–	–
	Wall (n = 79)	–	–	–	–	–		Wall (n = 324)	–	–	–	–	–
	Sink (n = 50)	–	–	–	–	–		Sink (n = 324)	–	–	–	–	–
	Top of sink plug (n = 20)	1 (5.0)	1	–	–	–		Top of sink plug (n = 322)	–	–	–	–	–
	Bottom of sink plug (n = 50)	4 (8.0)	5	–	–	–		Bottom of sink plug (n = 324)	–	–	–	–	–
ICU room	Nightstand (n = 20)	–	–	–	–	–	ICU room	Nightstand (n = 128)	–	–	–	–	–
	Wall (n = 20)	–	–	–	–	–		Wall (n = 150)	–	–	–	–	–
	Sink (n = 24)	1 (4.2)	1	–	–	–		Sink (n = 181)	–	–	–	–	–
	Top of sink plug (n = 24)	3 (8.3)	1	–	4	–		Top of sink plug (n = 181)	–	–	–	–	–
	Bottom of sink plug (n = 24)	11 (45.8)	3	4	20	–		Bottom of sink plug (n = 181)	–	–	–	–	–
Room with anteroom	Nightstand (n = 14)	–	–	–	–	–	Room with anteroom	Nightstand (n = 88)	–	–	–	–	–
	Table (n = 14)	–	–	–	–	–		Table (n = 93)	–	–	–	–	–
	Wall (n = 14)	–	–	–	–	–		Wall (n = 95)	–	–	–	–	–
	Sink (n = 14)	–	–	–	–	–		Sink (n = 95)	–	–	–	–	–
	Top of sink plug (n = 0)	–	–	–	–	–		Top of sink plug (n = 95)	–	–	–	–	–
	Bottom of sink plug (n = 14)	1 (7.1)	1	2	–	–		Bottom of sink plug (n = 95)	–	–	–	–	–
Shared bathroom	Toilet seat (n = 18)	–	–	–	–	–	Private bathroom	Toilet seat (n = 138)	1 (0.7)	–	–	–	1
	Shower chair (n = 17)	–	–	–	–	–		Shower chair (n = 138)	1 (0.7)	–	–	–	1
	Shower drain (n = 17)	2 (11.8)	2	3	–	–		Shower drain (n = 138)	3 (2.2)	1	3	–	1
	Door handle (n = 18)	–	–	–	–	–		Door handle (n = 138)	–	–	–	–	–
	Sink (n = 20)	–	–	–	–	–		Sink (n = 138)	–	–	–	–	–
	Top of sink plug (n = 5)	–	–	–	–	–		Top of sink plug (n = 126)	–	–	–	–	–
	Bottom of sink plug (n = 20)	1 (5.0)	2	–	–	–		Bottom of sink plug (n = 138)	–	–	–	–	–
Total	Sample sites (n = 724)	24 (3.3%)	16	9	24	–	Total	Sample sites (n = 4269)	5 (0.1%)	1	3	–	3

Abbreviations: CPE, carbapenemase-producing Enterobacterales; CP-PA, carbapenemase-producing *Pseudomonas aeruginosa*; ESBL-E, extended-spectrum β-lactamase-producing Enterobacterales; VRE, vancomycin-resistant *E. faecium*; ICU, Intensive Care Unit.

building was known since 2010, as a long-lasting multi-ward outbreak with the ICU as most affected ward (Van der Bij et al., 2011). A persistent presence of VIM-PA in the sink drains of the ICU was then identified, which is reflected by the results of our study (Pham et al., 2022; Pirzadian et al., 2020; Pirzadian et al., 2022; Van der Bij et al., 2012; Van der Bij et al., 2011; Voor In 't Holt et al., 2018). To contain this reservoir, a bundle of 'water free' patient care was introduced in the ICU in 2011 (Pham et al., 2022). This was discontinued in the ICU in the new building, although for bathing of patients pre-packed washcloths remained instead of water and soap. After relocating to the new hospital building, VIM-PA did not colonize the sink drains within the time frame of this study. All *P. aeruginosa* isolates identified in our study all belonged to the outbreak strain (ST111) (Pirzadian et al., 2020). When we analyzed the difference in environmental contamination with HRMO between the old and the new hospital building without the VIM-PA strains, there were still significantly less HRMO identified in the new hospital building ($P < 0.001$).

Sinks and sink drains are known and important reservoirs for HRMO, and often play a role in outbreaks (Decker and Palmore, 2013; Kizny Gordon et al., 2017). Where in the old building 89.8% of HRMO isolates were identified from sink plugs, in the new building, no HRMO were identified from this location. This difference cannot be explained by a change in material. In both the old and the new building, drains and drain plugs were made of stainless steel. When we exclude sink plugs from the comparison between the old hospital and the new hospital building, the difference in environmental contamination is no longer statistically significant ($P = 0.06$), although this could also be explained by a lack of statistical power. However, for our hospital's new building, the decision was made to keep sinks in the ICU patient rooms, as a facility for healthcare workers to wash their hands and arms in case of unexpected contact with body fluids of the patient, or for specific microorganisms that are less susceptible to alcohol-based hand rub. Thus, these potential reservoirs of HRMO were present in the new building, but over a period of three years of patient care, we showed that they did not emerge as reservoir for HRMO again.

Overall, the contamination rates with HRMO in both hospital buildings were low, especially when compared to other studies, where they showed contamination of HRMO in up to 55% of rooms (Chen et al., 2019; Mody et al., 2019; Shams et al., 2016; Tanner et al., 2021). An important explanation for these low contamination rates is the difference in prevalence of HRMO. Most studies have been conducted in the United States of America, where the prevalence of HRMO carriage among patients is higher than in the Netherlands, with consequently higher environmental contamination rates (CDC, 2019; De Greeff and Mouton, 2017; Gupta et al., 2019). Secondly, an explanation for the low contamination rates could be the chosen sample method. Based on our selection of sampled surfaces, we decided to sample with premoistened cotton swabs. While this method has some disadvantages, such as difficulty to standardize, they also come with several important advantages (Rawlinson et al., 2019). Cotton swabs have high recovery rates on wet surfaces, similar or better recovery rates compared to other sampling methods, and they can be used on all surfaces, including surfaces that are more difficult to sample such as door handles (Moore and Griffith, 2007; Rawlinson et al., 2019; Rose et al., 2004). Additionally, since the swabs were directly placed in a selective broth, we were able to identify HRMO in low concentrations. A third explanation could be that, while other studies focused mostly on "high-touch" surfaces (*e.g.* bed rails, call buttons) we sampled built-in surfaces, with the exception of the nightstand (Chen et al., 2019; Mody et al., 2019; Shams et al., 2016; Tanner et al., 2021). These locations might be less frequently contaminated, but since these surfaces are used by all room occupants, they are potentially a better indicator of differences between multiple-occupancy and single-occupancy rooms. Interestingly, no sink or shower drains were sampled in the other studies, while we identified almost all HRMO on these surfaces, and not on "dry" surfaces (*i.e.* nightstands, tables). Notwithstanding, the contamination rates observed in our study are low, even after considering the low prevalence of HRMO in the Netherlands and our chosen sample methods. Thus, it is likely that other factors, such as our cleaning protocol, have contributed to these low rates.

There are several explanations for the fluctuations over the three year follow-up period in CFU counts per cm^2. As expected, the CFU counts in single-occupancy rooms and bathrooms were significantly lower before transferring patients to the new hospital building. However, this was not observed for the ICU rooms or rooms with an anteroom. One explanation for this is the fact that, while the construction of the single-occupancy rooms was mostly finished during the sampling moments, construction of the ICU rooms and rooms with anterooms was still ongoing. Consequently, more construction workers were present in these rooms, leading to relatively higher contamination levels. The fluctuations in CFU counts during the three years most likely reflected the use of the rooms. CFU counts were compared with the CFU counts determined in the old hospital building one month before relocating patients, since we believed that this was more representative for the contamination than the values determined one week before relocating patients. One week before relocating, the number of admissions to the hospital was lower, to prepare for the transfer of patients, and thus locations were used less frequently. We did not correct for use or nonuse of the bathroom by the patient. It is unclear why the CFU counts nine months after opening were higher in single-occupancy rooms. There were no changes in sampling or lab protocol that could explain the increase, and on later sampling moments, this increase in CFU counts was not observed again. A possible explanation is that there were changes in indoor temperature, or in humidity, which can impact the bacterial load (Klassert et al., 2021). However, since we did not measure this, we cannot be sure about this. The final two sampling moments took place during the COVID-19 pandemic. The lower CFU count could be explained by enhanced cleaning and increased disinfection rates with 1000 ppm chlorine. Only four of the included single-occupancy rooms were dedicated for suspected COVID-19 patients, and two of the included isolation rooms were dedicated for COVID-19-care.

Other studies have suggested a cutoff value for the number of CFU for hand contact surfaces in the healthcare environment. Dancer et al. suggested 5 CFU/cm^2, however, due to our cutoff value of 100 CFU per RODAC, which translates to a maximum of 3.95 CFU/cm^2, we were unable to determine if this criteria was exceeded (Dancer, 2004). Griffith et al. suggested <2.5 CFU/cm^2 as a cutoff value, a value that they found was practicable for all sites after disinfection (Griffith et al., 2000; Malik et al., 2003). Nonetheless, CFU counts are not helpful to determine if a source is contaminated with HRMO. While we did not determine the correlation between CFU counts and HRMO presence, other studies have not shown a correlation between CFU counts and HRMO presence (Al-Hamad and Maxwell, 2008; Widmer et al., 2019).

WGS was performed on all identified isolates. No persistent contamination over time was identified in the new hospital building. Remarkably, in isolates that were considered to be genetically closely related, variation in the presence of AMR genes was detected. We believe this to be the result of plasmid gain/loss in strains of otherwise identical genetic background. Plasmid gain/loss as possible explanation for these observations fell beyond the scope of this study. Another interesting result is that one *K. pneumoniae* strain was of ST16 (Supplement 4). This strain is an important emergent lineage of *K. pneumoniae*, has caused multiple outbreaks within European hospitals, and is known to carry multiple carbapenem resistance genes (Boff et al., 2021; Espinal et al., 2019). However, the strain identified in the old hospital did not carry any gene encoding carbapenem resistance. Another interesting finding is that seven *E. hormaechei* strains, both from the old and the new hospital building, were of ST78 (Supplement 4). ST78 isolates are successful One Health clones, that are considered high risk and are of global interest (Cardoso et al., 2022). Additionally, nosocomial infections with this ST, both in Europe and Asia, are increasingly reported (Gomez-Simmonds et al., 2018; Villa et al., 2019). The ST78 isolates we identified from the hospital environment were CPE

and carried bla_{OXA-48}. As far as we know, these strains have not yet lead to nosocomial infection in our patients, but it is important to monitor presence of this strain.

4.1. Strengths and limitations

The main strength of this study is that we sampled the old and the new hospital building, with identical sampling methods and sampling locations. A second strength of our study is the follow-up period of three years in the new building. This follow-up period not only provided us with the opportunity to look at a situation where environmental contamination had developed, but also provided time for that contamination to build up further. Thirdly, we did not focus on environmental contamination with one type of HRMO, but looked at the presence of MRSA, ESBL-E, CPE, CP-PA, CR-AB, and VRE. Finally, we sampled a large number of rooms, on different wards, including isolation-, hematology-, and ICU rooms.

A limitation of our study is that we were not able to sample every room at every sample moment. When a patient was cared for in isolation, in a non-isolation room, we did not sample this room, but we sampled a nearby room instead. During the next sampling moment, the original room was sampled again. Secondly, it is likely that our study shows an underestimation of the environmental contamination. This could be due to our chosen sampling method or the selected sample sites. On the other hand, every sample method or selection of sampled surfaces will inherently introduce bias, and hence, it is unlikely that other studies have not shown an underestimation of the contamination rates. Thirdly, we only determine presence of HRMO, and not the abundance in which they were present. However, since the concentration of nosocomial pathogens is generally low, they are often only detectable with broth enrichment, which makes determining the abundance impossible (Otter et al., 2011). Fourth, we did not correct for the timing and compliance of cleaning or disinfection. During the three-year follow-up, rooms were sampled 15 times, and at different time points during the day. Some rooms were sampled directly after daily or final cleaning, while other rooms were sampled before cleaning. Since rooms were located throughout the hospital and thus cleaned at different moments, and we looked at the median CFU counts, we believe that our results are representative for the environmental contamination of our hospital. Finally, we did not determine how our results correlate with the incidence of healthcare-associated infections (HAI).

5. Conclusion

We observed significantly less HRMO in the single-occupancy rooms in the new hospital building over the three-year follow up, while CFUs were not impacted. This finding shows that, with regard to environmental contamination, single-occupancy rooms are favorable over multiple-occupancy rooms. These finding should be taken into account when considering hospital designs for renovations or the construction of hospitals. Future research should focus on the effect of changes in environmental contamination on the incidence of HAI. Additionally, the effect of single-occupancy rooms on environmental contamination in countries with higher HRMO prevalence should be determined. Finally, the impact of transitioning to single-occupancy rooms on other environmental aspects, such as the microbiome, should be studied further.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Author contributions

Conceived and designed the study: MV, JS, AV, MB, JH, DG. Collecting data: AS, AV. Analyzed the data: AS, AV, CK. Wrote the paper: AS, AV. All authors read and approved the final manuscript.

Declaration of competing interest

AS, JS, CK, DG, MB, JH, AV, and MV declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114106.

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