MOVEing Microorganisms
The effect of the built environment of the hospital and screening strategies on microbial safety

Adriënne S. van der Schoor

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MOVEing Microorganisms:
The Effect of the Built Environment of the Hospital and Screening Strategies on Microbial Safety

MOVEing micro-organismen: het effect van de ziekenhuisomgeving en screening strategieën op microbiologische veiligheid

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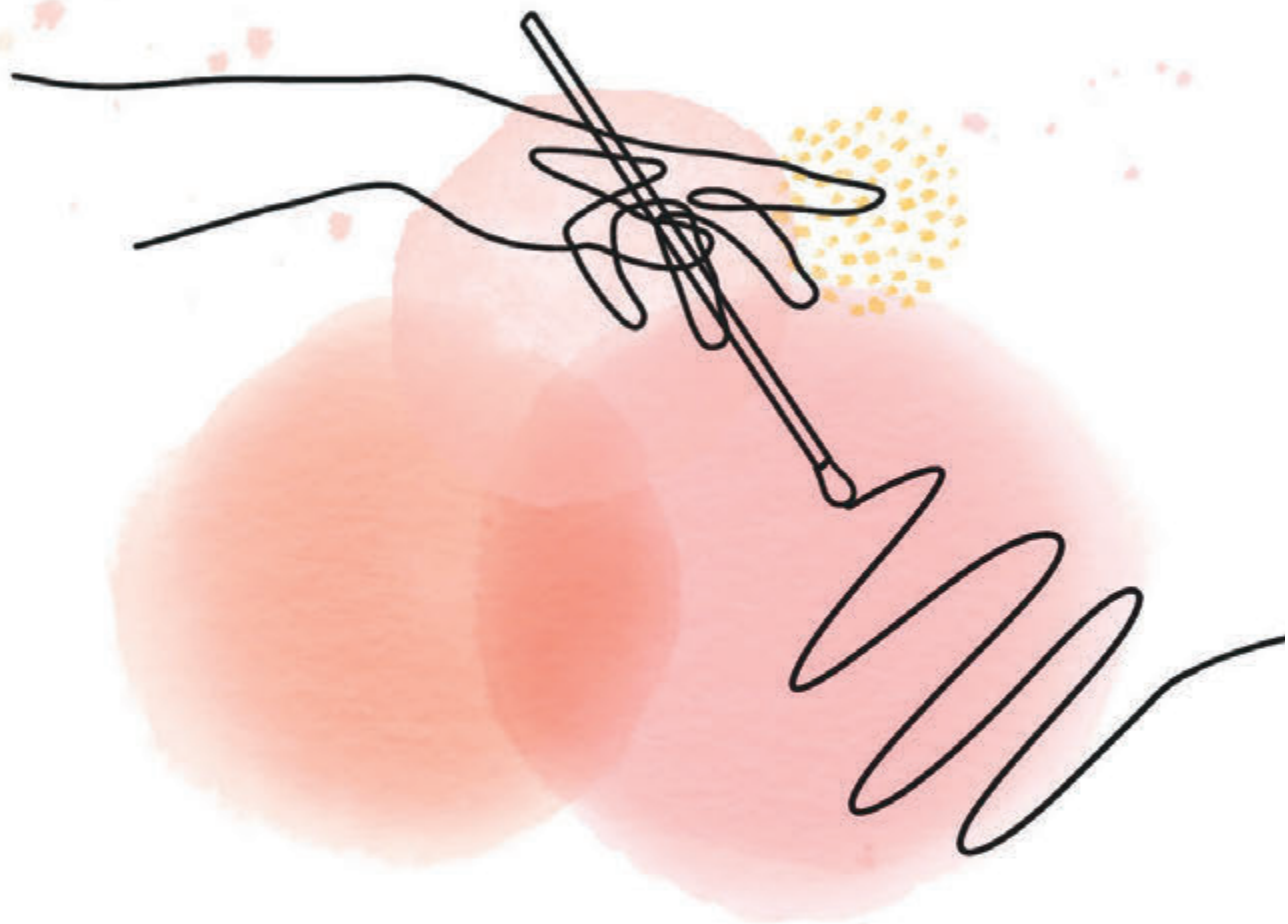
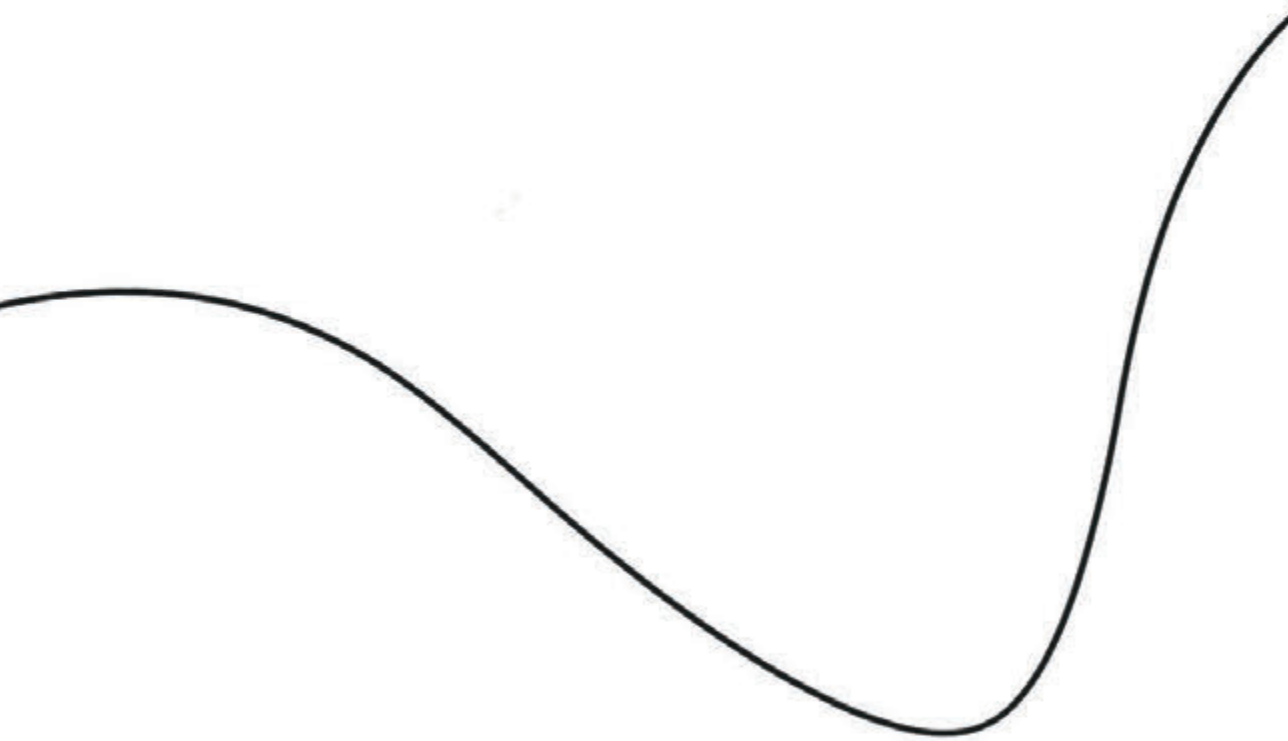


GENERAL INTRODUCTION AND OUTLINE OF THESIS



Multidrug-resistant microorganisms

A microorganism is defined as a multidrug-resistant microorganism (MDRO) when it is resistant to one or more classes of antimicrobial agents (1). MDRO are considered as an important threat to public health (2, 3). Examples of MDRO are extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E), carbapenemase-producing Enterobacterales (CPE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE) and carbapenemase-producing *Pseudomonas aeruginosa* (CP-PA). Among Enterobacterales, *Escherichia coli*, *Klebsiella pneumoniae*, and *Citrobacter freundii* are most frequently encountered. The worldwide increase in MDRO is causing increasing healthcare costs, morbidity, and mortality in patients (3, 4). The prevalence of MDRO differs per country and per type of MDRO, ranging from less than one percent to more than 50% of isolates. Within the Netherlands, the prevalence of MDRO is generally low, although it differs per type of microorganism. The prevalence of MRSA nasal carriage upon admission to the hospital ranged between 0.03-0.17% between 2010 and 2017 (5), while the prevalence of ESBL-E intestinal carriage in the Netherlands between 2011 and 2016 ranged between 4.5% and 8.6% (6-8).

MDRO are a common cause of healthcare-associated infections (HAI), which are infections caused by pathogens acquired by patients during their stay in a healthcare institution (9). HAI can be caused by microorganisms from an endogenous or exogenous source. Endogenous sources are body sites, including skin; exogenous sources are external sources, such as the hospital environment, its surfaces, or health care workers. This thesis is focused on endogenous sources by screening patients to identify microorganisms present upon admission to the hospital, and on exogenous sources, specifically the hospital innate environment and the transmission from the environment to the patient and vice versa.

Relocation of the Erasmus MC

The Erasmus MC University Medical Center (Erasmus MC) in Rotterdam, the Netherlands, is the largest academic hospital of the Netherlands. It includes the adult clinic ("Dijkzigt"), the Sophia Children's hospital, the Erasmus MC Cancer Institute and the Faculty of Medicine and Health sciences of the Erasmus University Rotterdam. From 1961 until May 18, 2018, the hospital was located in the Dijkzigt hospital building, while the Erasmus MC Cancer Institute was on a location named "Daniel den Hoed".

In 2009, the Erasmus MC started the construction of a new hospital building, directly next to the old hospital building (Figure 2) as replacement of the Dijkzigt hospital and the Erasmus MC Cancer Institute. At the beginning of the design process, it became clear that the relocation would be accompanied by a reduction in the number of beds due to expected changes in the organization of health care, resulting in less admission days. To optimize microbial safety and to make the most use out of the available number of beds, the decision

was made to implement 100% single-occupancy rooms with private bathrooms. Additionally, the implementation of 100% single-occupancy rooms was part of providing a safe and healing environment. The aim of a healing environment is to provide an environment for patients, staff, and visitors, that is calm, non-institutional, and can positively impact the recovery time of patients (10, 11). The decision for 100% single occupancy room was largely based on expert opinion, as evidence for its effects on infections and on other patient related outcomes was limited back then.

Figure 1a. The old hospital building of the Erasmus MC (the Dijkzigt hospital)

Figure 1b. The Daniel den Hoed Cancer Institute

Figure 2. The new hospital building of the Erasmus MC

The old hospital building

The old hospital building of the Erasmus MC (the Dijkzigt hospital building) had 1,125 beds, mainly divided over two- and four-person occupancy rooms (Figure 3), and 42 Intensive Care Unit (ICU) single-occupancy beds. Bathrooms were shared and located on the ward, with an average of four patients sharing a toilet, and seven patients sharing a shower. When a patient on the ward was placed in contact isolation, *i.e.,* standard precautions, and use of gloves and gowns, the other bed(s) in the room were blocked for admissions. For patients in isolation, a designated bathroom was appointed to that patient, or washing and toileting occurred on bed and by bedpan.

Exceptions to the multiple-occupancy rooms were the ICU, the isolation department, and the hematology departments. The ICU consisted of 100% single-occupancy rooms, of which some were designated isolation rooms with an anteroom and negative air pressure. The isolation department consisted of 100% single-occupancy rooms, all with anteroom, negative air pressure and private bathrooms. The hematology departments, which were located at both location Dijkzigt and location Daniel den Hoed, consisted of a number of two- and three-patient occupancy rooms, with attached bathrooms, but the majority of the rooms were single-occupancy rooms, all with anteroom, HEPA filtered air and private bathroom.

Figure 3. One side of a four-person occupancy room in the old hospital building of the Erasmus MC.

The new hospital building

The new hospital building was officially opened on May 18, 2018, when all patients were transferred in one day from the old hospital building to the new hospital building. All patients from the Daniel den Hoed were also relocated to the new hospital building on this date in a custom-made moving truck. The new hospital building has 525 beds, all single-occupancy rooms with private bathrooms and rooming-in facilities (Figure 4), and 56 ICU beds. Where in the old building there was an isolation department, the isolation rooms in the new hospital building are located at multiple wards.

Figure 4. A single-occupancy room in the new hospital building of the Erasmus MC.

Impact of single-occupancy rooms

The transition to 100% single-occupancy rooms was expected to have positive effects, among others on infection prevention and control (IPC). For example, research has shown that transitioning from two-person to single-occupancy rooms on an ICU decreased the number of patient transfers with 90%, and the number of medication errors with 67% (12). Other studies have found comparable effects on medication errors (13). Additionally, single-occupancy rooms are expected to improve patient sleep and social support, and potentially decrease the length of stay (11-13).

The relocation to the new building of the Erasmus MC provided the unique opportunity to determine the effect of transitioning to single-occupancy rooms on different aspects. For this purpose, the board of directors of the Erasmus MC funded the consortium Program Evaluating – Our New Erasmus (PE-ONE), which aimed to determine the transition to 100% single-occupancy rooms from a multidisciplinary point of view. PE-ONE consisted of three pillars: CHANGE, which looked at the transition from the old to the new building from a management point of view, WELCOME, which looked at experiences from patients and staff and evaluating work situations and efficiency, and MOVE, which looked at the effect of single-occupancy rooms on the microbial safety. The latter was subject of this thesis.

MOVE study; microorganisms in the environment of single- and multiple-occupancy rooms

The aim of the MOVE study was determining the effect of transitioning from an old hospital building with multiple-occupancy rooms and shared bathrooms to a newly constructed hospital building with single-occupancy rooms and private bathrooms on the microbial safety. We hypothesized that single-occupancy rooms would provide a microbial safer environment for patients compared to multiple-occupancy rooms (14). We define the environment of the new hospital microbial safer when the environmental contamination in

general and/or with MDRO is lower, and/or when acquisition and/or transmission of MDRO is lower compared to the old hospital.

This overarching hypothesis could be divided into several sub-hypotheses; first, 100% single-occupancy rooms will decrease the risk on the acquisition of MDRO during hospitalization as direct patient to patient transmission between roommates cannot occur in single-occupancy rooms. Research has indicated that there is a significant relation between being exposed to roommates and acquisition of microorganisms, especially for the same microorganism the roommate was colonized with (15, 16). Although a number of studies have been performed on the effect of single-occupancy rooms on acquisition of MDRO, and consequently the impact on HAI, literature is conflicted on the added benefit of single-occupancy rooms on IPC (14). While several studies showed a significant reduction in healthcare-associated colonization with MDRO after transitioning to mainly or only single-occupancy rooms (17-22), some studies showed no effect (23-25). The majority of the studies were performed on pediatric or adult ICUs or on a neonatology department (17-19, 21, 22, 24). Furthermore, only four studies studied the transition to 100% single-occupancy rooms (18-21). A recent study of McDonald et al., looked at the effect of relocating to a newly constructed building with only single-occupancy rooms on colonization and infection rates (26). They identified a decrease in colonization and infection rates with VRE and MRSA colonization, but not for MRSA and *Clostridioides difficile* infections. Overall, the effect of single-occupancy rooms on general wards on acquisition is lacking, specifically for ESBL-E and CPE.

Besides the elimination of transmission from roommates and the shared environment, introducing single-occupancy rooms eliminates specific reasons for intra-hospital patient transfers (*i.e.,* transferring patients from one patient room to another patient room in the same hospital). For example, intra-hospital patient transfers for small procedures, social circumstances, or for contact isolation will no longer be essential (27). The number of intra-hospital patient transfers on an ICU after transitioning to single-occupancy rooms was reduced by 90% (12). The hypothesis is that the number of intra-hospital patient transfers will decrease by the transition to 100% single-occupancy rooms. Limiting the number of intra-hospital patient transfers leads to less exposure of the patient to different hospital environments or in short; the patient is exposed to less square meters of the hospital environment. This potential reduction of exposure to different hospital environments, could also lead to a reduced risk of MDRO acquisition and transmission (28).

In this thesis (**chapter 2.1**), we aim to determine the effect of transitioning to 100% single-occupancy rooms on the odds on acquiring an ESBL-E during hospitalization. Additionally, we aim to determine the effect on the number of intra-hospital patient transfer.

A second hypothesis is that implementation of 100% single-occupancy rooms could lead to lower environmental contamination rates. This is based on the assumption that, since there

will only be one patient admitted to a room, only one patient can contaminate the environment. After the patient is discharged, the room can be cleaned and any contamination can be removed. However, there is no literature yet to support this assumption.

In this thesis (**chapter 3.2**), we aim to determine the differences in environmental contamination, for the total bioburden and the presence of MDRO, between multiple-occupancy rooms and single-occupancy rooms. Moreover, we will determine the change over time and potential build-up of environmental contamination in the new hospital building over a three-year follow up-period.

Universal risk assessment and risk-based screening

To prevent spread of MDRO throughout hospitals, transmission-based precautions are installed for known carriers of MDRO, in addition to standard precautions. These additional precautions differ per type of microorganism, *e.g.,* ESBL-E carriers are cared for in contact isolation (*i.e.,* single-occupancy room, gloves and gowns), while patients known to carry MRSA are cared for in strict isolation (*i.e.,* isolation room with ante room, gloves, gowns, surgical masks, and hair caps) (29).

In the Netherlands, patients are not routinely screened for MDRO colonization upon admission. Yet, the risk on being colonized with a MDRO upon admission is determined for all patients through the MDRO universal risk assessment and, when patients are deemed at risk, a risk-based screening (30). This nationally implemented risk assessment consists of six questions: i) Is the patient a known carrier of a MDRO, ii) has the patient recently been treated in or admitted to a healthcare institution abroad, iii) did the patient stay in a healthcare facility known with a MDRO outbreak in the past two months, and if yes, was the patient approached for screening, iv) has the patient lived in an institution for asylum seekers in the past two months, v) does the patient live or work where pigs, veal calves or broilers are kept commercially, and vi) is the patient a partner, housemate or caregiver of someone who is MRSA positive? Additionally, at the Erasmus MC, the question “are you a professional seafarer” is added due to the finding that the prevalence of MRSA is higher among seafarers who are frequently visiting our hospital as they come from the nearby located port of Rotterdam (31). When the universal risk assessment indicates that a patient is deemed high risk to be a carrier (*e.g.,* patient is a professional seafarer, or the housemate, caregiver or partner of a MRSA carrier), screening cultures (*i.e.,* nasal, throat, and perineal/rectal cultures) are obtained and the patient is cared for in strict isolation until the results of the screening cultures are known. When a patient is deemed low risk (*i.e.,* patient was admitted in a hospital abroad over two months ago, but did undergo surgery or had a wound), screening cultures are obtained, but the patient is not preemptively placed in isolation. When the patient is categorized as having no risk for MDRO carriage, no cultures are taken and the patient is not preemptively placed in isolation. The MDRO universal risk

assessment and risk-based screening were first developed to identify risk factors for MRSA carriage, later questions to determine carriage of other MDRO were added.

Recently, the efficacy of the MDRO universal risk assessment and risk-based screening is questioned (32). As timely identification and isolation of MDRO carriers is essential in preventing transmission throughout the hospital, improvement of the universal risk assessment should be considered. For instance, while in the universal risk assessment patients are asked if they have been hospitalized abroad, there is no question regarding recent travel history. Recently, literature has focused on the risk of HRMO acquisition during travelling, specifically to south-east Asia (33-35). However, the studies were performed with healthy travelers, and consequently cannot directly be extrapolated to patients admitted to our hospital. Besides improving the universal risk assessment and risk-based screening, other screening strategies should also be considered, such as a universal screening strategy. In order to generate further evidence for improving universal or risk-based risk assessment on colonization of HRMO, we performed two studies.

In this thesis (**chapter 2.2**), we assess if colonization with MDRO following international travel among patients is high enough to include this as a risk factor in the risk assessment. In **chapter 2.3** of this thesis, we aim to compare the yield of a universal screening strategy with the currently installed universal risk assessment and risk-based screening.

Environmental contamination

Surfaces in hospitals can act as reservoirs for pathogenic microorganisms, and hence for MDRO. The time period microorganisms are able to survive on surfaces differs per type of microorganism and can range from a few hours up to several months (36). Consequently, when a surface is not correctly cleaned and/or disinfected, the surface can be a lasting source for transmission. Studies determining the environmental contamination with MDRO in non-outbreak settings have showed contamination rates of up to 55%, even after terminal disinfection of the surface (37-40).

Environmental sampling practices

Environmental sampling can be performed for a number of reasons, but is mainly performed in outbreak situations to determine the source of the outbreak. Other reasons for environmental sampling could be evaluating cleaning/disinfection practices, routine sampling, or for research purposes. Environmental sampling methods can be divided into direct or indirect sampling methods. Direct sampling methods are methods that require no further processing, while extra processing is necessary for indirect methods (41). Examples of direct sampling methods are contact plates, dip slides and petriflm, examples of indirect sampling methods are sponges, wipes, and different types of swabs (*e.g.,* cotton swabs, flocked swabs, rayon swabs). Swabs are most often used when performing sampling of the

environment, which can be explained by the fact that they are easily available in a healthcare setting, easy in use, and associated costs are low (41). Results of environmental sampling can be reported as presence/absence, as the abundance in which a target microorganism is present, or the total bacterial load of a surface can be presented as the number of colony forming units (CFU). Currently, there are no guidelines on how to perform environmental sampling (41, 42).

In this thesis (**chapter 3.1**), we aim to determine what the current environmental sampling practices within Europe are, and if there is a consensus on how and when to sample the hospital environment.

Transmission from the hospital environment to patients

Transmission from the hospital environment to patients can take place through either direct contact with the contaminated surfaces, or indirect contact, *e.g.,* via the hands of healthcare workers (HCW). The crucial role of the hospital environment in outbreaks was highlighted by a study of Gastmeier et al., in which they identified the source of 1,561 published outbreaks (43). No source was identified for 37.1% of outbreaks. For the outbreaks where a source was identified, the source was an index patient (40.3%), equipment and devices (21.1%), personnel (15.8%), and the environment (19.8%) (43). The role of the environment in transmission is also highlighted by the study of Wu et al. (16). They determined that, when the previous roommate was colonized or infected with a MDRO, the current room occupant has a higher chance on becoming colonized or infected with that MDRO (16). Since there is no direct contact with a previous roommate, this transmission is most likely through the environment, either via direct or indirect contact.

Staphylococcus aureus is a well-known commensal, and an important cause of both community- and hospital-acquired bacteremia and other severe infections (44, 45). While the majority of nosocomial *S. aureus* infections (~80%) are endogenous, patients with exogenous infections, although not well understood, tend to have longer hospitalizations after bacteremia and a higher risk of mortality (46, 47). Furthermore, exogenous infections are, due to their origin, theoretically preventable. Consequently, it is important to strive for 100% prevention of spread of *S. aureus*. For this we have to understand the mode and factors of transmission to be able to install adequate IPC measures. Since *S. aureus* can survive on surfaces from hours up to several months, the hospital environment can be an important source of transmission (36, 48). Transmission of *S. aureus* from the hospital environment or hands of HCW to patients have been shown (49, 50). However, the dynamics of *S. aureus* in patients, in the hospital environment and between the hospital environment and patients are relatively unknown, specifically in non-outbreak settings.

In this thesis (**chapter 2.4**), we aim to determine colonization and acquisition rates of patients with *S. aureus* and environmental contamination with *S. aureus,* and subsequently

to determine if transmission of *S. aureus* occurred between patients and the hospital environment and vice versa.

To prevent transmission from the environment to the patient, correct cleaning and disinfection of hospital surfaces is needed. This is confirmed by the study of Chen et al., in which they report on frequent transmission between the hospital environment and patients, early in their admission (37). They suggest that room disinfection after discharge of patients might be inadequate in preventing transmission of MDRO. In the study of Chen et al., all rooms where disinfected after patient discharge. However, in the Netherlands, disinfection is only indicated after discharge of a patient that was a known carrier of a MDRO or other specific pathogens (*e.g., Clostridioides difficile*). In all other situations, rooms are dry cleaned and with damp microfiber cloths and not disinfected. This could result in a high environmental contamination rate, since not all MDRO carriers are identified, and thus their rooms are not disinfected upon discharge. However, the current environmental contamination rates are not known.

In this thesis (**chapter 3.2**) we aim to determine the environmental contamination with MDRO in patient rooms of the old and new hospital building.

Aim and outline of this thesis

The main aim of this thesis is to determine the effect of an intervention, the transition of the Erasmus MC to a new hospital building with 100% single-occupancy rooms and single occupancy rooms, on two elements of microbial safety; the odds on the acquisition of MDRO, and the extent of and effect on environmental contamination. A secondary aim of this thesis is to determine screening methods to identify patients colonized with MDRO upon admission.

This thesis is divided in two main chapters: patient related and hospital environment related research. In **chapter 2** we will focus on patients and acquisition of, colonization with, and screening for MDRO. In **chapter 2.1**, the effect of the intervention (the transition to 100% single-occupancy rooms) on the odds of acquisition of ESBL-E is determined in a prospective before-and-after study. The effect of the intervention on intra-hospital patient transfers is also investigated. In **chapter 2.2**, the travel behavior of patients is studied via a questionnaire upon admission, and the association between travel and MDRO colonization upon admission is determined. In **chapter 2.3**, we compare a universal screening strategy for MDRO upon admission to the currently installed universal risk assessment and risk-based screening upon admission for MDRO in a observational prospective cohort study. In **chapter 2.4**, we determine the carriage and acquisition rates of methicillin-susceptible *S. aureus* (MSSA) and MRSA in patients, and we determine transmissions between patients and the environment, and vice versa.

Chapter 3 will focus on the contamination of the hospital environment In **chapter 3.1**, we present the results of a web-based survey to determine surface sampling practices throughout Europe, and to determine if consensus regarding sampling practices exists. In **chapter 3.2**, we determine the effect of the intervention on environmental contamination, in a prospective before-and-after study, where we sampled the hospital environment over a three-year period. Finally, in **chapter 4** the data and findings presented in this thesis will be discussed in a summarizing discussion and future perspectives are provided.

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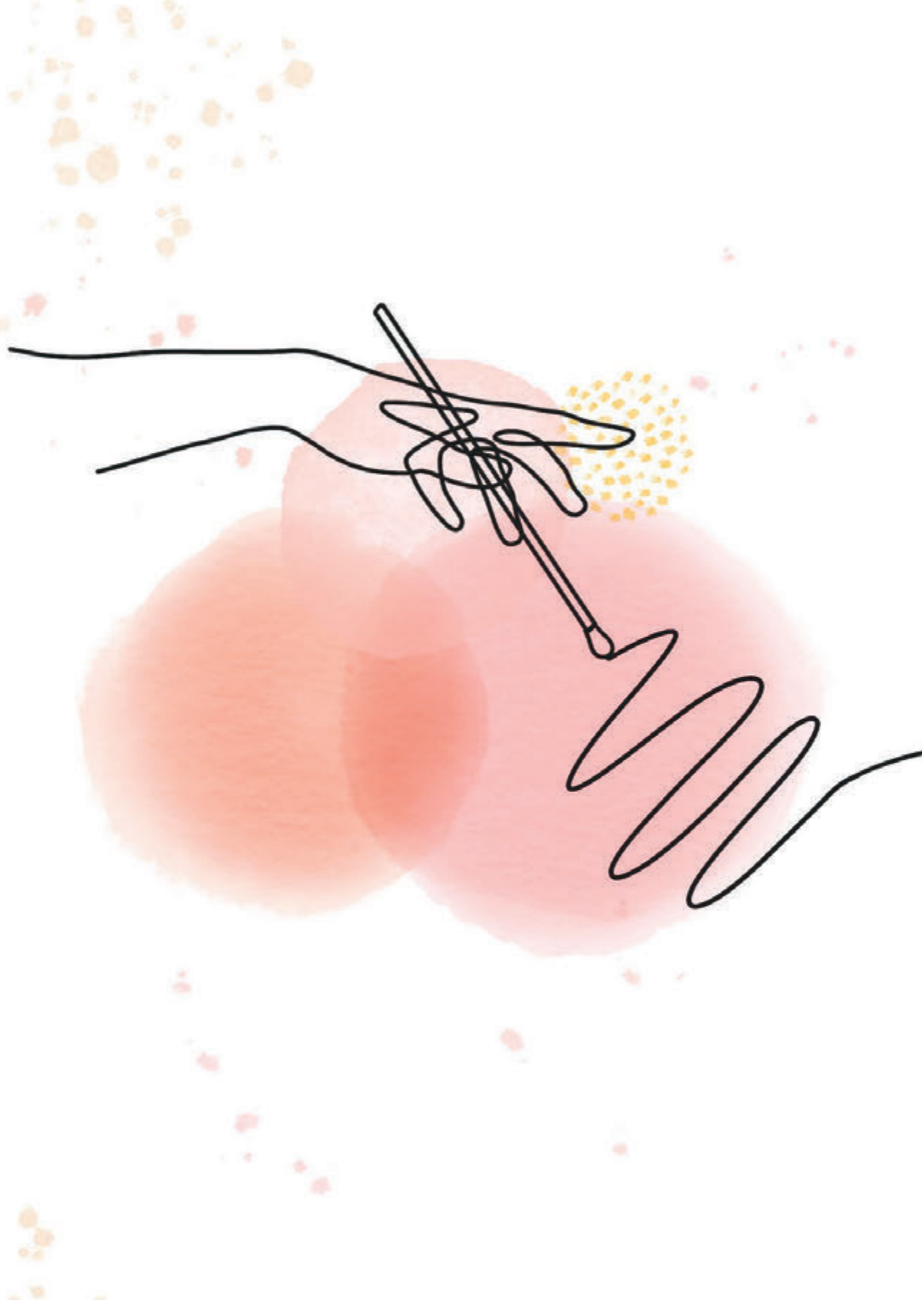
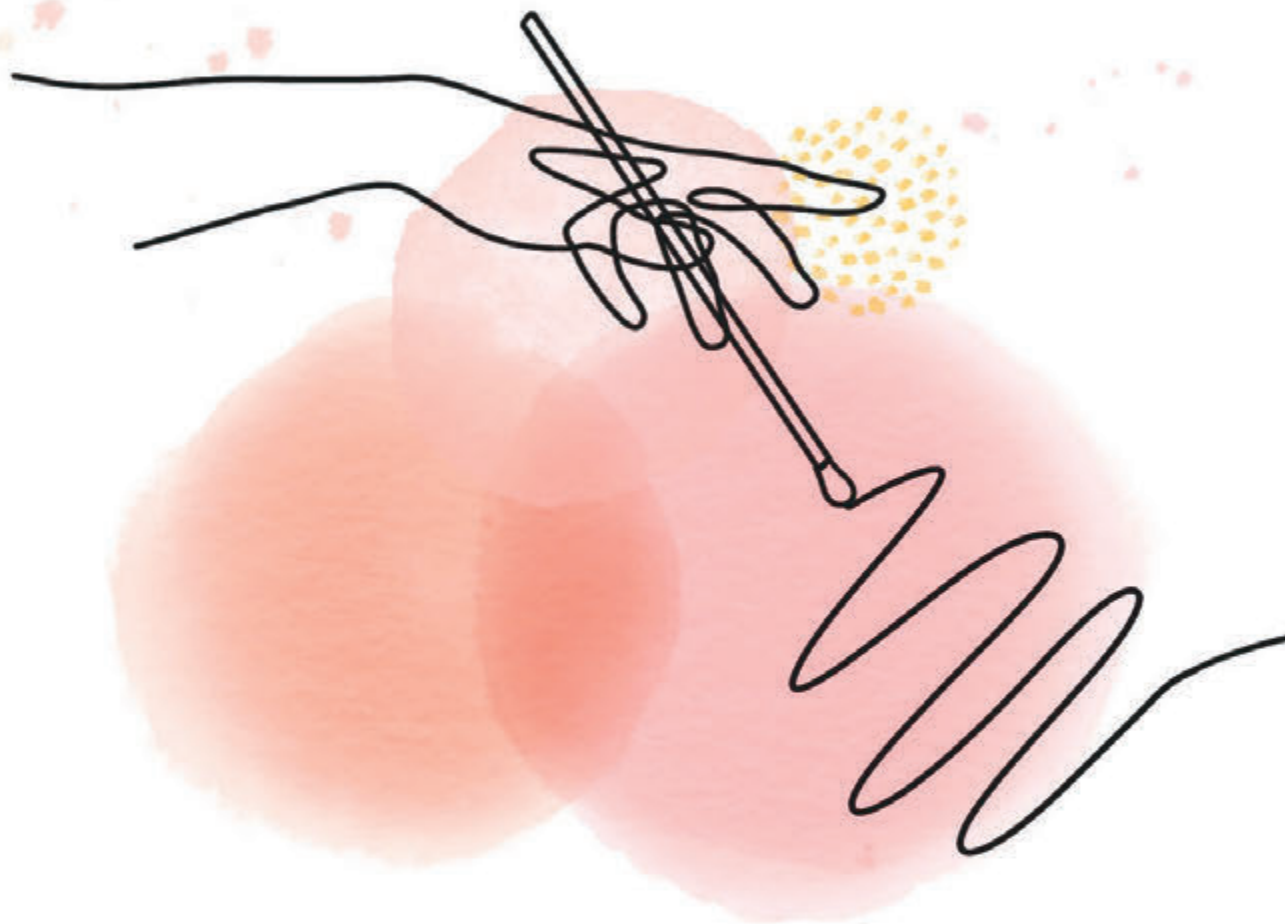


Chapter 2

PATIENTS

Chapter 2.1



THE EFFECT OF 100% SINGLE-OCCUPANCY ROOMS ON ACQUISITION OF EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERALES AND INTRA-HOSPITAL PATIENT TRANSFERS: A PROSPECTIVE BEFORE-AND-AFTER STUDY

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Abstract

Extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) are a well-known cause of healthcare-associated infections. The implementation of single-occupancy rooms is believed to decrease the spread of ESBL-E. Additionally, implementation of single-occupancy rooms is expected to reduce the need for intra-hospital patient transfers. We studied the impact of a new hospital with 100% single-occupancy rooms on the acquisition of ESBL-E and on intra-hospital patient transfers. In 2018, the Erasmus MC University Medical Center moved from an old, 1200-bed hospital with mainly multiple-occupancy rooms, to a newly constructed 522-bed hospital with 100% single-occupancy rooms. Adult patients admitted between January 2018 and September 2019 with an expected hospitalization of ≥48 hours were asked to participate in this study. Perianal samples were taken at admission and discharge. Patient characteristics and clinical information, including number of intra-hospital patient transfers, were collected from the patients' electronic health records. Five hundred and ninety-seven patients were included, 225 in the old and 372 in the new hospital building. Fifty-one (8.5%) ESBL-E carriers were identified. Thirty-four (66.7%) patients were already positive at admission, of which 23 without recent hospitalization. Twenty patients acquired an ESBL-E, seven (3.1%) in the old and 13 (3.5%) in the new hospital building (*P*=0.801). Forty-one (80.4%) carriers were only detected by the active screening performed during this study. Only 10 (19.6%) patients, six before and four during hospitalization, showed ESBL-E in a clinical sample taken on medical indication. Fifty-six (24.9%) patients were transferred to other rooms in the old hospital, compared to 53 (14.2%) in the new hospital building (*P*=0.001). Intra-hospital patient transfers were associated with ESBL-E acquisition (OR 3.18, 95%CI 1.27-7.98), with increasing odds when transferred twice or more. Transitioning to 100% single-occupancy rooms did not decrease ESBL-E acquisition, but did significantly decrease the number of intra-hospital patient transfers. The latter was associated with lower odds on ESBL-E acquisition. ESBL-E carriers remained largely unidentified through clinical samples.

Introduction

Highly resistant microorganisms (HRMO) are a common cause of healthcare-associated infections (HAI), and are a worldwide threat to public health and modern healthcare (1). Among HRMO, extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) are most frequently identified. Worldwide, the prevalence of ESBL-E in the community differs from 2% to 46% (2). In hospitals, this prevalence is higher and outbreaks with ESBL-E occur. Hospital design is thought to play an essential role in the spread of HRMO including ESBL-E (3-5). To decrease the spread of HRMO within hospitals, the Facility Guideline Institute recommends transitioning to 100% single-occupancy rooms for medical/surgical units (6). Moreover, their 2018 report advises 100% single patient rooms in adult critical care units (7). An added benefit of single-occupancy rooms is that they remove the necessity for intra-hospital patient transfers for small procedures, social circumstances (*e.g.* end-of-life care), or for an indication of contact isolation (8). By reducing the number of intra-hospital patient transfers, which leads to less exposure of the patient to different hospital environments, and by reducing the exposure to unidentified infected or colonized roommates, the implementation of 100% single-occupancy rooms is expected to reduce the risk of HRMO acquisition and transmission (9). However, current literature shows conflicting results for the effect of single-occupancy rooms on the acquisition of HRMO (4, 10, 11). Furthermore, literature on the effect of single-occupancy rooms on ESBL-E acquisition is limited to the comparison of ESBL-E acquisition between an intensive care unit (ICU) with an open plan and an ICU with single-occupancy rooms, which showed no significant difference (11).

In May 2018, the Erasmus MC University Medical Center (Erasmus MC) relocated from an old hospital building, with mainly multiple-occupancy rooms, to a newly constructed hospital building with 100% single-occupancy rooms. We used this unique opportunity to determine the effect of relocating to a new hospital with 100% single-occupancy rooms on the acquisition of ESBL-E by determining ESBL-E carriage in patients at admission and discharge in both buildings. Whole genome sequencing (WGS) was used to determine if strains at discharge were identical to those present at admission or the result of acquisition during hospitalization. Additionally, we aimed to determine the effect of intra-hospital patient transfers on ESBL-E acquisition, and to identify the percentage of ESBL-E carriers that remained undetected by clinical samples.

Methods

Study design and setting

This study was performed at the Erasmus MC, a university medical center located in Rotterdam, the Netherlands. On May 18, 2018, the adult clinic of the Erasmus MC relocated from an old, 1200-bed hospital building with mainly multiple-occupancy rooms and shared bathrooms, to a newly constructed 522-bed hospital building with 100% single-occupancy rooms and private bathrooms. To determine the prevalence of colonization with ESBL-E and the incidence of acquisition of ESBL-E in the old and new hospital building, a prospective before-and-after study was performed. Participating departments were cardiology,

gastroenterology and hepatology, general surgery, hematology, adult ICU, internal medicine, nephrology, neurology, neurosurgery, orthopedics, and plastic surgery, which do not always correspond to the admission specialization of the patients.

Room types

In the old building, almost all departments consisted of two- and four-patient rooms, and bathrooms were shared, with an average of four patients per toilet (range four to seven) and seven patients per shower (range five to nine). Exceptions were the isolation department, the adult ICU, and three hematology departments. The isolation department consisted of solely single-occupancy rooms with anterooms and private bathrooms, and the ICU consisted of solely single-occupancy rooms, some with anterooms but all without bathrooms. The three hematological departments consisted of 83.3, 80.0 and 69.2% single-occupancy rooms and private bathrooms. All multiple-occupancy rooms, two- or three-patient rooms, had attached shared 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 the same day. In the new hospital building, all departments consisted of only single-occupancy rooms with private bathrooms, with anterooms for hematology and isolation rooms.

Patient inclusion

From January 1, 2018 until September 1, 2019, all adult patients with an expected hospital stay of ≥48 h admitted to participating departments were asked to participate. Additionally, patients needed to understand and read Dutch. Patients who were admitted in the weekend or on holidays, via the emergency room, or who were cared for in airborne isolation were not approached for participation, as well as patients who were legally incapable in making decisions regarding participating, or patients who were in end-of-life stage. Patients with multiple hospitalizations during the study period were allowed to participate more than once. No additional information on HRMO risk factors were obtained before including patients (*i.e.* non-targeted screening). After obtaining written informed consent, perianal samples were collected within 24 h of admission, and on the day of discharge from the hospital. Patients who were admitted to the ICU during their hospital stay were considered as new admissions, even when they were already included in the study. Admission samples were taken on the day of admission to the ICU and discharge samples on the day patients were discharged from the ICU. Samples were either taken by trained members of the research team or patients could self-sample with clear verbal instructions of the members of the research team. Patients missed at discharge (*e.g.* unforeseen earlier discharge) received a letter asking them to take the sample at home, as well as a swab, swab-instructions with clear pictures and directions, and return-envelope. Patients admitted during the relocation of the hospital were asked for an additional swab, one day before relocation of the hospital. That sample was both the discharge sample for the old hospital building, and the admission sample for the new hospital building. ESBL-E colonization was defined as having a positive sample at admission. ESBL-E acquisition was defined as having a negative sample at admission and a positive sample for ESBL-E at discharge. It was also considered acquisition when patients were positive for a different ESBL-E at discharge. A different ESBL-E was defined as either being positive for a different

microorganism, or when WGS showed that the discharge isolate was not identical to the admission isolate. Results of the perianal sample were not communicated to medical staff or patients, were not registered in the electronic health records (EHR), and hence, no infection prevention measures were taken based on the results, as stated in the protocol approved by the medical ethical research committee of the Erasmus MC (MEC-2017-1011).

Microbiological methods

Perianal samples were taken with flocked swabs and transported in the accompanying 1mL Amies medium (e-Swabs (Copan Italia, Brescia, Italy)). Perianal samples collected from January 1, 2018, until January 19, 2019, were stored in a -80°C freezer before being processed. To prevent freezing/defrosting damage, 0.2 mL 99% glycerol was added to the samples before freezing (12). Samples taken after January 19, 2019 were processed directly. All samples, regardless of being frozen, were processed following the same protocol. Samples were vortexed for 10 s before 250µL of the sample was inoculated in a tryptic soy broth with vancomycin (50mg/L) and incubated overnight at 35°C. A *Brilliance*™ ESBL Agar (Oxoid, Basingstoke, UK) was inoculated from the broth with a 10 µl loop and incubated twice overnight at 35°C. Colonies were identified to species level using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF [Bruker Daltonics, Bremen, Germany]) and antibiotic susceptibility was tested with the VITEK®2 (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (13). All ESBL-E isolates were stored in a -80°C freezer.

Whole genome sequencing

WGS was performed for all identified ESBL-E isolates. Total genomic DNA was extracted using the MagNA Pure 96 platform (Roche Applied Science, Mannheim, Germany). 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 creating >100x coverage using Illumina technology (Novogene, HongKong, China). De novo genomic assemblies were generated using CLC Genomics Workbench v21 (Qiagen, Hilden Germany) using default parameters. Antimicrobial resistance (AMR) genes were detected and identified using the web-based Comprehensive Antibiotic Resistance Database (CARD) interface (https://card.mcmaster.ca/) restricted to perfect and strict hits (14). Conventional multi locus sequence types (MLST) and core-genome MLST cluster types were determined using each species' corresponding scheme (https://cgmlst.org/ncs) in SeqSphere+ v5 software (Ridom, Munster, Germany). The identity of all strains was verified by analyzing the genomic assemblies using the online TYGS platform (https://tygs.dsmz.de/) (15).

Data collection

Patient characteristics were collected from the EHR, including the demographic variables age at admission and sex. For the hospitalization period, data on admission specialization, all antibiotic usage, surgical procedures, ICU admission, length of hospital stay, and number of intra-hospital patient transfers were collected. Intra-hospital transfers were defined as being transferred to another patient room for ≥4 h, and did not include transfers to e.g. the

ICU, radiology, the operating theater, or the Post Anesthesia Care Unit, since the necessity of these transfers was not impacted by the transition to 100% single-occupancy rooms. Data on history of ESBL-E carriage up to 2013, bacteriological data of ESBL-E identified from clinical samples during hospitalization, and results of the hospital HRMO-screening risk-assessment score on admission was collected. This risk-assessment was performed and registered within the first 24-hours of hospitalization for every patient admitted to the hospital (16, 17). When patients were at risk according to the risk assessment, (e.g. having been admitted at a hospital abroad in the last 2 months; the complete assessment can be found in Additional file 3) cultures were taken and the patient was pre-emptively cared for in isolation until the results of the HRMO cultures were known (16, 17). Finally, to illustrate the exposure to the hospital environment, we calculated the square meters (m2) of patient rooms and bathrooms to which patients were exposed to in the old and new hospital setting (Supplement 1).

Statistical analyses

Patients were divided into three categories based on their admission specialization; medical, surgical or hematological. Medical patients were admitted to the specializations dermatology, endocrinology, geriatrics, immunology, infectious diseases, general internal medicine, gastroenterology and hepatology, nephrology, neurology, internal oncology, pain relief, radiology, or vascular medicine. Surgical patients were admitted to the specializations general, gastrointestinal, neurological, oncological, orthopedic, plastic, trauma, transplantation, or vascular surgery. Descriptive analyses were performed separately for these groups. For continuous variables, medians with range were presented. Normal distributed variables were analyzed with independent sample *t*-tests. The calculated m2 patients were exposed to were logarithmically-transformed and analyzed with independent sample *t*-tests. Categorical variables were presented as percentages and analyzed using a Chi-squared test. All *P*-values <0.05 were considered statistically significant. To determine correlations between variables, logistic regression analyses were performed and presented with odd ratios (OR) and 95% confidence intervals (95%CI). Continuous determinants in logistic regression analyses were categorized into four categories based on quartiles. When the 95%CI did not include 1.00, it 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.

Results

Inclusion study samples

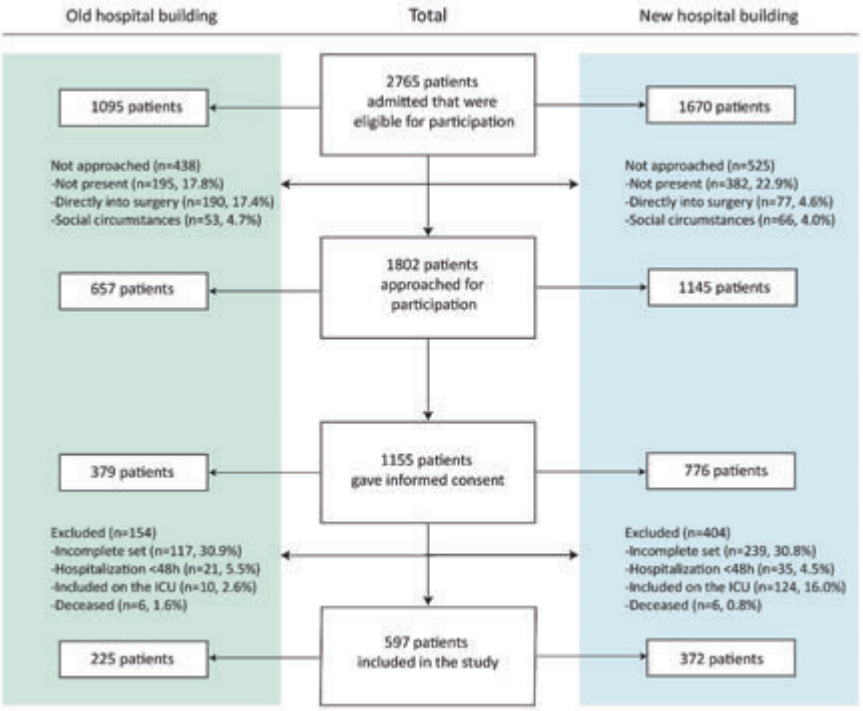
In total, 1095 patients in the old building, and 1670 patients in the new building were eligible for participation in the study (Fig. 1). Patients were not approached when they were in end of life stage, or when they were legally incapable to make a decision about participating (Fig. 1). In total, 1155 patients participated in the study, 379 (32.8%) in the old and 776 (67.2%) in the new building. Due to the unexpected result that samples of patients included on the ICU were incomplete (*i.e.* missing an admission or discharge sample) for all patients

in the old building (n=10) and nearly all patients in the new building (107 out of 124, 86.3%), all patients included on the ICU were excluded for further analysis (Fig. 1). After exclusions, 225 out of 379 (59.4%) patients in the old building, and 372 out of 776 (47.9%) patients in the new building were included (Fig. 1). In total, 511 patients were missed at discharge and received a self-sample request at their home address. Two-hundred and sixty (50.9%) patients returned a sample, with a median return time of eight days (2-45), 251 (49.1%) patients did not return a sample and were consequently excluded. Fifteen patients were included multiple times. In the old building, four patients were admitted twice, and in the new building eight patients were included twice and three patients were included three times. Four patients were admitted during the relocation of the hospital and were thus included in both the old and the new building. The majority of patients were admitted to a surgical department, 161 (71.6%) patients in the old building and 187 (50.2%) in the new building (Table 1). The proportion of patients admitted to a medical, surgical, and hematology specialization differed between the old building and the new hospital building (15.1 vs 21.2%, 71.5 vs 50.3%, and 13.3 vs 28.5%, respectively). Univariate analyses showed no statistically significant differences in patient characteristics of patients admitted to the old building and the new building (Table 1).

Carriage and acquisition of ESBL-producing Enterobacterales

Fifty-one out of 597 (8.5%) patients had at least one study sample positive for an ESBL-E, 16 out of 225 (7.1%) patients in the old building and 35 out of 372 (9.4%) patients in the new building (*P*=0.330). Thirty-four patients were ESBL-E colonized at admission, 10 (4.4%) patients in the old building, compared to 24 (6.5%) in the new building (*P*=0.305) (Table 2). Eleven out of 34 (32.4%) patients had been hospitalized in our hospital during the previous year, 23 (67.6%) patients were not hospitalized. Twelve patients, five (9.8%) in the old hospital building and 7 (13.7%) in the new hospital building, were positive at admission, but negative at discharge (*P*=0.774). In total, 20 (3.4%) patients, seven (3.1%) in the old building and 13 (3.5%) in the new building, acquired an ESBL-E during hospitalization (*P*=0.801) (Table 2). In total, 17 (3.0%) patients, six (2.7%) patients in the old building and 11 (3.0%) in the new building, were positive only at discharge. Additionally, one patient in the old building and one patient in the new building were positive for a different ESBL-E at discharge and one patient in the new building acquired an additional ESBL-E. *E. coli* and *K. pneumoniae were* most prevalent, at admission and discharge, and were also the ESBL-E most often acquired.

Fig. 1 Flowchart of the inclusion of patients



Intra-hospital patient transfers and exposure to square meters

One hundred and eight out of 597 (18.1%) patients were transferred during hospitalization. Fifty-six (24.9%) patients in the old building were transferred, compared to 52 (14.0%) in the new building (*P*=0.001). The number of patients not being transferred during hospitalization increased significantly for both medical (*P*=0.003) and hematological patients (*P*<0.001) in the new building (Table 3). Seventy-six out of 597 (12.7%) patients were transferred once, 42 (18.7%) in the old building and 34 (9.0%) in the new building (*P*=0.001). The number of medical patients transferred once decreased significantly (*P*=0.018) in the new building (Table 3). A decrease was also seen in the number of surgical and hematological patients transferred once, although not significantly (Table 3). Thirty-two (5.4%) patients were transferred at least twice, 14 (6.2%) patients in the old building and 18 (4.7%) in the new building (*P*=0.467). This decrease was seen for both medical and hematological patients, but not for surgical patients (Table 3).

In the new building, patients were exposed to less m2 during hospitalization than in the old building. Overall, the median m2 patients were exposed to in the old building was 43.3 m2 (21.9-177.9), compared to 22.9 m2 (22.9-114.6) in the new building (*P*<0.001) (Additional file 1).

Table 1. Characteristics of included medical, surgical, and hematological patients

Characteristics	Medical			Surgical			Hematological		
	Old building (n=34)	New building (n=79)	*P*-value	Old building (n=161)	New building (n=187)	*P*-value	Old building (n=30)	New building (n=106)	*P*-value
Male gender (%)	21 (61.8)	40 (50.6)	0.276	82 (50.9)	102 (54.5)	0.501	19 (63.3)	55 (51.9)	0.266
Age, median (range)	60 (19-78)	58 (20-89)	0.723	60 (18-85)	65 (24-87)	0.064	59 (33-76)	62 (20-81)	0.373
Dutch origin (%)*	28 (82.4)	68 (86.1)	0.612	143 (88.8)	164 (87.7)	0.431	26 (86.7)	97 (91.5)	0.332
Length of hospital stay, median (range)	3 (2-41)	4 (2-21)	0.374	5 (2 -43)	5 (2-72)	0.243	9.5 (2-52)	10.5 (2-146)	0.916
Surgery during hospitalization (%)	6 (17.6)	17 (21.5)	0.639	159 (98.8)	179 (95.7)	0.091	1 (3.3)	7 (6.6)	NA
ICU admission during hospitalization (%)	1 (2.9)	2 (2.5)	NA	20 (12.4)	14 (7.5)	0.122	0 (-)	2 (1.9)	NA
Antibiotic use during hospitalization (%)	14 (41.2)	27 (34.2)	0.478	147 (91.3)	171 (91.4)	0.963	26 (86.7)	92 (86.8)	0.986

ICU Intensive Care Unit. *NA* not applicable, *P*-values could not be calculated due to observed and expected values below 5 for one or both groups

*9 patients had missing data on country of origin

Intra-hospital patient transfers and acquisition of ESBL-E

Eight out of 108 (7.4%) transferred patients acquired an ESBL-E, compared to 12 out of 489 (2.3%) patients that were not transferred (OR 3.18, 95%CI 1.27-7.98). Five out of 32 (15.6%) patients that were transferred twice or more acquired an ESBL-E, compared to 15 out of 565 (2.7%) patients who were once or not transferred (OR 6.79, 95%CI 2.29-20.06). Patients who were transferred once did not have significantly higher odds for ESBL-E acquisition (OR 1.22, 95%CI 0.35-4.26). Having a hospitalization period of six to ten days was associated with higher odds on having intra-hospital patient transfers, compared to patients admitted two or three days (OR 3.01, 95%CI 1.53-5.91), as well as patients hospitalized ten days or longer (OR 3.75, 95%CI 1.97-7.14). Patients whom acquired an ESBL-E during hospitalization had a median length of stay of nine days (2-146), patients who did not acquire an ESBL-E had a median length of stay of 6 days (2-72). No significant association was identified between length of hospitalization and acquisition of ESBL-E.

Table 2. Number of patients who were positive for ESBL-producing Enterobacterales at admission, at discharge, and the number of patients who acquired an ESBL-producing Enterobacterales.

	Old hospital building (n=225)			**New hospital building (n=372)**		
	Admission (%)	**Discharge (%)**	**Acquisition (%)[4]**	**Admission (%)**	**Discharge (%)**	**Acquisition (%)**
No ESBL-E	215 (95.6)	214 (95.1)	NA	348 (93.5)	344 (92.5)	NA
ESBL-E[1,2]	10 (4.4)	11 (4.9)	7 (3.1)	24 (6.4)	28 (7.5)	13 (3.2)
Escherichia coli[3]	6 (2.7)	8 (3.5)	5 (2.2)	19 (5.1)	22 (5.9)	8 (2.2)
Klebsiella pneumoniae	1 (0.4)	3 (1.3)	2 (0.9)	2 (0.5)	5 (1.3)	3 (0.8)
Citrobacter freundii	2 (0.9)	0 (-)	NA	0 (-)	1 (0.3)	1 (0.3)
Proteus spp.	1 (0.4)	0 (-)	NA	2 (0.5)	0 (-)	NA
Enterobacter cloacae complex	0 (-)	0 (-)	NA	1 (0.3)	0 (-)	NA
Morganella morganii	0 (-)	1 (0.4)	1 (0.4)	0 (-)	0 (-)	NA
Klebsiella aerogenes	0 (-)	0 (-)	NA	1 (0.3)	2 (0.5)	1 (0.3)

ESBL extended-spectrum beta-lactamase, *ESBL-E* extended-spectrum beta-lactamase producing Enterobacterales, *NA* not applicable

[1] Five patients in the old building, and seven patients in the new building were ESBL-E positive at admission and ESBL-E negative at discharge.

[2] Non-significant difference between the old hospital setting and the new hospital setting for admission (*P*=0.305), for discharge (*P*=0.206), and for acquisition (*P*=0.801).

[3] Non-significant difference between the old hospital setting and the new hospital setting for admission (*P*=0.149), for discharge (*P*=0.156), and for acquisition (*P*=0.901).

[4] One patient was positive at admission but acquired a different ESBL-E during hospitalization and one patient acquired two ESBL-E in the old building. Consequently, there are seven patients who acquired an ESBL-E during hospitalization in the old building, but eight different ESBL-E.

[5] One *Proteus faecis*, one *Proteus terrae*, and one unknown *Proteus* spp.

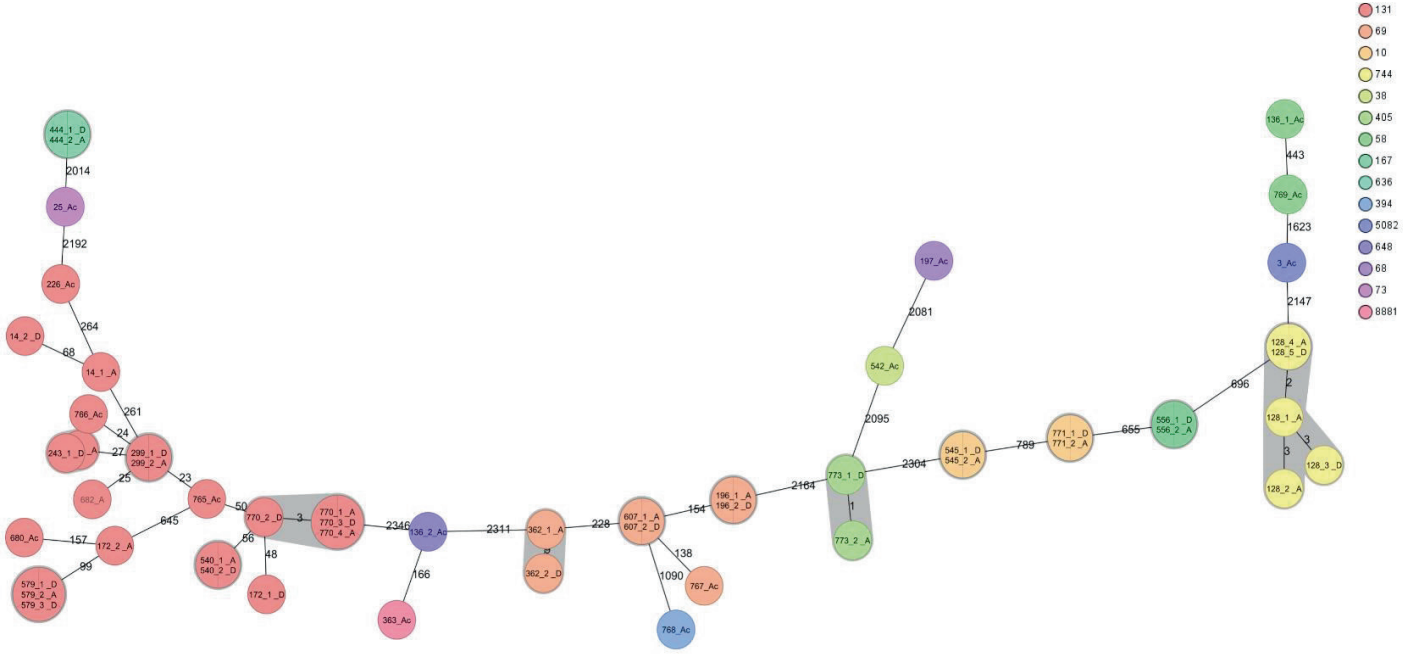
Table 3. Number of intra-hospital patient transfers for medical, surgical and hematological patients

	Medical			Surgical			Hematological		
	Old building (n=34)	New building (n=79)	*P*-value	Old building (n=161)	New building (n=187)	*P*-value	Old building (n=30)	New building (n=106)	*P*-value
Not transferred	22 (64.7)	70 (88.6)		127 (78.9)	152 (81.3)		20 (66.7)	98 (92.5)	
Transferred	12 (35.3)	9 (11.4)	**0.003**	34 (21.1)	35 (18.7)	0.575	10 (33.3)	8 (7.5)	**<0.001**
1	8 (23.5)	6 (7.6)	**0.018**	29 (18.0)	21 (11.2)	0.072	5 (16.7)	7 (6.6)	0.086
≥2	4 (11.8)	3 (3.8)	NA	5 (3.1)	14 (7.5)	0.073	5 (16.7)	1 (0.9)	NA

OR odds ratio, *95% CI* 95% confidence interval, *ESBL-E* extended-spectrum beta-lactamase producing Enterobacterales *NA* not applicable, *P*-values could not be calculated due to observed and expected values below 5 for one or both groups.

[a] Information about the overall number of patients positive for ESBL-E at admission and discharge, and patients who acquired an ESBL-E during hospitalization can be found in table 2.

Fig. 2 Core genome MLST analysis based on 2513 loci of *E. coli* isolates from patients positive at admission and discharge, and of *E. coli* isolates acquired during hospitalization. Node numbers represent isolate numbers and line numbers show the number of different alleles between the isolates. A cut-off value of >10 alleles difference (embedded in the SeqSphere software) was applied to consider strains to be different. Colors match the sequence types (ST). A: Admission, D: Discharge and Ac: Acquired



Core genome MLST and detection of AMR genes of ESBL-producing *E. coli* and *K. pneumoniae*

WGS was performed on all 82 strains isolated from 51 patients. The majority of strains were ESBL-producing *E. coli* isolates (61 strains from 39 patients) and ESBL-producing *K. pneumoniae* (12 strains from 7 patients). The *E. coli* isolates could be classified to 20 different sequence types (ST), with ST131 being the most frequently found (28, 39.4%) (Additional file 2). Patients positive for ESBL-producing *E. coli* at admission and discharge had identical STs, indicating persistent carriage. However, the discharge strains of 2 patients (172 and 14) were not identical to the admission strain, indicating acquisition during hospitalization (Fig. 2). Of the patients who acquired an ESBL-producing *E. coli*, one patient (136) acquired two different strains (Fig. 2). For *K. pneumoniae*, patients who were positive at both admission and discharge had identical strains at both moments. Detection of AMR genes confirmed the presence of beta-lactamases for all *E. coli* and *K. pneumoniae* strains, as well as the presence of other AMR genes. Detailed information on AMR genes can be found in Additional file 2.

HRMO risk assessment and unidentified carriers

The HRMO risk-assessment questions on admission were asked to 200 (88.9%) patients in the old hospital setting, compared to 341 (91.7%) in the new hospital setting (*P*=0.259). Six patients had a positive risk assessment, which led to pre-emptive isolation and active surveillance cultures taken in 100%. Five patients were known HRMO carriers, one patient had been admitted to a hospital abroad, but no HRMO were identified in cultures from this patient. Of the 51 ESBL-E carriers identified in our study, 49 (96.1%) carriers were not identified through the HRMO risk assessment.

Ten patients were identified through clinical samples (five in the old and five in the new building), six before (five within the past 6 months, one 18 months prior to hospitalization) and four during admission. However, 41 (80.4%) patients were not detected before or during hospitalization. The six patients that were already known to be a carrier of ESBL-E based on previous clinical cultures, had an electronic flag in their EHR and were cared for in isolation. Patients found to be ESBL-E positive during admission through clinical cultures, received an electronic flag during admission and were subsequently placed in isolation. Of the 41 unidentified carriers (6.9% of the 597 included patients), eleven out of 225 (4.9%) carriers were admitted to the old building and 30 out of 372 (8.1%) to the new building. Twenty-seven (65.8%) patients were positive at admission, of which 16 (61.5%) patients were also positive at discharge. Fourteen (34.2%) patients were only positive at discharge.

Discussion

In this prospective before-and-after study, we could not show that transitioning from a hospital facility with multi-occupancy rooms to a new hospital building with 100% single-occupancy rooms significantly decreases ESBL-E acquisition. However, as a result from this relocation to 100% single rooms, we did observe a significant decrease in the number of intra-hospital patient transfers, which was associated with higher odds on ESBL-E

acquisition, and a significant decrease in exposure to m^2. WGS showed that most patients that carried an ESBL-E at admission and discharge carried indistinguishable strains. Finally, we showed a high proportion of unknown ESBL-E carriers, of which the majority was already ESBL-E positive at admission.

Only a small number of studies have determined the effect of single-occupancy rooms on HRMO acquisition, with conflicting results (10, 11, 18, 19). The only study determining the effect of single-occupancy rooms on ESBL-E acquisition was performed by Levin et al. (11). They determined that transitioning from an open plan ICU to single-occupancy rooms did not significantly decrease ESBL-E acquisition, which is similar to our results. Both Vietri et al. (18) and Ellison et al. (19), who looked at methicillin-resistant *Staphylococcus aureus* (MRSA) colonization and HAI with MRSA or vancomycin-resistant enterococci (VRE) respectively, found no difference after the transition to mainly single-occupancy rooms. However, our hospital transitioned to 100% single-occupancy rooms. The study of McDonald et al. (10) is the only study who also determined the effect of transitioning to a newly constructed hospital with 100% single-occupancy rooms. They determined the effect on MRSA and VRE colonization and infection, and on *Clostridioides difficile* infection (CDI) rates and observed that the transition did not impact CDI or MRSA infection rates, but did significantly decrease VRE colonization and infection rates and MRSA colonization rates (10). Their results indicate that transitioning to 100% single-occupancy rooms can still positively impact the acquisition of other HRMO (10).

After relocating to the new hospital building, and thus after the transition to 100% single-occupancy rooms, the number of intra-hospital patient transfers decreased significantly. The biggest decrease was seen for hematological patients. Even though hematology wards already consisted of mainly single-occupancy rooms, patients in the old hospital were often first admitted to a multiple-occupancy room, and later transferred to a single-occupancy room. Additionally, we showed an association between intra-hospital transfers and ESBL-E acquisition, with higher odds for patients who were transferred at least twice. However, there could be other explanations for these increased odds, since the need for intra-hospital patient transfers could indicate the need for additional care. Consequently, these patients might have had contact with more healthcare workers, potentially had more intravenous or arterial catheters, and a higher antibiotic consumption, which are all potential risk factors for ESBL-E acquisition. Due to the small number of patients who acquired ESBL-E, we were unable to correct for these factors. An additional benefit of the reduction of transfers could be a reduction in workload, a decrease in cost, and a decrease in medical errors (8, 20-23).

As a result of the decrease in intra-hospital transfers, patients were exposed to less square meters of hospital environment in the new hospital building. Important is that not the intra-hospital patient transfers in itself, but the exposure to more, and different areas of, the hospital environment is a potential source for ESBL-E. However, since the exposure to the hospital environment is related to intra-hospital transfer, the number of intra-hospital transfers during hospitalization is an important risk factor for acquisition and should be included in future studies. In 19.8% of published outbreaks, the hospital environment was identified as the source (24). Additionally, studies have shown increased odds on HRMO acquisition when the prior room occupant was infected/colonized (25). While single-occupancy rooms in our hospital are cleaned after a patient is discharged, rooms are only

disinfected when a known HRMO carrier was admitted to the room. Our study identified a high percentage of unknown ESBL-E carriers, highlighting the fact that HRMO carriers are missed. Consequently, some rooms are only cleaned when disinfection would have been appropriate, potentially leaving HRMO reservoirs behind. Therefore, a decrease in exposure to the environment, means less exposure to pathogenic organisms of other patients. Since the exposure to the environment is an important factor for HRMO acquisition, the impact of the transition to single-occupancy rooms on the m2 patients were exposed to is an important outcome of this study.

While the majority of patients positive both at admission and discharge had indistinguishable strains, for two patients the discharge strain was not identical to the admission strain. This can be explained by acquisition of a different strain during hospitalization, or by carriage of multiple strain types, of which only one was detected at admission. To identify possible different strain types, or species, with ESBL-genes, it is recommended to pick and analyze multiple colonies, even when they are morphologically identical. Interspecies plasmid transfer in the gut is possible through plasmid carriers, which could possibly lead to phenotypic resistance, among which the ESBL phenotype. However we did not perform plasmid analyses in the strains from these two patients.

We determined a prevalence of ESBL-E at admission of 4.4% in the old building, and 6.5% in the new building, which is in agreement with previous reports on the prevalence of ESBL-E in the Netherlands, with ranges between 4.5% and 8.6% in 2018 (26-28). Of the 51 identified ESBL-E carriers, 34 were positive upon admission. The majority of these patients had no recent hospitalizations, suggesting that the majority of ESBL-E was community acquired. Twelve carriers were only positive at admission, indicating loss of the ESBL-E during hospitalization. A possible explanation is that they received antibiotic therapy during hospitalization, however, it is also possible that these were false-negative results. The high number of unidentified ESBL-E carriers can partly be explained by the fact that the risk-assessment questions asked at admission were unable to identify 49 out of the 51 (96.1%) ESBL-E carriers. Six of the 49 patients had already an electronic label in the EHR as being an ESBL-E carrier due to previous ESBL-E positive cultures and were thus known carriers to the hospital regardless of the risk-assessment outcome. Van Hout et al. (17) compared the observed prevalence of ESBL-E carriers newly identified via the risk assessment to the perceived ESBL-E carriage rate based on epidemiological studies in the Netherlands. They determined that the risk-assessment identified less than 1% of all ESBL-E carriers (17). A case control study in MRSA carriers without known risk factors found previously unknown risk factors, explaining 83% of the MRSA of unknown origin (29). Bastiaens et al (30) identified that active surveillance in patients hospitalized for ≥14 days can be used to identify asymptomatic HRMO colonization. Even though this added screening can help identify previously unknown carriers, after 14 days transmission to other patients or the environment could have already occurred within the hospital. Therefore, it should also be considered to determine additional risk factors for ESBL-E carriage, for example questions about travel history (31-34) or antibiotic usage in the last 90 days, specifically targeting use of fluoroquinolones and beta-lactams (28, 32, 35). An improved risk-assessment could help decrease the number of unidentified carriers at admission and hence prevent transmission to other patients within the hospital.

Strengths and limitations

The main strength of our study was that the relocation of the hospital provided us the opportunity to determine the difference in risk on acquisition of ESBL-E between multiple-occupancy rooms and single-occupancy rooms for patients from different departments and specializations. Additionally, performing WGS analyses provided us additional insights in ESBL-E colonization compared to only microbiological culture methods.

However, our study also has some limitations. The most important limitation was the low prevalence of ESBL-E, and the low incidence of ESBL-E acquisition. As a result, we did not have enough statistical power to perform multivariate analyses and were thus unable to correct for possible confounding factors, such as differences in hand hygiene compliance and cleaning protocols. Since we did not perform a sample-size calculation before the start of the study, it is possible that our study is underpowered. Additionally, we used perianal samples instead of rectal samples. While perianal swabs are less invasive then rectal swabs and might increase participation, it is known that the sensitivity of perianal swabs is lower compared to rectal swabs (36). By using selective broths and culture methods, we aimed to minimize the risk of false-negative results, but it is likely that ESBL-E carriers and ESBL-E acquisitions were missed. Additionally, repeated sampling throughout the hospitalization period would also have decreased the chance for false-negative samples. A final sampling limitation is that patients missed at discharge were asked to sample at home, which meant a delay in sampling. Therefore, not all discharge samples might be representative of the situation at discharge. Furthermore, we have introduced selection bias as a consequence of our inclusion criteria, and by the fact that the proportion of patients admitted to the different specializations was different in the old building compared to the new hospital building. An explanation for this is the fact that after the relocation of hematology patients from the Cancer Institute to the new hospital building, it was easier to approach and hence include these patients. Finally, we did not include all patients admitted to the participating departments. Therefore, we were unable to determine the exact dynamics of ESBL-E within and between departments.

Conclusion

Due to the design of the study, a significant decrease in ESBL-E acquisition after relocating to the new hospital could not be shown, but the transition to a hospital with 100% single-occupancy rooms was associated with a significant decrease in intra-hospital patient transfers and, hence, a significant decrease in exposure to square meters. By determining that transferred patients had higher odds on ESBL-E acquisition, we showed that the transition to 100% single-occupancy rooms can indirectly impact ESBL-E acquisition. Additionally, the large proportion of ESBL-E carriers that remains unidentified by clinical samples highlights the need for an improved risk-assessment screening at admission. Future research is needed to determine the impact of 100% single occupancy rooms on factors that could impact ESBL-E and HRMO acquisition, such as exposure to square meters as a measure for exposure to the hospital environment, and to develop an effective risk-assessment screening.

Abbreviations

95%CI	95% Confidence Interval
AMR	Antimicrobial resistance
CDI	*Clostridium difficile* infection
cgMLST	Core genome multi locus sequence type
EHR	Electronic health records
Erasmus MC	Erasmus MC University Medical Center
ESBL	Extended-spectrum beta-lactamase
ESBL-E	extended-spectrum beta-lactamase-producing Enterobacterales
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HAI	Healthcare associated infections
HRMO	Highly resistant microorganisms
ICU	Intensive Care Unit
m2	square meters
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry
MRSA	Methicillin-resistant *Staphylococcus aureus*
OR	Odds ratio
SPSS	Statistical Package for the Social Sciences Solutions
ST	Sequence type
VRE	Vancomycin-resistant enterococci
WGS	Whole genome sequencing

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Author contributions

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

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Declarations

Ethics approval and consent to participate

This study was approved by the medical ethical research committee of the Erasmus MC (MEC-2017-1011), and was not subject to the Medical Research Involving Human Subjects Act. Written informed consent was obtained from all participating patients. This study was registered in the Dutch National Trial Register (NL8406).

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due to privacy of the participating patients but are available from the corresponding author on reasonable request.

Competing interests

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

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Supplemental file 1: Calculating the square meters

To determine the surface area patients were exposed to, the square meters of the rooms were calculated. First, we standardized the square meters of patient rooms, because small differences in square meters did occur between different wards. The standardized surfaces we used were:

Old hospital building:

- Four-person room, excluding bathroom shared with ward mates: 46.83 m2
- Two-person room, excluding bathroom shared with ward mates: 17.75 m2
- Bathroom (i.e. toilet and shower) on the ward: 6.50 m2
- Single-occupancy room hematology, including private bathroom: 26.21 m2
- Two-person room hematology, including bathroom shared with roommates: 22.12m2
- Three-person room hematology, including bathroom shared with roommates: 43.62m2

New hospital building:

- Single-occupancy room including private bathroom: 26.21 m2

To calculate the total square meters a patient was exposed to during hospitalization, we included all rooms a patient was admitted to according to his/her electronic health records. In the old building bathrooms were shared with ward mates, with multiple available toilets and showers. When a patient was moved to a different room on the ward, but close to the previous room, no additional square meters for the bathroom were added. When a patient was relocated to the other side of the ward, we assumed that the patient would use a different bathroom then before and thus added additional square meters.

Results per specializations

In the new building, patients were exposed to less m2 during hospitalization than in the old building. Overall, the median m2 patients were exposed to in the old building was 43.3m2 (21.9-177.9), compared to 22.9m2 (22.9-114.6) in the new building (P<0.001). The median m2 was significantly lower for all medical specializations. For medical patients, the median m2 decreased from 52.4 m2 (22.9-77.9) in the old building to 26.6 m2 (22.9-68.7) in the new building (P<0.001), for surgical patients from 52.4 m2 (22.9-77.9) in the old building to 26.6 m2 (22.9-68.7) in the new building (P<0.001), and for hematological patients from 48.1 m2 (21.9-118.2) to 24.9 m2 (22.9-68.7) (P<0.001).

Supplemental file 2: Detected AMR genes and heatmaps for ESBL-producing Escherichia coli and K pneumoniae

Of the 16 patients included twice during the study period, two patients were positive for ESBL-E. Patient 128 was positive for an ESBL-producing E. coli at admission and discharge for both admissions. These E. coli strains were identical according to cgMLST, and no differences were observed in AMR genes. Patient 5 was positive for an ESBL-producing Citrobacter freundii at admission, but for an ESBL-producing K. pneumoniae at discharge. For the second hospitalization, the patient was positive for an ESBL-producing K. pneumoniae at admission and discharge.

Details for isolates of E. coli (A) and K. pneumoniae (B), including patient number, sampling moment, conventional MLST results, cgMLST cluster types determined by SeqSphere+ software (Ridom, Munster, Germany) and presence of antibiotic resistance genes (search restricted to perfect and strict matches) as determined using the CARD web-interface (https://card.mcmaster.ca/). Results shown are focused on different types of beta-lactamases and aminoglycoside modifying enzymes. A grey background indicates patients with strains having different CARD results between admission and discharge despite having an isogenic chromosomal background. A yellow background indicates patients with strains of a different genetic background between admission and discharge. Pink indicates patients only testing positive at discharge. A black background indicates presence of the gene. Numbers indicate percentage identity to the CARD reference sequence.

A

Patient nr.	Sampling moment	MLST	cgMLST cluster type	OXA-1	OXA-10	TEM-1	CTX-M-1	CTX-M-14	CTX-M-15	CTX-M-27	CTX-M-55	CTX-M-65	CTX-M-101	EC-15	EC-5	EC-8	aadA	aadA2	aadA5	AAC(3)-IId	AAC(3)-IIe	AAC(6')-Ib-cr4	AAC(6')-Ib-cr6	ANT(3'')-IIa	APH(3'')-Ib	APH(3')-Ia	APH(6)-Id
014	Admission	ST131	16101			100.0	100.0																				99.6
014	Discharge	ST131	16100			100.0	100.0																				99.6
128	Admission	ST744	2484								100.0														99.6	98.5	99.6
128	Admission	ST744	2484								100.0														99.6	98.5	99.6
128	Admission	ST744	2484								100.0														99.6	98.5	99.6
128	Discharge	ST744	2484								100.0														99.6	98.5	99.6
128	Discharge	ST744	2484								100.0														99.6	98.5	99.6
172	Admission	ST131	16102			100.0							100.0							100.0							
172	Discharge	ST131	16097	100.0		100.0			100.0											100.0			99.0				99.3
196	Admission	ST69	16093						100.0							98.7											
196	Discharge	ST69	16093						100.0							98.7											
243	Admission	ST131	16099							100.0																	
243	Discharge	ST131	16099							100.0																	
299	Admission	ST131	16091							100.0																	
299	Discharge	ST131	16091							100.0																	
362	Admission	ST69	16095		100.0							100.0				98.7							99.6				
362	Discharge	ST69	16095		100.0							100.0				98.7							99.6				
444	Admission	ST636	16088			100.0			100.0						98.9		99.6	100.0		100.0							99.6
444	Discharge	ST636	16088			100.0			100.0						98.9		99.6	100.0		100.0							99.6
540	Admission	ST131	3242	100.0					100.0														99.0		99.6		99.3
540	Discharge	ST131	3242	100.0					100.0													100.0			99.6		99.3
545	Admission	ST10	16098			100.0			100.0										100.0						99.6		99.3
545	Discharge	ST10	16098			100.0			100.0										100.0						99.6		99.3
556	Admission	ST167	16096	100.0					100.0					98.1					100.0				99.0				
556	Discharge	ST167	16096	100.0					100.0					98.1					100.0				99.0				
579	Admission	ST131	16089			100.0		100.0																	99.3		99.3
579	Discharge	ST131	16089			100.0		100.0																	99.3		99.3
579	Discharge	ST131	16089			100.0		100.0																	99.3		99.3
607	Admission	ST69	14297						100.0							98.7											
607	Discharge	ST69	14297						100.0							98.7											
682	Admission	ST131	16103							100.0									100.0						99.6		99.3
682	Discharge	ST131	16103							100.0									100.0						99.6		99.3
770	Admission	ST131	16090	100.0					100.0														99.0				
770	Admission	ST131	16090	100.0					100.0														99.0				
770	Discharge	ST131	16090	100.0					100.0														99.0				
770	Discharge	ST131	16090	100.0					100.0														99.0				
771	Admission	ST10	16092						100.0																		
771	Discharge	ST10	16092						100.0																		
773	Admission	ST405	16094	100.0					100.0							98.1			100.0		99.0		99.0				
773	Discharge	ST405	16094						100.0							98.1			100.0		99.0						

B

Patient nr.	Sampling moment	MLST	cgMLST cluster type	OXA-1	SHV-1	SHV-28	SHV-108	TEM-1	CTX-M-15	CTX-M-62	SHV-66	DHA-1	aadA2	aadA3	AAC(3)-IId	AAC(3)-IIe	AAC(6')-Ib10	AAC(6')-Ib-cr3	AAC(6')-Ib-cr6	APH(3'')-Ib	APH(6)-Id
5	admission	ST1565	7904	100.0							99.7	100.0		98.5				100.0			
5	discharge	ST1565	7904							100.0	99.7	100.0		98.5	100.0		96.7				
432	discharge	ST14	2080			100.0		100.0	100.0											99.6	99.6
561	discharge	ST14	7195			100.0		100.0	100.0											99.6	99.6
561	discharge	ST14	7195			100.0		100.0	100.0											99.6	99.6
613	discharge	ST4716	8006				100.0		100.0												
764	discharge	ST16	2350	100.0	100.0			100.0	100.0				100.0						99.0		
772	admisison	ST152	2703	100.0	100.0			100.0	100.0							99.0			99.0	99.6	99.6
772	discharge	ST152	2703	100.0	100.0			100.0	100.0							99.0			99.0	99.6	99.6

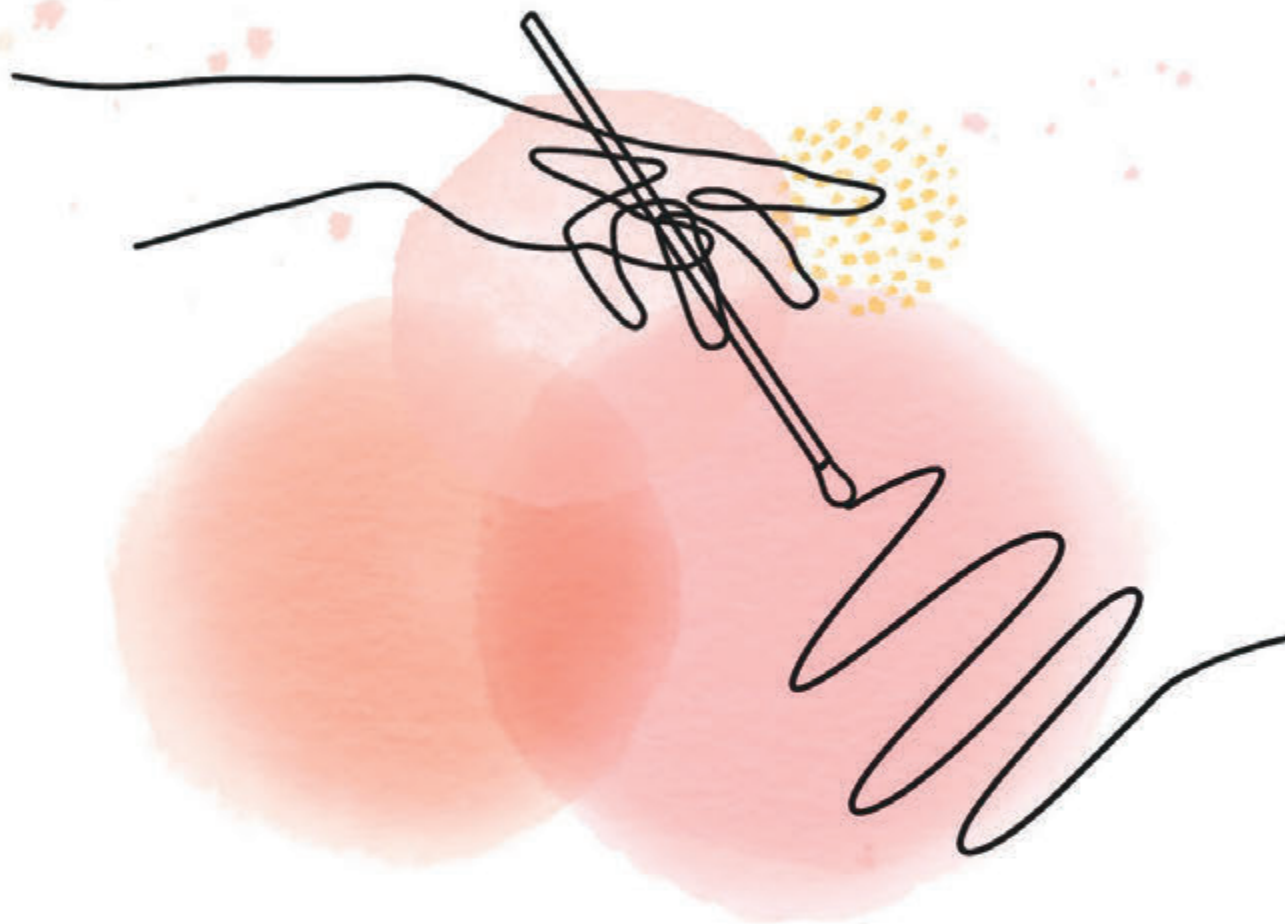
Legend

- beta-lactamase
- ESBL type beta-lactamase
- ampC type beta-lactamase
- aminoglycoside modifying enzyme

Supplemental file 3: HRMO screening risk assessment questions upon admission to the hospital

1. Is the patient/family/counselor available to answer questions?
2. Has the patient recently been treated in or admitted to a foreign healthcare institution?
3. Does the patient live or work where pigs, veal calves or broilers are kept commercially?
4. Is the patient a known carrier of an HRMO?
5. Is the patient a partner, housemate or caretaker of someone who is MRSA positive?
6. Did the patient stay in a healthcare facility known with an HRMO outbreak in the past 2 months, and if yes was the patient approached for screening?
7. Has the patient lived in an institution for asylum seekers in the past 2 months?
8. Is the patient a professional seafarer?

Chapter 2.2



PRE-COVID-19 INTERNATIONAL TRAVEL AND ADMISSION TO HOSPITAL WHEN BACK HOME: TRAVEL BEHAVIOR, CARRIAGE OF HIGHLY RESISTANT MICROORGANISMS, AND RISK PERCEPTION OF PATIENTS ADMITTED TO A LARGE TERTIARY CARE HOSPITAL

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Abstract

When people who recently travelled abroad are admitted to a hospital back home, there is a risk of introducing highly resistant microorganisms (HRMO) into the hospital. To minimize this risk, a feasible infection prevention strategy should be developed. In this study, we investigated patients' travel history and behavior during travel and analyzed whether this was correlated to HRMO carriage at admission. From May 2018 until August 2019, adult patients admitted to a large tertiary care center in the Netherlands were asked upon hospital admission to participate in the study. Included patients received a questionnaire about risk perception, travel history in the last year, and behavior during travel, and were screened for HRMO carriage at admission using a perianal swab. Six hundred and eight questionnaires were handed out, of which 247 were returned (40.6%). One hundred and thirty (52.6%) patients did not travel abroad in the last year, of whom eight (6.2%) were HRMO carrier at admission. One hundred seventeen (47.4%) patients travelled in the preceding year, of whom seven patients (6.0%) were HRMO carrier at admission. Thirty patients (12%) travelled outside of Europe; in this group HRMO prevalence was 13.3% (4 out of 30). The majority of patients (71.3%) were aware that international travel could lead to carriage of HRMO, and an even larger majority (89.5%) would support a screening strategy upon hospital admission in case of a travel history, to minimize the risk of introducing HRMO. We identified that half of admitted patients to a large tertiary care hospital travelled abroad in the last year, with only a small percentage outside Europe. We discuss several screening strategies and propose a strategy of screening and preemptive isolation of patients who travelled to Asia or Africa in the 2 months before their hospital admission; a strategy that patients would support.

Background

Before the start of the SARS-CoV-2 pandemic, international tourism was on the rise worldwide. Tourism increased from 25 million tourist arrivals in 1950 to over 1.4 billion international tourist arrivals in 2019 (1). Although the number of tourist arrivals has fallen to around 380 million in 2020, it is expected that it will return to the 2019 levels within 2.5 to 4 years (2). These international travelers do pick up microorganisms that they are exposed to during travel, among which antibiotic-resistant bacteria, and bring these microorganisms back home (3).

In recent years, it has been increasingly recognized that highly resistant microorganisms (HRMO) are a threat to human health, hampering antibiotic therapy, and increasing morbidity and mortality, especially in patients admitted to hospitals. Important risk factors for acquiring HRMO while travelling are exposure to healthcare abroad, experiencing travelers' diarrhea, and/or antibiotic use during travel. Travel to certain destinations is also a risk factor, specifically to Southern Asia; which is known as a region with high HRMO prevalence (4). A recent Dutch study amongst healthy travelers showed that 34.3% of included persons acquired extended-spectrum beta-lactamase (ESBL)-producing bacteria during travel, with an astonishing 75.1% in travelers traveling to Southern Asia (3). Other known risk factors include for example ice cream consumption, and consuming meals at street food stalls (4). Protective factors, although not well established, have also been identified; such as handwashing before meals, and having a vegetarian diet (3-5).

It is assumed that there is an increased risk of introducing HRMO into the hospital when people from countries with a low prevalence of HRMO are admitted to a hospital, after they have returned from travelling to countries with a high prevalence of HRMO. To contain this risk, a strategy that includes questions at admission about travel history, preemptive isolation, and screening for HRMO could be developed. However, it is unknown how many patients travel and to which destinations, and if they indeed carry HRMO at admission. Therefore, the primary aim of this study was to investigate the travel behavior of patients admitted to a large tertiary care hospital in a country with low prevalence of HRMO, and to correlate travel behavior to HRMO carriage of patients at admission. The secondary aim was to gain insight in the travel-related risk perception of patients, and about their opinion regarding measures hospitals can implement to prevent HRMO transmission due to undetected carriers. This knowledge can then be used in the future to develop policies or guidelines. Furthermore, we aimed to determine by whole genome sequencing (WGS) the sequence types and antimicrobial resistance genes in HRMO identified from traveling and non-traveling patients.

Methods

Study design

The Erasmus MC University Medical Center (Erasmus MC) Rotterdam, the Netherlands, is a tertiary care, university hospital, with all medical specialties available. In 2018, the Erasmus MC relocated to a newly constructed hospital building (*i.e.* for adult patients only), which

opened for admissions at May 18, 2018. The new hospital consisted of 522 single-occupancy rooms with private bathrooms.

This prospective cohort study included patients admitted from May 18, 2018 until September 1, 2019. Adult patients admitted to departments cardiology, gastroenterology and hepatology, general surgery, hematology, internal medicine, nephrology, neurology, neurosurgery, orthopedics, or plastic surgery with an expected stay of more than 48 h were asked to participate at admission. Patients with multiple hospitalizations during the study period were allowed to participate more than once. Participating patients received a questionnaire with accompanying return envelope, and a perianal swab (flocked swab [ESwab Copan Italia, Brescia, Italy] was obtained within 24 h of admission and transported in its accompanying 1mL Amies medium). Samples were taken by trained members of the research team, or patients could self-sample with instructions from the members of the research team.

Questionnaire

A questionnaire and a patient information form were designed in Dutch (see Additional file 1). The questionnaire was pilot tested on three persons and adjusted accordingly. The questionnaire included questions about risk perception (*i.e.* awareness and feelings about international travel and risk of acquiring HRMO), contact with domestic and farm animals, antibiotic use <1 year, antacid use <1 year, travel history <1 year of persons living in the same household, and travel history of the patient <1 year. If patients did travel, questions were asked about behavior during travel (*e.g.* pastry and ice cream consumption), use of malaria prophylaxis, experiencing travelers' diarrhea and/or vomiting, hospitalization, antibiotic use, and antacid use during travel.

Microbiological methods

Samples collected from May 18, 2018, until January 19, 2019, were stored in a -80°C freezer before being processed. To prevent freezing/defrosting damage, 0.2mL 99% glycerol was added to the samples before freezing. Samples taken after January 19, 2019 were processed directly. All samples, regardless of being frozen, were processed using the same procedure. Samples were screened for highly resistant *Pseudomonas aeruginosa*, -*Acinetobacter baumannii*, -*Enterococcus faecium*, and -Enterobacterales. First, 250µL was placed in an Enterococcosel Broth (BD diagnostics, Sparks, USA) with amoxicillin 8mg/L and incubated overnight at 35°C. From this broth, a Vancomycin Screen Agar (VSA, BD diagnostics, Sparks, USA) plate was inoculated and incubated twice overnight at 35°C. Second, 250µL was placed in a tryptic soy broth with vancomycin (50mg/L) and incubated overnight at 35°C. From the vancomycin broth, a ChromID Carba Smart plate (bioMérieux, Marcy l'Etoile, France) was inoculated on both sides and incubated overnight twice at 35°C. Additionally, from the vancomycin broth, a BrillianceTM ESBL Agar (Oxoid, Basingstoke, UK) was inoculated and incubated twice overnight at 35°C. For all plates, colonies were identified using MALDI-TOF MS (Bruker Daltonik, Bremen, Germany). In case of *P. aeruginosa*, isolates were tested for the presence of $bla_{\text{OXA-48}}$, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM} genes, using PCR, with use of established procedures. When negative, a Carbapenem Inactivation Method (CIM) test was performed (6). For *A. baumannii* isolates and for ESBL suspected colonies, antibiotic

susceptibility was tested using VITEK-2 (bioMérieux, Marcy l'Etoile, France). When *A. baumannii* isolates and ESBL suspected isolates were also suspected for carbapenemase production, a CIM test was performed. For isolates identified as *E. faecium*, a *vanA/vanB* PCR was performed (using established procedures, unpublished).

Genome sequencing and analysis

To assess sequence types and presence of antimicrobial resistance genes, WGS was performed for all detected HRMO.

DNA was extracted using MagNA pure 96 (Roche Applied Science, Mannheim, Germany). DNA sequencing was performed by Novogene (Beijing, China) using Illumina chemistry creating 150 bp paired end reads. Assemblies were created using Unicycler v0.4 with default parameters (7). Antimicrobial resistance genes were detected with RGI v5.1.0 using CARD database v3.0.5. Assembled genomes from *Escherichia coli* and *Klebsiella pneumoniae* were processed using the wgMLST scheme available in SeqSphere v5.1.0 (Ridom, Munster, Germany) (https://www.ridom.de/seqsphere/). Clustering trees and heatmaps were generated in R.

Statistical analysis

Data was presented as percentages, medians or means. In case of multiple visited regions, the region where the patient stayed the longest was used for analysis. The variable age was determined using date of birth and the date of filling out the questionnaire. Differences between groups were identified using the Chi-square statistic, T-test or if not normally distributed the independent-samples Mann-Whitney U test, using SPSS version 21 (IBM Corp., Armonk, New York, USA). *P*-values <0.05 were considered statistically significant.

Ethics statement

Written approval to conduct this study was received from the Medical Ethical Research Committee of the Erasmus MC (MEC-2017-1011). This study was not subjected to the Medical Research Involving Human Subjects Act. All patients participating in this study provided written informed consent. This study is registered in the Dutch National Trial Register (trial NL8406).

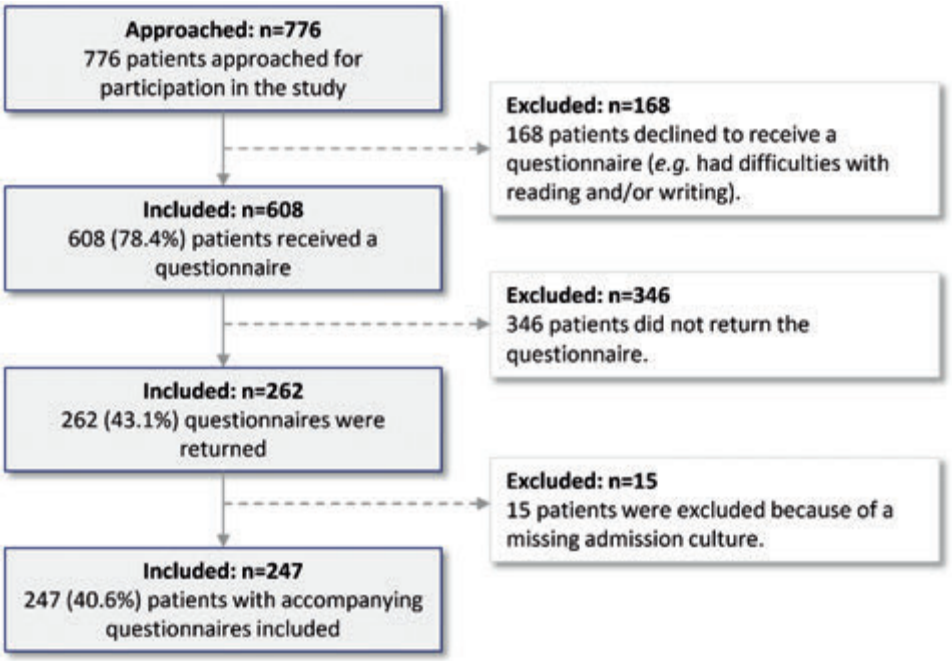
Results

Patient characteristics

From May 18, 2018 until August 1, 2019, 776 patients were approached for participation, of which 608 (78.4%) received a travel questionnaire (Fig. 1). Out of 608 handed out questionnaires, 262 were returned (43.1%). In 27 out of 262 returned questionnaires (10.3%), one or more answers were missing. Fifteen questionnaires from 15 patients (5.7%) were excluded because of a missing admission culture. Therefore, 247 patients with accompanying questionnaires (247 out of 608, 40.6%) were included in the current study (Fig. 1).

Of the included patients, 141 were male (57.1%), and the median age of all included patients was 64 years (Table 1). One hundred twenty-two patients (52.6%) used antibiotics in the last year, and 106 patients (44.2%) used antacids in the last year (Table 1). Overall, fifteen out of 247 (6.1%) patients were HRMO carrier at admission; n=12 (80%) carried ESBL-producing *E. coli*, n=2 (13.3%) carried ESBL-producing *K. pneumoniae*, and n=1 carried ESBL-producing *Proteus vulgaris* (6.7%). No other HRMO were detected. No significant differences were identified between characteristics of HRMO and non-HRMO carriers, including travelling abroad <1 year before admission (*p*-value 0.995) (Table 1).

Fig. 1 Flow diagram of patient inclusion



Non-traveling patients

Hundred-and-thirty (52.6%) patients did not travel in the year before admission. Eight (6.2%) of these patients were HRMO carrier at admission; *n*=5 carried an ESBL-producing *E. coli*, *n*=2 carried an ESBL-producing *K. pneumoniae*, and *n*=1 carried an ESBL-producing *P. vulgaris*. Non-traveling patients had significantly fewer household members that also travelled compared to patients that did travel (*p*-value 0.005, Table 2). Furthermore, non-traveling patients were significantly older compared to traveling patients (Table 2, Additional file 2).

Table 1. Characteristics of patients carrying HRMO and patients not carrying HRMO at admission

Patient characteristic	HRMO carrier; n=15	Not carrying HRMO; n=232	*p*-value
Male gender (%)	10 (66.7)	131 (56.5)	0.439
Age, median (IQR)	64 (26)	64 (18)	0.273
Travel <1y before admission (%)	7 (46.7)	110 (47.4)	0.955
Antibiotic use <1y (%)	7[a] (50)	115[b] (52.5)	0.855
Antacid use <1y (%)	9 (60)	97[c] (42.9)	0.197
Traveling household members <1y (%)	3 (20)	50 (21.6)	0.881
Animal contact [d] (%)	2 (13.3)	86 (37.1)	NA
Domestic animal contact	2 (13.3)	72[e] (31.4)	NA
Farm animal contact	0 (0)	5[e] (2.2)	NA

NA, not applicable, y, year, HRMO, highly resistant microorganism, IQR, interquartile range.

[a] One patient with missing information

[b] 13 patients with missing information

[c] six patients with missing information

[d] contact with farm or domestic animals more than 3 times a week, more than 1 hour each day

[e] three patients with missing information; these patients only stated they had animal contact, but not with which animal.

Traveling patients

Out of the 247 patients, 117 patients (47.4%) travelled in the year before admission. Out of these 117 travelers, most patients (n=87, 74.4%), travelled within Europe, and 30 patients (25.6%) travelled outside of Europe (Fig. 2). Of the 117 travelling patients, 54 patients (46.2%) traveled to multiple countries. Of these 54 patients, 38 patients (70.1%) traveled only within Europe, 15 patients (27.8%) traveled outside and inside Europe, and 1 patient (1.9%) traveled to multiple destinations outside of Europe. Most patients (n=105 out of 117, 89.7%) travelled for less than 1 month (Table 3).

In total, seven out of 117 traveling patients (6.0%) were HRMO carrier at hospital admission. All seven travelling patients carried an ESBL-producing E. coli, and travelled for less than a month (table 3). Thirty out of 117 patients (25.6%) travelled outside of Europe; in this group the HRMO prevalence was 13.3% (4 out of 30; all ESBL-positive *E. coli*). The highest carriage rates were observed in patients travelling to Northern Africa (50%), followed by travelling to Asia, to North America, and to South America 12.5%) (Figure 2), but overall carriage rates were low.

Out of 117 patients, 107 patients (91.5%) replied that the questionnaire was clear and easy, and 10 patients (8.5%) replied that they had difficulties to recall all the asked information (mainly the questions about use of antibiotics and antacids).

Table 2. Patient characteristics of traveling and non-traveling patients

Patient characteristic	**Traveling patient; n=117**	**Non-traveling patient; n=130**	***p*-value**
Male gender (%)	70 (59.8)	71 (54.6)	0.408
Age, median (IQR)	63 (21)	65 (15)	**0.006**
HRMO carrier at admission (%)	7 (6.0)	8 (6.2)	0.955
Antibiotic use <1y (%)	57 (51.8)[a]	65 (52.8)[a]	0.875
Antacid use <1y (%)	49 (43.0)[b]	57 (44.9)[b]	0.767
Traveling household members <1y (%)	34 (29.3)[c]	19 (14.6)	**0.005**
Animal contact (%)[d,e]	46 (39.3)	42 (32.3)	0.251
Domestic animal contact	39 (33.9)[f]	35 (27.1)[g,h]	0.272
Dogs	26 (22.6)	24 (18.8)	0.463
Cats	21 (18.3)	21 (16.4)	0.708
Birds	2 (1.7)	3 (2.3)	NA
Rabbits	1 (0.9)	1 (0.8)	NA
Farm animal contact	2 (1.7)[f]	3 (2.3)[g]	NA
Horses	2 (1.7)	2 (1.6)	NA
Goats	1 (0.9)	1 (0.8)	NA
Poultry	1 (0.9)	1 (0.8)	NA
Sheep	0 (0)	2 (1.6)	NA
Pigs	0 (0)	1 (0.8)	NA

Significant differences are indicated in bold text

NA, not applicable, HRMO, highly resistant microorganism, y, year

[a] seven patients with missing information

[b] three patients with missing information

[c] one patient with missing information

[d] contact with farm or domestic animals more than 3 times a week, more than 1 hour each day

[e] numbers do not add up because 20 patients had contact with multiple animals

[f] two patients with missing information about which animal

[g] one patient with missing information about which animal

[h] one patient reported domestic animal contact but missing information about which animal.

Behavior during travel

Overall, more than half of the travelling patients consumed ice cream and/or pastries during travel (Table 3). Traveling patients carrying HRMO at hospital admission experienced, with low numbers of patients however, more often diarrhea (14.3% vs. 4.6%), and used more often antibiotics during travel (14.3% vs. 4.6%) compared to patients not carrying HRMO at admission (Table 3). Vomiting during travel and the use of malaria prophylaxis were only described in HRMO-negative patients. Additionally, only HRMO-negative patients reported that they ate meals at street food stalls. HRMO carriage rates were higher for patients travelling outside of Europe, compared to patients travelling in Europe (13.3% vs. 3.4%, Table 3).

Fig. 2 Regions visited by patients admitted to the Erasmus MC University Medical Center. HRMO; highly resistant microorganism

Genomic analysis

WGS results confirmed the presence of beta-lactamases in the isolates from the 12 patients identified with an ESBL-producing *E. coli* (Additional Figure 3: Fig. 1, Additional file 4: Fig. 2). The beta-lactamases distribution in isolates was not associated with patient travelling (Additional Figure 3: Fig. 1). In two traveling and one non-traveling patient (patients 1, 2 and 3) $bla_{\text{OXA-1}}$ was detected. These three isolates also contained an $bla_{\text{CTX-M-15}}$ and *aac(6')-Ib-cr* gene. Additionally, multiple other aminoglycoside-modifying enzymes (AMEs) were present

in these 12 isolates with their presence being independent of traveling (Additional file 4: Fig. 2). We observed that isolates of patients 6 and 7 did not possess any AME, and the isolate of patient 8 that had only one AME (ANT(3")-IIa). The isolates of these three patients were of the same sequence type (ST)69. Other antimicrobial genes identified were *ampC*, *tet(A)*, *tet(B)*, and *tetR*, which were present in isolates from travelling and non-travelling patients. The isolates from one travelling patient (patient 4) and one non-travelling patient (patient 11) lacked these additional antimicrobial resistance genes (Additional file 4: Fig. 2). Two ESBL-producing *K. pneumoniae* isolates were found in non-travelling patients. One isolate belonged to ST465, and contained $bla_{\text{TEM-1}}$ $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-1}}$, and the other isolate belonged to ST1565 and contained $bla_{\text{OXA-1}}$, $bla_{\text{DHA-1}}$ and $bla_{\text{SHV-64}}$. For the ESBL-producing *P. vulgaris* no known ESBL genes were detected using the CARD database v3.0.5. However, using the disk diffusion ESBL kit (Rosco Diagnostica, Taastrup, Denmark), ESBL production was confirmed phenotypically.

Table 3. Travel behavior of traveling patients carrying HRMO at admission compared to not carrying HRMO at admission

Characteristic	Total n=117	HRMO-positive at admission, n=7	HRMO-negative at admission, n=110
Duration T <1 month	**105 (89.7)**	**7 (100)***	**98 (89.1)***
Duration T 1-3 months	9 (7.7)	0 (0)	9 (8.2)
Duration T 3-6 months	2 (1.7)	0 (0)	2 (1.8)
Duration T 6-12 months	1 (0.9)	0 (0)	1 (0.9)
Travelling outside of Europe <1y	**30 (25.6)**	**4 (57.1)**	**26 (23.6)****
Travelling within Europe <1y	**87 (74.4)**	**3 (42.9)**	**84 (76.4)**
Ice cream and pastry consumption (%)	64[c] (56.1)	3[a] (50)	61[b] (56.5)
Meals at street food stalls (%)	10[a] (8.6)	0 (0)	10[a] (9.2)
Experienced vomiting during travel (%)	3[a] (2.6)	0 (0)	3[a] (2.8)
Experienced diarrhea during travel (%)	6[a] (5.2)	1 (14.3)	5[a] (4.6)
Admitted to hospital during travel (%)	8 (6.8)	0 (0)	8 (7.3)
Antibiotic use during travel (%)	6[b] (5.2)	1 (14.3)	5[b] (4.6)
Antacid use during travel (%)	22[c] (19.3)	1[a] (16.7)	21[b] (19.4)
Used malaria prophylaxis during travel (%)	1[a] (0.9)	0 (0)	1[a] (0.9)

Relevant differences in percentages indicated in bold text

Abbreviations: Duration T; duration of travel, HRMO, highly resistant microorganism, y, year.

[a] One patient answered this question with 'unknown'

[b] two patients answered this question with 'unknown'

[c] three patients answered this question with 'unknown'

*_P_-value 0.356. ** Chi-square _P_-value 0.049, Fisher's exact test _P_-value 0.070.

Risk perception

The majority of patients (n=176 out of 247; 71.3%) were aware that international travel could lead to carriage of HRMO. The majority of patients (221 out of 243; 90.9%) supported the idea to screen for HRMO upon hospital admission in case of a travel history; 4 patients (1.6%) did not answer this question.

Traveling HRMO positive patients were less aware of the fact that traveling could lead to HRMO carriage (57.1% compared to 68.2%). Additionally, they were more careless with respect to perception of risk (Table 4). In both groups, approximately 86% supported the idea to screen for HRMO upon hospital admission in case of a travel history (Table 4).

Table 4. Risk perception of traveling patients in relation to HRMO positivity at admission

Opinion about risk of acquiring HRMO after travel	**HRMO-positive at admission, n=7**	**HRMO-negative at admission, n=110**
Aware that travel could lead to HRMO acquisition (%)	**4 (57.1)**	**75 (68.2)**
Risk of acquiring HRMO is no problem (%)	0 (0)	5 (4.5)
Aware that travel comes with risks (%)	**3 (42.9)**	**31 (28.2)**
Unpleasant, but will still travel (%)	**1 (14.3)**	**57 (51.8)**
Risk of acquiring HRMO is scary (%)	1 (14.3)	7 (6.4)
Other, or combination of answers (%)	2 (28.6)	10 (9.1)
Hospitals should screen for HRMO in case of a travel history (%)	6 (85.7)	94[a] (86.2)

Relevant differences in percentages indicated in bold text

HRMO; highly resistant microorganism

[a] one missing answer.

Discussion

Summary of evidence

Our study showed that almost 50% of the patients admitted to the hospital travel, both within and outside of Europe. Overall, we did not show a difference in carriage rates at admission between travelling <1y to any country abroad and non-travelling patients.

Multiple studies have determined the effect of travel on ESBL acquisition, and highlighted the importance of improved screening and efforts to reduce import (4). However, information on acquisition of HRMO during travel of patients is scarce; even more because other studies focused on people in settings outside hospitals, such as travel clinics. We found an overall carriage rate of 6.1%; 6.2% for non-travelers and 6.0% for traveling patients, which is comparable to the normal carriage rate of ESBL-producing Enterobacterales in the Netherlands (8). However, the majority of patients travelled within Europe. While the prevalence of HRMO is higher in Southern European countries compared to the Netherlands and countries in the Northern part of Europe, research has shown that travelling to countries in especially South East Asia is a risk factor (4). We showed that patients that did travel outside of Europe had higher carriage rates upon admission, compared to patients travelling in Europe, and compared to patients that did not travel (13.3% vs. 3.4% vs. 6.2%).

With regard to patients that did travel, experiencing diarrhea or vomiting during travel were rare, as was being admitted to a hospital abroad (*i.e.* less than 7%). Out of six patients using antibiotics abroad, only one carried an HRMO upon admittance. This in contrast to the study by Wuerz et al. that described that the risk of acquiring ESBL-producing Enterobacterales increases substantially when using antibiotics during travel (9). Overall, more than 50% of patients used antibiotics in the year before admission. This could be considered as high, especially higher compared to the study by Reuland et al, who took a representative sample of the general adult Dutch population and found rates between 14% and 26% (10). The difference between our findings and the findings by Reuland et al. could be explained by different populations included; in our study this population included patients of a tertiary care hospital. Additionally, the median age of included patients was 64 years old, ranging from 20 to 91, which is considerably older compared to the study by Reuland et al. (i.e. median age of cases 48 and controls 50 years old) and by Arcilla et al., (i.e. 51 years old, range 33 to 61). We assume that our older, tertiary-care hospital patients were less likely travelers outside of Europe.

We identified that two out of seven travelers carrying an ESBL-producing *E. coli* carried *E. coli* ST131, a common strain in the world, including in the Dutch community, and no carbapenemase-producing isolates were identified. In the study by Arcilla et al., and Peirano et al., $bla_{\text{CTX-M-15}}$ was the most frequently acquired ESBL-gene in travelers (>50%), as was in our study (6 out of 12 ESBL-producing *E. coli*, 50%; 4 travelling patients and 2 in non-travelling patients) (3, 11). CTX-M-15 (CTX-M-1 group) and CTX-M-27 (CTX-M-9 group) were previously identified as prevalent in the Netherlands, including in long-term care facilities, while CTX-M-14/65 (CTX-M-9 group and CTX-M-55 (CTX-M-1 group) are less present in the Dutch population (10, 12-14). In three patients, $bla_{\text{OXA-1}}$ was found, in combination with $bla_{\text{CTX-M-15}}$ and *aac(6')-Ib-cr*, which was also described as being a frequent combination in the UK (15). Of these, the *aac(6')-Ib-cr* is most worrisome, as this enzyme also confers resistance to ciprofloxacin and norfloxacin and its gene is known to be plasmid-mediated.

Towards a guideline – part 2

In a previous study, we described knowledge gaps that needed to be filled before national and international guidelines could be developed (4). First, we described that the proportion of patients with a recent travel history is unknown. With this current study, we identified that almost 50% of admitted patients traveled abroad in the last year, of which 25.6% traveled outside of Europe. Second, we previously described that it is unknown if strains carried by travelers spread in hospitals. In this study, we did not include ward mates nor did we sample the environment to assess spread in the hospital, so this knowledge gap is still unfilled. Third, the threshold of a carriage rate after travel that warrants screening and/or isolation was also an unresolved issue. In this study, we showed that carriage rates were higher in patients that travelled to Northern Africa, Asia, North America, and to South America in the last year, than the ESBL carriage rate in the Dutch community (i.e. 5.3%-9.9%) (8). In a study prospectively including healthy travelers, ESBL carriage rates observed among people traveling to Southeastern Asia (31.6%), followed by Southern Asia (21.5%), were higher than in the Dutch community(3). This could point to a strategy of only preemptively screening and isolating patients that have travelled to those countries.

A high majority of patients support the idea to screen for HRMO upon hospital admission in case of a travel history. However, although patients support screening, it is questionable if preemptive isolation and screening for around 12% (i.e. 30 out of 247 patients) of all admitted patients because of travelling outside of Europe in the last year is cost-effective, and even feasible in many hospitals with respect to isolation capacity. A screening-only (i.e. without preemptive isolation) policy could be considered, with as draw back that a contact investigation must be performed when an HRMO-positive patient is identified. We chose to ask for traveling in the year before hospital admission, however, also different cut-offs can be used (e.g. 1 month, 2 months, 3 months), since literature shows that the median elimination time of HRMO carriage after travel is quick (16). Therefore, we calculated the percentages of HRMO carriage when selecting more focused target populations for screening, primarily focusing on travelling to Asia or Africa, as previously defined destinations with high HRMO carriage upon return (3). Percentages of HRMO carriage increased when travel was closer to hospital admission, for patients traveling outside Europe and for patients traveling to Asia or Africa (i.e. travel outside Europe: 13% [n=4/30] if traveled <1 year before hospital admission to 29% [n=2/7] if <3 months to 40% [n=2/5] if <2 months to 67% [n=2/3] if <1 month; Travel to Asia or Africa: 14% [n=2/14] if traveled <1 year before hospital admission to 33% [n=1/3] if <3 months to 50% [n=1/2] if <2 months to 100% [n=1/1] if <1 month). Additionally, the numbers of patients included in these groups decrease rapidly. Antibiotic use during the year before hospital admission was not related to HRMO carriage. Considering the results of this current study and discussed literature, we would propose to target the patients that travelled more recently (i.e. <2 months) for screening and preemptive isolation. The travel destinations to include could be any country outside Europe based on our limited data, or travel to Asia or Africa, based on the broader picture from published data in combination with our data. A strategy with a more targeted patient population will be feasible for many hospitals, and most likely be cost-effective.

Strengths and limitations

A strength of our study is that we included a reasonable large number of patients with information on travel history with an accompanying admission culture. However, since we did not sample the patients before and after travel but at hospital admission, we do not know whether patients were already carrying an HRMO, or acquired the HRMO during travel. A second strength is that we asked for the perception of the patients towards this subject.

Potential limitations include this being a single center study in a tertiary care hospital, including a relatively older patient population with complicated medical histories who might travel less often compared to patients admitted to secondary care hospitals. Second, only a low number of HRMO were identified. This could mean that this study was underpowered and could therefore not identify meaningful differences between groups. Therefore, the results of this study should be confirmed by a larger study. Third, we could have encountered recall bias of patients with regard to questionnaire, and finally, we have introduced a language bias by providing the questionnaire in Dutch only.

Conclusions

With this study, we identified that half of admitted patients to a large tertiary care hospital travelled abroad in the last year, with only a small percentage outside Europe. We discussed that a strategy including screening and preemptive isolation of patients who travelled to Asia or Africa in the previous 2 months could be considered. Also, we learned that this strategy would be supported by patients. Some previously identified knowledge gaps have been filled and we are one step closer towards a guideline. However, before national or international guidelines can be developed, future research should focus on determining the burden of disease of travel-related HRMO carriage, and its transmissibility to other patients and to the environment, using a multi-center study design and taking cost-effectiveness into account. Finally, since this study was performed before the COVID-19 pandemic it is unknown if travel behavior changed because of this, and if travel destinations changed. Therefore, post-COVID studies still have to be performed, to assess the impact of the COVID-19 pandemic.

Abbreviations

AMEs; aminoglycoside-modifying enzymes

CIM; Carbapenem Inactivation Method

Erasmus MC; Erasmus MC University Medical Center, Rotterdam, The Netherlands

ESBL; extended-spectrum beta-lactamase

HRMO; highly resistant microorganisms

ST; sequence type

WGS; whole genome sequencing

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Authors' contributions

Conceptualization: JS, AV, MV, KM. Collecting data: AV, AS. Analyzed the data: AV, AS, NS. Interpretation of the data: AV, AS, NS, CK, MV, JS. Drafted the work: AV, AS, NS, JS. All authors read, reviewed, and approved the final manuscript, and all authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files, or can be provided by the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Written approval to conduct this study was received from the Medical Ethical Research Committee of the Erasmus MC University Medical Center (Erasmus MC), Rotterdam, the Netherlands (MEC-2017-1011), and was not subject to the Medical Research Involving Human Subjects Act. All patients participating in this study provided written informed consent.

Consent for publication

Not applicable

Competing interests

AV, AS, KM, NS, CK, MC, and JS declare that they have no competing interests. JS and CK recently collaborated with employees of bioMérieux on a research project that included whole-genome sequencing of bacterial isolates, which was performed by the company.

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results

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Supplemental file 1. Patient information form and questionnaire (in Dutch)

Naam:……

Geboortedatum:…….. **Man/Vrouw**

Datum invullen vragenlijst: …….-……..**-2019**

In de darmen van mensen komen veel bacteriën voor, dit wordt de darmflora genoemd. Bij reizen naar het buitenland kan de darmflora veranderen. Bacteriën die ongevoelig (resistent) zijn voor antibiotica komen in het buitenland meer voor dan in Nederland. Deze resistente bacteriën kunnen dan onderdeel worden van de darmflora, zonder dat je daar iets van merkt.

1) Wist u dat bij reizen naar het buitenland mensen ongemerkt drager kunnen worden van een resistente bacterie in de darmen?

□ Ja
□ Nee

2) Wat vindt u daarvan?

□ Geen probleem
□ Reizen heeft nu eenmaal risico's
□ Geen prettig idee, maar ik vind reizen leuk/belangrijk
□ Eng
□ Anders, namelijk: ……

3) Vindt u dat ziekenhuizen in Nederland patiënten hierop moeten controleren?
(Bijvoorbeeld vragen naar reizen en/of kweken van bacteriën uit de darm bij opname)

□ Ja
□ Nee

4) Heeft u meer dan 3x per week, meer dan 1 uur per dag contact met dieren?
(Bijvoorbeeld honden, katten, varkens, koeien, pluimvee)

□ Ja, namelijk met……
□ Nee

5) Heeft u in de laatste **12 maanden** antibiotica gebruikt? *(Zie vraag 15 voor voorbeelden)*

□ Ja
□ Nee
□ Weet ik niet

6) Heeft u in de laatste **12 maanden** maagzuurremmers gebruikt? *(Bijvoorbeeld omeprazol, Losec, Nexium*)

□ Ja
□ Nee
□ Weet ik niet

7) Heeft u huisgenoten die de laatste **12 maanden** zonder u naar het buitenland hebben gereisd?

□ Ja
□ Nee

8) Heeft u de afgelopen **12 maanden** naar het buitenland gereisd?

□ Ja → **Ga door naar vraag 9**
□ Nee → **Einde vragenlijst, hartelijk dank voor uw medewerking!**

9) Hoeveel vakanties/reizen heeft u de afgelopen 12 maanden gemaakt, welk(e) land(en) heeft u bezocht en wat was de vertrek- en terugkomstdatum?

- Heeft u meerdere landen bezocht gedurende 1 vakantie/reis, dan geldt dit als 1 vakantie/reis.
- Als u niet meer de exacte vertrek en terugkomstdatum weet, mag u ook het aantal dagen opschrijven.

Nr.	*Land(en)*	*Vertrekdatum*	*Terugkomstdatum*	*Soort reis**
1				
2				
3				
4				
5				

**Soort reis: bijvoorbeeld backpacken, groepsreis, strandvakantie, stedentrip, zakenreis, bezoek familie/vrienden, all-inclusive reis.*

10) Heeft u gebak of onverpakt ijs gegeten **tijdens uw vakantie/reis**?

□ Ja, bij vakantie/reis nummer(s)……
□ Nee
□ Weet ik niet

11) Heeft u **tijdens uw vakantie/reis** eten van straatstalletjes gegeten?
□ Ja, bij vakantie/reis nummer(s)……
□ Nee
□ Weet ik niet

12) Heeft u **tijdens uw vakantie/reis** last gehad van braken/overgeven?
□ Ja, bij vakantie/reis nummer(s)……
□ Nee
□ Weet ik niet

13) Heeft u **tijdens uw vakantie/reis** last gehad van diarree?
□ Ja, bij vakantie/reis nummer(s)……
□ Nee
□ Weet ik niet

14) Bent u in een ziekenhuis opgenomen geweest **tijdens uw vakantie/reis**?
□ Ja, bij vakantie/reis nummer(s)……, aantal dagen opgenomen geweest…….
□ Nee
□ Weet ik niet

15) Heeft u antibiotica gebruikt **tijdens uw vakantie/reis**?
□ Ja → vul onderstaande tabel in
□ Nee → ga door naar vraag **16**
□ Weet ik niet → ga door naar vraag **16**

Vakantie/reis nummer(s) zie vraag 9	*Naam antibioticum**	*Reden***	*Toediening****	*Hoe gekregen?*****

* *Bijvoorbeeld: Amoxicilline, Amoxicilline/clavulaanzuur (Augmentin), Ciprofloxacine (Ciproxin), Nitrofurantoïne (Furabid), Doxycycline, Claritromycine (Klacid), Azitromycine (Zithromax), Trimethoprim, Cotrimoxazol (Bactrimel).*

** *Bijvoorbeeld: Diarree, longontsteking, verkoudheid, huidinfectie, koorts.*

*** *Bijvoorbeeld: Tabletten, infuus, zalf, drankje.*
**** *Bijvoorbeeld: Voorgeschreven door arts in het buitenland, via een apotheek (zonder recept), via een drogist, van huis meegenomen naar het buitenland.*

16) Heeft u maagzuurremmers *(bijvoorbeeld omeprazol, Losec, Nexium*) gebruikt **tijdens uw vakantie/reis**? Dan wel van huis meegenomen, voorgeschreven gekregen in het buitenland of gekocht tijdens uw vakantie/reis.

□ Ja, bij vakantie/reis nummer(s)……
□ Nee
□ Weet ik niet

17) Heeft u malariaprofylaxe gebruikt? (Bijvoorbeeld Malarone, Lariam, Doxycycline)

□ Ja, bij vakantie/reis nummer(s)……
Welke profylaxe heeft u gebruikt? ……
□ Nee
□ Weet ik niet

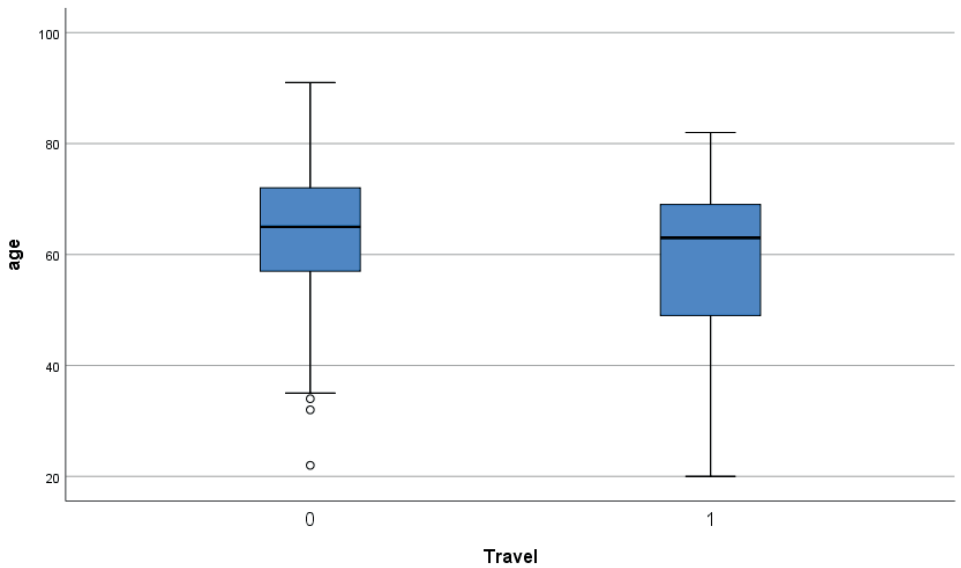
18) Vond u het lastig om antwoord te geven op de vragen in deze vragenlijst?

□ Nee
□ Ja, veel details, wist het niet meer precies
□ Ja, vragen onduidelijk, met name vraag/vragen……
□ Ja, omdat……

Einde vragenlijst. Hartelijk dank voor uw medewerking!

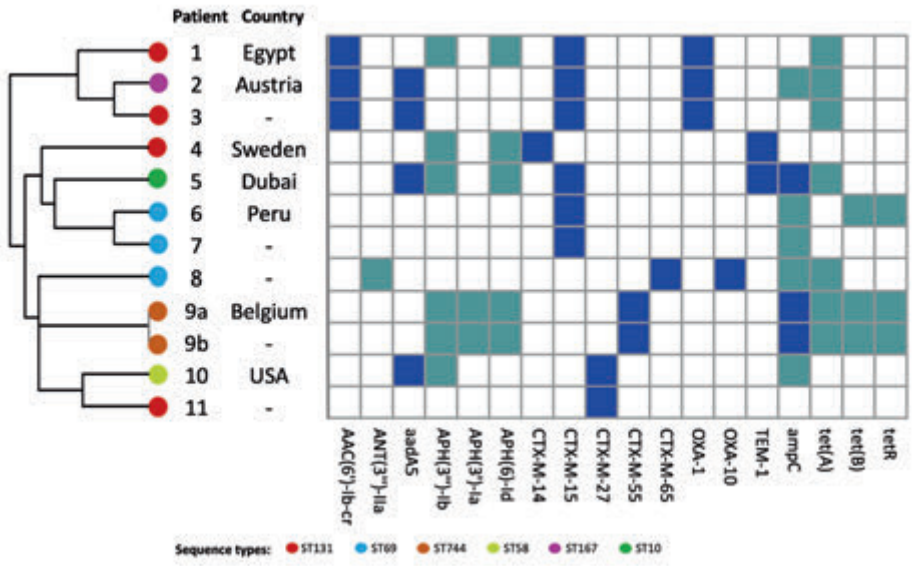
Supplemental file 2. Word file: Age distribution between travelling patients and non-travelling patients

Travel	Statistic	Value
No (0) n=130 patients	Mean	63.08
	95% Confidence interval for mean lower bound	60.88
	95% Confidence interval for mean upper bound	65.27
	Median	65.00
	Standard deviation	12.66
	Minimum	22
	Maximum	91
	Interquartile range	15
Yes (1) n=117 patients	Mean	57.87
	95% Confidence interval for mean lower bound	55.26
	95% Confidence interval for mean upper bound	60.49
	Median	63.00
	Standard deviation	14.28
	Minimum	20
	Maximum	82
	Interquartile range	21

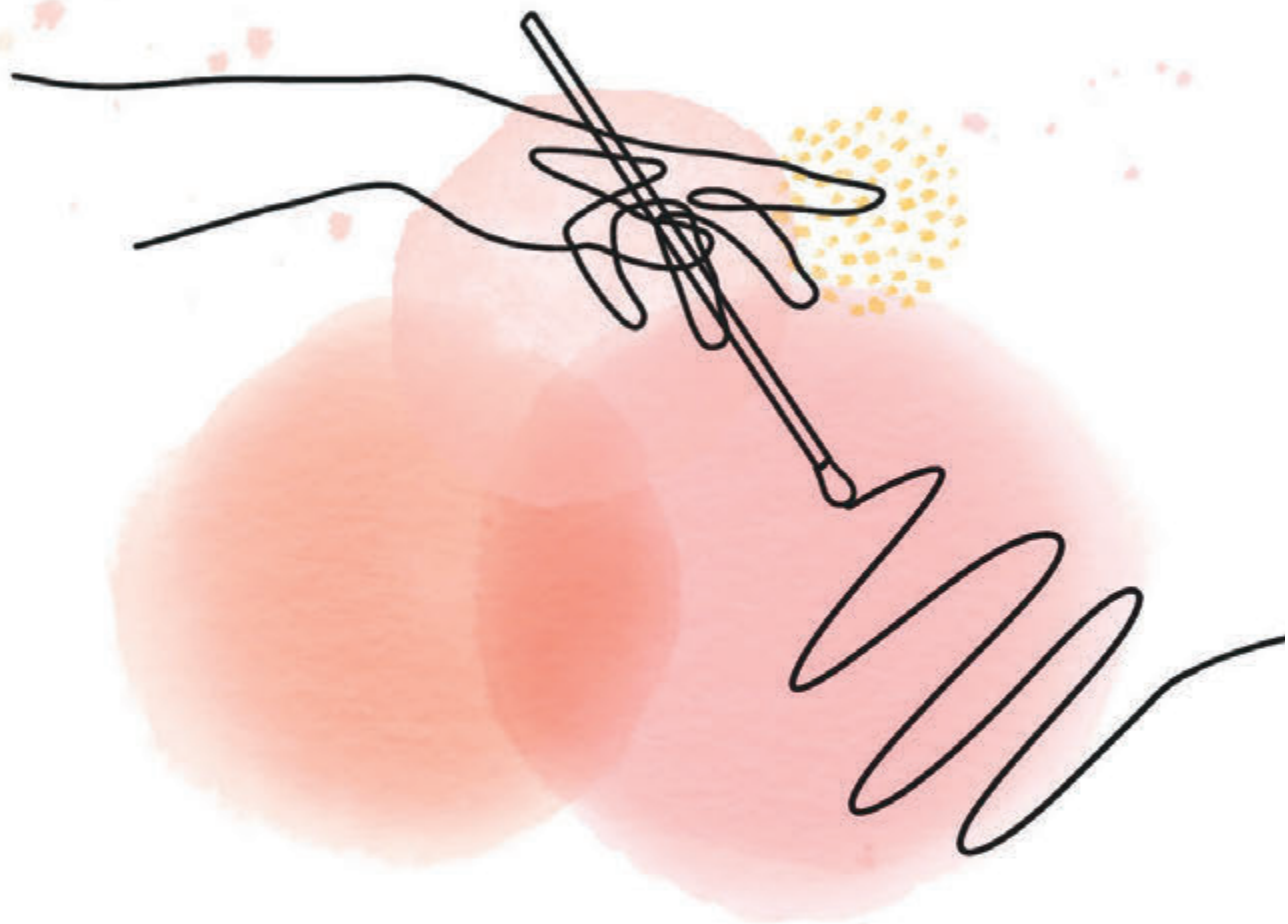


Supplemental file 3. Minimum spanning tree representing cgMLST analysis of the ESBL-producing *E. coli* strains. Node numbers correspond to patient numbers and line numbers indicate the number of different alleles between strains. Colors match the sequence types (ST). A grey background indicates genetically closely related isolates

Supplemental file 4. Distribution of selected antimicrobial resistance genes among the E. coli isolates. Isolates from patients are clustered based on similarities of presence and absence of the antimicrobial resistance genes. Blue represents a perfect hit to the reference sequence in the CARD database, teal represents a strict hit, and blank indicates absence of that gene in the isolate (8). Patient 9 was included twice in the study. ESBL-positive *E. coli* were cultured on both admissions (9a and 9b).



Chapter 2.3



UNIVERSAL SCREENING OR A UNIVERSAL RISK ASSESSMENT COMBINED WITH RISK-BASED SCREENING FOR MULTIDRUG-RESISTANT MICROORGANISMS UPON ADMISSION: COMPARING STRATEGIES

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Abstract

Timely identification of patients who carry multidrug-resistant microorganisms (MDRO) is needed to prevent nosocomial spread to other patients and to the hospital environment. We aimed to compare the yield of a universal screening strategy upon admission to the currently installed universal risk assessment combined with risk-based screening upon admission. This observational study was conducted within a prospective cohort study. From January 1, 2018, until September 1, 2019, patients admitted to our hospital were asked to participate. Nasal and perianal samples were taken upon admission and checked for the presence of MDRO. The results of the universal risk assessment and risk-based screening were collected retrospectively from electronic health records. In total, 1017 patients with 1069 separate hospital admissions participated in the study. Universal screening identified 38 (3.6%) unknown MDRO carriers upon admission (37 individual patients), all carrying extended-spectrum beta-lactamase-producing Enterobacterales. For 946 of 1069 (88.5%) patients, both the universal risk assessment and universal screening were performed. For 19 (2.0%) admissions, ≥1 risk factor was identified. The universal risk assessment identified one (0.1%) unknown carrier, compared to 37 out of 946 carriers for the universal screening ($P<0.001$). Of the 37 carriers identified through the universal screening, 35 (94.6%) reported no risk factors. Our results show that in our low endemic setting, a universal screening strategy identified significantly more MDRO carriers than the currently implemented universal risk-assessment. When implementing a universal risk-assessment, risk factors should be carefully selected to be able to identify ESBL-E carriers. While the universal screening identified more MDRO carriers, further research is needed to determine the cost-effectiveness of this strategy.

Background

Healthcare-associated infections (HAI), specifically those due to multidrug-resistant microorganisms (MDRO), are considered a worldwide threat to healthcare (1). In hospitals, infection prevention and control (IPC) measures are implemented to prevent the spread of MDRO. However, for these measures to be effective, timely identification of patients colonized with MDRO is essential. A common IPC measure to increase timely identification is targeted screening of patients based on a universal risk assessment upon admission, followed by risk-based screening (2). Upon admission, patients are asked several questions to determine the risk of being colonized with an MDRO and screened when they are considered at risk (2). Another strategy is universal screening upon admission. Universal screening strategies have been performed for methicillin-resistant *Staphylococcus aureus* (MRSA), however, with conflicting results. While some studies report the method not to be cost-effective, or only effective when having a high prevalence, it has also been reported that universal screening was effective in decreasing MRSA prevalence and incidence (3-6). Regarding carbapenemase-producing organisms, it was shown that universal screening might be a cost-effective strategy to reduce transmission (7-9). To our knowledge, the effect of a universal screening strategy for multiple MDRO upon admission has yet to be determined.

Recently, a Dutch study showed that the nationally implemented MDRO risk assessment only identifies a small portion of all MDRO carriers, while it was associated with a high workload for healthcare workers (10). The results of that study were confirmed by another Dutch hospital (11). Consequently, it should be considered if other strategies are more effective. In a previous large prospective cohort study (the MOVE study), we performed universal screening for MDRO upon admission (12). These patients were also screened with the universal MDRO risk assessment, in compliance with standard-of-care. Consequently, we are in the unique position to have patients of whom we have results of both universal screening and of the universal risk assessment combined with risk-based screening. We aimed to determine the yield of universal screening for MDRO and compare this to the yield of the currently installed universal risk assessment combined with risk-based screening, to determine the successfulness of both strategies to identify unknown MDRO carriers.

Methods

Study design and setting

The observational prospective cohort study (the MOVE study) was performed from January 1, 2018, until September 1, 2019, at the Erasmus MC University Medical Center (Erasmus MC) in Rotterdam, the Netherlands. The study design and setting were described previously (12). This study was approved by the medical ethical committee of the Erasmus MC (MEC-2017-1011) and was not subject to the Medical Research Involving Human Subjects Act. Written informed consent was obtained from all participating patients. The study was registered in the Dutch National Trial Register (NL8406) (12). Patients in the MOVE study were prospectively included. For these included patients, data on the universal risk

assessment and the results of the risk-based screening was retrospectively collected from the patient's electronic health records (EHR) between 2018 and 2022.

Inclusion of patients for universal screening

During the study period, adult patients admitted to the participating departments at the Erasmus MC with an expected hospitalization period of ≥48 hours, and who could speak and read in Dutch, were approached for participation in the MOVE study (12). Patients who were admitted multiple times during the study period were allowed to participate multiple times. Patients were not approached if they were admitted in the weekend/during holidays, if they were legally not able to decide about participating, or if they were in end-of-life stage (12). After obtaining written informed consent, a nose and perianal sample were taken on the day of admission by trained members of the study team, or by the patient, with clear verbal instructions from trained members (11). For patients admitted directly to the intensive care unit (ICU), passive informed consent was accepted (i.e., information regarding the study was provided to the patient or their family, and consent was assumed if they or their family did not explicitly object). The result of the admission screening as part of the MOVE study was not shared with the patient, nor with the treating physician, as approved by the medical ethical committee. Consequently, a positive universal screening culture did not result in isolation or otherwise change of care.

Universal risk assessment combined with risk-based screening

The universal risk assessment combined with risk-based screening is a national mandatory assessment (2). All patients admitted to Dutch hospitals are asked several questions upon admission to determine their risk of being colonized with MDRO (Supplementary file 1). These questions are 1) is the patient a known carrier of a MDRO, 2) has the patient recently been treated in or admitted to a healthcare institution abroad, 3) did the patient stay in a healthcare facility known with a MDRO outbreak in the past two months, and if yes, was the patient approached for screening, 4) has the patient lived in an institution for asylum seekers in the past two months, 5) does the patient live or work where pigs, veal calves or broilers are kept commercially, and 6) is the patient a partner, housemate or caregiver of someone who is MRSA positive? Additionally, in the Erasmus MC, the question "Is the patient a professional seafarer?" was added, after identifying that seafarers from the nearby harbor had higher carriage rates (13). When patients are deemed at risk according to the assessment, screening cultures (i.e., nasal, throat, and perianal for MRSA; throat and rectal samples for other MDRO) are taken. Additionally, the patient is places in pre-emptive isolation. When a patient has had an hospitalization abroad that was more than two months ago, but has undergone surgery there or a wound is still present, screening cultures are taken, but the patient is not placed in isolation (Supplementary file 1). When the risk-based screening cultures are negative, pre-emptive isolation measures are lifted. When the screening cultures are positive, pre-emptive isolation measures are adapted to the type of MDRO. When an MDRO is identified, isolation with additional IPC measures are always initiated according to the Dutch national MDRO guideline (14). Results of the risk assessment, results of screening cultures, and consequent implications for isolation measures, were reported in the patient's electronic health records (EHR). These results were retrospectively collected from the EHR from patients included in the MOVE study.

Consequently, from patients who were at risk according to the universal risk assessment, two types of cultures were taken: risk-based cultures and universal screening cultures as part of the MOVE study.

Microbiological methods

Risk-based screening samples (*i.e.* nasal, throat, perineal, and rectal) were taken with cotton swabs. Nasal, throat, and perineal samples were screened for the presence of MRSA; rectal samples for vancomycin-resistant *Enterococcus faecium* (VRE), multidrug-resistant *Pseudomonas* spp., multidrug-resistant *Acinetobacter calcoaceticus-baumannii* complex (*A. baumannii*), extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales (ESBL-E), and carbapenemase-producing Enterobacterales (CPE). Presence of these MDRO was determined using standard microbiological procedures (Supplementary file 2).

Universal screening samples, both nasal and perianal, were taken with flocked swabs (Copan Italia, Brescia, Italy). Nasal samples were screened for MRSA, and perianal samples were screened for VRE, highly resistant *P. aeruginosa,* highly resistant *A. baumannii*, CPE, and ESBL-E using standard microbiological procedures (Supplementary file 2). Whole genome sequencing (WGS) was performed on all isolates identified through universal screening to identify the presence of antimicrobial resistance genes (Supplementary file 2). Also, multi locus sequence types (MLST) were inferred from the WGS data. In case of discrepancy between WGS and phenotypic ESBL detection, the phenotypic test result was used, to mimic best the standard-of-care.

Data collection and analysis

Patient data, including results from the universal risk assessment and risk-based screening, and installed isolation measures, were retrospectively collected from the EHR. Additionally, data on admission specialization was collected. Admission specializations were categorized into surgical, medical, hematological or ICU admissions (12). Descriptive analyses were performed, and the yield of screening strategies were compared using Fisher's exact test using IBM Statistical Package for the Social Sciences Solutions (SPSS) version 28 (IBM Corp., Armonk, New York, USA). Data was processed pseudonymized, AS and AV had access to information that could identify individual patients.

Results

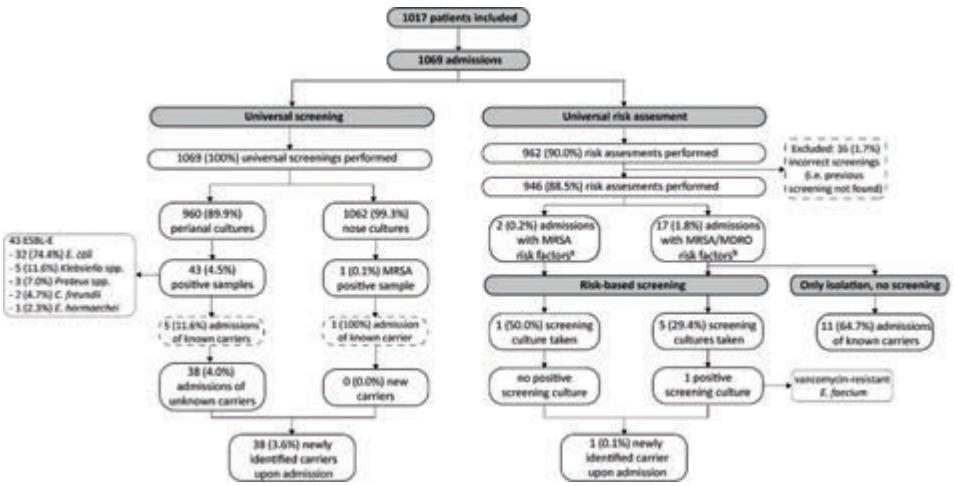
Universal screening

In total, 1069 admission cultures were taken from 1017 patients (Figure 1). Forty-eight (4.7%) patients were admitted more than once, 44 patients were admitted twice and four patients three times. Only a nasal sample was taken for 109 (10.2%) admissions of 109 patients, and only a perianal sample was taken for seven patients (Figure 1). The median age upon admission was 61 (range 18-90). Forty-four (4.1%) cultures of 42 patients were positive for MDRO (Figure 1), 43 (4.5%) perianal cultures were positive for ESBL-E and one

(0.1%) nasal culture was positive for MRSA. The majority of identified ESBL-E were Escherichia coli (74.4%) (Figure 1).

Six (13.6%) cultures of six patients were taken from known carriers according to their EHR, and were thus cared for in isolation. For one of 44 (2.2%) admissions, the patient was labelled as a carrier of *Citrobacter freundii* and *Enterobacter cloacae* in the EHR, both ESBL-producing, but identified as an unknown ESBL-producing *Klebsiella pneumoniae* carrier through the universal screening and thus considered as newly identified. This patient was included twice, and twice identified as a carrier of a previously unknown MDRO. Consequently, 38 out of 1069 (3.6%) admission cultures from 37 out of 1017 (3.6%) patients identified unknown carriers at the moment of hospitalization, all ESBL-E carriers. For 26 (2.4%) admissions of 26 patients, MDRO were identified from the cultures, although patients were labelled as MDRO carrier in the EHR. Through universal screening, no carriers of VRE, CPE, highly resistant *P. aeruginosa* or *–A. baumannii* were identified. The result of WGS are presented in Supplementary file 3.

Figure 1. Flowchart of universal screening and universal risk assessment, with screening results.



Universal risk assessment combined with risk-based screening

The risk assessment was performed for 946 (88.5%) admissions of 900 individual patients (Figure 1). For 107 (10.0%) admissions, no risk assessment could be found in the EHR. Additionally, for 16 (1.7%) admissions, the EHR referred to a previous screening, but no previous screening was found (Figure 1). Risk factors for MDRO including the specific risk factors for MRSA were present at 19 (2.0%) admissions (Figure 1). Eleven patients were known carriers from which cultures were recently taken, and thus no screening cultures were taken, but patients were placed in isolation. For two (10.5%) admissions, risk-based screening was not performed, although risk factors were present; one patient was a roommate of or caregiver for a MRSA carrier, and one patient had been hospitalized abroad.

For both patients, the risk assessment referred to a previous risk assessment, in which the risk factors were reported. The risk-based screening identified one (0.1%) new carrier. This patient was a known carrier, which was shown in cultures taken at another hospital where an outbreak occurred when the patient was hospitalized. This carrier was not identified through the universal screening. Of the 946 patients, 32 (3.4%) were a known carrier according to their EHR and were placed in isolation upon admission. Of these patients, 12 (37.5%) answered that they were a known carrier.

Comparing screening strategies

For 946 admissions, both the universal screening and the universal risk assessment were performed. The universal screening identified 37 carriers, of which 31 new carriers, compared to the universal risk-assessment combined with risk-based screening which identified one new carrier (P<0.001). Thirty-five out of 37 (94.6%) carriers identified through the universal screening reported no risk factors upon admissions, two (5.4%) patients stated that they were a known carrier.

The yield of the universal screening was highest for ICU and medical patients (Table 1). These patients had the lowest percentages of performed universal risk assessments, but the highest percentages of risk factors (Table 1).

Table 1. Characteristics and outcome of screening strategies for the 1017 included medical, surgical, hematological, and ICU patients, with 1069 separate admissions.

	Medical (n=203)	Surgical (n=583)	Hematological (n=239)	ICU (n=44)	Total (n=1069)
Female (%)	94 (46.3)	265 (45.5)	100 (41.8)	17 (38.6)	476 (44.5)
Age, median (range)	58 (19-90)	63 (18-89)	61 (20-81)	51 (25-85)	61 (18-90)
Labelled as MDRO carrier (%)	12 (5.9)	10 (1.7)	9 (3.8)	2 (4.5)	33 (3.1)
Universal nasal sample (%)	203 (100)	581 (99.7)	238 (99.6)	40 (90.9)	1062 (99.3)
Positive (%)	1 (0.5)	0 (-)	0 (-)	0 (-)	1 (0.1)
Universal perianal sample (%)	178 (87.7)	520 (89.2)	223 (93.3)	39 (88.6)	960 (89.8)
Positive (%)	11 (6.2)	17 (3.3)	12 (5.4)	3 (7.7)	43 (4.5)
Universal risk assessment (%)	189 (93.1)	506 (86.8)	220 (92.1)	31 (70.5)	946 (88.5)
1 risk factor (%)	6 (3.2)	7 (1.4)	4 (1.8)	1 (3.2)	18 (1.9)
≥2 risk factors (%)	1 (0.5)	0 (-)	0 (-)	0 (-)	1 (0.1)
Risk-based screening (%)	1 (0.5)	4 (0.7)	1 (0.4)	0 (-)	6 (0.6)
Positive (%)	1 (100)	0 (-)	0 (-)	0 (-)	1 (16.7)

Abbreviations: ICU, Intensive Care Unit, MDRO, multidrug-resistant microorganisms

Discussion

Our results show that in a low endemic setting, a universal screening strategy identifies significantly more MDRO carriers than through the currently implemented universal risk assessment combined with risk-based screening.

The result that a universal screening strategy identifies more carriers in our low endemic setting is not surprising. This could be explained by the fact that more patients are microbiologically screened than through the risk-based screening. Secondly, the universal risk assessment only includes questions regarding a limited number of risk factors, and some MDRO carriers do not have any of the predefined risk factors, as shown for MRSA (15). Since we did not identify new MRSA, CPE, highly resistant *P. aeruginosa* or *A. baumannii*, our discussion will be focused on ESBL-E. The question remains what the added benefit of identifying these MDRO (in our setting all ESBL-E) carriers would be. Several studies have studied the effect of isolation practices for known ESBL-E carriers (16, 17). Kluytmans-van den Bergh et al. showed that transmission from index patients was higher for patients with unprotected ward stay, compared to patients who were cared for under contact precautions directly upon admission, although not significantly. This highlights the importance of timely identification and isolation (16). Our previous study showed that most ESBL-E carriers also remain unidentified through clinical cultures throughout their hospitalization (12). This, in combination with the high percentage of unidentified carriers upon admission, raises concern for unidentified transmissions throughout the hospital and the potential clinical implications. While we did not identify CPE, the study of Phee et al. highlighted the key role universal screening has in identifying the true prevalence of carbapenemase-producing organisms (7). Some of the sequence types (ST) that were found among ESBL-E. coli in our study have been reported to spread in hospitals with a *bla*$_{NDM}$ gene (ST10 in Mexico and ST167 in Denmark) (18, 19).

Our findings regarding the yield of the universal risk assessment and risk-based screening were in agreement with the study by Van Hout et al. (10) and Vainio and Bril (11). Both our results and the results of Van Hout et al. identified that the currently installed strategy is unable to identify most carriers, and that the highest yield is through the question “are you a known MDRO carrier?”. Consequently, Van Hout et al. proposed abandoning the risk-based screening, and only installing transmission-based precautions for (previously) known carriers (10). Vainio and Bril identified that the question about hospitalization abroad substantially contributed to the yield, and consequently they suggest a simplified risk assessment, only asking about known MDRO carrier status and recent hospitalization abroad (11). However, according to our results, most known carriers do not report they are a known carrier. This could be deliberate, to prevent being cared for in isolation, or it could be that the patient is not aware of or does not completely understand their own MDRO status (20). Also, due to frequent inter-hospital patient transfers, communication on the current MDRO status of a patient may be delayed. This could lead to a delay in installing isolation practices, and consequently could lead to transmission to other patients and to the hospital environment.

It is important to notice that due to transmission in the population of ESBL-E, it is difficult to implement an effective risk factor screening strategy. Not all ESBL-E carriers have

(known) risk factors, which is seen in our study and in the study of Vianio and Bril, who reported that almost 80% of MDRO carriers are unexpected findings (11). However, as shown for MRSA by Lekkerkerk et al., new risk factors can be identified (21). Consequently, it could be worthwhile to investigate the effect of adding additional risk factors to the universal risk assessment, or to identify new risk factors for ESBL-E carriage.

Strengths and limitations

The main strength of this study is the active sampling of patients upon admission to the hospital, regardless of risk factors or MDRO status. This study also has some limitations. The main limitation of this study is that it is a single center study in a low prevalence country. Therefore, the generalizability of our work is limited, especially to countries with a higher MDRO prevalence. A second limitation is that different cultures were taken for the universal screening compared to the risk-based screening. For example, perianal samples instead of rectal samples were taken for the universal screening. Perianal samples may be less sensitive than rectal samples for detection of MDRO, therefore, the true carriage rate upon admission may be higher than our results indicate. This could also explain why the observed carriage rate of 4.6% for ESBL-E is lower than observed in other studies in the Netherlands (16). Additionally, it could explain why the newly identified VRE carrier was not detected in the universal screening, although it is also known that VRE colonization may be missed when only one culture is taken (22). In general, detection of MDRO is challenging as antibiotic use of the patient or sampling error play a role, which may result in false-negative results. However, for most MDRO, we used enrichment broths to overcome this as much as possible. Another limitation is that we were not able to sample all patients admitted to the hospital. For example, patients admitted during the weekend were not approached for participation. Therefore, our results are not complete. Finally, we only included patients in the study who could speak and read Dutch.

Future studies

Our results highlight the need for improvement of the universal risk assessment. It should be considered to add questions regarding travel history to the risk assessment, as this is a known risk factor for MDRO carriage (23-25). Other well-known but more general risk factors, such as antibiotic usage, could be of additional value as well. To further identify risk factors and to tailor the questions, a study with MDRO carriers from multiple hospitals in countries with low prevalence of MDRO is needed. Future studies should determine if adding additional questions improves the risk assessment for patients admitted to a hospital in a low-prevalence country and if this strategy is cost-effective. Additionally, cost-effectiveness combined with the risk of transmission of identified MDRO, especially ESBL-producing *E. coli*, should be studied. However, as stated by Van Hout et al. (10) and by Vainio and Bril (11), the universal risk assessment is associated with a high workload for healthcare workers, and adding questions would increase this workload, which needs to be considered. A prediction system, based on data available in EHR of patients across multiple healthcare facilities, including pharmacies and general practitioners, would be a solution to overcome this in the future.

Additionally, to determine if a universal screening strategy could be an alternative strategy, the cost-effectiveness needs to be evaluated, preferably for different MDRO prevalence rates. Moreover, even though our results do not clearly show that universal screening is more effective for specific patient populations, the added benefit of universal screening for specific patient populations (e.g., ICU) should be evaluated. Our results can also be used for modelling studies to identify the best approach.

Conclusion

Overall, our results indicate that the currently installed universal risk assessment combined with risk-based screening in a tertiary care center in the Netherlands is not successful in identifying MDRO carriers upon admission. The universal screening strategy identified significantly more new carriers. In our opinion, to improve the yield of the universal risk assessment, an updated version of the universal risk assessment would be the best approach in settings similar to ours, as the current risk factors are not identifying all ESBL-E carriers. Cost-effectiveness studies need to be performed to determine if a universal screening strategy could be a valid alternative strategy.

Abbreviations

CPE	Carbapenemase-producing Enterobacterales
EHR	Electronic health record
Erasmus MC	Erasmus MC University Medical Center
ESBL	Extended-spectrum beta-lactamase
ESBL-E	Extended-spectrum beta-lactamase-producing Enterobacterales
HAI	Healthcare-associated infections
ICU	Intensive care unit
IPC	Infection prevention and control
MDRO	Multidrug-resistant microorganisms
MIC	Minimal inhibitory concentration
MLST	multi locus sequence type
MRSA	Methicillin-resistant *Staphylococcus aureus*
ST	Sequence types
VRE	Vancomycin-resistant *Enterococcus faecium*
WGS	Whole genome sequencing

Transparency declaration

Conflict of interest statement

All authors have no conflicts to declare.

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Author contributions

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

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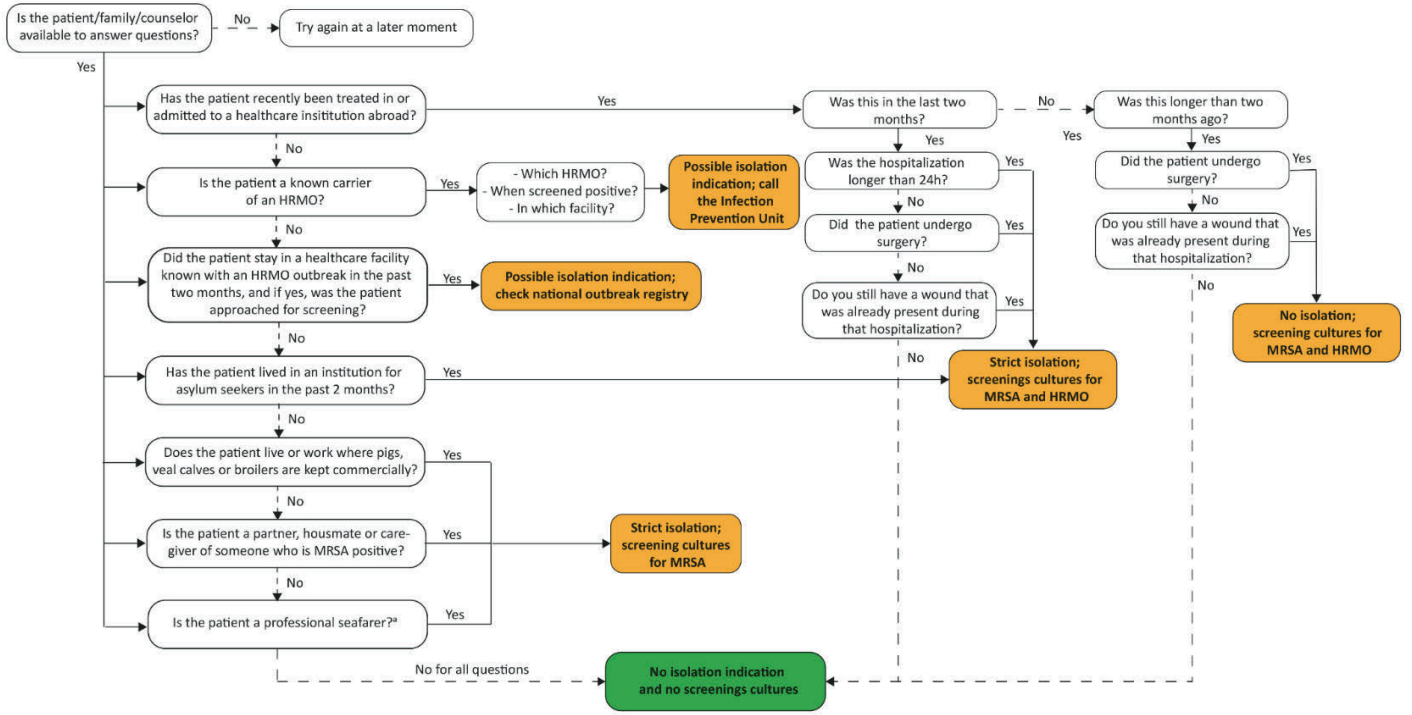
Supplement 1: risk-based screening strategy questions upon admission to the hospital



Abbreviations: HRMO highly resistant microorganisms. MRSA meticillin-resistant *Staphylococcus aureus*

[a] Question specific for the Erasmus MC as shown by Lekkerkerk et al. (Lekkerkerk WS, van Genderen PJ, Severin JA, Peper JP, Storm EF, Vos MC. Letter to the editor: seafarers: a new risk group for meticillin-resistant *Staphylococcus aureus* (MRSA). Euro Surveill. 2013 Oct 24;18(43):20618.)

Supplement 2. Microbiological methods and whole genome sequencing

Microbiological methods

Risk based screening samples

For methicillin-resistant *Staphylococcus aureus* (MRSA), nose, throat, and perineal samples were taken using cotton swabs (Copan Italia, Brescia, Italy). For vancomycin-resistant *Enterococcus faecium* (VRE), a rectal swab was taken. For the Gram-negative antibiotic-resistant bacteria, throat and rectal samples were taken, also with cotton swabs.

To determine the presence of MRSA, the swab was placed in a tryptic soy broth (TSB) with 6.5% NaCl and incubated for 24 h at 35°C. Subsequently, 10 µl of broth was subcultured on a BBL-CHROMagar MRSA (BD diagnostics, Sparks, USA) which was incubated for 48 h at 35°C. Plates were checked at 24 h and 48 h. Selected pink and purple colonies were identified with the Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, Bremen, Germany). A suspension of 0.5 McFarland was made from *S. aureus* isolates, and used to perform a cefoxitin disk diffusion (30 µg; Oxoid, Basingstoke, UK) on a Mueller Hinton agar (BD diagnostics, Sparks, USA). A growth inhibition zone of <22mm after 18 to 24 h was considered resistant. To confirm presence of MRSA, a *mecA/mecC* PCR was performed, using established procedures.

To determine the presence of VRE, the swab was placed in an Enterococcosel broth (BD diagnostics, Sparks, USA) with 8 mg/L amoxicillin and incubated overnight at 35°C. From the broth, a *Brilliance*™ VRE (Oxoid, Basingstoke, UK) was inoculated and incubated twice overnight at 35°C. Selected blue/purple colonies were identified using the MALDI-TOF and antibiotic susceptibility was determined with the VITEK®2 (bioMérieux, Marcy l'Etoile, France). For *E. faecium* colonies resistant for amoxicillin, an Etest for vancomycin, an Etest for teicoplanin, and a *vanA/vanB* PCR using established procedures, were performed to confirm the presence of VRE.

To determine the presence of extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E), a *Brilliance*™ ESBL agar (Oxoid, Basingstoke, UK) was inoculated and incubated twice overnight at 35°C. To determine the presence of carbapenemase-producing Gram-negative bacteria, a ChromID CarbaSmart agar (bioMérieux, Marcy l'Etoile, France) was inoculated and incubated twice overnight at 35°C. All colonies were identified using the MALDI-TOF and antibiotic susceptibility was determined with the VITEK®2. ESBL production was confirmed phenotypically, using double disk diffusion test. Carbapenemase production was tested with the carbapenem inactivation method (CIM) test, presence of carbapenemase genes with a multiplex PCR for bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{KPC} and $bla_{\text{OXA-48-like}}$ (1).

To determine the presence of $bla_{\text{OXA-48}}$-positive Gram-negative rods, the swab was placed in a TSB with 0.25 mg/L ertapenem and 50 mg/L vancomycin and incubated overnight at 35°C. From the broth, a PCR for $bla_{\text{OXA-48}}$ was performed. In case of a positive PCR result, the broth was subcultured on a ChromID CarbaSmart agar and a MacConkey agar (Biomerieux,

Marcy l'Etoile, France) and these were incubated twice overnight at 35°C. Colonies were identified using the MALDI-TOF and antibiotic susceptibility was determined with the VITEK®2.

To determine the presence of highly resistant *Acinetobacter calcoaceticus-baumannii* complex (*A. baumannii)*, the swab was placed in a TSB with 2 mg/L ceftazidime and 50 mg/L vancomycin, and incubated overnight at 35°C. From the broth, a MacConkey agar and a ChromID CarbaSmart agar were inoculated, and incubated twice overnight at 35°C. Suspected colonies were identified using the MALDI-TOF and antibiotic susceptibility was determined with the VITEK®2. To confirm the presence of highly resistant *A. baumannii*, an Etest (usually for meropenem and imipenem) or disk diffusion was performed, which was decided by the supervising clinical microbiologist.

Universal screening samples

Samples were taken with flocked swabs (Copan). Nasal samples were screened for MRSA, and perianal samples were screened for VRE, highly resistant *Pseudomonas aeruginosa,* highly resistant *A. baumannii*, carbapenemase-producing Enterobacterales (CPE), and ESBL-E.

Nasal samples were placed in the accompanying 2mL 2.5% NaCl TSB medium (Copan). Of the TSB medium, 800 µL was pipetted in a 6.5% NaCl TSB and incubated for 24 hours at 35°C. A *nuc* gene PCR was performed to identify the presence of *S. aureus* using established procedures. When the PCR was positive, a blood agar (BD diagnostics, Sparks, USA) was inoculated and incubated twice overnight at 35°C. Colonies were identified using the MALDI-TOF. To determine beta-lactam antibiotic resistance, a cefoxitin disk diffusion (30 µg; Oxoid, Basingstoke, UK) was performed. A growth inhibition zone of <22mm after 18 to 24 hours was considered resistant. For cefoxitin-resistant isolates, a multiplex PCR to detect *mecA* and *mecC* genes was performed using established procedures. All MRSA strains were stored in -80°C.

Perianal samples were placed in the accompanying 1 mL Amies medium ((e-Swabs (Copan)). Of the Amies medium, 250µL was pipetted in an Enterococcosel broth with 8 mg/L amoxicillin, and 250µL in a TSB with 50 mg/L vancomycin. From the amoxicillin broth, a *Brilliance*™ VRE was inoculated and incubated twice overnight at 35°C to screen for VRE. From the vancomycin broth, a ChromID CarbaSmart plate was inoculated on both sides and incubated twice overnight at 35°C to screen for CPE, highly resistant *P. aeruginosa*, and highly resistant *A. baumannii*. Additionally, a *Brilliance*™ ESBL agar plate was inoculated from the vancomycin broth, to screen for ESBL-E, highly resistant *P. aeruginosa*, and highly resistant *A. baumannii*. All colonies were identified to species level using the MALDI-TOF. For suspected VRE and ESBL-E, based on growth on the *Brilliance*™ VRE or ESBL agar plate, respectively, antibiotic susceptibility was determined with the VITEK®2. For suspected carbapenemase-producing bacteria, based on growth on the ChromID Carba Smart, 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 that were negative for these carbapenemase genes, a CIM test was performed All identified colonies were stored at -80°C.

Whole genome sequencing

WGS was performed for all identified highly resistant *P. aeruginosa, -A. baumannii*, CPE, ESBL-E, MRSA, and VRE isolates from universal screening samples. Total genomic DNA was extracted using the MagNA Pure 96 platform (Roche Applied Science, Mannheim, Germany). Genomic DNA was sent to Novogene (HongKong, China) where it 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 creating >100x coverage using Illumina technology. Fastq data were provided and de novo genomic assemblies were generated using CLC Genomics Workbench (Qiagen, Hilden, Germany) with default parameters (2, 3). Presence of antimicrobial resistance (AMR) genes was determined using the web-based comprehensive antimicrobial resistance database (CARD) (including perfect and strict hits)(https://card.mcmaster.ca/analyze/rgi) (4). Conventional multi locus sequence types (MLST) and core genome multi locus sequence type (cgMLST) were determined based on each species' corresponding (cg)MLST scheme (https://cgmlst.org/ncs) available in SeqSphere+ software (Ridom, Munster, Germany). Isolates were identified to the species level by analysing their de-novo assemblies using the Tyge Strain Genome Server (TYGS - https://tygs.dsmz.de/) (5).

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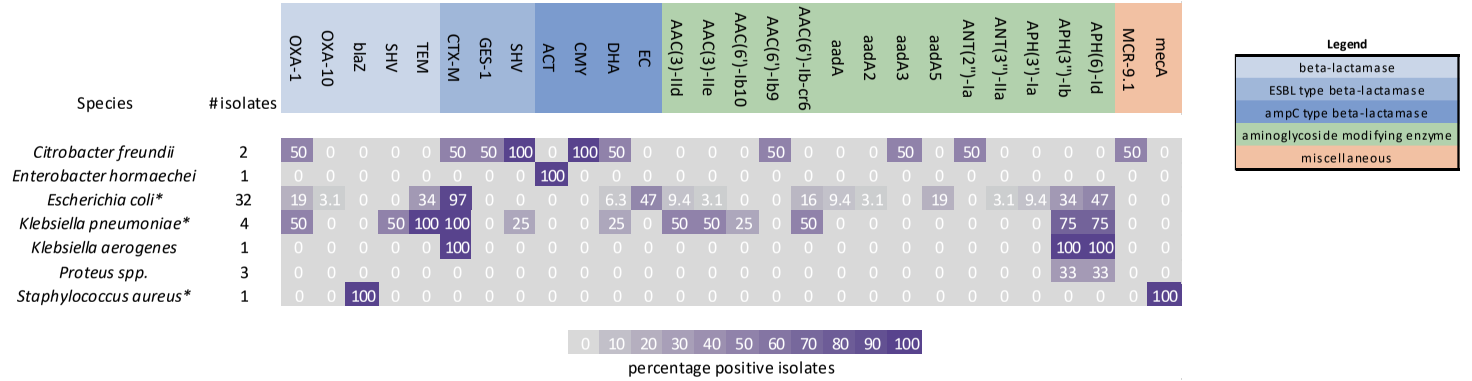
Supplement 3. Whole genome sequencing results

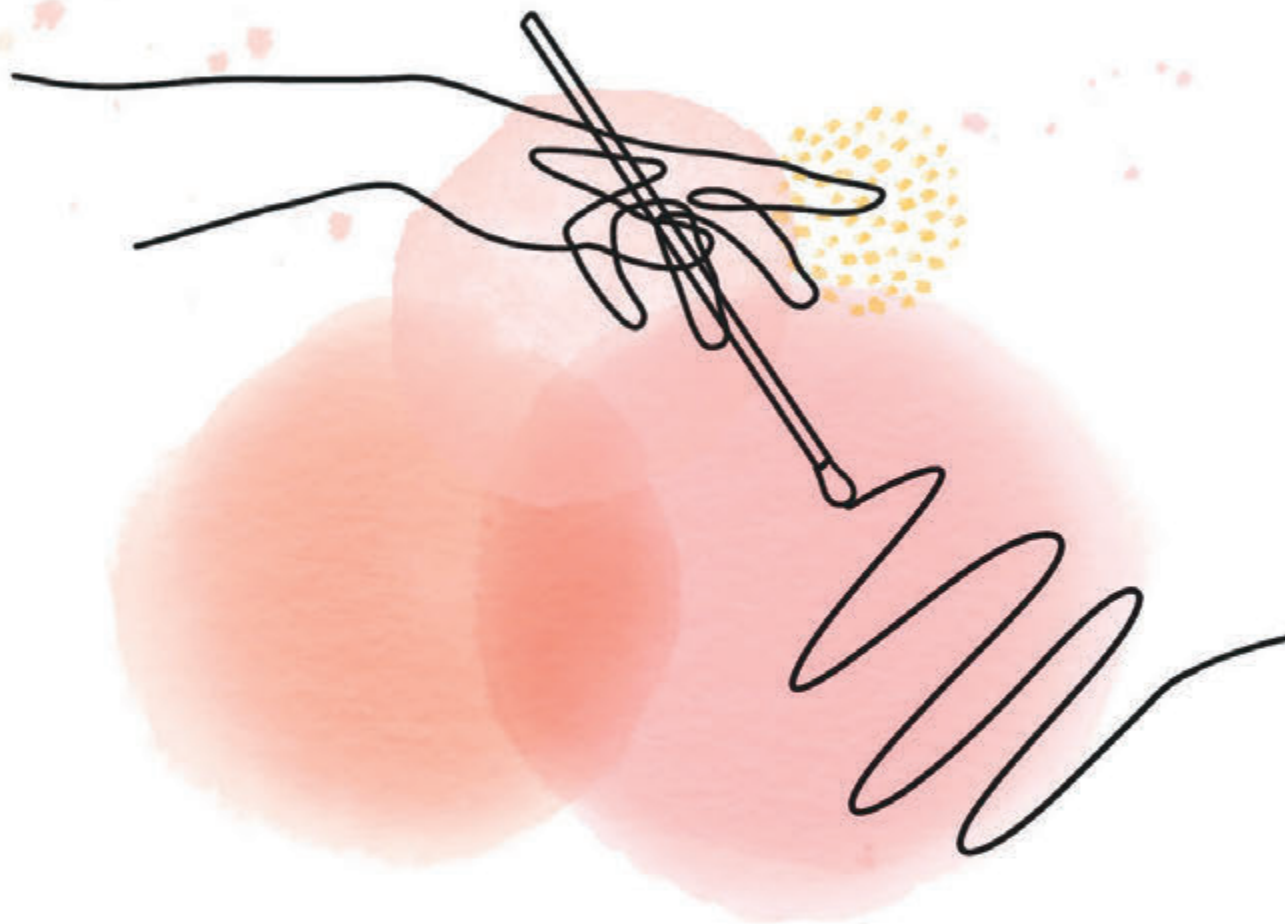
WGS showed that the majority of Escherichia coli (97%) and Klebsiella spp. (100%) isolates were CTX-M positive (Supplementary file 3). Of the 32 ESBL-E. coli, 11 (34.4%) belonged to sequence type (ST) 131. One Citrobacter freundii carried an mcr-9 gene (with a colistin minimal inhibitory concentration (MIC) of 0.5 µg/mL as measured by Vitek2). While the Proteus spp. phenotypically showed ESBL activity, no beta-lactamase resistance genes were identified (Supplementary file 3).



Prevalence of antimicrobial resistance genes among the different species as determined using the CARD web-interface (https://card.mcmaster.ca/). The analysis (restricted to perfect and strict hits only) was focused on different types of beta-lactamases and aminoglycoside modifying enzymes.

*sequence types involved: E.coli ST131 (n=11), ST10/ST69 (n=4), ST405/ST744 (n=2), ST38/ST58/ST75/ST88/ST120/ST167/ST224/ST636/ST13164 (n=1); K. pneumoniae ST152/ST307/ST464/ST561 (n=1); S. aureus ST6 (n=1)

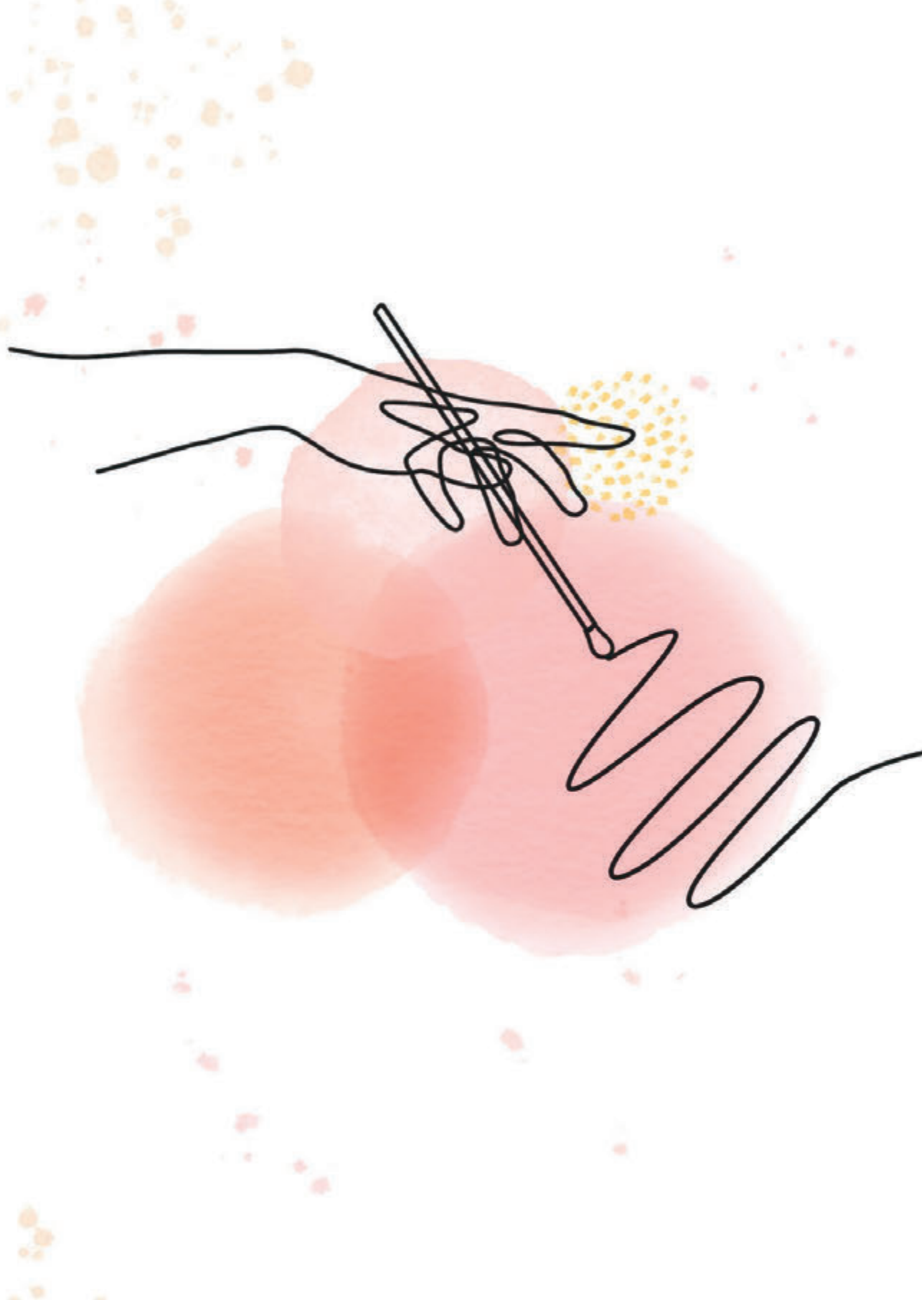
Chapter 2.4



DYNAMICS OF STAPHYLOCOCCUS AUREUS IN PATIENTS AND THE HOSPITAL ENVIRONMENT IN A TERTIARY CARE HOSPITAL IN THE NETHERLANDS

Adriënne S. van der Schoor, Anne F. Voor in 't holt, Willemien H.A. Zandijk, Marco J. Bruno, Diederik Gommers, Johannes P.C. van den Akker, Joke M. Hendriks, Juliëtte A. Severin, Corné H.W. Klaassen, Margreet C. Vos

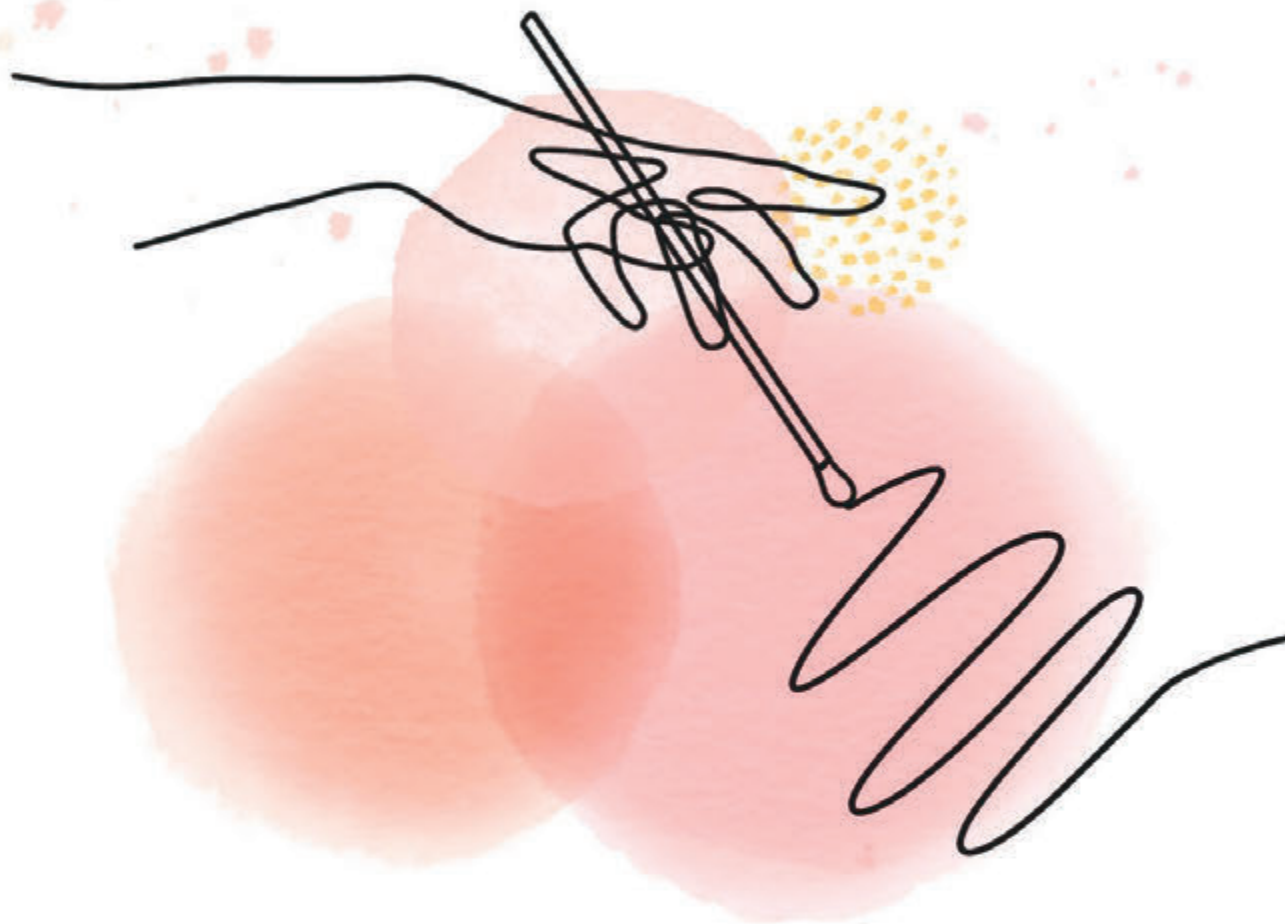
Submitted



Chapter 3

ENVIRONMENTAL SAMPLING AND CONTAMINATION

Chapter 3.1



ENVIRONMENTAL SAMPLING PRACTICES OF INNATE HOSPITAL SURFACES: A SURVEY OF CURRENT PRACTICES AND THE NEED FOR GUIDELINES

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J Hosp Infect. 2022 Oct;128:92-95

Abstract

Surfaces in healthcare facilities can act as reservoirs of infection. Currently, no standardized protocol on when and how to sample hospital surfaces exists. A web-based questionnaire was devised to gain insight into current sampling practices and was distributed by email to a targeted infection prevention and control (IPC) audience. The survey consisted of 26 questions on sample collection and processing for a number of healthcare relevant bacterial species. The majority of respondents were clinical microbiologists or IPC practitioners, and 57.3% were from either the Netherlands, the United Kingdom, or Ireland. Respondents had high self-reported knowledge, but this was not consistent with response to certain questions. There was no consensus on sample sites, either within or between countries. Indirect sampling methods were preferred for all target microorganisms, and cotton and flocked swabs were the most popular methods. The results of our survey highlight the inconsistences in environmental sampling between and within countries, and the need for guidance and consensus.

Introduction

Inanimate surfaces in hospitals may be contaminated with nosocomial pathogens, such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp. (1). These pathogens play an important role in the acquisition of healthcare associated infections (HAI) via direct or indirect contact with the contaminated surface (1, 2). An analysis of 1561 nosocomial outbreaks showed that the hospital environment was the source in almost 20% of those outbreaks, highlighting the importance of the environment (3). Next to identifying the source of an outbreak and apart from sampling for research aims, monitoring the environment can be used to routinely determine the presence of nosocomial pathogens, or to evaluate cleaning efficacy. Nevertheless, there are no national or international guidelines on when and how to perform environmental sampling (4, 5). Therefore, with this current survey study, we aimed to provide insights on current environmental sampling practices of the innate environment and the laboratory methods used to process these samples.

Methods

Study design

A web-based survey in the English language was developed and opened for responses between August 6th, 2021, and December 20th, 2021. Before releasing the survey, it was piloted in two centers. The survey was distributed digitally amongst members of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Nosocomial Infections (ESGNI), the Healthcare Infection Society (HIS), and members of the European Network to Promote Infection Prevention for Patient Safety (EUNETIPS), who forwarded the survey to the members of their respective societies.

Survey questions

The survey consisted of three sections and asked specifically about sampling practices for MRSA, VRE, CPE, *Pseudomonas* spp., and *Acinetobacter* spp. (Supplementary Appendix). The first section focused on the respondent and their role in environmental sampling, the second section on sampling practices, and the third section on sample processing methods. A distinction was made between indirect and direct sampling methods. Indirect methods included sponges, wipes, cotton swabs, flocked swabs and cotton swabs; direct methods included contact plates, dip slides and petrifilm. Before proceeding to the second and third sections, respondents were asked if they could answer these questions. If they answered ‘no’, they were redirected to the next section of the survey. It was not mandatory to answer all questions. All questions consisted of multiple answer options from which to choose one, except for one question where the answer was in free text.

Statistical analyses

Responses to the survey were analyzed in total, and within and between countries. Regarding analyses between countries, the following categories were used: 1) the Netherlands, 2) the United Kingdom (UK) and Ireland, and 3) other countries. Response rates differed for each question. Unanswered questions were categorized as 'not applicable', 'missing' or 'no' based on the question involved. For example, when a respondent reported not using direct sampling methods, any answers to questions regarding which direct methods were used were not included, as the respondent had already indicated that this method was not used. All analyses were performed in SPSS version 28 (IBM Corp., Armonk, New York, USA).

Results

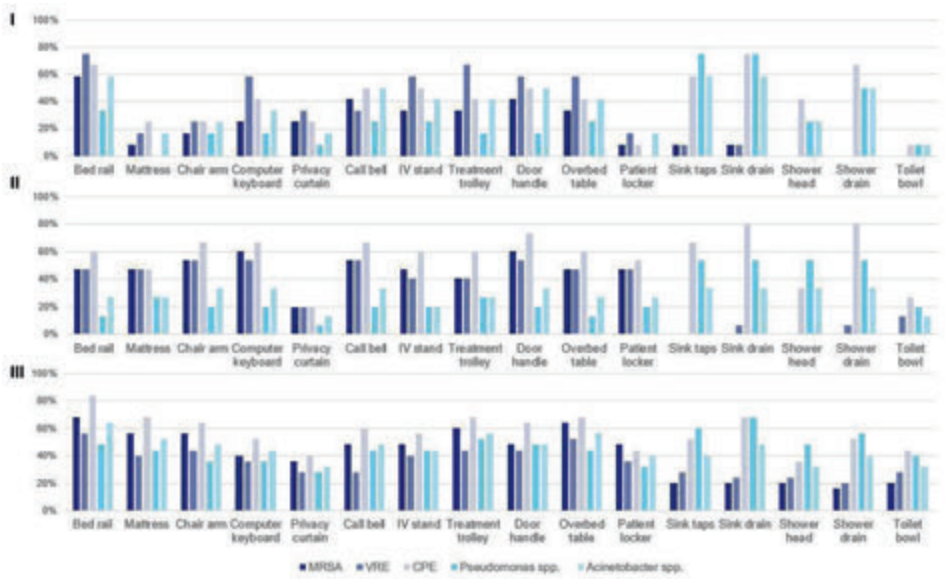
Eighty-nine respondents completed at least part of the survey. Forty-six respondents (51.7%) were clinical microbiologists, and 35 respondents (39.3%) were infection prevention and control (IPC) practitioners. Eight respondents had another role. Eighty-eight respondents (98.9%) worked in an acute care or specialized hospital, one respondent (1.1%) worked in a health centre. Respondents were from 21 different countries, with a range of one to 22 respondents from any one country. The majority of respondents (57.3%) were from the Netherlands (n=22, 24.7%), the UK (n=17, 19.1%), and Ireland (n=12, 13.5%). Six out of 89 respondents were from non-European countries (Hong Kong N=3, India N=2, United States of America N=1).

Most respondents self-reported having good to excellent knowledge on sample collection (73/89, 82.0%), and questions in the section regarding sampling practices were answered by 58 (65.2%) respondents. Thirty-two of 58 (55.2%) respondents sampled the environment to find the source of an ongoing outbreak, 13/58 (22.4%) routinely sampled the environment for monitoring reasons, and 2/58 (3.4%) respondents never sampled the environment. Regarding sampling protocols, 42/56 (75.0%) respondents reported that they always or usually had a sampling protocol. Respondents reported that areas to be sampled were determined both prior to entering the area and while in the area to be sampled, instead of solely prior to or while in the area (30/56, 53.6%).

Sample locations

No sample was universally sampled for any target microorganism (Figure 1). However, for certain sites, there was consensus within countries not to sample certain locations for a target microorganism. UK respondents never sampled the privacy curtain for any microorganisms, and Dutch respondents never sampled the mattress and patient locker for *Pseudomonas* spp. Amongst Dutch, UK, and Irish respondents, there was consensus not to sample the showerhead, shower drain, and toilet bowl for MRSA. Other countries did report sampling these sites. Dry sites were mainly sampled for CPE, except in the Netherlands, where these sites were most frequently sampled for VRE. Wet or damp sites were mainly assessed for the presence of CPE in the UK and Ireland, and to detect both CPE and *Pseudomonas* spp. in the Netherlands and in other countries (Figure 1).

Figure 1. Percentage of respondents that sampled specific sites for the target microorganism per country. I) The Netherlands (n=12), II) United Kingdom and Ireland (n=15), III) Other countries (n=25).



Sample methods

Indirect methods were preferred for all target microorganisms, but differed between countries. Dutch respondents preferred flocked swabs, and never used sponges or rayon swabs. UK and Irish respondents preferred cotton or flocked swabs and sponges, and never used rayon swabs or wipes. Other countries preferred cotton swabs. Direct methods were rarely used and only reported to detect MRSA or VRE. No respondents reported the use of dip slides.

Laboratory processing

The majority of respondents reported having good to excellent knowledge on sample processing (72/89, 81.8%). Questions on processing methods were answered by 39 (54.2%) respondents. Indirect culture methods were preferred for MRSA, VRE, and CPE, and direct culture methods for *Pseudomonas* spp. and *Acinetobacter* spp. For MRSA and VRE, selective enrichment broths were preferred; for CPE and *Pseudomonas* spp., non-selective enrichment broths were preferred; and for *Acinetobacter* spp. broths were not preferred. Samples were vortexed before plating (16/38, 42.1%), and direct swabbing was the commonest plating technique (20/38, 52.6%).

Discussion

Through our survey, we sought to gain insight into current environmental sampling practices. The results indicate that there is great variability in sampling practices, both within and between countries. Whereas the literature is focused mainly on sampling to identify the source of an ongoing outbreak, specifically for outbreaks caused by multidrug resistant microorganisms, respondents also indicated that routine environmental sampling takes place. Eighty-nine respondents filled in the survey, but response rates differed with each question. The highest sampling rates were found for CPE, with the exception of the Netherlands, perhaps because CPE is less prevalent there than in other countries (6).

Though it was to be expected that, without current guidelines, there would be differences in sampling procedures between countries, there was still a lot of diversity even within countries, specifically for which sample sites to be assessed. Although there was some consensus within countries on which sites were never sampled (*e.g*. the privacy curtain), there was no consensus on which sites needed to be sampled. A possible explanation could be that the majority of respondents decided on locations to be sampled prior to entering the area, but also then changed some of these or added others while in that area. Consequently, sampling practices may differ with each sampling occasion. It may be that for this survey, respondents only reported locations that are determined prior to entering the area.

Flocked and cotton swabs were the most preferred sampling method, which is unsurprising, since they are the most frequently used sampling method in the literature (5). This could be explained by the fact that they can be used to sample every type of surface, their affordability, and because they are readily available in most hospitals. However, standardization of sampling methods is difficult, leading to variations in recovery rates and non-comparable results (7).

Sampling was most common for CPE, and this may be explained by national epidemiology, e.g. in Ireland, a national public health emergency was declared in 2017 to address CPE and acute hospitals undertake a nationally mandated programme of extensive patient screening to prevent CPE becoming endemic (8, 9). However, sampling rates in the Netherlands were highest for VRE. This could be explained by the low prevalence of VRE in the Netherlands compared to other countries. In 2020, 0.5% of *Enterococcus faecium* isolates were resistant to vancomycin, compared to 35.9% in Ireland (10). Additionally, outbreaks with VRE have occurred in the Netherlands, whereas outbreaks with CPE are less common. Therefore, VRE is a greater priority for IPC measures in the Netherlands to maintain a low prevalence compared to other countries. For CPE, the prevalence throughout Europe is of concern, and consequently a priority for IPC teams (10).

We observed a distinct difference between self-reported knowledge and objective knowledge. The majority of respondents claimed good to excellent knowledge at the start of the survey, but a substantial proportion of these respondents were not able to answer the relevant questions. This could indicate that the respondents expected different questions, or that the respondents were not aware about gaps in their knowledge regarding environmental sampling processes.

An important strength of this study is that, to our knowledge, this is the first study to determine environmental sampling practices. This study has, however, several limitations. First, despite being distributed to a large network of relevant professionals, a relatively small number of respondents replied, and the majority were from three countries. Second, most respondents were either IPC practitioners or clinical microbiologists, and only one was a scientist. Third, we do not know the total number of individuals to whom the survey was sent, as it was distributed by various professional societies and groups. Furthermore, we were unable to determine variations according to professional background and the size of hospital. Therefore, the limited perspective captured by this survey may not be representative of true practices.

The results of our study highlight the diversity and lack of consensus regarding environmental sampling practices and laboratory processing, both within and between countries. There is a need for national and/or international guidelines or advice regarding environmental sampling practices, to provide some consistency in sampling. Currently, there are guidelines on surface sampling in the food industry (11). However, there are obvious differences between the surfaces in healthcare buildings and in the food industry and the activities that occur in both settings. A standard of <5 colony forming units/cm^2 for aerobic bacteria has been suggested for surfaces in hospitals, but this has not been universally agreed (12). Nonetheless, guidelines might optimize the benefits of environmental sampling, including a focus on what to sample and for what purpose, and how to minimize unnecessary costs. Then environmental sampling might be more effective and the results would be more comparable at a national and international level. However, perhaps information about environmental sampling on a larger scale is needed first. We also need to have a greater understanding of the motivation behind sampling the environment, what information is being sought by investigators, and how the results inform and shape IPC measures.

Acknowledgements

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Author contributions

AS, MB, AV, MC and HH developed the survey. AS and MB collected and analyzed the data, and drafted the manuscript. All authors have approved the final version of the manuscript.

Conflict of interest statement

In recent years, HH has received research grants from Pfizer and Astellas and has been a recipient of a consultancy fee from Pfizer. MV has received unrestricted research grants or support of Olympus, Pentax and 3M for studying endoscope contaminations. All other authors have no conflicts to declare.

Funding sources

The preparation of the survey, piloting it, conducting it analyzing the data, and drafting the manuscript were not the subject of any funding.

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Supplemental file 1: Survey of environmental sampling practices in healthcare facilities

Survey of environmental sampling practices

Survey of environmental sampling practices in healthcare facilities

This is a survey about environmental sampling in healthcare facilities. The questions asked cover your role in the hospital and in sampling, how samples are taken and processed and what happens to the results.

1. Where do you work?
    - Acute care hospital
    - Specialised hospital
    - Nursing home
    - Long stay care home
    - Health centre

2. In what country are you based? (If non-European, please select 'other')

3. Name of hospital

4. What is your current role?
    - Clinical microbiologist
    - Infection control practicioner
    - Medical laboratory scientist
    - Scientist (other)
    - Other

5. With regard to environmental sampling, what is our role? (tick all which apply)
    - Sample collection
    - Sample processing
    - Interpretation of results
    - Dissemination of the results
    - Making decisions about policy and process
    - Other (please specify)

6. What is your level of understanding of these within you own institution?

	Poor	Fair	Good	Very Good	Excellent
How samples are collected	○	○	○	○	○
How samples are processed	○	○	○	○	○
How the results are interpreted	○	○	○	○	○
How the results are disseminated	○	○	○	○	○
How the results inform infection prevention and control	○	○	○	○	○

7. Is there collaboration between clinical microbiologists, infection control team (ICT) and laboratory?
    - Yes, between all three
    - Yes, only between microbiologists and ICT
    - Yes, only between microbiologists and laboratory
    - Yes, only between ICT and the laboratory
    - No
    - I don't know

Next page.

1. Could you answer questions about how the environment is sampled?
    - Yes, I can answer questions on sampling
    - No, I will skip the questions on sampling (continue to questions about laboratory)

Next page.

How is environmental sampling of your healthcare facility done?

1. When is the environment sampled?
    - Routine
    - Every outbreak
    - To find the source of an ongoing outbreak
    - To check disinfection protocols
    - Never

- I don't know

2. Is there a standardized sampling protocol?
   - Always
   - Usually
   - Sometimes
   - Rarely
   - Never
   - I don't know

3. When are areas to be sampled determined?
   - Prior to entering the area to be sampled
   - While in the area to be sampled
   - Combination of the two
   - I don't know

4. If you sample dry areas, which areas are sampled?

	MRSA	VRE	CPE	Pseudomonas	Acinetobacter
Bed rail	☐	☐	☐	☐	☐
Mattress	☐	☐	☐	☐	☐
Chair arm	☐	☐	☐	☐	☐
Computer keyboard	☐	☐	☐	☐	☐
Privacy curtain	☐	☐	☐	☐	☐
Call bell	☐	☐	☐	☐	☐
IV stand	☐	☐	☐	☐	☐
Treatment trolley	☐	☐	☐	☐	☐
Door handle	☐	☐	☐	☐	☐
Overbed table	☐	☐	☐	☐	☐
Patient locker	☐	☐	☐	☐	☐

5. If you sample wet areas, which areas are sampled?

	MRSA	VRE	CPE	Pseudomonas	Acinetobacter
Sink taps	☐	☐	☐	☐	☐
Sink drain	☐	☐	☐	☐	☐
Shower head	☐	☐	☐	☐	☐
Shower drain	☐	☐	☐	☐	☐
Toilet bowl	☐	☐	☐	☐	☐

6. Do you typically use direct (contact plates, dipslides) or indirect (swabs, wipes, sponges) to sample the environment?

	MRSA	VRE	CPE	Pseudomonas	Acinetobacter
Direct	☐	☐	☐	☐	☐
Indirect	☐	☐	☐	☐	☐

7. If you use direct sampling methods, which methods do you use?

	MRSA	VRE	CPE	Pseudomonas	Acinetobacter
Contact plates	☐	☐	☐	☐	☐
Dipslides	☐	☐	☐	☐	☐
Petrifilm	☐	☐	☐	☐	☐

8. If you use indirect sampling methods, which methods do you use?

	MRSA	VRE	CPE	Pseudomonas	Acinetobacter
Wipes	☐	☐	☐	☐	☐
Rayon swab	☐	☐	☐	☐	☐
Cotton swab	☐	☐	☐	☐	☐
Flocked swab (e-swab)	☐	☐	☐	☐	☐
Sponge	☐	☐	☐	☐	☐

9. If you use one of these indirect sampling methods, do you moisten the sampler and if yes, what is used?

	PBS	Transport/Growth media	Water	Neutalizer	No moistener used	I don't know
Wipes	○	○	○	○	○	○
Rayon Swab	○	○	○	○	○	○
Cotton Swab	○	○	○	○	○	○
Flocked Swab	○	○	○	○	○	○
Sponge	○	○	○	○	○	○

Next page.

1. Could you answer questions about how the samples are processed in the laboratory?
    - Yes, I can answer the questions on laboratory processing
    - No, I will skip the questions on laboratory processing. (proceed to "what happens to the results of environmental sampling")

Next page.

How samples are processed in the laboratory.

1. The culture method used is:

	Selective	Non-Selective	Don't know
MRSA	○	○	○
VRE	○	○	○
CPE	○	○	○
Pseudomonas	○	○	○
Acinetobacter	○	○	○

2. Is broth enrichment used?

	Selective	Non-Selective	No
MRSA	○	○	○
VRE	○	○	○
CPE	○	○	○
Pseudomonas	○	○	○
Acinetobacter	○	○	○

3. Before plating sample liquid is
    - Vortexed
    - Sonicated
    - Shaken
    - None of the above
    - I don't know

4. Plating technique
    - Spread plate
    - Drop count
    - Direct swabbing
    - N/A
    - I don't know

5. How are the results reported?
    - CFU/sample
    - CFU/area
    - Presence/absence
    - I don't know

6. Are molecular methods used?
    - Yes, to detect from a sample after culture
    - Yes, to detect directly from a sample
    - Yes, for another reason
    - No
    - I don't know

Next page.

What happens to the results of environmental sampling?

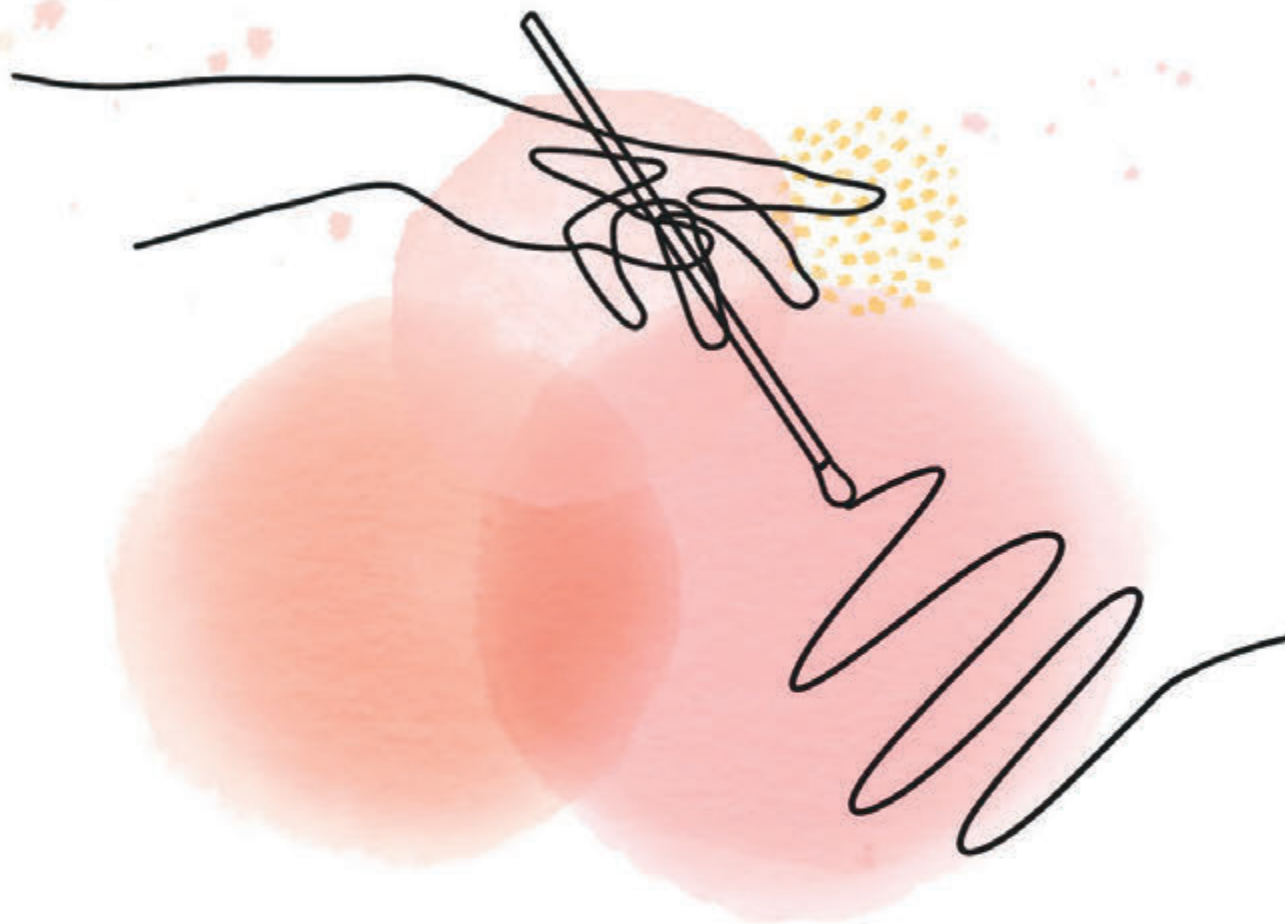
1. Who receives the results? (Check all that apply)
    - Infection control team
    - Clinical microbiologist
    - Clinical staff
    - Chief executive
    - Other (please specify)

Next page.

1. Is there any additional information about your surface sampling approach you would like to include? (Open field).

End of questionnaire.

Chapter 3.2



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

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. 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. 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. 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.

Introduction

Inanimate surfaces in hospitals, especially in patient rooms and bathrooms, can be a reservoir for pathogenic and possibly highly resistant microorganisms (HRMO) (1). From these environmental reservoirs, microorganisms can be transmitted to patients. Depending on the species, microorganisms are able to survive in the environment for long periods of time, ranging from a few hours up to several months or even years (2, 3). Environmental contamination of patient rooms can therefore be a prolonged source of pathogens. A review of 1,561 published outbreaks has identified that the hospital environment was the source in almost one fifth of the studied outbreaks (4). 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 (5, 6). 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* (7). 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 (8, 9). 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.

Methods

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 (Figure 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 (Figure 1).

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

Study setting

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.

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 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.

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.

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 100cm^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 100cm^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).

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 hours 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 <22mm 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 $bla_{\text{OXA-48-like}}$ 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 (10). All identified MRSA, VRE, ESBL-E, CPE, CP-PA and CR-AB isolates were stored at -80°C.

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 (Hong Kong, 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 (11, 12). 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 (13) Identification confirmation was performed using the Type strain genome server (TYGS) (https://tygs.dsmz.de)/ (14). 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.

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.

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.

Results

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 (Figure 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 (Figure 2, Table 1).

Figure 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.

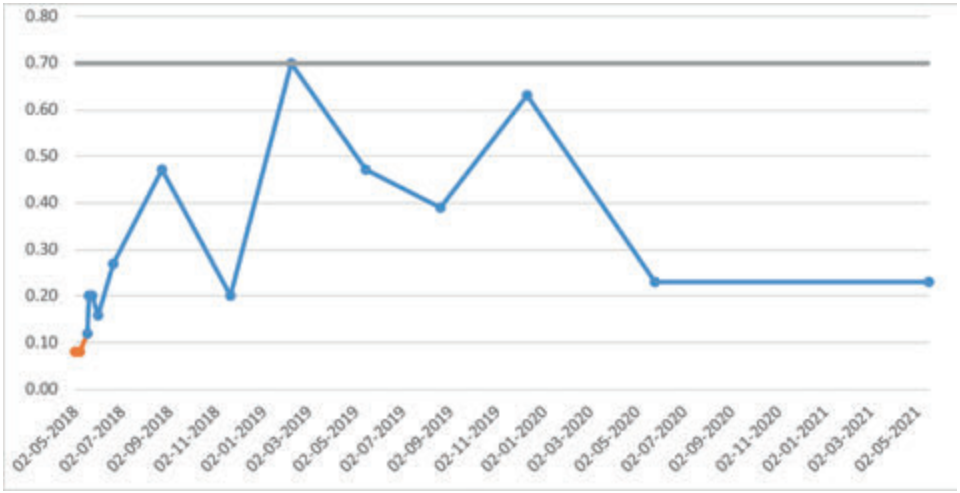

Table 1. The median CFU count per cm^2 determined in the new hospital building compared to the median CFU count per cm^2 determined in the old hospital building one month before relocating.

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	0.08	0.12	0.20	0.12	0.16	0.23	0.51	0.08	1.13	0.55	0.31	0.63	0.16	0.12
			↓89%	↓89%	↓84%	↓73%	↓84%	↓78%	↓69%	↓31%	↓89%	↑52%	↓26%	↓58%	↓15%	↓78%	↓84%
	Nightstand	1.46	0.20	0.23	0.39	0.66	0.59	1.05	0.86	1.37	0.41	3.4	1.09	0.94	2.19	0.7	0.43
			↓86%	↓84%	↓73%	↓55%	↓60%	↓28%	↓41%	↓6%	↓72%	↑133%	↓25%	↓36%	↑50%	↓52%	↓71%
	Table	1.29	0.04	0.12	0.10	0.20	0.39	0.51	0.66	0.78	0.47	3.13	0.78	0.31	2.19	0.27	0.39
			↓97%	↓91%	↓92%	↓84%	↓70%	↓60%	↓49%	↓40%	↓64%	↑143%	↓40%	↓76%	↑70%	↓79%	↓70%
	Wall	0.12	0	0	0	0	0.04	0	0	0.04	0.04	0.47	0.12	0	0.08	0.04	0
			↓100%	↓100%	↓100%	↓100%	↓67%	↓100%	↓100%	↓67%	↓67%	↑292%	0%	↓100%	↓33%	↓67%	↓100%
	Sink	0.31	0.12	0.04	0.12	0.20	0.08	0.08	0.27	0.39	0.04	0.59	0.31	0.59	0.27	0.16	0.23
			↓61%	↓87%	↓61%	↓35%	↓74%	↓74%	↓13%	↑26%	↓87%	↑90%	0%	↑90%	↓13%	↓48%	↓26%
	Top of sink plug	0.39	0	0.04	0.12	0.20	0.39	0.27	0.12	0.63	0	0.23	0.63	0.59	0.63	0.08	0.12
			↓100%	↓90%	↓69%	↓49%	0%	↓31%	↓69%	↑62%	↓100%	↓41%	↑62%	↑51%	↑62%	↓79%	↓69%
ICU room	Overall	0.25	0.04	0.12	0.18	0.18	0.20	0.08	0.08	0.12	0.21	0.08	0.14	0.12	0.33	0.10	0.08
			↓84%	↓62%	↓28%	↓28%	↓20%	↓68%	↓68%	↓62%	↓16%	↓68%	↓44%	↓62%	↑32%	↓60%	↓68%
	Nightstand	0.25	-	-	0.57	0.55	0.59	0.08	0.12	0.23	0.59	0.47	0.23	0.35	0.63	0.55	0.53
					↑128%	↑120%	↑136%	↓68%	↓52%	↓8%	↑136%	↑88%	↓8%	↑40%	↑152%	↑120%	↑112%
	Wall	0.06	0	0.04	0	0	0.04	0	0.02	0.02	0.04	0.04	0	0.06	0.27	0.10	0.04
			↓100%	↓33%	↓100%	↓100%	↓33%	↓100%	↓66%	↓66%	↓33%	↓33%	↓100%	0%	↑350%	↑67%	↓33%

Room with anteroom	Sink	1.56	0.18	0.20	0.33	0.45	0.20	0.21	0.21	0.47	0.29	0.14	0.20	0.16	0.23	0.06	0.12
			↓88%	↓87%	↓79%	↓71%	↓87%	↓87%	↓87%	↓70%	↓81%	↓91%	↓87%	↓90%	↓85%	↓96%	↓92%
	Top of sink plug	0.41	0.02	0.10	0.14	0.06	0.31	0.16	0.08	0.68	0.47	0	0.16	0.08	0.39	0	0.04
			↓95%	↓76%	↓66%	↓85%	↓24%	↓61%	↓80%	↑66%	↑15%	↓100%	↓61%	↓80%	↓5%	↓100%	↓90%
	Overall	0.23	0.20	0.12	0.47	0.04	0.12	0.12	0.23	0.16	0.16	0.18	0.16	0.23	0.39	0.16	0.20
			↓13%	↓48%	↑104%	↓83%	↓48%	↓48%	0%	↓30%	↓30%	↓22%	↓30%	0%	↑70%	↓30%	↓13%
	Nightstand	0.51	0.47	0.27	0.35	0.63	0.39	1.56	1.17	1.56	0.59	1.29	0.27	0.39	1.17	1.88	1.84
			↓8%	↓47%	↓15%	↑24%	↓24%	↑206%	↑129%	↑206%	↑16%	↑153%	↓47%	↓24%	↑129%	↑269%	↑261%
	Table	0.35	0.20	0.20	0.47	1.37	0.31	0.59	0.27	0.35	2.73	1.48	0.55	1.25	2.46	0.51	0.55
			↓43%	↓43%	↑34%	↑291%	↓11%	↑69%	↓23%	0%	↑680%	↑323%	↑57%	↑257%	↑603%	↑46%	↑57%
	Wall	0.04	0.12	0.04	0	0	0	0	0	0.04	0	0.16	0	0.04	0.08	0	0.12
			↑200%	0%	↓100%	↓100%	↓100%	↓100%	↓100%	0%	↓100%	↑300%	↓100%	0%	↑100%	↓100%	↑200%
	Sink	0.23	0.16	0.12	0.47	0	0.04	0.08	0.16	0.04	0.04	0.04	0.08	0.23	0.12	0.08	0.04
			↓30%	↓48%	↑104%	↓100%	↓83%	↓65%	↓30%	↓83%	↓83%	↓83%	↓65%	0%	↓48%	↓65%	↓83%
	Top of sink plug	0.2	0.20	0.08	0.04	0.04	0.20	0.08	0.27	0.35	0.08	0.08	0.04	0.08	0.39	0.08	0.08
			0%	↓60%	↓80%	↓80%	0%	↓60%	↑35%	↑75%	↓60%	↓60%	↓80%	↓60%	↑95%	↓60%	↓60%
Bathroom	Overall	1.76	0.08	0.08	0.10	0.39	0.51	0.55	1.02	1.76	1.13	3.81	1.52	3.24	3.40	1.64	1.31
			↓95%	↓95%	↓94%	↓78%	↓71%	↓69%	↓42%	0%	↓36%	↑116%	↓14%	↑84%	↑104%	↓7%	↓26%
	Toilet seat	1.56	0.10	0.16	0.12	0.27	0.98	1.15	1.04	1.52	2.58	3.05	1.09	2.25	1.66	2.13	1.27
			↓94%	↓90%	↓92%	↓83%	↓37%	↓26%	↓33%	↓3%	↑65%	↑96%	↓30%	↑44%	↑6%	↑37%	↓19%
	Shower chair	2.11	0.04	0.12	0.59	3.52	1.17	1.89	2.3	2.56	0.59	3.95	1.07	3.95	3.40	1.97	3.14
			↓98%	↓94%	↓72%	↑67%	↓45%	↓10%	↑9%	↑21%	↓72%	↑87%	↓49%	↑87%	↑61%	↓7%	↑49%

Shower drain	3.95	0.57	0.06	0.12	3.13	0.68	1.35	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95	3.95
		↓86%	↓98%	↓97%	↓21%	↓83%	↓66%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Door handle	2.07	0.08	0.10	0.08	0.20	0.25	0.57	0.25	0.86	0.45	2.77	1.33	0.82	1.66	0.47	0.20
		↓96%	↓95%	↓96%	↓90%	↓88%	↓72%	↓88%	↓58%	↓78%	↑34%	↓36%	↓60%	↓20%	↓77%	↓90%
Sink	0.94	0.06	0.14	0.04	0.31	0.20	0.10	0.66	1.17	0.57	3.40	1.04	2.25	2.27	0.80	0.61
		↓94%	↓85%	↓96%	↓67%	↓79%	↓89%	↓30%	↑24%	↓39%	↑262%	↑11%	↑139%	↑141%	↓15%	↓35%
Top of sink plug	1.76	0.04	0.04	0	0.47	0.27	0.04	0.37	1.56	0.41	3.67	0.66	2.64	0.51	1.68	0.82
		↓98%	↓98%	↓100%	↓73%	↓85%	↓98%	↓79%	↓11%	↓77%	↑109%	↓62%	↑50%	↓71%	↓5%	↓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.

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 a *mcr-9* variant gene, but this involved several clonally related isolates. Upon clone correction this involved 3 strains. Three *mcr*-9 positive isolates had a minimum inhibitory concentration (MIC) of 0.5 µg/mL, and four strains had an MIC of 8 µg/mL, as measured by Vitek2. No other *mcr* genes were detected. In isolates that were considered to be genetically closely related, variation in the presence of AMR genes was detected.

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

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 building was known since 2010, as a long-lasting multi-ward outbreak with the ICU as most affected ward (15). 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 (15-20). To contain this reservoir, a bundle of 'water free' patient care was introduced in the ICU in 2011 (20). 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) (18). 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 (21, 22). 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 (7, 23-25). 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 (26-28). 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 (29). 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 (29-31). 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 (7, 23-25). 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 (32). 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 (33). 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 (34, 35). 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 (36, 37).

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 (38, 39). 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 (40). Additionally, nosocomial infections with this ST, both in Europe and Asia, are increasingly reported (41, 42). The ST78 isolates we identified from the hospital environment were CPE and carried *bla*$_{\text{OXA-48}}$. 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.

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 (43). 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).

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.

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

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

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

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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.

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Supplemental file 1. Included patient rooms and their specialties

Both in the old and the new hospital building, we sampled three hematology rooms, four isolation rooms and 10 intensive care unit (ICU) rooms. The other rooms were from different medical specializations, which are shown in the tables below (Table 1 and table 2). Additionally, bathrooms were sampled from different specializations. In the old building, each department belonged to a specific specialization; in the new hospital building, specializations were combined into care cores. This was to organize care around the clinical picture or syndromes, instead of specializations. There are five care cores in the Erasmus MC; Movement, Nephrology and vascular disease, Systemic diseases, Gastroenterology including oncology, and Gastroenterology and hepatology. Care core Movement consists of the specializations Dermatology, Trauma, Orthopedics, and Plastic surgery. Nephrology and vascular disease consists of Dermatology, General surgery, and Internal surgery. Systemic diseases from Dermatology, Internal medicine, and Rheumatology. Gastroenterology including oncology, consists of Dermatology, General surgery, and Gastroenterology and hepatology. The care core Gastroenterology and hepatology consists of the specializations General surgery, and Gastroenterology and hepatology.

Supplementary Table 1.1. Sampled rooms and their respective specialization in the old hospital building

Room type	Room number	Specialty
Four-person room	Room #1	Gastroenterology and hepatology
Four-person room	Room #2	Gastroenterology and hepatology
Four-person room	Room #3	Nephrology
Four-person room	Room #4	Internal medicine
Four-person room	Room #5	Rheumatology
Four-person room	Room #6	Neurosurgery
Four-person room	Room #7	Neurosurgery
Four-person room	Room #8	Neurosurgery
Four-person room	Room #9	Orthopedics
Four-person room	Room #10	Orthopedics
Four-person room	Room #11	Plastic surgery
Four-person room	Room #12	General surgery
Four-person room	Room #13	General surgery
Four-person room	Room #14	General surgery
Four-person room	Room #15	General surgery
Two-person room	Room #1	Gastroenterology and hepatology
Two-person room	Room #2	Internal medicine
Two-person room	Room #3	Nephrology
Two-person room	Room #4	Neurosurgery
Two-person room	Room #5	Neurosurgery

Two-person room	Room #6	Orthopedics
Two-person room	Room #7	General surgery
Two-person room	Room #8	General surgery
Two-person room	Room #9	General surgery
Two-person room	Room #10	General surgery
Bathroom	Room #1	Gastroenterology and hepatology
Bathroom	Room #2	Rheumatology
Bathroom	Room #3	Orthopedics
Bathroom	Room #4	Neurosurgery
Bathroom	Room #5	General surgery
Bathroom	Room #6	General surgery
Bathroom	Room #7	Isolation
Bathroom	Room #8	Hematology
Bathroom	Room #9	Hematology

Supplementary Table 1.2. Sampled rooms and their respective specialization in the new hospital building.

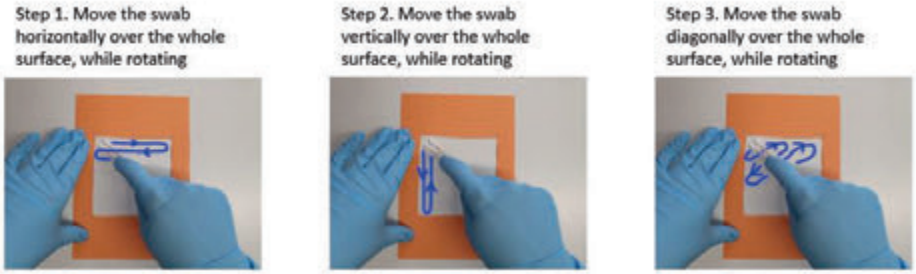
Room type	**Room number**	**Specialty or care core**
Single-occupancy room	Room #1	Neurology
Single-occupancy room	Room #2	Neuro surgery
Single-occupancy room	Room #3	Neuro surgery
Single-occupancy room	Room #4	Movement
Single-occupancy room	Room #5	Movement
Single-occupancy room	Room #6	Nephrology and vascular disease
Single-occupancy room	Room #7	Nephrology and vascular disease
Single-occupancy room	Room #8	Systemic diseases
Single-occupancy room	Room #9	Systemic diseases
Single-occupancy room	Room #10	Systemic diseases
Single-occupancy room	Room #11	Gastroenterology, including oncology
Single-occupancy room	Room #12	Gastroenterology, including oncology
Single-occupancy room	Room #13	Gastroenterology and hepatology
Single-occupancy room	Room #14	Gastroenterology and hepatology
Single-occupancy room	Room #15	Gastroenterology and hepatology
Single-occupancy room with sampled bathroom	Room #1	Neurology, stroke unit
Single-occupancy room with sampled bathroom	Room #2	Neuro surgery
Single-occupancy room with sampled bathroom	Room #3	Hematology
Single-occupancy room with sampled bathroom	Room #4	Isolation

Single-occupancy room with sampled bathroom	Room #5	Movement
Single-occupancy room with sampled bathroom	Room #6	Nephrology and vascular disease
Single-occupancy room with sampled bathroom	Room #7	Systemic diseases
Single-occupancy room with sampled bathroom	Room #8	Gastroenterology, including oncology
Single-occupancy room with sampled bathroom	Room #9	Gastroenterology, including oncology
Single-occupancy room with sampled bathroom	Room #10	Gastroenterology and hepatology

Supplemental file 2. Sampled locations

Both in the old and the new building, swabs were taken from all locations. Two cotton swabs were used per location, and a standardized surface of 100 cm2 was sampled. For the nightstand, table, wall, sink, shower chair and the sink in the bathroom, we used a frame of 10 by 10 cm2. To accommodate for the toilet seat, frames of 5 by 20 cm2 were used. Due to the shape of the top and bottom of the sink plug, and the shower drain and door handle, no frames were used to sample these locations. Instead, the whole surface was sampled.

Supplementary figure 2.1. Taking the swab.



Supplementary figure 2.2. The sink plug

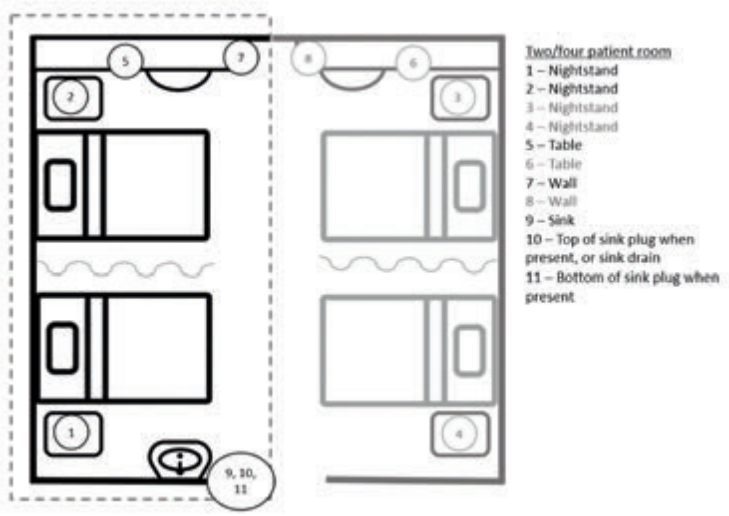
Sampled locations in the old building

Patient room (N)	Bathroom (N)
Nightstand (97)	Toilet seat (9)
Table (47)	Shower chair (9)
Wall (57)	Shower drain (9)
Sink (44)	Door handle on the inside (9)
Top of sink plug or grid of sink drain (44)	Sink (9)
Bottom of sink plug (22)	Top of sink plug or grid of sink drain (9)
	Bottom of sink plug (1)

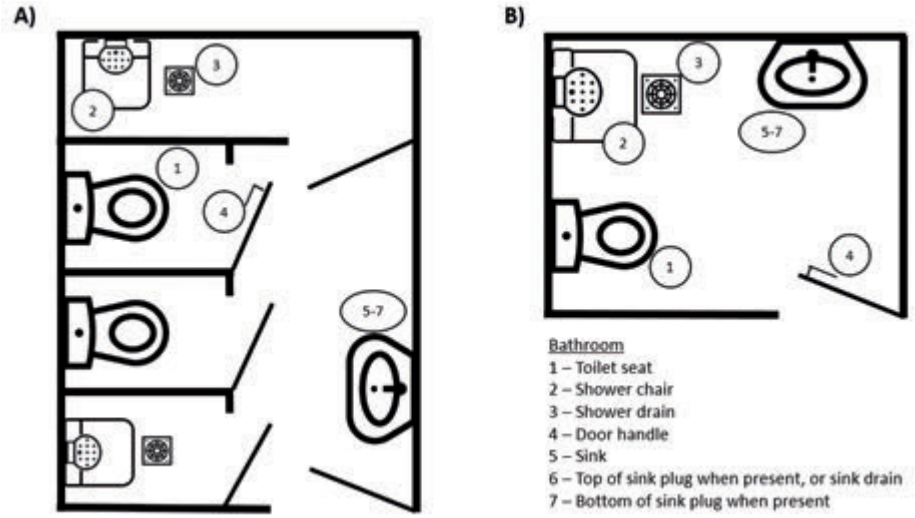
Sampled locations in the new building

Patient room (N)	Bathroom (N)
Nightstand (40)	Toilet seat (10)
Table (30)	Shower chair (10)
Wall (40)	Shower drain (10)
Sink (42)	Door handle on the inside (10)
Top of sink plug or grid of sink drain (42)	Sink (10)
Bottom of sink plug (42)	Top of sink plug or grid of sink drain (10)
	Bottom of sink plug (9)

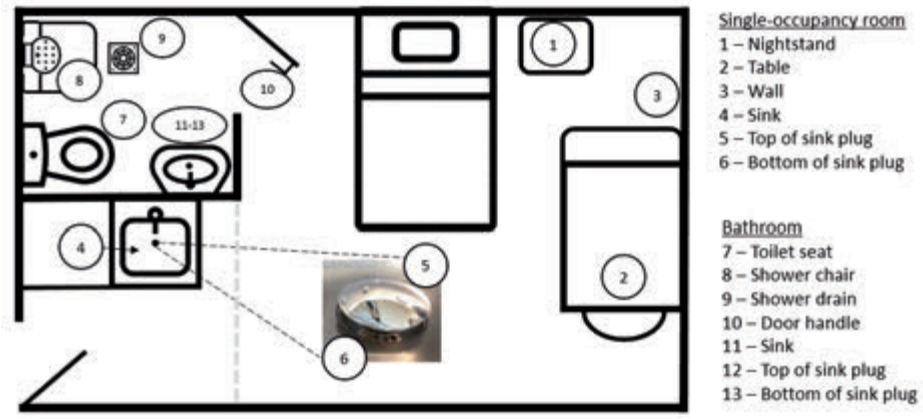
Supplementary figure 2.3. Floorplan of two- and four patient room in the old hospital building with sampled locations. Additional sampling locations for four patient rooms are indicated in grey.



Supplementary figure 2.4. Floorplan of bathrooms in the old hospital building, with sampled locations



Supplementary figure 2.5. Floorplan of single-occupancy room in the new building, with sampled locations. The light grey line indicates where the door of the ante room is located, when an ante room is present.



Supplemental file 3. The median CFU counts per cm^2 per sampled surface in the old hospital building

Supplementary Table 3.1. The median CFU counts per cm^2 per sampled surface in the five room types in the old hospital building, one month and one week before relocating patients to the new hospital building.

Room type	Sample location	CFUs per cm^2 one month before relocating patients	CFUs per cm^2 one week before relocating patients	*P*-value
Two-bed patient rooms (n=10)	Overall	0.57	0.51	0.334
	Nightstand	1.99	0.78	**0.005**
	Table	1.37	0.64	0.393
	Wall	0.23	0.10	0.218
	Sink	0.18	0.55	0.063
	Top of sink plug	0.29	1.07	0.315
Four-bed patient rooms (n=15)	Overall	0.76	0.51	**0.003**
	Nightstand	1.33	0.78	**0.001**
	Table	1.25	0.55	**<0.001**
	Wall	0.12	0.10	0.273
	Sink	0.70	0.51	0.775
	Top of sink plug	0.39	0.98	0.126
ICU rooms (n=10)	Overall	0.25	0.12	0.070
	Nightstand	0.25	0.12	0.075
	Wall	0.06	0.06	0.853
	Sink	1.56	0.21	0.478
	Top of sink plug	0.41	0.06	0.347
Rooms with anteroom (n=7)	Overall	0.23	0.12	0.638
	Nightstand	0.51	0.78	0.318
	Table	0.35	0.27	0.902
	Wall	0.04	0.04	0.902
	Sink	0.23	0.20	0.710
	Top of sink plug	0.20	0.70	0.620
Bathrooms (n=9)	Overall	1.76	1.56	0.822
	Toilet seat	1.56	1.56	0.673
	Shower chair	2.11	3.95	0.931
	Shower drain	3.95	3.95	0.094
	Door handle	2.07	1.17	0.423

	Sink	0.94	0.78	0.503
	Top of sink plug	1.76	1.29	0.766

Abbreviations: CFU, colony forming units; ICU, intensive care unit. Bold *P*-values are significant+

Supplemental file 4. Results of whole genome sequencing

Details of sequenced isolates showing their identification, isolation location, conventional sequence type, cg/wgMLST clustering info and a selection of antibiotic resistance genes detected by CARD analysis. The CARD analysis was focused on different types of beta-lactamases (illustrated with different colors) and aminoglycoside modifying enzymes. A black background indicates presence of the gene. Numbers indicate the percentage of similarity to the CARD reference sequence.

Legend	
	Beta-lactamase
	ESBL type beta-lactamase
	ampC type beta-lactamase
	Carbapenemase
	Aminoglycoside modifying enzyme
	Miscellaneous

Species	Sample nr.	Building	MLST	cg/wgMLST Cluster	AAC(6')-Ii	aad(6)	APH(3')-IIIa	vanB	Plasmid replicon types
E. faecium	M-026	new	ST117	A	98.9	100.0	100.0	99.4	rep2, rep11a, repUS15
E. faecium	M-027	new	ST117	A	98.9	100.0	100.0	99.4	rep2, rep11a, repUS15
E. faecium	M-030	new	ST117	A	98.9	100.0	100.0	99.4	rep2, rep11a, repUS15

Species	Sample nr.	Building	MLST	cg/wgMLST Cluster	CARB-3	OXA-395	PDC-3	VIM-2	AAC(6')-29a	AAC(6')-29b	AAC(6')-Ib'	AAC(6')-Ib9	aadA3	APH(3')-IIb	Plasmid replicon types
P. aeruginosa	M-112	old	ST111	A	99.7	100.0	100.0	100.0	#	100.0		98.0	98.5	98.5	N.A.
P. aeruginosa	M-115	old	ST111	A	99.7	100.0	100.0	100.0	#	99.3		98.0	98.5	98.5	N.A.
P. aeruginosa	M-117	old	ST111	A	99.7	100.0	100.0	100.0	99.2	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-119	old	ST111	A	99.7	100.0	100.0	100.0	#	100.0		99.5	98.5	98.5	N.A.
P. aeruginosa	M-140	old	ST111	A	99.7	100.0	100.0	100.0	#	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-141	old	ST111	A	99.7	100.0	100.0	100.0	#	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-143	old	ST111	A	99.7	100.0	100.0	100.0	#	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-149	old	ST111	A	99.7	100.0	100.0	100.0	100.0	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-154	old	ST111	A	99.7	100.0	100.0	100.0	99.2	#		99.5	98.5	98.5	N.A.
P. aeruginosa	M-156	old	ST111	A	99.7	100.0	100.0	100.0	#	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-1484	old	ST111	A	99.7	100.0	100.0	100.0	99.2	#		99.5	98.5	98.5	N.A.
P. aeruginosa	M-1487	old	ST111	A	99.7	100.0	100.0	100.0	#	100.0		99.5	98.5	98.5	N.A.
P. aeruginosa	M-1493	old	ST111	A	99.7	100.0	100.0	100.0	99.2	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-110	old	ST111	B	99.7	100.0	100.0	100.0	100.0	#		98.0	98.5	98.5	N.A.
P. aeruginosa	M-111	old	ST111	B	99.7	100.0	100.0	100.0	100.0	#	100.0		98.5	98.5	N.A.
P. aeruginosa	M-139	old	ST111	B		100.0	100.0	100.0	#	#				98.5	N.A.
P. aeruginosa	M-155	old	ST111	B		100.0	100.0	100.0	#	100.0				98.5	N.A.
P. aeruginosa	M-157	old	ST111	B		100.0	100.0	100.0	100.0	#				98.5	N.A.
P. aeruginosa	M-158	old	ST111	B		100.0	100.0	100.0	100.0	#				98.5	N.A.
P. aeruginosa	M-109	old	ST111	C		100.0	100.0	100.0	100.0	#				98.5	N.A.
P. aeruginosa	M-130	old	ST111	C		100.0	100.0	100.0	#	#				98.5	N.A.
P. aeruginosa	M-147	old	ST111	C		100.0	100.0	100.0	#	#				98.5	N.A.
P. aeruginosa	M-134	old	ST111	unique		100.0	100.0	100.0	#	100.0				98.5	N.A.
P. aeruginosa	M-145	old	ST111	unique	99.7	100.0	100.0	100.0	#	#		99.5	98.5	98.5	N.A.

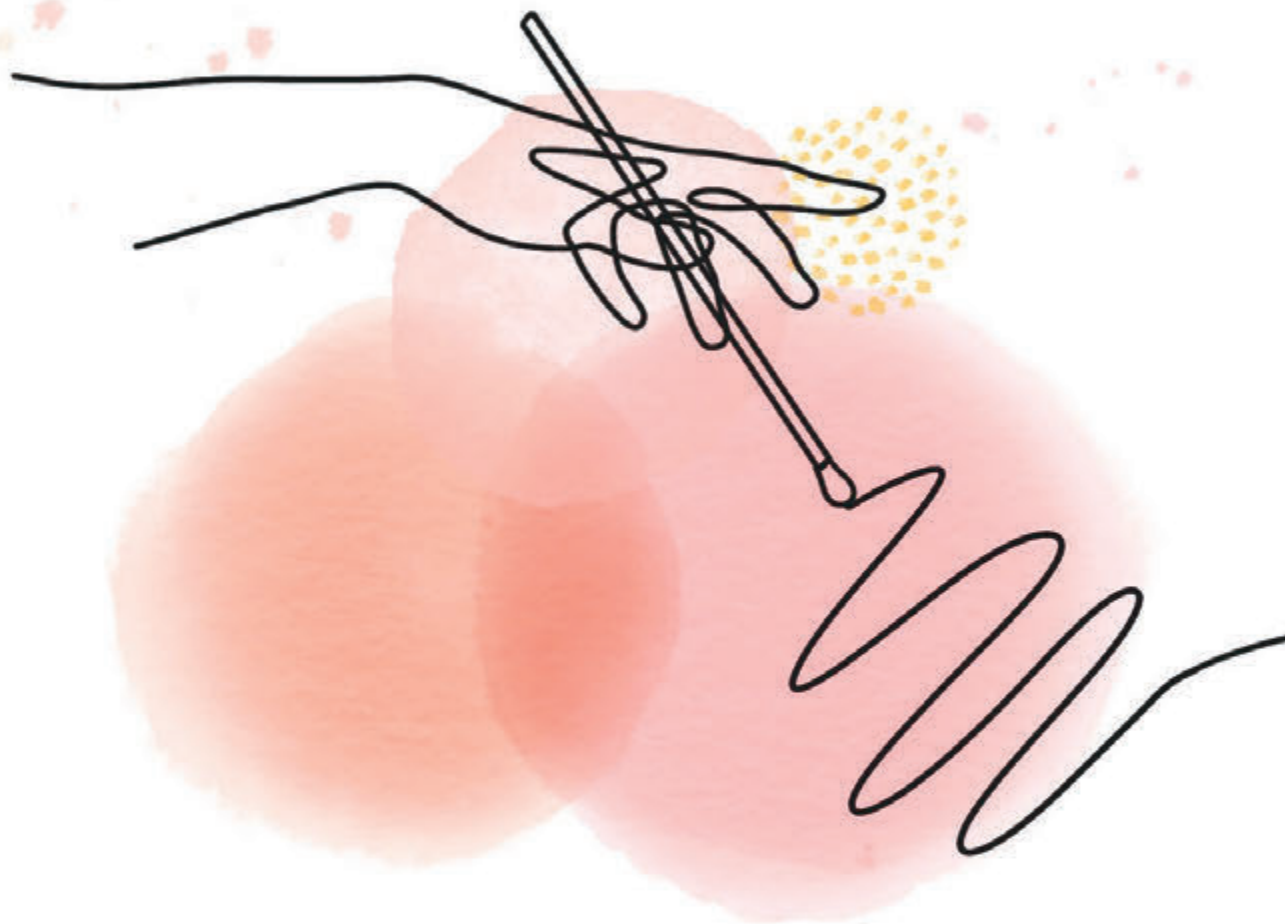
Species	Sample nr.	Building	MLST	cg/wgMLST Cluster	OXA-1	OXA-10	TEM-1	CTX-M-15	CTX-M-238	SHV-66	CMY-48	CMY-65	CMY-81	KPC-2	AAC(3)-IId	AAC(3)-IIe	AAC(6')-Ib8	AAC(6')-Ib-cr6	AAC(6')-If	aadA2	aadA3	ANT(2'')-Ia	ANT(3'')-IIa	APH(3')-Ia	MCR-9.1	Plasmid replicon types
C. amalonaticus	M-123	old	N.A.	N.A.	100.0				100.0							99.0		99.0								IncFII(K)
C. freundii	M-116	old	ST169	A			100.0					100.0		100.0	100.0		98.2			100.0						IncFII(pHCM2), IncR, IncX5
C. freundii	M-129	old	ST169	A								100.0		100.0												IncFII(pHCM2), IncR, IncX5
C. freundii	M-1490	old	ST169	A								100.0		100.0												IncFII(pHCM2), IncR, IncX5
C. freundii	M-1494	old	ST169	A								100.0		100.0												IncFII(pHCM2), IncR, IncX5
C. freundii	M-035	old	ST311	B						99.7			100.0												100.0	IncX3, pKPC-CAV1321
C. freundii	M-137	old	ST311	B						99.7			100.0												100.0	IncX3, pKPC-CAV1321
C. freundii	M-118	old	ST111	unique	100.0	100.0	100.0	100.0								99.0		99.0				99.6	100.0			IncC, IncR
C. freundii	M-150	old	ST125	unique							100.0								97.2		98.5	100.0				IncFII(SARC14)

Species	Sample nr.	Building	MLST	cg/wgMLST Cluster	LAP-2	OXA-1	OXA-9	TEM-1	CTX-M-9	CTX-M-15	GES-1	SHV-66	ACT-6	ACT-17	ACT-24	ACT-57	MIR-20	MIR-23	OXA-48	AAC(3)-IIe	AAC(6')-Ib10	AAC(6')-Ib4	AAC(6')-Ib-cr4	AAC(6')-Ib-cr6	aadA	aadA2	aadA3	ANT(2'')-Ia	ANT(3'')-IIa	APH(3'')-Ib	APH(6)-Id	MCR-9.1	Plasmid replicon types
E. asburiae	M-132	old	ST484	A		100.0		100.0		100.0			99.0							99.0					99.6					99.6	99.6	100.0	IncFIB(pECLA), IncFII(pECLA)
E. asburiae	M-144	old	ST484	A		100.0				100.0			99.0							99.0					99.6					99.6	99.6	100.0	IncFIB(pECLA), IncFII(pECLA)
E. asburiae	M-142	old	ST484	A				100.0		100.0			99.0															99.6	99.6	99.6	100.0	100.0	IncFIB(pECLA), IncFII(pECLA)
E. asburiae	M-146	old	ST484	A		100.0		100.0		100.0			99.0										100.0						99.6	99.6	99.6	100.0	IncFIB(pECLA), IncFII(pECLA)
E. asburiae	M-152	old	ST25	unique								99.7				100.0				99.0	96.7							98.5	100.0			100.0	IncFIB(pECLA), IncFII(pECLA), IncHI2, IncHI2A
E. hormaechei subsp. hoffmannii	M-125	old	ST78	C	100.0						100.0	100.0		100.0					100.0			98.4											Col(pHAD28), IncFIB(pHCM2), IncL, IncQ2
E. hormaechei subsp. hoffmannii	M-138	old	ST78	C	100.0						100.0	100.0		100.0					100.0			98.4											Col(pHAD28), IncFIB(pHCM2), IncL, IncQ2
E. hormaechei subsp. hoffmannii	M-1488	old	ST78	C	100.0						100.0	100.0		100.0					100.0			98.4											Col(pHAD28), IncFIB(pHCM2), IncL, IncQ2
E. hormaechei subsp. hoffmannii	M-1491	old	ST78	C	100.0						100.0	100.0		100.0					100.0			98.4											Col(pHAD28), IncFIB(pHCM2), IncL, IncQ2
E. hormaechei subsp. hoffmannii	M-1456	new	ST78	D	100.0						100.0	100.0		100.0					100.0			98.4											Col(pHAD28), IncFIB(pHCM2), IncL, IncQ2
E. hormaechei subsp. hoffmannii	M-1458	new	ST78	D	100.0						100.0	100.0		100.0					100.0														Col(pHAD28), IncFIB(pHCM2), IncL
E. hormaechei subsp. hoffmannii	M-1459	new	ST78	D	100.0						100.0	100.0		100.0					100.0														Col(pHAD28), IncFIB(pHCM2), IncL
E. hormaechei subsp. steigerwaltii	M-135	old	ST45	unique				100.0						100.0				100.0											99.6	99.6			Col(pHAD28), IncFIB(pECLA)
E. roggenkampii	M-121	old	new	B														100.0															none
E. roggenkampii	M-131	old	new	B														100.0															none
E. roggenkampii	M-148	old	ST1576	unique		100.0		100.0		100.0								100.0		99.0					100.0		100.0	99.6					IncFIB(pECLA), IncFII(pECLA), IncN

Species	Sample nr.	Building	MLST	cg/wgMLST Cluster	OXA-1	SHV-1	SHV-201	TEM-1	CTX-M-14	CTX-M-15	DHA-1	AAC(3)-IId	AAC(3)-IIe	AAC(6')-Ib-cr6	aadA2	APH(3')-Ia	APH(3'')-Ib	APH(6)-Id	Plasmid replicon types
K. aerogenes	M-151	old	N.A.	N.A.				100.0		100.0		100.0							ColRNAI, IncI1-I(Alpha), IncX3
K. pneumoniae	M-083	new	ST45	unique	100.0	100.0		100.0	100.0		100.0			99.0		98.5			Col156, IncFIB(AP001918), IncFIB(K), IncFII(29), IncFII(K), IncFII(pKP91), IncR
K. pneumoniae	M-114	old	ST1564	unique	100.0		100.0	100.0		100.0			99.0	99.0			99.6	99.6	IncFIB(K)
K. pneumoniae	M-133	old	ST16	unique	100.0	100.0		100.0		100.0				99.0	100.0				Col440II, IncFIB(K), IncFII(K)

N.A. Not applicable. # In P. aeruginosa ST111 the VIM-2 gene is reported to be flanked by AAC(6')-29a and AAC(6')-29b elements. Due to the limitations of short-read sequencing only, these do not always end up in the assembly and thus remain unnoticed by CARD analysis. Presence of these elements was verified by a resequencing approach.

Chapter 4



SUMMARIZING DISCUSSION AND FUTURE PERSPECTIVES

Multidrug-resistant microorganisms (MDRO) are increasing worldwide and are leading to increased healthcare costs, morbidity, and mortality (1, 2). They are a frequent source of healthcare-associated infections (HAI), hindering antimicrobial treatment (3). HAI can have an endogenous source, *i.e.,* body sites such as the skin, or an exogenous source, *e.g.,* the hospital environment, its surfaces, or healthcare workers. This thesis focused on endogenous sources by screening patients upon admission to the hospital, and on exogenous sources, specifically the hospital environment, of MDRO and *Staphylococcus aureus*. We aimed to determine the effect of transitioning to a newly constructed hospital with 100% single-occupancy rooms and private bathrooms on the microbial safety of the hospital. We consider the microbial safety of the new hospital as improved when the environmental contamination in general and/or with MDRO is lower, and/or when the acquisition and/or transmission of MDRO is lower compared to the old hospital. The studies in this thesis were divided into patient related and environmental related research. In the patient related research chapter (**Chapter 2**), we determined the effect on acquisition of ESBL-E (**Chapter 2.1**). Additionally, we aimed to determine screening methods to identify patients colonized with MDRO upon admission (**Chapter 2.2** and **Chapter 2.3**). Furthermore, we aimed to determine the dynamics between patients and the hospital environment for *Staphylococcus aureus* (**Chapter 2.4**). In the environmental related research chapter (**Chapter 3**), we performed a survey to determine current sampling practices throughout Europe (**Chapter 3.1**). Finally, we determined the effect of the new environment, *i.e.,* single rooms, on environmental contamination of the patient room and bathroom (**Chapter 3.2**).

Dynamics of MDRO and *S. aureus* colonization during hospitalization

The main goal of infection prevention and control is to prevent or to stop spread of nosocomial MDRO throughout the hospital. For example, patients who are known carriers are cared for in isolation, and patients at risk for being a MDRO carrier are screened and cared for in isolation depending on the type of MDRO at risk. Upon identification of a new carrier, contact tracing can be performed to determine if transmission occurred. While these are important practices, there are still many knowledge gaps, including colonization rates upon admission to the hospital, and the dynamics of colonization during hospitalization.

In the Netherlands, patients are not routinely screened for MDRO colonization upon admission to the hospital. Consequently, the true carriage rate upon admission is not known. Additionally, this makes it likely that MDRO carriers are not identified, and as a consequence, transmissions within the hospital go unrecognized. In **Chapter 2.1** and **Chapter 2.2**, we have determined the carriage rates of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales (ESBL-E) upon admission to the hospital; in **Chapter 2.3** we determined the colonization rate for MDRO upon admission; and in **Chapter 2.4** we have determined the carriage rates for *S. aureus* upon hospitalization. We identified a carriage rate for ESBL-E between 4.4% and 6.5%, with no significant differences between the old and the new hospital building (4, 5). While these prevalence rates are low, they are not unexpected and are comparable to prevalence rates identified in other studies in the

Netherlands. Arcilla et al., identified a carriage rate of 6.1% in healthy volunteers and Kluytmans-van den Bergh et al, identified a carriage rate at admission to the hospital between 6.4 and 7.4% (6, 7). Additionally, in a trans-European cross-sectional study, a carriage rate of 6% was identified (8). Interestingly, the carriage rates in the Netherlands remained almost constant over the years. When compared to surveillance data for *E. coli*, the percentage of resistant isolates was between ranged between 6.4% and 7.5% between 2016 and 2021, with a prevalence of 6.6% in 2021 (9). Among the 1155 included patients, we did not identify patients positive for vancomycin-resistant *E. faecium* (VRE), multi-drug resistant *Pseudomonas aeruginosa*, multi-drug resistant *Acinetobacter baumannii*, or carbapenemase-producing Enterobacterales upon admission. The prevalence of these MDRO are low in the Netherlands, and consequently it is not unexpected that we did not identify carriers (9). One patient (0.1%) was positive for methicillin-resistant *S. aureus* (MRSA) upon admission to the hospital, which is in line with the prevalence of 0.07-0.13% (10, 11). Carriage rates for methicillin-susceptible *S. aureus* (MSSA) was 27.0%, which is in line with other studies (10, 11).

During hospitalization, acquisition or loss of MDRO and/or *S. aureus* can occur. These dynamics are relatively unknown, as patients are not routinely screened for colonization upon admission and during hospitalization. We have determined these dynamics for ESBL-E in **Chapter 2.1** and for *S. aureus* in **Chapter 2.4**. We observed that 19 out of 597 (3.2%) of patients acquired an ESBL during hospitalization, of which 18 patients (94.7%) were not identified through clinical cultures. Whole genome sequencing (WGS) was performed on all available isolates, and showed no transmission between identified patients. Consequently, the source was either a patient who was not included in the MOVE-study, the hospital environment, or less probable, health care workers or visitors. Another possibility is that the admission culture was a false-negative culture due to very low abundance which was increased by antibiotic use during admission period and therefore became detectable at discharge.

Other studies determining transmission of ESBL-E during hospitalization showed transmission rates of 1.5% to 5.4% (7, 12-15). In contrast to our study, the ESBL-E status as described in the published studies, were known by healthcare workers, and therefore, in most studies patients were cared for in isolation upon identification of ESBL-E. Consequently, their transmission rates cannot be directly extrapolated to unidentified carriers who are not cared for in isolation during their hospitalization, as in our study. Although our observed acquisition rate of 3.2% is comparable to the transmission rates in the other studies, we have not shown transmission between patients. It is not likely that no transmission between patients occurred. Our inability to show transmission is a consequence of the fact that we were not able to include all patients admitted to the hospital. It is reasonable to conclude that transmissions between patients were missed.

In **Chapter 2.4**, we determined the dynamics of *S. aureus* during hospitalization. We showed acquisition in 15 out of 673 (2.2%) patients. Additionally, we determined if *S. aureus* identified in study samples were identical to clinical samples, or otherwise if they were

assumed to be from an exogenous source. From 19 patients, clinical samples were taken during the same hospitalization period as the study sample was taken. For three of these 19 patients (15.9%), the *S. aureus* was not identical to the clinical strain, indicating a potential exogenous source. It is important to prevent acquisition of *S. aureus,* as non-carriers have a higher mortality following bacteremia compared to carriers (16). It is estimated that 80% of nosocomial *S. aureus* infections are endogenous, and approximately 20% exogenous (16, 17). This is in line with our finding that 15.9% of patients had a potential exogenous source. Another explanation for the difference between the nasal and clinical strain could be that patients carried multiple strains upon admission, of which we only identified one (11, 18). However, only 6.6-10% of *S. aureus* carriers carry multiple strains simultaneously. While morphologically different *S. aureus* from one nasal sample were analyzed, we did not identify multiple *S. aureus* strains in any of the nasal samples. Besides acquisition, we showed that 35.2% of patients positive for MSSA upon admission were negative at discharge. Although we did not determine the use of antibiotics, antibiotic use could explain the relatively large proportion no longer colonized or not cultivable upon discharge.

Overall, our results regarding prevalence of MSSA, MRSA, and ESBL-E are in line with previous Dutch studies, but we identified that transmission within the hospital often remains unidentified. We showed that transmission in the hospital is invisible, which highlights gaps in the current surveillance methods. In total, during the study period, we observed acquisition of ESBL-E in 20 patients, and of MSSA in 15 patients, which theoretically could have been prevented. Consequently, **we conclude** that there is a need for improving or adjusting current surveillance methods within the hospital.

Screening of patients upon admission

In **Chapter 2.1** we observed that 91.6% of ESBL-E carriers were unknown carriers upon admission, and remained unidentified throughout their hospitalization (4). Consequently, these patients were not cared for in isolation, increasing the chances on transmission to other patients. The majority of these patients remained unidentified carriers throughout their hospitalization.

The high percentage of unidentified carriers highlights the need for effective screening methods upon admission. Currently, there is a nationally implemented universal risk assessment combined with risk-based screening upon admission to the hospital. This assessment consists of a number of questions determining the risk on being colonized with a MDRO (19). When a patient is deemed at risk, risk-based screening is performed and, depending on if the patient is considered to be at high or low risk, the patient is preemptively placed in isolation. However, van Hout et al. showed that the currently nationally installed universal risk assessment combined with risk-based screening is not effective in identifying MDRO carriers upon admission (20). They identified that the majority of carriers identified through the risk assessment were identified through the question: “are you a known carrier of a MDRO?”. Consequently, they propose abandoning the currently

installed risk-assessment and transfer to a system where (previously) known MDRO carriers are cared for in isolation. However, we feel that more research is needed before such a drastic change in policy is made. For this, we advocate to improve the risk assessment or to consider the possibility of different screening strategies. This could be done by adding questions about risk factors to the risk-assessment, or by evaluating other screening strategies, such as a universal screening strategy upon admission (Table 1).

Table 1. Overview of current risk assessment and proposed improvements and other screening strategies

Current risk-assessment of the Erasmus MC
1) Is the patient a known carrier of a MDRO?
2) Has the patient recently been treated in or admitted to a healthcare institution abroad?
3) Did the patient stay in a healthcare facility known with a MDRO outbreak in the past two months, and if yes, was the patient approached for screening?
4) Has the patient lived in an institution for asylum seekers in the past two months?
5) Does the patient live or work where pigs, veal calves or broilers are kept commercially?
6) Is the patient a partner, housemate or caregiver of someone who is MRSA positive?
7) Is the patient a professional seafarer?
Proposal for additional questions in the risk-assessment
8) Did you recently travel to South East Asia?
9) Did you recently consume antibiotics, and if yes, what types?
10) ….
Alternative strategy
Universal screening upon admission
Screening of specific patient population upon admission

The universal risk assessment does not ask about all known risk factors, such as antibiotic usage in the last 90 days, specifically use of fluoroquinolones and beta-lactams (6, 21, 22), or recent travel history (6, 23-25). However, studies determining MDRO colonization after travel are often performed in healthy volunteers and cannot be directly extrapolated to patients admitted to our hospital. In **Chapter 2.2**, we determined if patients admitted to our hospital recently traveled, and if yes, if they were colonized with a MDRO upon admission (5). We observed that almost 47.4% of patients travelled in the year before hospitalization, the majority of which within Europe and only a small number of patients outside of Europe. Colonization rates among travelers was 6.0%, compared to 6.2% among non-travelers. Although numbers were small and differences were non-significant, carriage rates were lower among travelers inside of Europe (3.4%) compared to travelers outside of Europe (13.3%). Our results indicate that the carriage rate increased when we looked at patients who travelled outside Europe <3 months before hospitalization (29%), <2 months (40%), and <1 month (67%). For patients who travelled to Asia or Africa, carriage rates were even

higher. This is in line with the study of Arcilla et al., who showed the highest carriage rates for ESBL-E upon returning from Southern Asia, central and eastern Asia, and Northern Africa among healthy volunteers (23). Additionally, Voor in 't holt et al. showed the highest carriage rates of multidrug resistant Enterobacterales after travel to Southern Asia and Northern Africa (25). While more research into the burden of travel-related MDRO carriage and possible consequences within the hospital environment, our results show that including questions regarding recent travel to Asia or Africa could be an addition to the universal risk assessment. According to our study, the majority of patients (90.9%) would support a screening strategy based on travel history. Therefore, **we conclude** that adding a question regarding recent travel behavior to the universal risk-assessment would be accepted by patients and would be beneficial in identifying ESBL-E carriers.

While adding questions to the universal risk assessment might increase the identification of MDRO carriers, implementing this would not be without consequences. Van Hout et al. determined that the universal risk-assessment currently is associated with a high workload (20). They estimated that during the four-and-a-half years of their study, 160 working weeks of 36h were spent on performing and administration of the risk assessment. Adding questions to the risk assessment would only add to the current workload. Additionally, the question remains if it is necessary to ask about all risk factors. Besides increasing the workload for healthcare workers, the questions could also become a burden for patients. For example, Lekkerkerk et al. identified that having at least one foreign parent is a risk factor for MRSA carriage (26). This type of personal questions could be an imposition for patients. Furthermore, the yield of an improved risk assessment still needs to be determined. It is an illusion that a risk-based screening strategy could be 100% effective in identifying all carriers, as not all carriers will have a risk factor. Moreover, it is debatable if it is necessary to identify all MDRO carriers. As stated before, the majority of ESBL-E carriers identified in **Chapter 2.1** were and remained unidentified through clinical cultures and were not regularly screened for carriage. Therefore, it could be concluded that identifying these carriers was not of clinical importance. However, we also showed acquisition in 19 patients. Consequently, transmission within the hospital occurs, also if identification was not of clinical importance for the index patients. As a hospital, you aim to provide a microbial safe environment for your patients, and as part of this, it is important to have a screening strategy with the highest possible yield, but with a balanced outcome and effort. Therefore, if we want to increase the yield of screening strategies upon admission, other screening strategies should also be considered. These strategies could potentially lead to a decrease in workload and pose less of a burden for patients.

We were in the unique position to have admission cultures from a large group of patients, independent from the universal risk assessment combined with risk-based screening. Consequently, we were able to compare the yield of universal screening strategy with the yield of the universal risk assessment combined with risk-based screening, of which the results were presented in **Chapter 2.3**. We found that a universal screening strategy identified more MDRO compared to the universal risk assessment, although the risk-based

screening identified a vancomycin-resistant *Enterococcus faecium* that was missed through the universal screening. An explanation for this could be that we used perianal samples instead of rectal samples, which have a higher recovery rate (27). That universal screening identified more carriers was expected and in agreement with other studies. Phee et al. even showed that a universal screening strategy for carbapenemase-producing organisms identified a higher local incidence than the reported average in the United Kingdom (28). When conducting the administration of the universal risk assessment, healthcare workers can select "the previous risk assessment is still up to date". This is associated with several challenges. Within our study, the electronic health records (EHR) of 16 patients (1.7%) referred to a previous risk assessment, while no previous risk assessment was taken. Consequently, for these patients the risk on MDRO colonization was not determined. Another challenge is when a previous risk assessment was performed, and that the patient was deemed at risk. When the consequent risk assessment refers to the risk assessment where a patient was at risk, the patient should again be screened for MDRO colonization. However, in this situation the patient is often not screened. An added benefit of a universal screening strategy is that this method will also identify patients who acquired a MDRO from transmission in the community, thus without risk-factors. We did not determine if a universal screening strategy would be a cost-effective strategy, however, several studies have been performed. Regarding the identification of MRSA, some studies reported that a universal screening strategy is not effective, or only effective when there is a high prevalence (29-31). On the other hand, it has been reported that universal screening decreased the incidence and prevalence of MRSA, and even to be cost-effective in the study of Borg et al. (30, 32). For carbapenemase-producing organisms, universal screening to reduce transmission appeared to be a cost-effective strategy (28, 33, 34). The cost-effectiveness of a universal screening strategy for MDRO in general still needs to be determined.

Considering the number of unidentified carriers (n=49) and the number of unidentified acquisitions (n=19) within the hospital identified in **Chapter 2.1,** there is a need for improved screening for MDRO colonization upon admission. The yield of the currently installed universal risk assessment combined with risk-based screening is low. Additionally, the current screening is time consuming, not complete in asking about known risk factors, consequently not complete in identifying MDRO carriers upon admission, and at last prone to administrative mistakes**.** It is possible to adapt the risk assessment, but this could increase the burden for both healthcare workers and patients. Therefore**, we conclude** that in theory a universal screening strategy including rectal and nose swabs appears to be an effective method to increase the yield of the current screening strategy. However, it is importance to note that this strategy would not only be labor intensive, but also could be a financial burden for the hospital, at least on the short-term. Such a strategy would not only increase the number of microbiological cultures, but also the number of patients that need to be cared for in isolation. This is associated with an average price between 28 and 41 euros per day per patient (35). Additionally, an increase in the number of isolated patients is associated with an added workload for healthcare workers. Consequently, it should be

considered to only install universal screening for specific patient populations, such as surgical or intensive care patients, next to implementing the extended risk assessment as described above. Carefully checking the compliance to this assessment is highly recommended to ensure optimal detection.

The contamination of the innate hospital environment

Pathogens can survive on surfaces for longer periods, ranging from a few hours up to several months (36). Therefore, surfaces can be a lasting source for transmission when they are not appropriately cleaned and/or disinfected. Consequently, it is not surprising that the hospital environment plays an important role in transmission and outbreaks. The importance of the hospital environment in outbreaks has been shown in several studies (37-39).

When an outbreak has no clear source, and or cannot be controlled, environmental sampling can be performed to identify the source. To perform environmental sampling, direct (*e.g.,* contact plates, dip slides, petri film) and indirect (*e.g.,* sponges, wipes, cotton swabs, flocked swabs, cotton swabs) sampling methods exist. However, currently, no guidelines on how and when (indication) to perform environmental sampling exist. In **Chapter 3.1** we aimed to determine current environmental sampling practices by performing an online survey (40). Our results show that currently, there is no consensus on how to perform environmental sampling, even within countries. While sampling practices depend on the target microorganism and the nature of the outbreak, it was surprising that differences in locations sampled for specific microorganisms within countries. Additionally, some locations were never sampled for specific target microorganisms, while these locations are reported in other studies. For example, the Netherlands did not report sampling the shower drain for VRE, while in **Chapter 3.2** of this study we reported identifying vancomycin resistant *E. faecium* on the shower chair (41). This highlights the need for guidelines, while tailoring of these guidelines will remain necessary for specific situations. Regarding sampling method, the respondents mainly reported the use of swabs, either flocked or cotton. This was as expected, as swabs are readily available in the healthcare environment, are low in cost, and can be used to sample all types and shapes of surfaces (42). However, sampling with swabs is difficult to standardize (*e.g.,* pressure on the swab during sampling, the sampling pattern, the angle of the swab during sampling), which causes variability in recovery rates (43). This again indicates to the need for sampling guidelines. Interestingly, our results indicated a difference between the self-reported knowledge of the respondents and the objective knowledge. While over-estimation of knowledge is to be expected (44, 45), the respondents to our survey were mainly infection prevention and control practitioners and clinical microbiologists. These are the professionals that are responsible for either performing environmental sampling, or evaluating the results and deciding how to proceed. It is worrisome that these professionals are not aware of the gaps in their knowledge, and again highlights the need for standardization and guidelines. **We conclude** that guidelines for how and when to perform environmental sampling are necessary, both on a national and an international level. These guidelines could help standardize sampling, provide a focus on what to sample and why,

and consequently might reduce costs associated with sampling. Additionally, standardization of environmental sampling could help to make national and international results more comparable.

Since environmental sampling is mainly performed during (ongoing) outbreaks, information on bacterial contamination of the hospital environment during non-outbreak settings is rare. However, several studies have been conducted in non-outbreak settings, identifying that up to 55% of rooms had at least one surface contaminated with a MDRO (38, 46-48). In **Chapter 3.2**, we report the results of three-years of environmental sampling (41). In the old hospital building, we identified the presence of MDRO on 3.3% of all sampled surfaces, and in the new hospital building on 0.1% of all sampled surfaces. This contamination rate is very low compared to the other studies. An important factor is the difference in prevalence rates of MDRO between the countries. The Netherlands has a very low prevalence of MDRO, while the majority of studies were performed in the United States, which has a higher MDRO prevalence (49-51). Besides contamination with MDRO, we also have determined the contamination rates with *S. aureus* in **Chapter 2.4**. It is important to note that, while the low prevalence of MDRO might explain the low contamination rates, the prevalence of MSSA carriage is not lower in the Netherlands and should thus not impact environmental contamination rates (10, 11). The observed environmental contamination rates were low, with 3.0% of surfaces positive for methicillin-susceptible *S. aureus* (MSSA), in the old building, and 2.8% of surfaces in the new hospital building, and no methicillin-resistant *S. aureus* (MRSA) identified in both buildings. A study in the UK identified MSSA/MRSA on 5.3-16.1% of the sampled surfaces (52). We identified that when multiple locations in one patient room were present at the same moment, these strains belonged to the same *spa*-type and most likely had the same source. Locations that were MSSA positive over time differed in *spa*-type. From this, **we conclude** that environmental contamination with *S. aureus* is temporarily. It is known that *S. aureus* is able to survive up to seven months on surfaces (36). Consequently, it is likely that the temporary contamination is not due to the *S. aureus*, but due to external factors, such as our such as our sampling protocol, the presence of dry biofilm, and our cleaning activities. It is likely that these factors also contributed to the low contamination rates found with MDRO.

First, our sampling protocol; all sampling methods come with disadvantages and none have a recovery rate of 100%. Consequently, results of environmental sampling will per definition show an underestimation of the true environmental contamination. Therefore, it is key to choose a sampling method best suited to the specific situation. As we sampled different types and different shaped surfaces, both wet and dry locations, and we were interested in multiple target microorganisms, we decided to use cotton swabs. These swabs were premoistened with PBS before sampling a standardized surface of 100 cm^2 (with the exception locations with a deviating shape, such as the doorknob, the shower drain, and the top and bottom of the sink plug, for which the entire surface was sampled). To standardize sampling, we first swabbed horizontally, then vertically, and finally diagonally, while rotating the swab. Because we chose this sampling method, we could easily sample

all surfaces, even those with a deviating shape. Additionally, cotton swabs have higher recovery rates on wet surfaces, and have similar or better recovery rates when compared to other sampling methods (42, 53, 54). However, recovery rates of swabs remain low, for example for *S. aureus* in vitro recovery rates range between 22 and 58% (42, 43). This is mainly due to the difficulty in standardization of sampling (42). Finally, this sampling method provided us the opportunity to use selective broths. Because of this, we were able to detect MDRO that were present in low abundance.

Second, the presence of dry biofilm could also be an important explanation for our low contamination rates, which are present on all types of surfaces (55). Even when no planktonic bacteria were identified on a surface, viable bacteria were identified in biofilms (56-58). Hu et al. showed that MDROs were found on ICU surfaces, even after terminal disinfection was performed (58). Besides the fact that the presence of dry biofilm decreases detection of bacteria, they also hamper cleaning and disinfection (59, 60). Almatroudi et al. showed that live *S. aureus* were present, even after the majority of biofilm was removed by sodium hypochlorite at 20,000 ppm (60). Parvin et al. showed that while a single wiping action was able to remove planktonic *S. aureus*, 50 wipes were necessary to remove biofilm (59).

In our hospital, daily cleaning is performed with microfiber cloths, dampened with water, without a cleaning or disinfection solution. Disinfection is only performed when indicated. While cleaning is essential in keeping hospitals microbiologically safe and could help in preventing healthcare-associated infections, cleaning protocols vary widely and literature on evidence-based practices for hospital cleaning is scarce (61). However, Berendt et al. showed that swiping plastic surfaces with any type of moist wipes decreases the bioburden, and that saline wipes can be just as effective as disinfectant wipes when used appropriately (62). Additionally, Rutala et al. identified that sterile water was effective at removing more than 95% of the test bacteria (63). This supports our standard protocol of only cleaning with damp microfiber cloths. Based on the fact that sampling methods and the presence of dry biofilm will also lead to underestimation of environmental contamination rates in other countries and hospitals, **we conclude** that our cleaning protocol is an important contributor to the low contamination rates with MDRO (0.1% of surfaces) observed in the new building in the three years after relocating (**Chapter 3.2**). However, it is important to consider that our hospital is only three years old and thus that low contamination rates could be due to a relatively new building. However, how fast contamination rates increases and stabilize is not known.

In **Chapter 2.4**, we compared the results of study samples, clinical samples, and environmental samples, to determine if we could show transmission from and to the hospital environment. We were unable to determine if the environment was the source of the acquired *S. aureus*. However, we identified patients with MSSA positive clinical samples who had an epidemiological link to the ward of a room positive for MSSA. From these patients, we identified 16 potential transmissions to or from the hospital environment. Four patients were admitted during sampling, and were consequently the most likely source of

environmental contamination. Two patients were discharged before environmental sampling was performed, but were still the most likely source. One patient was admitted 61 days after environmental sampling, indicating potential transmission from the environment to the patient. However, due to the time frame this does not seem likely. Nine patients were admitted to the ward, but no to the contaminated room. This could indicate transmission on the ward. Overall, **we conclude** that the patient admitted to the room is the most likely source of environmental contamination with *S. aureus* as measured during stay of that particular patient. The study of Chen et al. determined transfers of MDRO from patients to the hospital environment and vice versa (38). They identified that in one third of cases, the patient was the source of environmental contamination, that in one third the environment was the source for the strain acquired by the patient, and that in the final one third, the direction could not be determined. Interestingly, the two transfers with MRSA were either from the patient to the environment or not determined. However, their results highlight that, even though we observed that the patient was the likely source, this does not diminish the importance of the hospital environment in transmission of MDRO.

Besides determining the contamination with MDRO and *S. aureus*, we also determined the total bioburden of surfaces the number of colony forming units (CFU) in **Chapter 3.2**. We observed fluctuating levels of the CFU counts over the three year follow up, with lower levels during the COVID-19 pandemic. This could be related to the enhanced cleaning and disinfection performed during this period. In literature, suggestions have been made for cutoff values for CFU counts on hand contact surfaces in healthcare facilities. Dancer et al. suggested a cutoff value of <5 CFU/cm^2, while Griffith et al. suggested a cutoff value of <2.5 CFU/cm^2 (64, 65). Due to our method, we were unable to determine if the cutoff value of <5 CFU/cm^2 was exceeded, but the cutoff value of <2.5 CFU/cm^2 was exceeded, especially in the bathroom. While CFU counts provide a good indication of the total bacterial load of a surface, they do not provide information about what bacteria are present. When we focus on MDRO, other studies have not identified a correlation between CFU counts and the presence of MDRO (66, 67). In general, nosocomial pathogens are present in low concentrations (68). Consequently, **we conclude** that CFU counts only provide limited information about the environmental contamination and should not be used on its own when determining environmental contamination. While CFU counts provide a good indication of the overall bioburden and could be used to determine cleaning efficacy, when the aim of environmental sampling is to determine environmental colonization with specific target bacteria, other sample methods (*e.g.,* targeted screening) are more effective.

Effect of transitioning to 100% single-occupancy rooms and private bathrooms

One of the research questions of this thesis was if the transition to a newly constructed hospital with 100% single-occupancy rooms and private bathrooms would lead to a microbiologically safer hospital. We determined if ESBL-E acquisition during hospitalization was impacted by the relocation to the new hospital building (**Chapter 2.1**), and if the bacterial contamination of the hospital environment was impacted (**Chapter 3.2**). Regarding the acquisition of ESBL-E during hospitalization, we did not see a significant difference between the old hospital building with multiple-occupancy rooms, and the new hospital building with 100% single-occupancy rooms. However, we did observe a significant decrease in the number of intra-hospital patient transfers. Additionally, we determined a significant correlation between being transferred during hospitalization, and acquiring an ESBL-E. This association was also found by Pasricha et al. (15). Consequently, the transition to single-occupancy rooms did seem to impact the acquisition of ESBL-E through the effect on intra-hospital patient transfers. The reason why these transfers decreased from 24.9% of patients to 14.0% of patients, is that introducing 100% single-occupancy rooms eliminates a number of reasons for these transfers. For example, relocating due to social circumstances, when a patient needs to be placed in contact isolation, or for small procedures (69). The impact of transitioning to single-occupancy rooms on intra-hospital patient transfers was already shown for an intensive care unit, and our research has confirmed that this effect is also observed on other departments (4, 70).

Previous research has been inconclusive about the effect of single-occupancy rooms. Some studies showed that acquisition of MDRO was significantly impacted by the relocation, while other studies found no effect (71-79). The majority of these studies were performed on either an ICU or a neonatology intensive care unit. Additionally, only four observed the effect of transitioning to 100% single-occupancy rooms instead of mainly single-occupancy rooms (72-75). All of these studies showed that single-occupancy rooms could decrease nosocomial infections. The impact of transitioning to single-occupancy rooms on ESBL-E was only determined in an ICU by Levin et al. (76). They did not show that transitioning to single-occupancy rooms impacted acquisition of ESBL-E. However, they only showed acquisition in a very small number of patients and the study did not have the power to determine statistical differences. They did observe a decrease of 8% of patients to 2% of patients who acquired an ESBL-E (76). In 2019, the study of McDonald et al. looked at the effect of transitioning to a hospital with 100% single-occupancy rooms (80). They identified that this relocation was associated with a decrease in newly identified colonization with MRSA and with VRE, and with a decrease in VRE infections. The relocation did not seem to impact MRSA infection rates, or infections with *Clostridioides difficile*. Due to the low observed prevalence of MDRO colonization upon admission and low acquisition rates during hospitalization in our hospital, we could only determine prevalence and acquisition rates for ESBL-E. However, considering the results of previous research, is likely that the transition could impact the transmission and acquisition of other types of MDRO.

Literature on the impact of 100% single-occupancy rooms on environmental contamination with MDRO is lacking. However, as stated in the general introduction of this thesis, the hypothesis is that, since there will only be one patient admitted to a room, the contamination will be lower. The transition to 100% single-occupancy rooms also meant that a final cleaning after discharge could be introduced. While patient rooms were always cleaned after discharged, in multiple-occupancy rooms this would mean that only the patient specific surfaces were cleaned, as there were still patients admitted to the room. Although we did not observe a difference in the total bacterial contamination of the hospital environment, we did show a significant reduction in the environmental contamination with MDRO (41). Interestingly, sink drains were the most contaminated location in the old hospital building, but no contamination was observed in the new hospital building. This is partly explained by the fact that the old building had persistent colonization of the sink drains with VIM-positive *Pseudomonas aeruginosa* following an outbreak (81). However, not only *P. aeruginosa* was identified in sink drains in the old hospital building. This seems to be an effect of the relocation of the hospital building, where sinks and sink drains are new, and sinks had a different design compared to those in the old building, and potentially of awareness of usage. Additionally, we introduced sink plugs on all sink drains in the new hospital building and screens next to the sinks where needed. The sink plugs were installed in some sinks in the old hospital building, to decrease transmission of *P. aeruginosa* from sink drains to patients (82, 83). It is important to note that the difference in environmental contamination is not the result of differences in prevalence, as we did not observe differences in colonization rates upon admission to the hospital building between the old and the new hospital building (4). The decrease in environmental contamination is important, as the hospital environment is an important source of outbreaks (37). The introduction of 100% single-occupancy rooms will not eliminate the risk of transmission from the hospital environment to patients, but when the contamination rates are lower, it is likely that the frequency of transmission decreases.

The COVID-19 pandemic has highlighted the need for pandemic preparedness. It is important to evaluate the added benefit of single-occupancy rooms in this light. During the COVID-19 pandemic, scarcity of rooms suitable for isolation was an important concern for many hospitals. However, in our hospital, every room was available as an isolation room. Additionally, with the emergence of other MDRO and *Candida auris* single-occupancy rooms are also a necessity, as the guideline is to isolate patients in single-occupancy rooms. It is possible that, with future epidemics or pandemics, 100% single-occupancy rooms may even become a necessity to care for large numbers of infectious patients. It is important to acknowledge that the introduction of 100% single-occupancy rooms affects more than just the microbial safety of the hospital environment. Therefore, the other studies within the consortium PE-ONE will determine the effects from a management point of view, on experiences from patients and staff, and evaluating work situations and efficiency.

Multidisciplinary consortia like PE-ONE are essential and rely heavily on the generosity of funders, in our specific case the board of directors of the Erasmus MC. To ensure the future of these consortia, this generosity of funders is pivotal, and we would like to acknowledge and thank the board of directors for funding PE-ONE. **We conclude** that single-occupancy rooms and private bathrooms provide a microbial safer environment compared to multiple-occupancy rooms and shared bathrooms. This is based on the decrease in intra-hospital patient transfers and their association with acquisition of ESBL-E, and on the observed decrease in environmental contamination and the anticipated effects this will have on transmission from the hospital environment to patients. Consequently, our recommendation for architects is to include 100% single-occupancy rooms in the design of future hospitals.

Future perspectives

This thesis provides important insights in the effect of transitioning to 100% single-occupancy rooms and the impact on the microbial safety, on alternatives for screening practices for MDRO upon admission to the hospital, and on the need for guidelines for environmental sampling. However, there are still important gaps that need to be filled.

Dynamics of MDRO within the hospital

The true dynamics of MDRO within the hospital environment remain unknown. To gain more insight, **we recommend** performing a prospective study in which all patients admitted to a single, or multiple, departments are screened upon admission and upon discharge. Patients who have a long hospitalization period (>1 week) should be screened weekly during hospitalization. Additionally, environmental samples should be taken. The results of these samples should be used to determine transmission and transmission routes within the department. Additionally, this data could help identify which proportion of transmissions in the hospital currently remains unidentified, and how infection prevention and control interventions could be tailored to prevent these.

Screening upon admission

Our results identified gaps in the currently installed universal risk assessment combined with risk-based screening for MDRO upon admission. **We recommend** that a multi-center study should be performed, to identify the added value of universal screening upon admission. Simultaneously, risk factors for MDRO colonization need to be determined, for example by a questionnaire upon admission. With these data and the results of the universal risk assessment, the most important risk factors for MDRO colonization upon admission can be determined. Additionally, this will show the percentage of MDRO carriers who do not have any risk factors. These data could then be used to decide if the universal risk assessment should be updated, or if it is cost-efficient to introduce universal screening upon admission, or screening upon admission for specific patient populations. The ultimate goal would be a prediction model embedded within the electronic health records. Upon

admission, this prediction model, based on several risk factors, could determine the chance on MDRO carriage and determine if a patient should be screened and placed in pre-emptive isolation. However, to make such a model effective, data from different hospitals and general practitioners needs to be combined. Otherwise, gaps regarding risk factors will remain, limiting the added benefit of the model. Consequently, to make this model effective, a national electronic health record should be implemented.

The hospital environment

Our results of the environmental contamination seem to indicate that the cleaning protocol in our hospital is efficient. However, cleaning protocols vary between countries. **We recommend** performing an international survey to determine current cleaning protocols in hospitals, for both isolation and non-isolation rooms, and determine the impact on environmental contamination. With the information from this survey, the most used cleaning methods should be evaluated for efficiency. This information can then be used for a recommendation for guidelines for how to clean the hospital environment.

As shown by our survey, there is no consensus for environmental sampling practices. In order to make environmental sampling more effective and results more comparable, **we recommend** evaluating the best sampling methods for different target microorganisms. The ESCMID study group of nosocomial infections (ESGNI), together with the Hospital Infection Society (HIS) are currently studying the role of sampling in outbreak management. A literature review should help identify the most commonly contaminated surfaces. This information can be used to determine which locations always need to be sampled. This is a first step towards a guideline.

In recent years, the impact on single-occupancy rooms on the hospital-associated microbiota has been determined. However, there are still many knowledge gaps to be filled regarding this topic. **We recommend** determining the effect of single-occupancy rooms on the hospital-associated microbiota, and its consequent impact on transmission of HAI within the hospital environment. More insight into hospital-associated microbiota could help identifying important environmental niches for infection prevention and control and could provide more insight into the dissemination of resistance genes. Currently, the ENEMI study, as part of the PE-ONE consortium, is determining the effect of 100% single-occupancy rooms on the hospital-associated microbiota.

The impact of 100% single-occupancy rooms

To further determine the impact of 100% single-occupancy rooms, multiple things merit from additional research. First, our results reflect the effect of the transition to 100% single-occupancy rooms during the three years after relocating. However, it is possible that the effect will change over time. Consequently, **we recommend** performing environmental sampling 5 and 10 years after relocating, to determine if the observed effects on environmental contamination were partly explained by the follow-up time, or if it is truly a long-lasting effect of the relocation. Secondly, literature on the impact of introducing 100%

single-occupancy rooms on outbreaks in the hospital is lacking. **We recommend** that a retrospective study determining the number of outbreaks in the old hospital building and the number of outbreaks in the new hospital building. This data could show if the relocation impacted transmission of MDRO. It should also be considered to determine the differences in the number and the size of contact tracing investigations that needed to be performed after identifying a MDRO carrier. This data could be used to determine the costs and workload associated with the outbreaks, to determine the cost-effectiveness of the relocation from an infection prevention and control point of view. Thirdly, our results show the impact of 100% single-occupancy rooms in a country with a low prevalence of MDRO. Due to the variability in prevalence of MDRO, **we recommend** to evaluate the impact of 100% single-occupancy rooms in countries with a difference MDRO prevalence. And fourth, our results show the effect for an adult population. **We recommend** that the impact of 100% single-occupancy rooms on acquisition, environmental contamination, and on outbreaks, should also be evaluated in a children's hospital.

As stated before, **we recommend** that architects design future hospitals with 100% single-occupancy rooms. To reach this, it is important that architects work together with medical microbiologists, epidemiologists, infection prevention and control practitioners, and other healthcare workers. In current practice, this is not (yet) occurring. Combining the expertise of these professions with the expertise of architects would increase the microbial safety of future hospitals. Additionally, including the people who will work in the building (*e.g.,* nurses, health and safety officers, supportive staff) other bottlenecks could be identified early in the design phase, making adaptations more feasible. To quickly start the collaborations, a network proposal to the EU-COST program (Hospital Preparedness for Epidemics: network for designing safe and healthy healthcare environments (HoPE)) has been submitted. The purpose of this network is to create an international multidisciplinary research network with a focus of improving safety of hospital by design. This could be a first step in improving collaborations between architects and healthcare workers.

On conclusion, given the results of this thesis, we recommend that new hospital buildings should be built with 100% single-occupancy rooms, to maximize microbial safety. We had two parameters for microbial safety. The first was met: Environmental contamination in the 100% single-occupancy rooms was significantly lower. The second was partially met: While we did not show a decrease in acquisition, we showed that patients are transferred less in a hospital with 100% single-occupancy rooms. This decreased the exposure to the hospital environment and was associated with lower odds on ESBL-E acquisition, and potentially other MDRO. Consequently, we believe that our hospital with 100% single-occupancy rooms provides a microbial safer hospital. Given the results in our low endemic setting, the impact of introducing 100% single-occupancy rooms might even be more substantial in countries with a higher prevalence of MDRO. Regarding the screening methods upon admission, a universal screening strategy seems to be a good strategy in theory. However, adding questions, such as about recent travel history, to the risk assessment is more feasible and will improve the detection of carriers upon admission.

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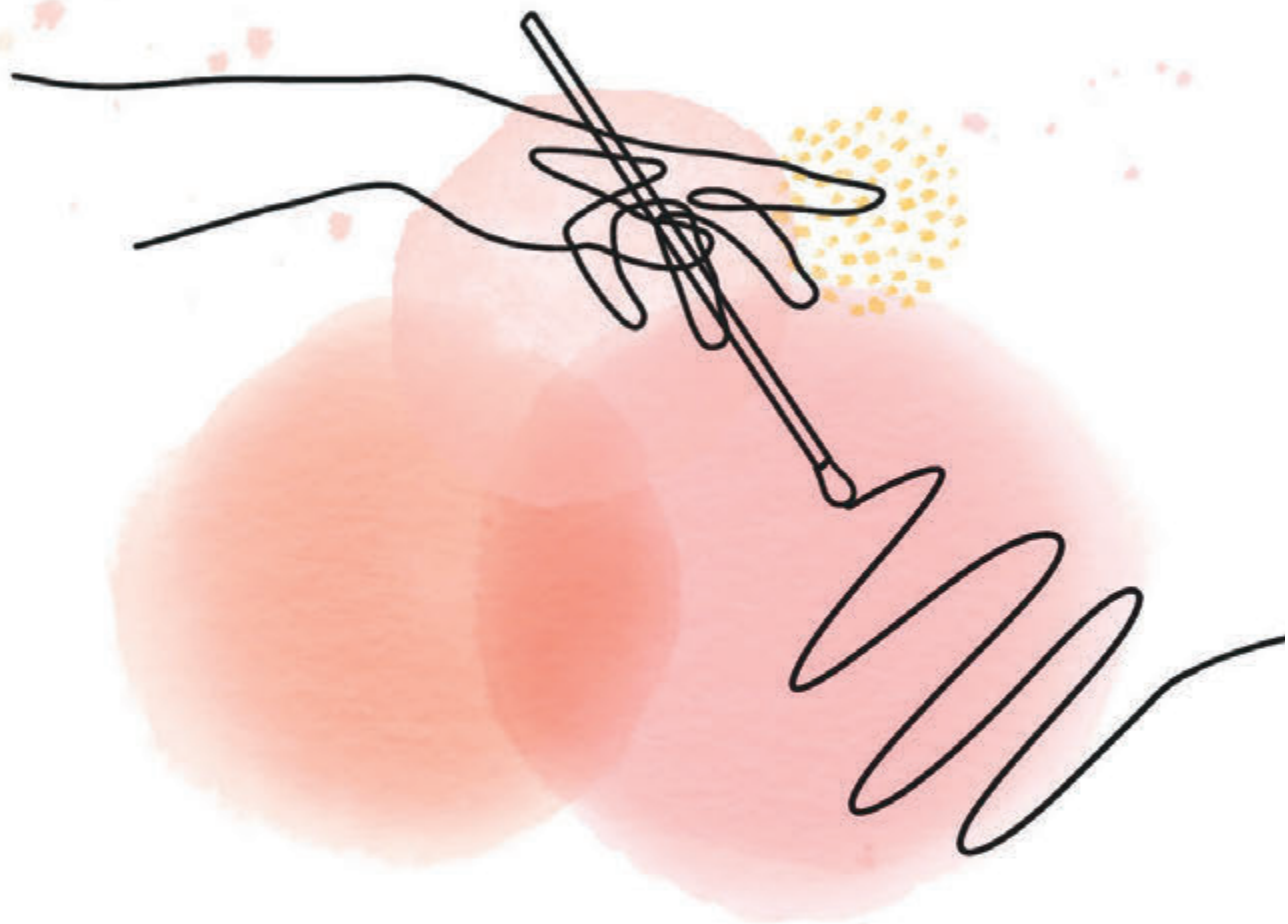
Chapter 5



NEDERLANDSE SAMENVATTING

Op 18 mei 2018 verhuisde het Erasmus MC Universitair Medisch Centrum van een oud ziekenhuisgebouw met voornamelijk twee- en vierpersoonskamers en gedeelde badkamers naar een nieuw ziekenhuisgebouw met 100% éénpersoonskamers en privé badkamers. Het doel van dit proefschrift was bepalen of dit nieuwe ziekenhuis en daarmee de overgang naar 100% éénpersoonskamers, heeft gezorgd voor een microbiologisch veiliger ziekenhuis. Wij beschouwden het nieuwe ziekenhuis als microbiologisch veiliger wanneer de omgevingscontaminatie in het algemeen en/of met bijzonder resistente micro-organismen (BRMO) lager is en wanneer er minder acquisitie/transmissie van BRMO plaatsvindt in vergelijking met de oudbouw. Tevens is er in dit proefschrift onderzocht hoe screenings methoden voor BRMO bij opname verbeterd kunnen worden en wat de huidige samplingsmethoden voor detectie van contaminatie in de omgeving in Europa zijn.

Dynamiek van BRMO en *Staphylococcus aureus* kolonisatie tijdens opname

BRMO zijn micro-organismen die resistent zijn tegen eerste keus antibiotica, of tegen meerdere groepen antibiotica. In Nederland is de prevalentie van BRMO laag, maar verschilt per soort. Ondanks deze lage prevalentie komen infecties met BRMO voor bij patiënten. Deze infecties zijn lastiger te behandelen en leiden tot een hogere morbiditeit en mortaliteit. Het doel van infectiepreventie in het ziekenhuis is het voorkomen of stoppen van transmissie van BRMO in het ziekenhuis. Een voorbeeld van een maatregel die transmissie voorkomt is het verplegen van bekende dragers van BRMO in isolatie. Er zijn echter nog belangrijke kennishiaten, waaronder het efficiënt aantonen van gekoloniseerde patiënten bij opname in het ziekenhuis en de dynamiek van dragerschap in de patiënt en transmissie tussen de patiënten tijdens de opname.

In **hoofdstuk 2.1** en **2.2** hebben wij het percentage dragers van de BRMO extended-spectrum beta-lactamase (ESBL)-producerende Enterobacterales (ESBL-E) bij opname van 597 patiënten bepaald, in **hoofdstuk 2.3** het percentage voor BRMO-dragers bij opname, en in **hoofdstuk 2.4** het percentage *Staphylococcus aureus* dragers bij opname. Het dragerschapspercentage voor ESBL-E was 4.4%-6.5%, wat overeenkomt met wat eerder bekend was over het dragerschapspercentage in Nederland. Naast de ESBL-E dragers, hebben we één methicilline resistente *S. aureus* (MRSA) drager geïdentificeerd. We hebben geen dragers van vancomycine-resistente *Enterococcus faecium* (VRE), multidrug resistente *Pseudomonas aeruginosa*, multidrug resistente *Acinetobacter baumannii*, of carbapenemase producerende Enterobacterales geïdentificeerd bij opname. Aangezien zowel de prevalentie van MRSA als van de andere BRMO laag is in Nederland, was dit niet onverwacht. Wat betreft dragerschap van methicilline gevoelige *S. aureus* (MSSA), hebben wij een dragerschapspercentage van 27.0% gevonden, wat ook overeenkwam met andere studies.

Tijdens een ziekenhuisopname kunnen patiënten een BRMO of MSSA oplopen, of verliezen. Deze dynamiek is relatief onbekend, aangezien patiënten niet standaard voor en gedurende een opname gescreend worden voor dragerschap van BRMO en/of MSSA. In **hoofdstuk 2.1** hebben wij deze dynamieken onderzocht voor ESBL-E, en in **hoofdstuk 2.4** voor MSSA. In

hoofdstuk 2.1 hebben wij bij 3.2% van patiënten een ESBL-E bij ontslag, maar niet bij opname aangetoond. Het overgrote deel van dit dragerschap bij deze patiënten (94.7%) werd niet geïdentificeerd door klinische samples tijdens de ziekenhuis opname. We hebben geen transmissie tussen patiënten kunnen identificeren, waardoor de bron van transmissie waarschijnlijk een niet geïncludeerde patiënt of de ziekenhuis omgeving was, of, minder waarschijnlijk, zorgmedewerkers of bezoekers. Doordat wij niet alle patiënten, die opgenomen waren in het ziekenhuis, hebben kunnen includeren is het aannemelijk dat patiënt-naar-patiënt transmissies gemist zijn. Een andere verklaring kan zijn dat bijvoorbeeld door selectie door antibiotica gebruik het verschil tussen de opbrengst van de kweek bij opname en bij ontslag beïnvloed kan zijn. In **hoofdstuk 2.4** hebben we aangetoond dat 2.2% van patiënten een MSSA opliep tijdens de ziekenhuisopname. Ook hebben we hier de resultaten van de neuskweken afgenomen voor de studie vergeleken met de resultaten van klinische kweken. Van de patiënten die een positieve neuskweek en een positieve klinische kweek hadden, was voor 15.9% de studiekweek niet identiek aan de klinische kweek. Dit wijst erop dat deze patiënten een MSSA hebben opgelopen tijdens de opname of dat de neuskweek bij opname MSSA types heeft gemist.

Onze resultaten laten zien dat transmissie in het ziekenhuis vaak onopgemerkt blijft, wat hiaten in de huidige screeningsmethoden laat zien. We hebben acquisitie van ESBL-E bij twintig patiënten en van MSSA bij 15 patiënten geobserveerd, die in theorie voorkomen hadden kunnen worden. We concluderen dat de huidige screenings en surveillance methoden in het ziekenhuis verbeterd moeten worden.

Screening van patiënten bij opname

Hoewel patiënten in Nederland niet standaard gescreend worden voor BRMO/MRSA bij opname, is er wel een landelijk ingevoerd risico assessment bij opname om zodoende risico geleid te screenen. Dit risico assessment bestaat uit zeven vragen, om te bepalen wat het risico is op het dragen van een BRMO (Tabel 1). In het Erasmus MC is er nog een achtste vraag toegevoegd, namelijk “bent u professioneel zeevaarder”. Als patiënten volgens het assessment een risico hebben om drager van een BRMO te zijn, worden zij in isolatie geplaatst en worden er screeningskweken afgenomen om te bepalen of zij inderdaad drager zijn. Het risico assessment is echter niet compleet. Zo is reizen bij gezonde vrijwilligers een bekende risicofactor voor het oplopen van BRMO, specifiek reizen naar Zuidoost-Azië. Op dit moment bevat het risico assessment nog geen vraag over recente reisgeschiedenis van patiënten. In **hoofdstuk 2.2** hebben wij onderzocht of patiënten bij opname drager waren van een BRMO, en hebben wij aan hen een questionnaire gegeven waarin gevraagd werd of zij in het afgelopen jaar op reis zijn geweest. Uit de resultaten bleek dat de helft van de opgenomen patiënten in het afgelopen jaar op reis is geweest, maar dat slechts een klein percentage van de patiënten buiten Europa was geweest. Bij de patiënten die binnen Europa op reis waren geweest was 3.4% drager, vergeleken met 13.3% van patiënten die buiten Europa op reis waren geweest. De patiënten in onze studie gaven aan dat zij een beleid van screenen en isoleren van patiënten die op reis zijn geweest steunen. Wij concluderen dat het aanvullen van het BRMO-risico assessment met een vraag over recente

reisgeschiedenis geaccepteerd zou worden door patiënten en zou helpen om meer ESBL-E dragers te identificeren.

Tabel 1. Overzicht van de huidige risico assessment en voorgestelde aanpassingen en alternatieve strategieën.

Huidige risico assessment in het Erasmus MC
1) Is de patiënt recent behandeld of opgenomen geweest in een buitenlandse zorginstelling? 2) Woont/werkt de patiënt daar waar bedrijfsmatig varkens, vleeskalveren of vleeskuikens worden gehouden? 3) Is de patiënt aangetoond drager van MRSA/BRMO? 4) Is de patiënt een partner, huisgenoot of verzorgende van iemand die MRSA positief is? 5) Verbleef de patiënt de afgelopen 2 maanden in een zorginstelling met een MRSA/BRMO uitbraak, en is de patiënt benaderd voor kweekonderzoek? 6) Is de patiënt de afgelopen 2 maanden woonachtig geweest in een instelling voor asielzoekers? 7) Bent u beroepsmatig zeevarende?
Voorgestelde vragen voor aanvulling van risico assessment
8) Bent u recent naar Zuidoost Azië geweest? 9) Heeft u recent antibiotica gebruikt, en zo ja, welke? 10) ….
Alternatieve strategie
Universele screening bij opname Screening van specifieke patiëntpopulaties bij opname

Recent onderzoek van Van Hout et al. heeft aangetoond dat het BRMO-risico assessment en de risico screenings kweken niet efficiënt zijn in het identificeren van nieuwe BRMO dragers (van Hout et al., J Hosp Infect. 2021;109:32-9). Hoewel het toevoegen van een vraag over reisgedrag deze efficiëntie zou kunnen vergroten, vergroot dit ook de werklast die geassocieerd is met het afnemen van het BRMO-risico assessment. Wij waren in de unieke positie dat wij opname kweken hadden van een grote groep patiënten, los van het BRMO-risico assessment. In **hoofdstuk 2.3** vergeleken we de uitkomst van deze universele opname kweken met de uitkomst van het BRMO-risico assessment en risico screenings kweken. We vonden dat we significant meer dragers identificeerden door middel van de universele (bij iedere patiënt) opname kweken. We vonden ook dat het BRMO-risico assessment leidde tot incidentele administratieve fouten, bijvoorbeeld verwijzen naar een eerder assessment terwijl die niet bestaat, of verwijzen naar een eerder assessment waarbij de patiënt risicofactoren had voor het dragen van een BRMO, maar er geen kweken waren afgenomen. Gezien de ongeïdentificeerde dragers uit **hoofdstuk 2.1**, en de niet geïdentificeerde transmissies, is er behoefte aan een verbeterde screening bij opname. Wij concluderen dat

een universele screenings strategie met rectum en neus kweken een effectieve alternatieve strategie zou zijn, aangezien de universele screening significant meer dragers identificeerde. Een belangrijke kanttekening is dat deze strategie wellicht niet kosteneffectief is en zeker wel arbeidsintensief. Toekomstig onderzoek zou moeten bepalen of de voorgestelde strategie kosteneffectief is. Alternatief is de strategie alleen te implementeren voor specifieke nader te duiden patiëntengroepen.

Contaminatie van de ziekenhuisomgeving

Het is bekend dat micro-organismen voor lange tijd op oppervlakten kunnen overleven, van enkele uren tot een aantal maanden (Kramer et al., BMC Infect Dis. 2006;6:130). Als gevolg kunnen oppervlaktes in het ziekenhuis, wanneer zij niet goed schoongemaakt en/of gedesinfecteerd worden, voor lange tijd een bron van transmissie zijn. Het is dan ook niet verassend dat de ziekenhuisomgeving een belangrijke rol speelt in transmissie en tijdens uitbraken.

Wanneer de bron van een uitbraak niet gevonden wordt, of wanneer de uitbraak niet onder controle gekregen kan worden, kunnen er omgevingssamples afgenomen worden. Dit kan door middel van directe (bijv. contactplaten) of indirecte (bijv. swabs) samplingsmethoden. Op dit moment bestaan er geen nationale of internationale richtlijnen over hoe en wanneer omgevingssamples afgenomen moeten worden. Dit bracht ons ertoe om een internationaal onderzoek hiernaar op te starten; wat zijn de huidige methoden? In **hoofdstuk 3.1** worden de resultaten van een survey over omgevingssampling gewoontes en methoden besproken. Door middel van deze survey wilden wij een inzicht krijgen in de manier waarop er op dit moment omgevingssamples worden afgenomen in Europa. De resultaten van de survey laten duidelijk zien dat er op dit moment geen overeenkomst is over hoe de omgevingskweken afgenomen moeten worden, welke plekken gesampled moeten worden voor specifieke target micro-organismen, en hoe de samples in het lab verwerkt moeten worden. Wel werd duidelijk dat swabs de meest gebruikte sample methoden zijn, al verschilt het type swab per land (bijv. katoenen swabs, flocked swabs). Onze resultaten maakten duidelijk dat er een verschil zat tussen de gerapporteerde kennis en de objectief gemeten kennis over omgevingssample methoden van de respondenten. Hoewel het overschatten van kennis te verwachten valt in een survey, is deze uitkomst zorgwekkend omdat onze respondenten voornamelijk deskundigen infectiepreventie en arts-microbiologen waren. Dit zijn de beroepsgroepen die (deels) verantwoordelijk zijn voor het uitvoeren van het samplen, interpreteren van de resultaten, en het besluiten hoe verder te gaan. We concluderen dat er vraag is naar een duidelijke richtlijn die beschrijft wanneer en hoe je omgevingssamples af moet nemen. Dit kan leiden tot het standaardiseren van samplingsmethoden, waardoor resultaten beter te vergelijken zijn. Ook zou het een focus voor sampling aan kunnen brengen, wat de kosten geassocieerd met omgevingssamples zou kunnen verlagen.

Omgevingssamples worden bijna uitsluitend afgenomen tijdens uitbraken. Daarom is er weinig informatie over de bacteriële contaminatie van de ziekenhuisomgeving wanneer er

geen uitbraak plaatsvindt. In **hoofdstuk 3.2** hebben wij de bacteriële contaminatie van de omgeving in het oude en nieuwe ziekenhuisomgeving bepaald, nadat wij over een periode van 3 jaar omgevingssamples hebben afgenomen. We vonden dat slechts 3.3% van de oppervlakten in de oudbouw en 0.1% van de oppervlakten in de nieuwbouw gecontamineerd waren met BRMO, terwijl studies uit andere landen vonden dat tot wel 55% van patiëntenkamers gecontamineerd was. In **hoofdstuk 2.4** hebben we de contaminatie van de ziekenhuis omgeving met *S. aureus* bepaald. De contaminatie met MSSA was met 3.0% van de oppervlakten in de oudbouw en 2.8% van de oppervlakte in de nieuwbouw ook laag. Een studie in het Verenigd Koninkrijk vond MSSA/MRSA op 5.3%-16.1% van de gesamplede oppervlakten (Dancer et al., Int J Environ Health Res. 2008 Oct;18(5):357-64). Ook vonden we dat oppervlakten niet langdurig gecontamineerd waren met hetzelfde micro-organisme. De lage omgevingscontaminatie kan deels verklaard worden door de lage BRMO-prevalentie in Nederland, maar aangezien de prevalentie van MSSA in Nederland niet lager is vergeleken met andere landen, lijkt het dat andere factoren een belangrijke rol spelen in de lage omgevingscontaminatie. Een verklarende factor zou onze sampling methode met katoenen swabs kunnen zijn. Hoewel samplen met swabs een aantal voordelen heeft, zoals dat elk type oppervlak gesampled kan worden, kan het ook leiden tot een onderschatting van de contaminatie. Aan de andere kant zorgt geen enkele samplingmethode voor 100% detectie, en dus leidt elke methode automatisch tot een onderschatting. Een tweede verklaring kan zijn dat er droog biofilm aanwezig is op de gesamplede oppervlaktes. Droog biofilm zorgt dat bacteriën minder goed gedetecteerd kunnen worden. Als laatste kan onze schoonmaakactiviteiten invloed hebben op de lage contaminatie. In ons ziekenhuis maken we dagelijks schoon met een klam vochtige microvezel doek, zonder toegevoegde schoonmaak of desinfectieproducten. In de nieuwbouw is er een eindschoonmaak geïntroduceerd, na ontslag van de patiënt. Desinfectie wordt alleen toegepast op indicatie, bijvoorbeeld wanneer een patiënt die bekend drager is van MRSA wordt ontslagen. Aangezien alle sample methoden tot een onderschatting van de omgevingscontaminatie leidt, en elk ziekenhuis droog biofilm op oppervlakte heeft, achten wij het schoonmaak protocol de meest voor de hand liggende verklaring voor de lage geobserveerde contaminatie. Wel is het belangrijk dat ons ziekenhuis pas drie jaar oud is en de lage contaminatie ook het gevolg kan zijn van het relatief nieuwe gebouw. Het is echter niet bekend hoe snel contaminatie plaatsvindt en wanneer dit stabiliseert.

In **hoofdstuk 2.4** hebben we ook gekeken of we transmissie van *S. aureus* van patiënten naar de omgeving en van de omgeving naar patiënten vast konden stellen. Hiervoor hebben we *S. aureus* uit klinische kweken van patiënten met een epidemiologische link met een *S. aureus* positieve kamer en *S. aureus* uit de omgevingskweken met elkaar vergeleken. Met een epidemiologische link bedoelen we dat de patiënt binnen drie maanden voor of na het afnemen van omgevingssamples opgenomen was op de afdeling waar de *S. aureus* positieve kamer was. Van de 16 patiënten met een epidemiologische link was het merendeel van de patiënten waarbij de *S. aureus* uit de klinische kweken identiek was aan *S. aureus* uit de omgeving was opgenomen tijdens het samplen van de omgeving, of was kort daarvoor

ontslagen. Negen patiënten waren opgenomen in een andere kamer op de afdeling dan de gecontamineerde kamer, wat kan wijzen op transmissie op de afdeling. We concluderen dat transmissie meestal plaatsvindt van de patiënt naar de omgeving, en dat omgevingscontaminatie vaak afkomstig is van de patiënt die opgenomen ligt op het moment van samplen.

Naast contaminatie met BRMO of *S. aureus*, hebben we in **hoofdstuk 3.2** ook gekeken naar de totale bacteriële contaminatie van de omgeving. Hiervoor hebben we contactplaten afgenomen (RODACs), waarop het aantal groeiende koloniën geteld werd. Deze koloniën per cm^2 (CFU/cm^2) geven een beeld van de totale contaminatie. We hebben deze CFU/cm^2 bepaald over een periode van 3 jaar in de nieuwbouw, waarbij ze tijdens de eerste drie maanden stegen en het daarna fluctueerde. Twee en drie jaar na de verhuizing, tijdens de COVID-19 pandemie, waren de CFU/cm^2 significant lager in vergelijking met de oudbouw. Dit zou een gevolg kunnen zijn van de extra aandacht die er in deze periode was voor schoonmaken en desinfecteren. In de literatuur wordt een afkapwaarde voorgesteld van <2.5 of <5 CFU/cm^2. De afkapwaarde van 2.5 CFU/cm^2 werd voor het merendeel van de locaties overschreden, door onze meetmethoden hebben wij niet kunnen bepalen of de waarde van 5 CFU/cm^2 overschreden werd. Aangezien studies geen correlatie aan hebben getoond tussen CFU/cm^2 en de aanwezigheid van BRMO, concluderen wij dat alleen het bepalen van CFU/cm^2 niet voldoende informatie geeft over belangrijke en relevante contaminatie van de omgeving (Widmer et al., J Hosp Infect. 2019 Feb;101(2):240-244). CFUs kunnen wel extra informatie geven om bijvoorbeeld de effectiviteit van schoonmaak te bepalen.

Effect van overgang naar 100% éénpersoonskamers en privé badkamers

De overgang van twee- en vierpersoonskamers naar éénpersoonskamers was onderdeel van het creëren van een helende omgeving. Daarnaast was de verwachting dat het ook positieve gevolgen zou hebben voor infectiepreventie. De verwachting was dat patiënten minder BRMO op zouden lopen, onder andere doordat er geen direct contact meer met kamergenoten is. Ook was de verwachting dat patiënten minder verplaatst zouden worden, omdat het introduceren van 100% éénpersoonskamers veel redenen voor verplaatsingen elimineert, zoals verplaatsen voor contact isolatie of voor sociale indicaties. Daarnaast was de verwachting dat de omgevingscontaminatie met BRMO lager zou zijn in de éénpersoonskamers.

Om te bepalen of éénpersoonskamers effect hadden op het oplopen van BRMO, hebben wij in **hoofdstuk 2.1** gekeken of er een verschil zat in het aantal patiënten wat een ESBL-E opliep terwijl zij opgenomen lagen op een meer-persoonskamer en het aantal patiënten wat een ESBL-E opliep op een éénpersoonskamer. Uit onze resultaten is gebleken dat de overgang naar éénpersoonskamers geen effect had op het aantal patiënten wat een ESBL-E opliep. Wel bleek dat de overgang naar éénpersoonskamers het aantal verplaatsingen van patiënten van kamer naar kamer significant verminderde. Verder vonden wij dat patiënten die verplaatst werden een grotere kans hadden op het oplopen van een ESBL-E in

vergelijking met patiënten die niet verplaatst werden (OR 3.18, 95%CI 1.27-7.98). Deze kans werd groter als een patiënt twee keer of vaker verplaatst werd (OR 6.79, 95%CI 2.29-20.06). Doordat het aantal verplaatsingen in de nieuwbouw significant lager is, zullen naar verwachting minder patiënten het risico lopen een ESBL-E op te lopen. Daardoor is er wel een indirect effect van de overgang op éénpersoonskamers op het oplopen van ESBL-E. Als laatste vonden wij dat 96.1% van de patiënten die positief waren voor ESBL-E bij opname niet geïdentificeerd waren door het BRMO-risico assessment die bij opname gesteld worden. Wij konden niet aantonen dat de éénpersoonskamers direct effect hadden op acquisitie van MRSA, VRE, CPE, of multidrug resistente *P. aeruginosa* of *A. baumannii*. Andere studies hebben gevonden dat éénpersoonskamers voor verminderde acquisitie tijdens de ziekenhuisopname zorgden (McDonald et al., JAMA Internal Medicine 2019;179(11):1501-1506, Stiller et al., Antimicrob Resist Infect Control 2016 Nov 29;5:51). Het is mogelijk dat dit effect ook in ons ziekenhuis heeft plaatsgevonden, maar dat wij dit door de lage prevalentie niet hebben kunnen observeren.

Het verschil in omgevingscontaminatie tussen twee- en vierpersoonskamers en éénpersoonskamers hebben we bepaald in **hoofdstuk 3.2**. Hierbij vonden wij significant minder BRMO in de omgeving van éénpersoonskamers gedurende de eerste drie jaar na de verhuizing. Dit kan deels verklaard worden doordat wij een groot aantal VIM-positieve *P. aeruginosa* hebben geïdentificeerd in wasbakken op de afdeling intensive care in de oudbouw, een gevolg van een uitbraak uit het verleden (Van der Bij et al., Int J. Antimicrob Agents 2011;37(6):513-8). Wanneer we deze niet meenemen is er echter nog steeds een significant verschil. Opvallend is dat bijna alle BRMO in de oudbouw geïdentificeerd zijn in wasbakken, terwijl er geen BRMO in wasbakken in de nieuwbouw zijn gevonden. Dit zou een gevolg kunnen zijn van het installeren van spatdeksels in bijna alle wasbakken en zo nodig schermen naast de wasbakken in de nieuwbouw, en van bewustzijn bij gebruik. De spatdeksels zijn geïntroduceerd in de oudbouw, en waren essentieel in het voorkomen van transmissie van wasbakken naar patiënten (Pirzadian et al., PLoS One 2023 Mar 24;18(3):e0282090). De afname in omgevingscontaminatie met BRMO is belangrijk voor het verminderen van transmissie via de ziekenhuisomgeving.

Naast het effect van éénpersoonskamers voor de microbiologische veiligheid, is het ook belangrijk om te kijken naar deze overgang in het licht van pandemische paraatheid. Tijdens de COVID-19 pandemie was er in veel ziekenhuizen een tekort aan éénpersoonskamers. Door de overgang naar éénpersoonskamers was in ons ziekenhuis elke kamer geschikt als isolatiekamer. De vraag naar kamers geschikt voor isolatie, zal in de toekomst wellicht groter worden, bijvoorbeeld door de opkomst van *Candida auris* en nieuwe BRMO. Het is zeker dat en toekomstige pandemie zo mogelijk 100% éénpersoonskamers vereist om zo efficiënt mogelijk te kunnen werken met grote aantallen besmettelijke patiënten. Het is belangrijk om te beseffen dat de overgang naar éénpersoonskamers niet alleen effect heeft op de microbiologische veiligheid. Binnen ons consortium Program Evaluating Our New Erasmus (PE-ONE) wordt daarom ook gekeken naar de effecten vanuit een management oogpunt, effecten voor patiënten en medewerkers, en worden situaties en efficiëntie van

processen geëvalueerd. Wij concluderen dat de overgang naar éénpersoonskamers en privé-badkamers heeft gezorgd voor een microbiologisch veiliger ziekenhuis. Deze conclusie is gebaseerd op de afname in verplaatsingen van patiënten, de associatie tussen deze verplaatsingen en het oplopen van ESBL-E, en de lagere omgevingscontaminatie en de daardoor te verwachten effecten voor transmissie. Daarom is onze aanbeveling voor architecten van ziekenhuizen om toekomstige ziekenhuizen te ontwerpen met 100% éénpersoonskamers.

Toekomst

De studies in dit proefschrift geven inzicht in het effect van het overgaan op 100% éénpersoonskamers en de bijbehorende effecten op de microbiologische veiligheid, mogelijke manieren om screening voor BRMO bij opname te verbeteren, en maken de noodzaak voor richtlijnen voor het afnemen van omgevingssamples duidelijk. Er zijn echter ook vervolgstudies die nodig zijn om bestaande en nieuwe kennishiaten te dichten.

Bij een volgende studie met als doel de **dynamieken van BRMO** in het ziekenhuis beter in kaart te brengen raden we aan om alle patiënten opgenomen op één (of meerdere) afdelingen te includeren, en te samplen bij opname, ontslag, en bij verblijf langer dan een week, wekelijks. In combinatie met omgevingskweken kan dit informatie geven over transmissieroutes op een afdeling, en over hoeveel transmissies momenteel onzichtbaar zijn, wat ervoor kan zorgen infectiepreventie strategieën verbeterd kunnen worden.

Om de **screening voor BRMO bij patiënten bij opname** te optimaliseren raden wij aan om een multicenter studie uit te voeren, om te bepalen wat de toegevoegde waarde van universele screenings strategie is. De kosteneffectiviteit van deze strategie moet ook bepaald worden. Tegelijkertijd moeten risico factoren voor dragerschap in kaart gebracht worden, waarmee de BRMO-risico assessment verbeterd zou kunnen worden. Het uiteindelijke doel zou kunnen zijn om een predictie model in te bedden in de elektronische dossiers, waarbij op basis van een aantal risicofactoren, het risico op BRMO-dragerschap berekend wordt. Om zo'n predictiemodel efficiënt te maken en een teveel aan missende informatie te voorkomen, is een gecombineerd dossier vanuit huisartsenpraktijken en ziekenhuizen nodig.

Om de **contaminatie van de ziekenhuis omgeving** beter in kaart te brengen, raden wij ten eerste aan om een internationale survey uit te voeren, waarbij huidige schoonmaak protocollen in kaart gebracht worden. Deze schoonmaak protocollen kunnen geëvalueerd worden op hun effectiviteit en deze informatie kan gebruikt worden om een richtlijn voor het schoonmaken van ziekenhuizen op te stellen. Ten tweede raden wij aan om te bepalen welke sampling methoden het meest geschikt is voor specifieke micro-organismen. De rol van sampling bij uitbraakmanagement wordt momenteel al door ESGNI en HIS leden opgepakt middels een reviewstudie. Dit is een mooi vervolg op de eerdergenoemde sampling studie. Ook kan een literatuurstudie uitgevoerd worden om te bepalen welke oppervlakte regelmatig gecontamineerd zijn. Al deze informatie tezamen kan gebruikt

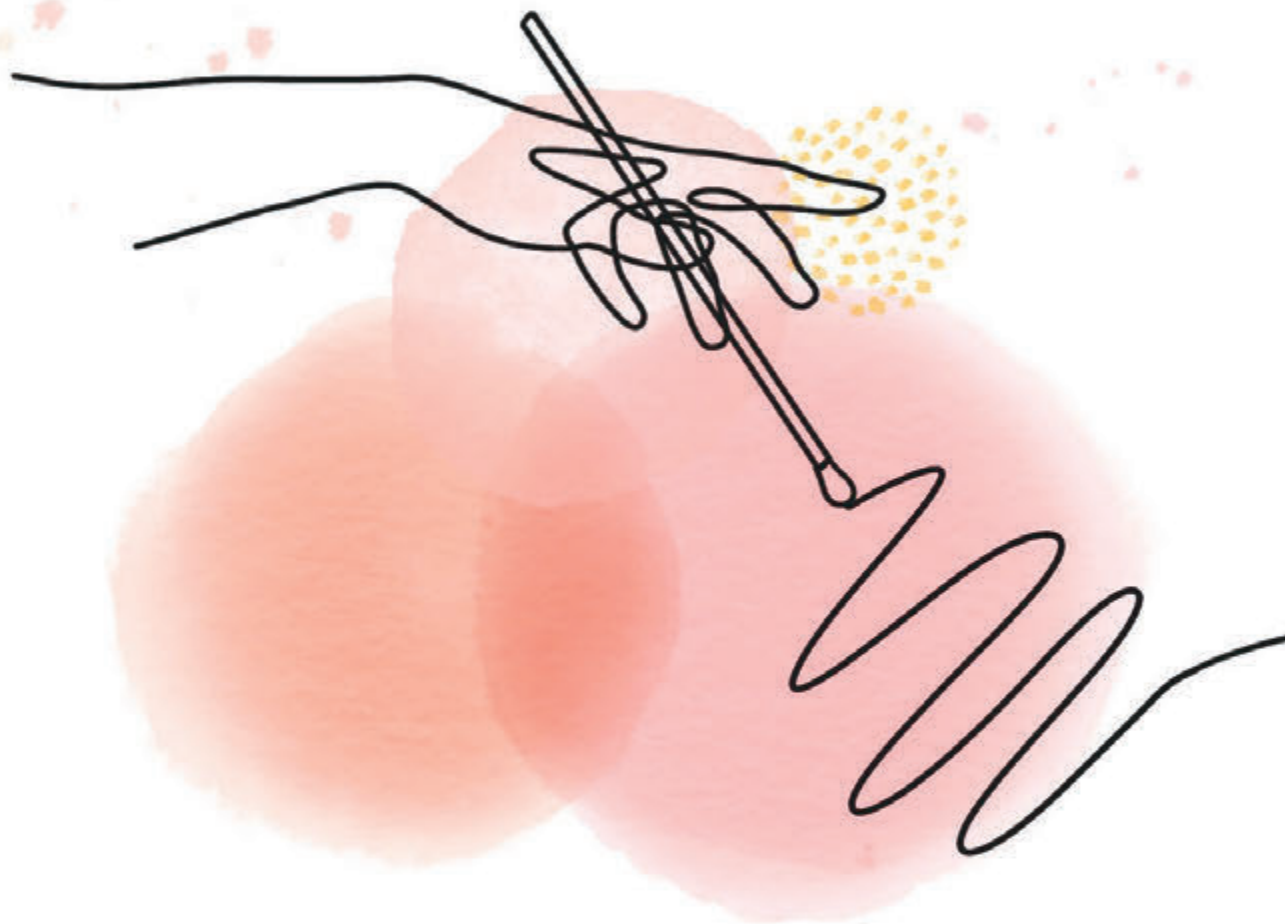
worden bij het opstellen van een richtlijn voor het uitvoeren en de indicatiestelling van omgevingssampling. Ten derde raden we aan om het effect van éénpersoonskamers op het microbioom van de ziekenhuisomgeving te bepalen, en de mogelijke gevolgen voor transmissie van BRMO. Hiertoe loopt reeds een onderzoek dat kijkt naar het effect van 100% éénpersoonskamers op het microbioom van de ziekenhuisomgeving. Deze informatie kan bijdragen aan het verbeteren van infectiepreventie strategieën en aan het in kaart brengen van de verspreiding van resistentiegenen.

Om het **effect van de overgang naar éénpersoonskamers en privé-badkamers** beter in kaart te brengen, raden wij ten eerste aan om de omgevingscontaminatie ook te bepalen na 5 en 10 jaar, om het lange termijneffect van de overgang te bepalen. Ten tweede raden wij aan om retrospectief te kijken of er verschil zit in het aantal uitbraken in de oudbouw en de nieuwbouw. Hierbij moet ook gekeken worden naar het bijvoorbeeld het aantal contact onderzoeken wat uitgezet is. Hiermee kan o.a. bepaald worden of de verhuizing kosteneffectief was vanuit infectiepreventie oogpunt. Ten derde raden wij aan om het effect van éénpersoonskamers te bepalen in een land met een hogere prevalentie van BRMO. Als laatste raden wij aan om het effect van éénpersoonskamers op acquisitie, omgevingscontaminatie en op uitbraken te bepalen in een kinderziekenhuis.

Voor het ontwerpen van toekomstige ziekenhuizen raden wij architecten aan om 100% éénpersoonskamers te implementeren in hun ontwerp. Daarnaast raden wij met klem aan dat architecten nauwer samenwerken met artsen-microbioloog, deskundigen infectiepreventie, artsen, verpleegkundigen en facilitair medewerkers, inclusief schoonmaakpersoneel. Door de verschillende expertises te combineren kan gezorgd worden dat het toekomstige ziekenhuis zo efficiënt mogelijk gebruikt kan worden. Om hier een eerste start voor te maken is er een netwerkvoorstel ingediend in het EU-COST programma (Hospital Preparedness for Epidemics: network for designing safe and healthy healthcare environments, HoPE), met als doel een multidisciplinair netwerk voor het verbeteren van de veiligheid van ziekenhuizen door ontwerp op te zetten.

Concluderend; gebaseerd op de resultaten van dit proefschrift raden wij aan dat nieuwe ziekenhuizen gebouwd worden met 100% éénpersoonskamers om de microbiologische veiligheid te verbeteren. De omgevingscontaminatie met BRMO was significant lager in de éénpersoonskamers. Hoewel we niet een direct effect op acquisitie aan hebben kunnen tonen, zorgt de geassocieerde verlaging in verplaatsingen binnen het ziekenhuis voor minder acquisitie van ESBL-E, en mogelijk ook andere BRMO. Wij concluderen dat de éénpersoonskamers bijdragen aan een microbiologisch veiliger ziekenhuis. Gezien de resultaten in onze situatie met een lage prevalentie van BRMO, kan de impact van éénpersoonskamers in een situatie met een hogere prevalentie potentieel groter zijn. Wat betreft de screening van patiënten bij opname concluderen wij dat, hoewel een universele screening strategie in theorie effectief is, het redelijk is om het risico assessment te verbeteren. Dit kan door bijvoorbeeld extra vragen over risico factoren zoals recent reisgedrag toe te voegen, waardoor de identificatie van dragers bij opname verhoogd zal worden.

Appendices



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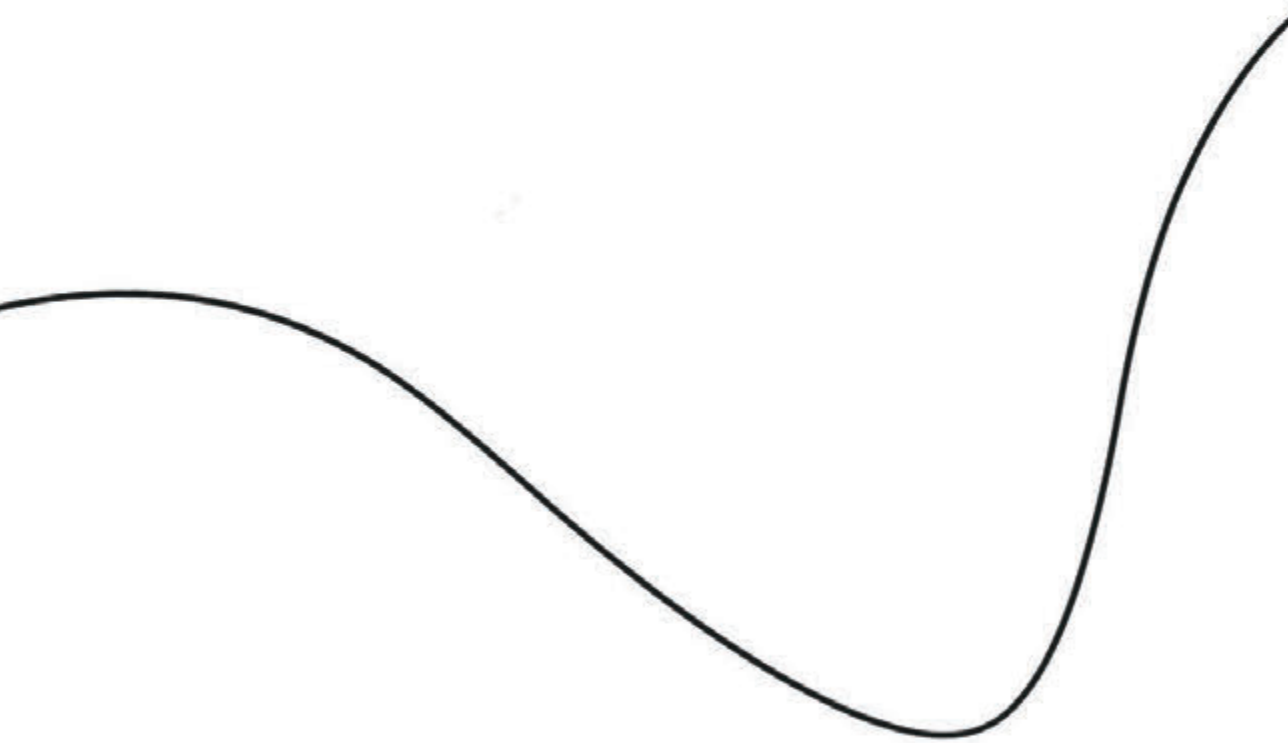

Dankwoord

Tegen de tijd van mijn verdediging ben ik 2,185 dagen met mijn proefschrift bezig geweest, ofwel 19.7% van mijn leven. Zes jaar (min zes dagen), waarin ik genoeg swabs voor mijn hele leven heb afgenomen (bijna 20,000), en die het beste te beschrijven zijn als leerzaam, frustrerend, uitdagend, en een rollercoaster van emoties. Gelukkig schrijf je een proefschrift nooit écht alleen en heb ik de afgelopen jaren een ontzettend lieve groep van mensen om me heen mogen verzamelen, zowel op werk, als privé. Nu is HET moment om deze mensen te bedanken en ze in het zonnetje te zetten voor wat ze voor mij hebben betekend.

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Curriculum vitae

Adriënne Stephanie van der Schoor was born on the 17th of June, in 1993, in Noordwijk, the Netherlands. After finishing the gymnasium at the Teylingen College, location Leeuwenhorst, Noordwijkerhout, in 2011, she started with the bachelor Health and Life Sciences at the Vrije Universiteit Amsterdam. She obtained her bachelor's degree in 2015 with a major in health sciences, after finishing her bachelor thesis at the Institute of Public Health and the Environment. She presented the results of her bachelor thesis at the 8th EUPHA in Milan, Italy. Directly after obtaining her bachelor's degree, she started with the master Health Sciences at the Vrije Universiteit Amsterdam, with a specialization in Infectious Diseases. After finishing her master thesis at the tropical institute in Antwerp, Belgium, she obtained her master's degree in 2016. Hereafter, she took a gap year and went to Valencia, Spain for two months to improve her Spanish. Upon returning, she obtained a PhD position at the department of Medical Microbiology and Infectious Diseases at the Erasmus MC University Medical Center, under the supervision of Prof. dr. Margreet C. Vos, dr. Anne F. Voor in 't holt, and dr. Juliëtte A. Severin, of which the results are presented in this thesis. During her PhD, Adriënne has presented her research at multiple international conferences. She has been involved in organizing the departmental team building activity, and in organizing the weekly departmental research meeting. In April 2023, she started working at the municipal health services (region Haaglanden and Hollands-Midden) to formulate the program for infectious disease control for the Academic Workplace Public Health NZH.

List of publications

Voor In 't Holt AF, Mourik K, Beishuizen B, **van der Schoor AS**, Verbon A, Vos MC, Severin JA. Acquisition of multidrug-resistant Enterobacterales during international travel: a systematic review of clinical and microbiological characteristics and meta-analyses of risk factors. Antimicrob Resist Infect Control. 2020 May 20;9(1):71.

van der Schoor AS, Severin JA, van der Weg AS, Strepis N, Klaassen CHW, van den Akker JPC, Bruno MJ, Hendriks JM, Vos MC*, Voor In 't Holt AF*. The effect of 100% single-occupancy rooms on acquisition of extended-spectrum beta-lactamase-producing Enterobacterales and intra-hospital patient transfers: a prospective before-and-after study. Antimicrob Resist Infect Control. 2022 Jun 2;11(1):76.

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van der Schoor AS*, Boyle M*, Voor In 't Holt AF, Vos MC**, Humphreys H**; ESCMID Study Group for Nosocomial Infections. Environmental sampling of innate hospital surfaces: a survey of current practices and the need for guidelines. J Hosp Infect. 2022 Oct;128:92-95.

van der Schoor AS, Severin JA, Klaassen CHW, Gommers D, Bruno MJ, Hendriks JM, Voor In 't Holt AF, Vos MC. 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. Int J Hyg Environ Health. 2023 Jan 6;248:114106. doi: 10.1016/j.ijheh.2022.114106.

van der Schoor AS, Severin JA, Klaassen CHW, van den Akker JPC, Bruno MJ, Hendriks JM, Vos MC, Voor In 't Holt AF. Universal screening or a universal risk assessment combined with risk-based screening for multidrug-resistant microorganisms upon admission: Comparing strategies. PLoS One. 2023 Jul 25;18(7):e0289163. doi: 10.1371/journal.pone.0289163.

*authors contributed equally

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PhD portfolio

Name	Adriënne Stephanie van der Schoor
Institute	Erasmus MC University Medical Center
Department	Medical Microbiology and Infectious Diseases
PhD period	2017-2023
Promotor	Prof.dr. Margreet C. Vos
Co-promotor	Dr. Anne F. Voor in 't holt
	Dr. Juliëtte A. Severin

Courses

The Course on R, 1,8 ECTS	2018
The Workshop on Microsoft Excel 2010: Advanced, 0,3 ECTS	2019
The Photoshop and Illustrator CC 2019 Workshop for PhD-students and other researchers, 0,15 ECTS	2019
Research Integrity, 0,3 ECTS	2019
The Workshop on How to Design and Pitch a Good Poster, 0,15 ECTS	2020
The Workshop on InDesign CC 2019 for PhD-students and other researchers, 0,15 ECTS	2020
The Biomedical English Writing Course for MSc and PhD students, 2,5 ECTS	2021
The Excel Visual Basic, 0,30 ECTS	2021

National & international conferences, meetings, & presentations

Conferences

The 29th ECCMID, Amsterdam, the Netherlands	2019
Two paper posters (first author)	
One oral presentation (co-author)	
The 30th ECCMID, Paris, France - *cancelled*	2020
One paper poster (first author)	
FIS/HIS International - *digital*	2020
One oral presentation (first author)	
One digital poster (co-author)	
Decennial 2020, 6th International Conference on Healthcare Associated Infections, Atlanta, USA - *cancelled*	2020
Two poster presentations, first author	
The 31st ECCMID – *digital*	2021
One oral presentation (first author)	
One mini oral flash (first author)	
Two digital posters (first author)	
ICPIC, Geneva, Switzerland	2021
The 32nd ECCMID, Lisbon, Portugal	2022
One oral presentation (first author)	
ARCH22, Delft, the Netherlands	2022
One oral presentation (first author)	

Meetings & Seminars

HIP Symposium – Wees ruim(te) denkend in infectiepreventie, Den Haag, the Netherlands	2019
Evolutie of revolutie? Praat mee over de wetenschapper van 2030, Den Haag, the Netherlands	2019
Inspiratie dag patiëntveiligheid,, Rotterdam, the Netherlands Invited speaker	2019
Weekly departmental Research meetings	2017 - 2022
Weekly departmental Journal club meetings	2017 - 2022
Science day Medical Microbiology and Infectious Diseases	2017, 2019

Teaching activities

Supervision of Tija Ivanovic, third-year Biology and Medical Laboratory Research student	2018
Supervision of medical student team	2018 - 2019
Supervision of Anna-Sjoukje van der Weg, MSc student Health Sciences	2020
Lecture “Antimicrobial resistance in returning travelers” for master I&I	2022

Grants

Personal grants

31st ECCMID Travel Grant	2020

Research grants

Erasmus MC Grant, pilot study Environmental Microbiome: Identifying the Hidden Hell of a Hospital Room co applicant	2018

Other activities

Review of articles

Canadian journal of infectious diseases and medical microbiology	2020
Journal of Hospital Infection	2020
International Journal of Environmental Research and Public Health	2020

Extracurricular activities

Organization of team building activity	2019 - 2020
Organization of weekly departmental research meeting	2020 - 2021