

Microbial ecology of protective isolation room: Air and Surfaces

Écologie microbienne des chambres d'isolement protecteur: Air et surfaces

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ABSTRACT

Introduction: Healthcare-associated infections pose a significant public health burden, leading to morbidity, mortality, prolonged hospital stays, and substantial social and economic costs. Immunocompromised patients are at a heightened risk of nosocomial infections.

Aim: This prospective study conducted at Mohammed VI University Hospital of Oujda aimed to assess the microbial ecology of surfaces and air in an immunosuppressed patient room compared to a double hospitalization room.

Methods: Microbiological air purity tests were conducted employing both the sedimentation method and the collision method with the assistance of Microflow Alpha. The sedimentation method used Mueller Hinton with 5% human blood, facilitating the free fall of contaminated dust particles. The collection program employed was set for 10 minutes per 1 m³. For surface sampling, swabs were taken from a 25 cm² surface. The swabs were immediately forwarded to the Microbiology Laboratory. We carried out both macroscopic and microscopic identification of colonies, followed by definitive biochemical identification using the BD phoenixTM system. Antibiotic susceptibility was assessed through agar diffusion on Muller Hinton medium coupled with the determination of the minimum inhibitory concentration.

Results: The results revealed a decreased bacterial count within the protective isolation room, in contrast to the standard hospital room. We noted the predominance of coagulase-negative Staphylococcus spp and Bacillus spp. Staphylococcus aureus and Aspergillus spp, common pathogens in healthcare-associated infections, were notably absent in the protective isolation room. The findings underline the pivotal role of hospital environments in the transmission of healthcare-associated infections.

Conclusion: The protective isolation room demonstrated effective control of microbial contamination, with fewer and less resistant germs. The study highlighted the significance of air treatment systems in preventing the spread of opportunistic infections. Our study underscored the critical role of microbiological cleanliness in preventing nosocomial infections.

Key words: microbial ecology, protective isolation room, immunocompromised patient, healthcare-associated infections.

RÉSUMÉ

Introduction: Les infections associées aux soins représentent un grand fardeau pour la santé publique, entraînant morbidité, mortalité, séjours prolongés à l'hôpital et coûts sociaux et économiques substantiels. Les patients immunodéprimés présentent un risque accru d'infections nosocomiales.

Objectif: Cette étude prospective menée au CHU Mohammed VI d'Oujda avait pour objectif d'évaluer l'écologie microbienne des surfaces et de l'air dans une chambre d'isolement protecteur comparée à une chambre d'hospitalisation double.

Méthodes: Le prélèvement de l'air a été réalisé en utilisant la méthode de sédimentation et de collision avec l'aide de Microflow Alpha. Le programme de collecte utilisé était fixé à 10 minutes pour 1 m³. Pour l'échantillonnage de surface, des écouvillons ont été prélevés sur une surface de 25 cm². Ils ont été immédiatement acheminés au laboratoire de microbiologie. Nous avons procédé à l'identification macroscopique et microscopique des colonies avant l'identification biochimique définitive par l'automate BD phoenixTM. L'étude de la sensibilité aux antibiotiques a été réalisée par diffusion sur gélose Muller Hinton et par la détermination de la concentration minimale inhibitrice.

Résultats: Les résultats ont révélé un nombre réduit de bactéries dans la chambre d'isolement protecteur par rapport à la chambre d'hospitalisation ordinaire. Nous avons pu noter la prédominance de staphylocoques à coagulase négative et de Bacillus spp. Staphylococcus aureus et Aspergillus spp, des agents pathogènes courants dans les infections associées aux soins, étaient notablement absents dans la chambre d'isolement protecteur. Ces résultats mettent en évidence l'importance des environnements hospitaliers dans la transmission des infections associées aux soins de santé.

Conclusion: La chambre d'isolement protecteur a démontré un contrôle efficace de la contamination microbienne, avec des germes moins nombreux et moins résistants. L'étude a mis en évidence l'importance des systèmes de traitement de l'air dans la prévention de la propagation des infections opportunistes. Notre étude souligne le rôle critique de la propreté microbiologique dans la prévention des infections nosocomiales.

Mots clés: écologie microbienne, chambre d'isolement protecteur, patient immunodéprimé, infections associées aux soins.

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INTRODUCTION

Healthcare-associated infections represent a major public health burden (1). These infections are responsible for morbidity and mortality in hospitalized patients, as well as prolonged length of stay and all the resulting social and economic costs (2) Individuals admitted to hospitals often experience a decline in their immune defenses, rendering them more vulnerable to infections compared to those in good health. Various treatments administered during hospitalization can further compromise their immune resistance. The World Health Organization (WHO) characterizes adverse events, encompassing nosocomial infections, as harm inflicted during or due to treatment, unrelated to the inherent progression of the disease or the patient's health status. Such events can manifest at any point during the hospital stay (3). Protective isolation rooms are specifically designed for immunocompromised patients requiring safeguarding against potential infections, with nosocomial infections being particularly life-threatening for this vulnerable group. Regular checks, primarily microbiological are essential to identify any contamination, assess the microbial ecology within the hospital, and implement preventive and corrective measures, protocols, and procedures. This rigorous approach is fundamental for managing infectious risks within the hospital system and ensuring the safety of immunocompromised patients (4). Microbiological testing of the hospital environment is advised during an epidemic outbreak when the environment is suspected to be the primary source of a rapidly spreading microorganism (5). Conversely, routine microbiological testing of the environment is a subject of debate, primarily attributed to the considerable financial expenses involved and concerns regarding the smear method's perceived low and inconsistent sensitivity when used for testing surfaces (5,6).

Our aim is to determine the microbial ecology of germs present on hospital surfaces and in the air in an immunosuppressed patient room, compare them to a double hospitalization room to detect any germs that could be responsible for nosocomial infection and highlight the value of the protective isolation room in providing a healthy climate against opportunistic infections that can affect patients in very vulnerable situations.

METHODS

It was a prospective study carried out at Mohammed VI University Hospital in Oujda. The samples were taken on the same day by the doctor. We included in our study a room dedicated to immunocompromised patients and a room dedicated to the hospitalization of two immunocompetent patients. The first room is subject to very strict hygiene rules and is equipped with a laminar flow air treatment system: Airdrop 1800. Visits are very limited, and personal protective equipment must be worn by nursing staff. The second room is an ordinary hospital room, cleaned daily in accordance with zone 2

protocols.

We have included air and surface sampling in our study. The surfaces concerned were: the patient's bed, the table, the door handle, the wall, the chair, and the sofa. The collected material underwent analysis at the Clinical Microbiology Laboratory. Microbiological air purity tests were conducted employing both the sedimentation method and the collision method with the assistance of Microflow Alpha. The sedimentation method utilized blood Agar, facilitating the free fall of contaminated dust particles. The collection program employed was set for 10 minutes per 1 m³. For surface sampling, swabs were taken from a 25 cm² surface. The swabs were immediately forwarded to the Microbiology Laboratory. Inoculation of swabs was performed in quadrants on blood agar and incubated at 35 ± 2 °C for 48 hours until the colonies were grown on the media. The purification was performed by taking the bacterial colonies that have different characteristics/aspects in each petri dish. The obtained isolates were identified using the BD Phoenix™. An antibiogram was carried out to study the sensitivity of the bacteria to the different families of antibiotics according to the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST 2023)(7). We studied antibiotic sensitivity using the minimum inhibitory concentration method for coagulase-negative staphylococci and the agar diffusion method for staphylococcus aureus. An interpretative reading according to the recommendations of the French Microbiology Society's Antibiogram Committee (CASFM) was carried out.

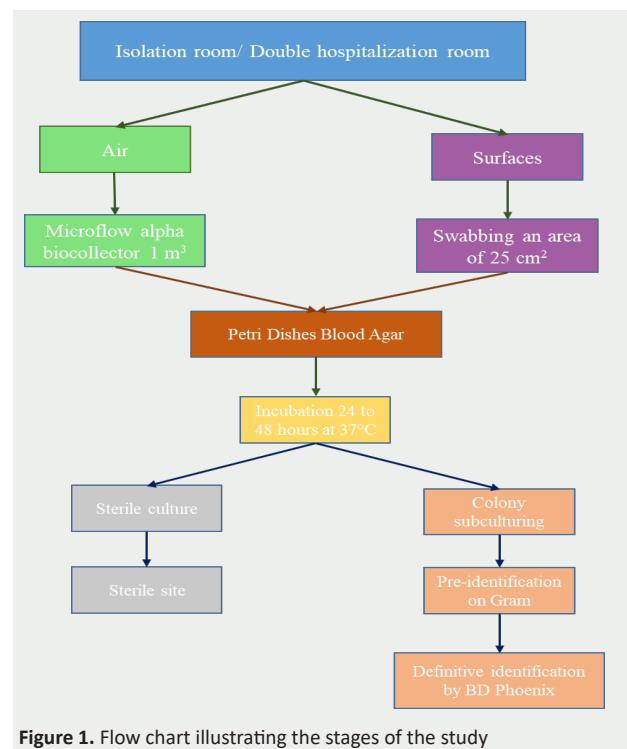


Figure 1. Flow chart illustrating the stages of the study

RESULTS

The results of our study varied according to the chamber concerned and the site sampled. The protective isolation

room was inferior to the double hospitalization room. All the results are shown in Tables 1 and 2. The percentage of bacteria identified by the automated ranged between 90% and 99%.

Table 1. Enumeration and identification of germs in the protective isolation chamber

Site	Colony count UFC / 25 cm ²	Identified germs
Air	83*	<i>Staphylococcus pettenkoferi</i> <i>Kocuria varians</i> <i>Streptococcus oralis</i> <i>Bacillus threngiensis</i> <i>Bacillus circulans</i>
Surfaces: Wall	0	
Surfaces: Door handle	3	<i>Brevudimonas vesicularis</i>
Surfaces: Bed	9	<i>Micrococcus lylae</i> <i>Corynebacterium urealyticum</i>
Surfaces : Table	45	<i>Bacillus circulans</i> <i>Kocuria varians</i>
Surfaces : chair	61	<i>Bacillus circulans</i> <i>Staphylococcus hominis</i> <i>Rodentibacter pneumotropicus</i> <i>Gemella morbillorum</i>
Surfaces : Sofa	209	<i>Staphylococcus warneri</i>

*Unit used for air: UFC/m³

Table 2. Enumeration and identification of germs in the double hospitalization room

Site	Colony count UFC / 25 cm ²	Identified germs
Air	167 *	<i>Staphylococcus lugdunensis</i> <i>Staphylococcus hemolyticus</i> <i>Staphylococcus capitis</i> <i>Micrococcus luteus</i> <i>Arcanobacterium haemolyticum</i> <i>Bacillus cereus</i> <i>Aspergillus fumigatus</i> <i>Mucor mucor</i>
Surfaces : Door handle	4	<i>Staphylococcus epidermidis</i> <i>Staphylococcus hominis</i>
Surfaces : interrupteur	10	<i>Staphylococcus aureus</i> <i>Mannheimia hemolytica</i>
Surfaces : Bed 1	26	<i>Staphylococcus saprophyticus</i> <i>Staphylococcus hemolyticus</i> <i>Staphylococcus epidermidis</i> <i>Kocuria varians</i> <i>Corynebacterium urealyticum</i>
Surfaces : Bed 2	16	<i>Staphylococcus hominis</i> <i>Kocuria varians</i>
Surfaces : Wall	10	<i>Staphylococcus warneri</i> <i>Gemella morbillorum</i>
Surfaces : Table 1	36	<i>Staphylococcus aureus</i> <i>Staphylococcus hominis</i> <i>Pantoea agglomerans</i> <i>Bacillus coagulans</i> <i>Candida guilliermondii</i>
Surfaces : Table 2	41	<i>Staphylococcus gallinam</i> <i>Staphylococcus hominis</i> <i>Micrococcus luteus</i> <i>Bacillus subtilis</i> <i>Bacillus licheniformis</i>
Surfaces : Chair	25	<i>Staphylococcus hemolyticus</i> <i>Staphylococcus equorum</i> <i>Micrococcus luteus</i>

*Unit used for air: UFC/m³

We performed an antibiogram for staphylococcus aureus on agar medium with 30 µg cefoxitin disc, determining it to be methicillin-resistant staphylococcus aureus (MRSA).

Additionally, an antibiotic susceptibility study was conducted for coagulase-negative staphylococci (Figure 2), given their prevalence among the isolated germs and their involvement in healthcare-associated infections due to their multi-resistance to antibiotics. While these germs were once considered to be little or no pathogenic significance, they are now increasingly recognized as contributors to healthcare-associated infections.

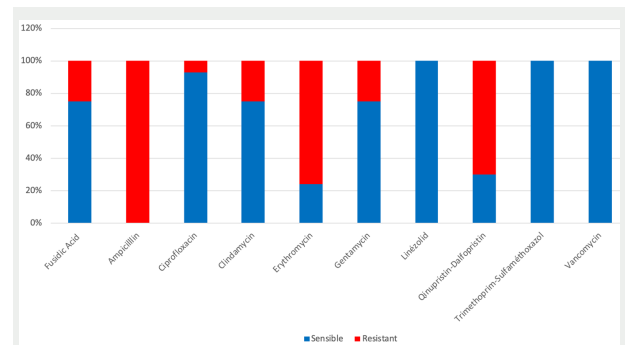


Figure 2. Antibiogram of coagulase-negative *Staphylococci*

DISCUSSION

Contamination of an anatomical site by micro-organisms and their multiplication leads to colonization and subsequently to the infection responsible for the symptoms. In the hospital environment, patients are exposed to nosocomial germs responsible for healthcare-associated infections.

Two types of bacteria can be found in patients' environments: bacteria of human origin (skin, mucous membranes), including multi-drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and vancomycin-resistant *Enterococcus* (8), and those originating from environmental sources. The most common bacteria found were coagulase-negative *Staphylococci* at 36% and *Bacillus* at 16%. *Staphylococcus aureus* was found in the double hospital room and represented 5% of all bacteria that were found in this chamber, at two sites: the light switch and the patient's table. However, it was not isolated in the protective isolation room, which may be explained by the fact that caregivers and visitors must wear personal protective equipment including a mask covering the nose and mouth, and the germ was not isolated. According to a study carried out in Europe, this bacterium is characterized by nasal carriage in 40% of the population (9). Hence the importance of screening for nasal colonization by *S. aureus* on patients. A study was carried out in 2017 at Craiova Hospital, Romania by Anca Ungureanu et al. where 322 pharyngeal exudates and 142 nasal exudates in inpatients and outpatients for screening purposes were received.

The rates of pharyngeal carriage were 27.06% for *Staphylococcus aureus*, 11.55% for methicillin-resistant *Staphylococcus aureus* (MRSA), and 5.61% for methicillin-oxacillin-resistant *Staphylococcus aureus* (MORSA). Meanwhile, the rates of nasal carriage were 35.38% for *S. aureus*, 18.46% for MRSA, and 13.85% for MORSA

(10). Bacteria of environmental origin, some of which exhibit frequent natural resistance to antibiotics, notably Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Burkholderia cepacia*, *Legionella pneumophila* or atypical mycobacteria, were not found in either chamber. We also noted the absence of *Aspergillus* in the protective isolation chamber, but we found it in the ordinary room; *Aspergillus fumigatus* and *Mucor mucor*, which is not equipped with an air treatment system. These results can only confirm the effectiveness of the protective isolation chamber air treatment system. Yeasts and especially environmental filamentous fungi (*Aspergillus spp.*) are very well adapted to survival and multiplication in the environment and will be responsible for nosocomial infections mainly in immunocompromised patients (11). Viruses can also contaminate the environment, most often from the human reservoir formed by patients and hospital staff. Their importance is certainly underestimated, as their detection is technically difficult to carry out (12,13). When patients are colonized, and especially when there is a patent infection, their immediate environment is usually heavily contaminated with these micro-organisms (14-16).

The survival and eventual multiplication of bacteria determine the nature and extent of environmental colonization, and the environment's capacity to become a reservoir in which the micro-organism can be transmitted. This survival in the environment, favored by the formation of biofilms on surfaces, varies according to the bacteria and the nature of the contaminated surfaces (4). *Staphylococcus aureus* and *Acinetobacter baumannii* are among the most resistant species to desiccation and can survive for several weeks on dry surfaces, ahead of *Pseudomonas aeruginosa*, some *Enterobacterales*, and *Enterococci*, which can survive for more than a week (17-22). *Escherichia coli*, the most common *Enterobacteriaceae* in hospital-acquired infections, is much less resistant to desiccation (18-22). Particularly long survival times of over 6 months have been described, especially with certain epidemic strains of methicillin-resistant *S. aureus* (23). In humid conditions and in the presence of organic matter, survival is even longer (18). The ability of some bacteria, such as *Clostridium difficile*, to sporulate ensures very long persistence in the environment. Our results are comparable to those described in the literature, notably the study carried out by Kanga Hortense Gonsu and colleagues at two referral hospitals in Yaoundé-Cameroon and Tagnoukam, which showed the predominance of 57% coagulase-negative *Staphylococcus spp* (24), and the Moroccan study carried out by Saouide el ayne. N and al. at a hospital in kénitra, which showed the predominance of 26 % coagulase-negative *Staphylococcus spp* and 27% *Bacillus spp* (25). We isolated methicillin-resistant *S. aureus*. In recent decades, due to bacterial evolution and antibiotic abuse, drug resistance in *S. aureus* has progressively increased, the rate of MRSA infection has risen worldwide, and clinical anti-infective treatment of MRSA has become more difficult.

Diseases constitute the second leading cause of

global human mortality. *Staphylococcus aureus*, a prevalent pathogenic microorganism in humans, has the potential to instigate various infectious conditions, including skin and soft tissue infections, endocarditis, osteomyelitis, bacteremia, and fatal pneumonia. (26). Antibiotic susceptibility testing for coagulase-negative *Staphylococci* showed zero sensitivity to ampicillin, and 100% sensitivity to trimethoprim-sulfamethoxazole, linezolid, and vancomycin. Sensitivity was over 70% for fucidic acid, ciprofloxacin, clindamycin and gentamycin. Sensitivity below 30% was reported for erythromycin and quinupristin-dalfopristin.

In our study, we compared the protective isolation room with the ordinary hospital room, the number of colonies was significantly lower, and the germs isolated were by far the least resistant to treatment, while we did not find the germs responsible for healthcare-associated infections. This underlines the interest and importance of protective isolation rooms in ensuring the best health care for immunocompromised patients.

The new cleaning technologies demonstrate a high level of efficiency compared with those used in the past, which reduced the microbial load but did not definitively eradicate bacteria. These new methods include technologies that are both microbiologically effective and safe to use, such as hydrogen peroxide vapor and UV light decontamination for terminal cleaning, as well as ultra-microfibers combined with a copper-based biocide (27). Hydrogen peroxide vapor and UV light can reduce the amount of bacterial cells by at least four orders of magnitude, significantly reducing patients' risk of contracting multidrug-resistant bacterial infection (28). Identifying and prioritizing the factors that contribute to healthcare-associated infections can help to guide and better target actions to prevent and combat HCAs. The factors contributing to the occurrence of HCAI are numerous and interrelated. They can be grouped under three main headings: patient-related factors, exposure to infectious risks associated with diagnostic and therapeutic procedures, and shortcomings in the organization of care (29). The actions of the hospital-acquired infection control committee cannot act on the intrinsic factors that concern the patient, but can very well act on the other two headings. First and foremost, they focus on raising the awareness of care staff, applying hygiene rules, and limiting the use of invasive care methods when the clinical context allows. They also ensure periodic microbiological monitoring of the hospital environment, as well as the management of patient movements within the hospital, the management of visits and the management of medical and pharmaceutical waste.

CONCLUSION

Maintaining microbiological cleanliness in hospitals is a crucial factor in preventing nosocomial infections. Our research suggests that the predominant microorganisms in hospital environments are Gram-positive, with relatively low pathogenic potential, including *Micrococcus spp*, *Bacillus spp*, and *Staphylococcus spp* (excluding *S.*

aureus). These microorganisms are commonly present in the air and on dry surfaces. Despite their perceived lower pathogenicity, even these microorganisms can pose a risk to patients with severe immunodeficiency. The physical environment in healthcare facilities plays a crucial role in reducing and preventing the spread of healthcare-associated infections. Proper design and construction of health care buildings, including ventilation and air conditioning systems, help prevent infection spread between functional areas. Antimicrobial materials, cleaning and disinfection protocols, and personal hygiene practices, such as hand hygiene, are key factors in infection control. The positioning of hand hygiene stations is also essential to improve compliance among healthcare professionals.

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