Universidade de Lisboa
Faculdade de Farmácia

Microbiological Assessment of Air and Surfaces of Surgery Rooms from a Lisbon Hospital

Beatriz Isabel Mendes Almeida

Mestrado Integrado em Ciências Farmacêuticas

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Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

O presente trabalho surgiu no âmbito de uma parceria entre o Departamento de Qualidade do Hospital da Ordem Terceira e o Laboratório de Controlo Microbiológico da Universidade de Lisboa - Associação para o Desenvolvimento e Ensino da Microbiologia (ADEIM) que teve como objetivo avaliar anualmente a qualidade microbiológica do ar e das superfícies dos blocos operatórios e das respetivas salas de recobro deste hospital. O estudo foi iniciado em 2011, ano em que se realizou o controlo microbiológico do bloco operatório oftalmológico (Sala Lasik) e da respetiva sala de recobro. A partir do ano 2014 o estudo foi alargado aos 5 blocos operatórios gerais e à sala de recobro geral. Como complemento ao estudo, a partir de 2015 passou a ser feita a pesquisa de *Legionella pneumophila* nos ductos do ar condicionado dos blocos operatórios.

A contaminação microbiológica do ar e das superfícies durante os procedimentos cirúrgicos é um percursor das infeções do local cirúrgico, particularmente das infeções incisionais superficiais. Estas infeções implicam uma proliferação bacteriana e subsequente reação inflamatória nas zonas associadas à cirurgia. Nas suas expressões mais graves pode condicionar uma reação inflamatória sistémica com disfunção ou falência multiorgânica, associada a um aumento de mortalidade e morbilidade. A avaliação microbiológica periódica e sistemática do ar interior e das superfícies dos blocos operatórios é assim essencial para garantir níveis mínimos de qualidade e assegurar a saúde e segurança não só dos doentes, mas também dos profissionais de saúde.

No contexto da monitorização anual da contaminação microbiológica do ar e das superfícies dos blocos operatórios e das salas de recobro do Hospital da Ordem Terceira, pretendeu-se realizar um estudo preventivo com o objetivo principal de determinar pontos críticos de contaminação e verificar se os procedimentos de higienização e desinfeção implementados estavam de facto a ser eficazes.

No que diz respeito à metodologia utilizada, as amostras de ar foram recolhidas através do método de impactação com o equipamento MAS-100 (Merck®) e as amostras de superfície foram recolhidas com placas de contacto (superfícies regulares) ou com zaragatoas (superfícies irregulares ou de difícil acesso). Depois de processadas as amostras, e após os respetivos tempos de incubação, foi feita a contagem do número de microrganismos aeróbicos totais (TAMC) e do número de fungos e leveduras totais (TYMC) e foram identificadas as estirpes suspeitas.

A pesquisa de *Legionella pneumophila* foi feita nos ductos do ar condicionado dos blocos operatórios através da recolha da água de condensação e utilizando o método da zaragatoa. Esta bactéria pode persistir por longos períodos de tempo na água e é encontrada frequentemente em biofilmes no interior das canalizações. É o agente etiológico da Doença dos Legionários pelo que é fundamental garantir a sua ausência em ambiente hospitalar.

Os resultados obtidos no presente trabalho demonstraram que das 230 amostras de ar colhidas nos blocos operatórios e nas salas de recobro entre 2011 e 2016, apenas em 11% não se verificou qualquer crescimento microbiológico, no entanto em nenhuma amostra se observou um número incontável de colónias. A amostra mais contaminada (241 CFU/500L) foi colhida em frente ao Bloco Operatório 3 no ano 2015. Como seria espectável, a contaminação do ar no corredor em frente aos blocos operatórios foi superior ao interior dos blocos operatórios.

Relativamente às amostras de superfícies, foram colhidas 980 amostras das quais 48% não revelaram qualquer crescimento microbiológico. Os locais de amostragem que estavam diretamente em contacto com o doente ou com os profissionais de saúde foram os que revelaram maior contaminação microbiológica. Entre eles destacaram-se os interruptores, o carro de anestesia, a marquesa operatória, a calculadora e o microscópio.

Nos diversos anos de estudo as salas de recobro revelaram níveis de contaminação do ar e de superfícies superiores ao dos blocos operatórios. Estes resultados estão de acordo com o esperado uma vez que os blocos operatórios têm procedimentos de limpeza e desinfeção mais frequentes e rigorosos.

As bactérias isoladas nos blocos operatórios e nas salas de recobro foram *Staphylococcus* spp. e *Bacillus* spp.. No que diz respeito aos fungos e leveduras, foram identificados *Cladosporium* spp., *Penicilium* spp., *Aspergillus* spp., *Alternaria* spp., *Phoma* spp., *Rhizopus* spp. e *Rhodotorulla* spp..

Na origem dos picos de contaminação observados esporadicamente em determinados pontos de amostragem poderão estar os seguintes fatores: procedimento de higienização e desinfeção inadequados, falha nos sistemas de ventilação ou alterações ambientais (temperatura e humidade).

Em nenhuma das amostras recolhidas nos dois anos de estudo foi detetada a presença de *Legionella pneumophila* nos ductos do ar condicionado.

A partir dos resultados obtidos conclui-se que a monitorização microbiológica periódica do ar e das superfícies dos blocos operatórios em conjunto com procedimentos de limpeza e desinfeção é fundamental para manter padrões de qualidade e consequentemente melhorar o serviço ao utente e prevenir infeções do local cirúrgico.

De facto, a monitorização do ambiente hospitalar tem sido objeto de interesse e debate nos últimos anos, porém, ainda não existem normas internacionais no que respeita aos níveis de contaminação mínimos admissíveis e não há uniformização nos métodos de colheita das amostras, o que dificulta a comparação de resultados. Alguns países têm legislação própria, como é o caso de França e Inglaterra, no entanto, em Portugal a única legislação existente diz respeito à qualidade do ar interior de edifícios de comércio e serviços, que não exigem limites de contaminação tão restritos.

Palavras-chave: avaliação microbiológica; bloco operatório; contaminação do ar; contaminação das superfícies

Abstract

The Quality Management Department of the Ordem Terceira Hospital, in collaboration with the Microbiological Quality Control Laboratory of the University of Lisbon, initiated, in 2011, an annual study concerning the microbiological evaluation of the air and (inanimate) surfaces in the ophthalmology operating room (Lasik) and recovery room. As from 2014, the study was extended to include the five general operating rooms and general recovery room.

The main aim of this study was to identify critical contamination points within the operating and recovery rooms of this hospital in order to verify whether the methods of hygienization and disinfection implemented were in fact effective.

The impaction method using Merck™ MAS 100 equipment was used for the collection of indoor air, while contact plates and the Swab Test were used for the evaluation of surfaces and equipment. For all the samples collected, the total microbial aerobic count was performed for the quantification of bacteria, and the total yeast and mould count was performed for the quantification of yeast and moulds.

As a complement to this study, monitoring for the presence of *Legionella pneumophila* in the ducts of the operating room air conditioners was also carried out, starting in 2015. This investigation was conducted using samples of condensed water from the air conditioners and the Swab Test.

The results obtained revealed that in 11% of the 230 air samples collected from 2011 to 2016 there was no microbial growth, while 48% of the 980 samples collected in the same period did not reveal microbial growth. *Legionella pneumophila* was not detected in any of the samples collected.

A periodic review of the microbiological quality of the air and surfaces within operating rooms (and other high risk areas), in conjunction with cleaning and disinfection plans, is essential for maintaining the quality standards of these rooms and subsequent prevention of surgical site infections.

Keywords: indoor air quality; microbiological evaluation; operating room; surface contamination; surveillance

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Finally, any errors or inadequacies that may appear in this work are, of course, of my entire responsibility.

List of Abbreviations

CFU	Colony-Forming Units
GVPC	Glycerol Vancomycin Polymyxin Cycloheximide
OR	Operating Room
SAB CHLOR	Sabouraud Chloramphenicol Agar
TSA	Trypto-Casein Soy Agar
TSB	Trypto-Casein Soy Broth
TAMC	Total Aerobic Bacteria Count
TYMC	Total Yeast and Fungi Count

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1 Introduction

The hospital environment, colonized by many microorganisms, is composed of true ecological niches (1). Several studies carried out over the past years (2–6) have highlighted the importance of air and surface contamination in the acquisition of patient infections and occupational diseases.

Already in the 1960's, the sanitary control of the hospital environment was widely accepted as a desirable step in infection-prevention programs, even though the precise relationship between environmental contamination and hospital-acquired (nosocomial) infections was not yet clear (7). At that time, there was still a shortage of reliable information concerning the relative concentrations of all microorganisms in hospital air and the factors which influence the level of contamination (8).

Since then research has provided evidence of the importance of establishing environmental monitoring programs to control and maintain an aseptic hospital environment, especially in operating rooms (ORs), which are important hospital wards where most surgeries take place, and in other controlled areas such as recovery rooms (8,9).

Nowadays, OR complexes are divided into four different zones, based on the level of cleanliness, with the bacterial burden decreasing from the outer to the inner zones (10):

- a protective area that includes the changing rooms for the medical personnel, administrative staff rooms, pre- and post-operative rooms and the sterile and non-sterile stores;
- a clean area that connects the protective area to the aseptic zone;
- an aseptic zone which includes the ORs;
- a disposal area for each OR.

These zones are maintained by a differential decreasing positive pressure to prevent unfiltered air flow toward the inside of the ORs (11–13).

In order to control environmental factors such as temperature, relative humidity and air flow, contemporary ORs are equipped with heating, ventilation and air conditioning systems. The ventilation systems (with vertical flow, horizontal flow, or exponential laminar flow) are equipped with different filters according to the surgical procedures performed (13).

Studies carried out in the United States (14–16) have reported that *Staphylococcus aureus*, which is a typical skin-associated microbe, is a commonly isolated microorganism from OR environments. It can live for weeks or months on surfaces that are not kept clean. In two studies (16–18), it was observed that there is a larger number of these bacteria in the critical zones (areas which are in close proximity to the patient) than in the intermediate and peripheral zones of the ORs (16,19). Other microorganisms which have been isolated from ORs include: coagulase-negative staphylococci, *Escherichia coli*, *Enterococcus faecalis, Bacillus spp, Micrococcus* spp., *Pseudomonas* spp., *Klebsiella* spp..(5,20–22)

When suitable control measures are implemented in these areas, the level of contamination can be diminished and kept low, thus reducing the incidence of certain hospital-acquired infections (23,24)

Surgical site infections (SSIs) are infections that occur after surgery in the part of the body where the surgery took place and account for about 14–20% of all hospital-acquired (nosocomial) infections (13). Some are superficial, involving only the skin, while others are more serious and can involve tissues under the skin, organs, or implanted material. Factors causing SSIs are known to be multifarious. Superficial SSIs are most often associated with environmental factors, such as environmental contamination by fungi and bacteria, surface contamination, humidity, differential pressure and temperature of the OR, while factors that determine deep and organ/space SSIs are more often associated with patient characteristics (age, sex, transfusion, nasogastric feeding and nutrition, as measured by the level of albumin in the blood), type of intervention and preoperative stay (2).

SSIs continue to be a major problem in modern medicine, with both individual and economic consequences. They are the most frequent nosocomial infections in low- and middle-income countries, affecting up to one third of patients who have undergone a surgical procedure, while in high-income countries they are the second most frequent type (16). They can result in significant patient illnesses and may be life threatening, especially among the elderly patients or those with chronic and immunocompromising conditions, and are due to the emergence of antibiotic-resistant microorganisms. From an economical point of view, these infections have important consequences on the healthcare systems of countries due to direct hospital costs associated with prolonged hospital stays and additional expenditure with medical staff and treatment. In addition, there are economic impacts resulting from diminished worker productivity or the loss of life (25,26). In a recent review, Badia et al. (26)

highlighted the need for renewed efforts from European countries to improve quality of care and consequently reduce the financial burden of SSIs.

Inadequate infection control measures and microbial contamination in the ORs may explain why these are "hot zones" for the emergence and spread of microbial resistance. Indeed, studies in Europe (9,27–32) have shown that the implementation of training and sanitary education programs result in an improvement of the environmental parameters and a significant reduction in the level of microbial contamination in ORs, and consequently in the reduction of the incidence of SSIs.

The first ever Global guidelines for the prevention of SSIs were published by the World Health Organization (WHO) (16) in 2016 and were elaborated in accordance with research carried out over the past years. Measures indicated include the maintenance of safe and salubrious ORs in which all sources of pollution and any micro-environmental alterations are kept strictly under control. This encompasses the maintenance of low contamination levels in the OR environment by employing strict cleaning procedures such as sterilization, disinfection and removal of contaminants (e.g. dust and organic waste). Cleaning and maintenance schedules should be implemented according to the surgical procedures performed. All ORs should be cleaned at the beginning of the day, between each surgical procedure, and at the end of the day, followed by a weekly or each second week total clean-up of the entire OR, including walls, floor and ventilation system (19). In addition, restricted staff entry, personnel hygiene, appropriate staff attire, adequate pre-operative preparation of the patient, the practice of optimal surgical techniques, appropriate use of peri-operative antimicrobial prophylaxis, a surgical wound surveillance programme, are also pointed out. Other recommendations include laminar flow, hight-efficiency particulate absorbing filters, daily exposure to ultraviolet radiation and air renewal (2).

Legionella pneumophila, a Gram-negative bacterium, is a causative agent of Legionnaires' disease, which can be acquired in hospitals and result in morbidity and mortality (33,34). Legionnaires' disease was first described in association with an outbreak of pneumonia at an American Legion convention in Philadelphia, Pennsylvania, in 1976 (34,35). This bacteria is normally found in man-made water systems, such as water of cooling towers of hospital air conditioners (36,37). Legionnaire's disease can be acquired by inhalation of aerosols containing legionella, or by micro-aspiration of contaminated drinking water (33).

Microbial monitoring of ORs (and also of other controlled environments, such as recovery rooms) is therefore essential to obtain representative estimates of the bioburden of the environment. It includes quantitation of the microbial content of room air, compressor air that enters the critical area, surfaces, equipment, sanitization containers, floors, walls and personnel garments and also the search for *Legionella pneumophila* in the water systems. Information gathered from the data compiled and analysed can then be useful in the investigation of the source of the contamination and the subsequent adoption of preventive measures.

The present work was realized in collaboration with the Quality Management Department of the Ordem Terceira Hospital and the Microbiological Quality Control Laboratory of the University of Lisbon, with the purpose of improving health care services and guaranteeing quality standards by the control and maintenance of environmental quality.

The Ordem Terceira Hospital is situated in Lisbon and is a Catholic institution founded in 1972. It is a reference hospital for ophthalmology, having a last generation Excimer Laser which permits a precise and secure correction of the majority of refractive errors. Apart from the ophthalmology OR, this hospital has five other general operating rooms, where a wide range of surgical procedures are performed.

This study was initiated in 2011 and comprised the microbiological evaluation of air and surfaces within the ORs and recovery rooms, carried out annually in the ophthalmology OR (Lasik) and corresponding recovery room. As from the year 2014, the study was extended to include the five general ORs and general recovery room. As a complement to this study, in 2015, an investigation concerning the existence of *Legionella pneumophila* in the ducts of the OR air conditioners was also undertaken.

The specific objectives are:

- to determine the bacteriological load on air and surfaces within the ORs and recovery rooms;
- to isolate and identify pathogenic strains of bacteria on equipments and contact surfaces;
- to determine the level of bacteriologic contamination of contact surfaces and equipments;
- to determine whether *Legionella pneumophila* was present in the ducts of the OR air conditioners.

The investigations carried out provided data from which estimates could be made of the levels of contamination in various sites within the ORs and recovery rooms and it is hoped that the data obtained will contribute to the overall knowledge of these environments and, subsequently, to the control of SSIs by the adoption of appropriate measures where necessary, by the staff and health professionals.

2 Material and Methods

2.1 Preanalytical Phase

2.1.1 Culture media

For the evaluation of air and surfaces, Trypto-Casein Soy Agar (TSA), which is a universal nutrient medium, was used for the growth and isolation of both aerobic and anaerobic bacteria, while Sabouraud Chloramphenicol Agar was used for the isolation of yeast and fungi. Trypto-Casein Soy Broth (TSB) was used as a transport medium between the Ordem Terceira Hospital and the ADEIM Laboratory for the surface samples collected using swabs. All these culture media were prepared in the ADEIM Laboratory on the day before sampling was carried out.

Concerning the *Legionella pneumophila* investigation, for each Air Handling Unit (AHU), one polystyrene vial with sodium thiosulphate (António Cruz™) and one 90 mm plate of Glycerol Vancomycin Polymyxin Cycloheximide (GVPC) (PVL™) were used. Both were obtained commercially ready-to-use. GVPC is a selective medium for the isolation and presumptive identification of Legionella species from water and other environmental samples.

Polystyrene vials with sodium thiosulphate were used for collecting the condensed water. In order to neutralize disinfectants present in the water, 0,5 mL of 0,1N sodium thiosulfate was added to each 1 litre sample, as recommended.

The culture media were stored in a refrigerator of the ADEIM Laboratory, at a temperature of 2-8ºC, and transported between this laboratory and the Ordem Terceira Hospital in a cooler with a storage water heater, also at 2-8ºC.

2.1.1.1 Preparation of Trypto-Casein Soy Agar

To prepare the TSA culture medium, 40,0 g of dehydrated media (Biokar Diagnostic™) was weighed using an analytical balance (Sartorius™), a scoopula and weighing paper. The powder was dissolved in 100-200 mL of hot sterile bidistilled water and the volume was then completed with sterile bidistilled water. When necessary, the solution was slowly brought to the boil and stirred with constant agitation until completely dissolved. Next, the culture medium was sterilized in a vertical autoclave (Amaro 200™) at 121ºC for 15 minutes.

Finally, the molten media, held at a temperature of 44-47ºC in a vertical laminar flow chamber BioIIA (TellStar™): 1190x580x700, was poured into sterile contact plates or 90 mm Petri plates (António Cruz™) and allowed to solidify on a cold, flat surface.

Before sterilisation, the pH of the medium, measured with a pH 1000L meter (VWR™) at 25ºC, was 7.3+/- 0.2.

It is important to refer that Tween 80 was added to the medium in the preparation of the contact plates in order to neutralize disinfectants that might exist in the sampling site.

2.1.1.2 Preparation of Sabouraud Chloramphenicol Agar

To prepare the Sabouraud Chloramphenicol Agar culture medium, 42,5 g of dehydrated medium (BIO-RAD™) was weighed using an analytical balance (Sartorius™), a scoopula and weighing paper. The powder was dissolved in 100-200 mL of hot sterile bidistilled water and the volume was then completed with sterile bidistilled water. When necessary, the solution was slowly brought to the boil and stirred with constant agitation until completely dissolved. Next, the culture medium was sterilized in a vertical autoclave (Amaro 200™) at a temperature of 121ºC for 20 minutes. Finally, the molten media held at 44-47ºC in a vertical laminar flow chamber BioIIA (TellStar™): 1190x580x700, was poured into contact or 90 mm sterile Petri plates (António Cruz™) and allowed to solidify on a cold, flat surface.

Before sterilization, the pH of the medium, measured with a pH 1000 L meter (VWR™), at 20ºC, was 5,8.

It is important to refer that Tween 80 was added to the medium in the preparation of the contact plates in order to neutralize disinfectants that might exist in the sampling site.

2.1.1.3 Preparation of Trypto-Casein Soy Broth

To prepare the TSB culture medium, 30,0g of dehydrated medium (Biokar Diagnostic™) was weighed using an analytical balance (Sartorius™), a scoopula and weighing paper. The powder was dissolved in 100-200 mL of hot sterile bidistilled water and slowly stirred until a complete dissolution was obtained. Next, the volume was complete with sterile bidistilled water lot. The medium was dispensed in several tubes (10 mL in each), sterilised in a vertical autoclave (Amaro 200™) at 121ºC for 15 minutes and cooled to room temperature.

Before sterilisation, the pH of the medium, measured with a pH 1000 L meter (VWR™) at 25ºC, was 7,3+/-0,2.

2.2 Collection Phase

The surface and air samples were taken after adequate cleaning and disinfection of the operating rooms and prior to the entry of the surgery and support team, when the OR was not in use. Cleaning and disinfection procedures in the Ordem Terceira Hospital are as follows:

In the morning, before the surgical journey

- all horizontal surfaces and the cialitic lamp are damp-wiped and the floor is damp-mopped.

Between surgeries

- all dirty materials are removed and the respective transport bags are closed
- all surgery support elements are removed and substituted
- all horizontal surfaces (operating table, instrument table, supports, electric scalpel accessories, …) are damp-wiped with ANIOS D.D.S.H
- the floor is washed with Surfanios Premium (Laboratoires ANIOS™), a disinfectant detergent which acts efficiently on bacteria and moulds present in hospital environments
- before reusing the room, the floor is allowed to dry

After surgical journey

- the procedures specified for between surgeries are performed
- all fixed horizontal surfaces and walls are cleaned and disinfected with ANIOS D.D.S.H (Laboratoires ANIOS™), a spray foam efficient on bacteria and moulds present in the hospital environment
- the mobile material is transported, if possible to clean areas, for subsequent cleaning and disinfection with the same product ANIOS D.D.S.H
- complementary disinfection is performed by using spray or by aerial means
- adjoining support rooms, anaesthetic induction room, recovery room, material room and disinfection room … are cleaned

- cleaning materials are stored

Weekly cleaning

- procedures specified for the end of the surgical journey are carried out
- doors and ventilation ducts are cleaned and disinfected with ANIOS D.D.S.H
- the floor may be cleaned with DETERG'ANIOS (Laboratoires ANIOS™), a bacteriostatic and fungistatic agent
- the rate and frequency for cleaning the ceiling should be adjusted to its architecture and speciality
- as a complement, aerial disinfection is performed

Suitable precautions were taken to minimize contamination of media by the sampler operators. Before entering the restricted area of the ORs, scrubs, hair coverings, face masks, gloves and shoe covers were put on and all the material/equipment were cleansed with 70% isopropanol with water for injection (Crystel Gold-Sterile™). In addition, conversations and movements were minimized during and immediately prior to sampling.

The choice of sampling points was made after consultation with the Quality Control Department Engineer and targeted the most critical sites in the operating and recovery rooms. It should be pointed out that during the course of the study, there was an adjustment of the sampling points in the sense that those in which no contamination was observed were excluded and other potential critical ones were included.

After all the samples were collected and properly labelled, they were immediately transported to the ADEIM Laboratory for processing.

2.2.1 Air Sampling by Impaction Method

The impaction method using MAS-100 equipment (Merck™) was used for the collection of indoor air. The MAS-100 is a high-performance instrument that is based on the principle of the Andersen air sampler, which aspirates air through a perforated plate. The resulting airflow is directed onto a 90 mm Petri dish containing agar.

For each air sample collection, the MAS-100 equipment was placed on a firm support at about 1 m above the ground, the perforated lid was opened by rotating to the right and cleaned with isopropanol. Next, a closed 90 mm Petri dish filled with agar was placed on

top of the dish support, the lid was taken off the Petri dish, the MAS-100 perforated lid was closed, the angle of the sampling head was adjusted and the equipment programmed to aspire 500 L of air (flow rate of 100L/min). After the collection cycle, the sampling head was opened and the Petri dish was closed with the Petri dish cover and placed in the cooler to later be transported to the laboratory. Two collection cycles were realized in each critical point, one for the TSA and another for the Sabouraud Chloramphenicol Agar.

Since concentrations of airborne bacteria differ from location to location in a given area, sampling was always carried out at three or more fixed points in each of the rooms under study. These points included the air at the patient entrance door and the air at the end of the room in the direction of the operating table. Air samples were also collected in the corridor adjacent to each general operating room and general recovery room, in front of the entrance doors (see pages 35-39).

2.2.2 Surface Sampling by Contact Plate Method and Swab Test

The samples were drawn from equipment/materials and furniture in the ORs and recovery rooms with which patients and health care professionals had greater contact. These include mechanical respirators, infusion pumps, heart rate monitors, stethoscopes, bed rails, inner handle of the entry and exit door, bedside table handle, clinical outcome table and phone (for more details see pages 38-44).

The Contact Plate Method was used for collecting samples from regular surfaces while the Swab Test was used for collecting samples from irregular or hard-to-reach surfaces where the use of contact plates is inefficient/difficult.

The Contact Plate Method consists in taking the lid off the contact Petri dish, pressing for 10 seconds, the entire surface of the culture medium against the surface area of approximately 20 cm^2, and finally closing the Petri dish with the Petri dish cover. In our study, two samples were collected from each critical point using this method, one for the TSA medium and another for the Sabouraud Chloramphenicol Agar medium.

Some sites where the Swab Test was used included ball point pens, schwins button, operating table handle, microscope objective and ocular, round button next to door, door handle, incubator, telephone, sink tap, light switches, computer keyboard, trolley, respiratory ventilator, surgical light button, computer mouse, intercom, serum stand, Aliseo equipment buttons, mobile phone, equipment controls and calculator.

The Swab Test consists in soaking the sterile cotton swab (Gardening SRL™) in the TSB medium contained in a tube, rubbing and rolling the swab firmly in all directions (horizontally, vertically and diagonally) across the sampling area of about 10 cm x 10 cm, several times, during 10 seconds, and finally returning the swab into the tube.

2.2.3 Condensed Water Samples and Swab Samples Collected in the Ducts of the Air Conditioner

The search for *Legionella pneumophila* in the ducts of the Air Handling Units (AHU) of the five general ORs and general recovery room was conducted using samples of condensed water from the air conditioners and also using the Swab Test.

Condensed water was collected from a duct of each into a vial with sodium thioglycolate and, next, a biofilm was collected with a transport swab. The swab was removed from the tube containing activated charcoal, rubbed and rolled firmly several times in all directions (horizontally, vertically and diagonally) in the interior of the ducts during 10 seconds, and finally returned into the tube.

2.3 Laboratory Phase

2.3.1 Air and Surface Samples

After all the samples were collected and properly labelled, they were immediately transported to the ADEIM Laboratory, in a cooler with a storage water heater at 2-8ºC, for processing.

All the air and surface samples collected for both 90 mm and contact Petri dishes containing TSA were incubated at 32,5±2,5 ºC for 48 to 72H, under aerobic conditions, in a BE500 incubator (Memmert™) for maintenance of bacterial strains and performance of viable counts the bacterial cultures. The air and surface samples collected for both 90 mm or contact Petri dishes containing Sabouraud Chloramphenicol Agar medium were incubated at 22,5±2,5 ºC for 5 days in a Heraus incubator for the maintenance of yeast and fungi strains and performance of viable counts of yeast and fungi cultures.

With respect to the swabs samples collected from the hard-to-reach surfaces in sealed vertical laminar flow chambers - NU-437-400E (Nuaire™): 1178x597x724 and with the chamber flow turned on, the swab was streaked in a Petri plate containing TSA and in another containing

Sabouraud Chloramphenicol Agar media. These culture plates were incubated at 32,5±2,5 ºC for 48 to 72H in a BE500 incubator (Memmert™) and 22,5±2,5 ºC for 5 days in a Heraus incubator, respectively.

After the respective incubation times, the Total Aerobic Bacteria Count (TAMC) and the Total Yeast and Fungi Count (TYMC) were performed with a manual colony counter and the results were expressed as colony-forming units (CFU)/500L for the air samples and as CFU/plate for the surface samples. The suspected colonies were identified.

The bacterial and yeast species were identified using API™ and BBL™ identification systems, while the fungal species were identified by microscopic and macroscopic observation.

2.3.2 Samples Collected in Ducts of Air Conditioners

With regard to the condensed water samples collected for the vials containing sodium thioglycolate, the membrane filtration method was used.

For each condensed water sample 100 mL, 10mL and 1 mL were put in three 1L flasks and then sterile water was added for a final volume of 1L (dilution 1:10, 1:100 and 1:1000). Each of these diluted solutions was filtered in a 0.45 µm membrane filter (Sartorius Stedim Biotech GmbH™) with a vacuum pump (JP Recirculating Water Aspirator - Velp Científica™) and a ramp filter (PALL Scientific™). After each filtration the membrane filter was placed with tweezers in a Petri dish (Deltalab™) containing GVPC (for *Legionella pneumophilla)*. Next, 1mL of the condensed water sample was filtered and the membrane filter placed in a Petri dish containing TSA medium. This procedure is important for comparison with contaminating flora. Finally, the remaining condensed water was filtered and the membrane filter transferred to a Petri dish containing GVPC medium. All these Petri dishes were incubated in an anaerobic jar in a BE500 incubator (Memmert™) at 32,5ºC during 5-7 days.

With respect to the swab samples in sealed vertical laminar flow chambers - NU-437-400E (Nuaire): 1178x597x724 and with the chamber flow turned on, the swab was streaked in two Petri plate containing GVPC medium. The Petri dishes were incubated in a microaerophilic atmosphere at 32,5ºC during 5-7 days.

3 Results and Discussion

During the three years of our study, 1210 air and surface samples were collected and analysed. All these samples were taken after adequate cleaning, disinfection and sterilization of the operating and recovery rooms and when these were not being used, in the morning before the surgical journey or between surgeries.

After the respective incubation times, the Total Aerobic Microbial Count (TAMC) and the Total Yeast and Fungi Count (TYMC) were performed and the results were expressed as colony-forming units (CFU)/500L for the air samples and as CFU/plate for the surface samples.

The TAMC is considered to be equal to the number of colonies of bacteria found using TSA medium while the TYMC is considered to be equal to the number of colonies of yeast and fungi found using Sabouraud Chloramphenicol Agar medium.

3.1 Microbiological Evaluation of Air

A total of 230 air samples were collected from 2011 to 2016, 155 for the Sabouraud Chloramphenicol Agar medium and another 155 for the TSA medium. In 11% of these samples there was no microbial growth (see Figure 3.1).

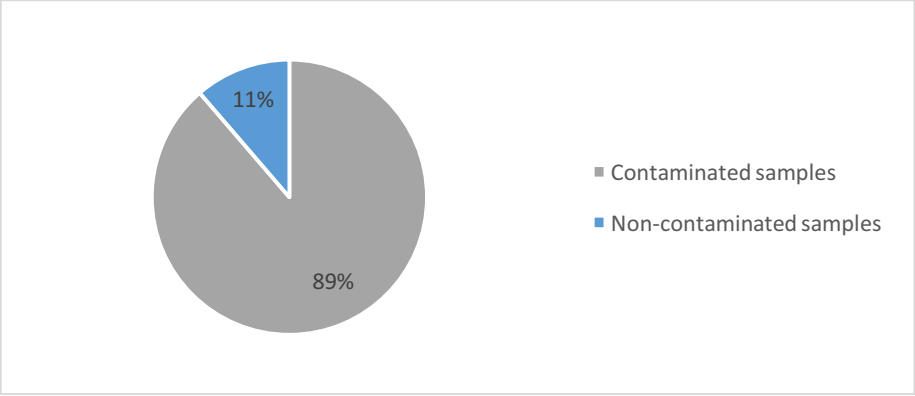

Figure 3.1 - Percentage of air samples collected for TSA and Sabouraud Chloramphenicol Agar media with microbial growth and percentage without microbial growth

Since concentrations of airborne bacteria differ from location to location in a given area (8), in order to characterize airborne microbial concentration, sampling was always carried out at three or more fixed potential critical points inside each room. These included air samples collected at the patient entrance door and at the end of the room in the direction of the operating table. Moreover, samples were also collected outside the rooms, in the corridor adjacent to each general OR and general recovery room, in front of the entrance doors.

As might be expected, the results revealed an effective reduction in the concentration of microorganisms from outdoor to indoor in the general ORs and general recovery room. In fact, almost all the samples collected in the corridors revealed TAMC and TYMC higher than the samples collect inside the rooms.

None of the air samples collected revealed an uncountable CFU number (all were below 300 CFU/500L).

Table 3.1 –TAMC and TYMC averages (expressed in CFU/500L) of the air samples collected in each general OR and each recovery room, for the years 2014-2016. The Impaction Method was used. Trypto-Casein Soy Agar was used for isolation of bacteria and Sabouraud Chloramphenicol Agar media was used for isolation of yeast and fungi.

	2014		2015		2016		2014-2016	
	Mean TAMC (CFU/500L)	**Mean TYMC** (CFU/500L)	**Mean TAMC** (CFU/500L)	**Mean TYMC** (CFU/500L)	**Mean TAMC** (CFU/500L)	**Mean TYMC** (CFU/500L)	**Mean TAMC** (CFU/500L)	**Mean TYMC** (CFU/500L)
Lasik Recovery Room	63,3	22,3	39,3	9,0	69,0	62,3	57,2	31,2
General Recovery Room	6,0	0,8	105,4	17,6	115,5	2,3	75,6	6,9
Lasik Room	6,3	2,3	17,3	4,7	19,0	6,7	14,2	4,6
OR1	16,0	4,0	35,5	2,5	26,3	1,5	25,9	2,7
OR2	19,6	17,0	21,4	4,8	42,0	2,0	27,7	7,9
OR3	34,5	2,8	97,3	30,0	26,3	0,3	52,7	11,0
OR4	45,8	4,3	37,5	9,5	25,0	0,8	36,1	4,8
OR5	15,0	18,8	76,5	46,5	18,8	1,3	36,8	22,2

A total of 42 air samples were collected from 2011 to 2013 in the Lasik room and Lasik recovery room. From 2014, the study was extended to include the five general ORs and the general recovery room. A total of 188 air samples were collected in all the rooms in the years 2014-2016.

Taking only into account the samples collected in the period 2014-2016, the highest airborne bacterial concentration averages occurred in the general recovery room (75,6 CFU/500L) and in the Lasik recovery room (57,2 CFU/500L). The highest airborne TYMC average was 31,2 CFU/500L and also occurred in the general recovery room. Lower TAMC averages were observed in the Lasik room (14,2 CFU/500L) and in OR1 (25,9 CFU/500L). The lowest airborne TYMC averages were observed in OR1 (2,7 CFU/500L) and in the Lasik room (4,6 CFU/500L) (see Table 3.1). These results were as one might expect, since operating rooms are high risk areas subjected to strict cleaning and sterilization procedures.

Concerning the air samples collected, the average TAMC was lower in the ophthalmology (Lasik) room than in the general ORs. Similar findings were obtained by Anjali et al. (38) as well as by Najotra et al (39), which may be explained by the fact that eye surgery is a clean surgery procedure.

The TAMC and TYMC averages for the air samples collected in all ORs in the period 2011-2016 were 16,7 CFU/500L and 6,0 CFU/500L, respectively. Similar values (average TAMC=26,9 CFU/500L and average TYMC=6 CFU/500L) were obtained in a study carried out in 29 ORs over a three-year period at the University Hospital of Parma in Italy. As in the present study, the air samples were also collected in empty ORs using an impactor air sampler, TSA and Sabouraud dextrose agar with chloramphenicol media (40).

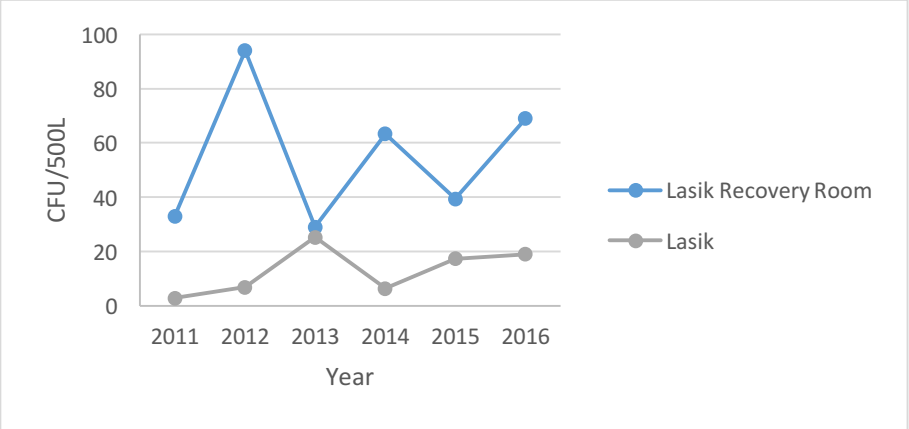

Figure 3.2 – TAMC averages (expressed in CFU/500L) of the air samples collected in the Lasik room and the Lasik recovery room, for the years 2011-2016. Trypto-Casein Soy Agar medium and the Impaction Method were used.

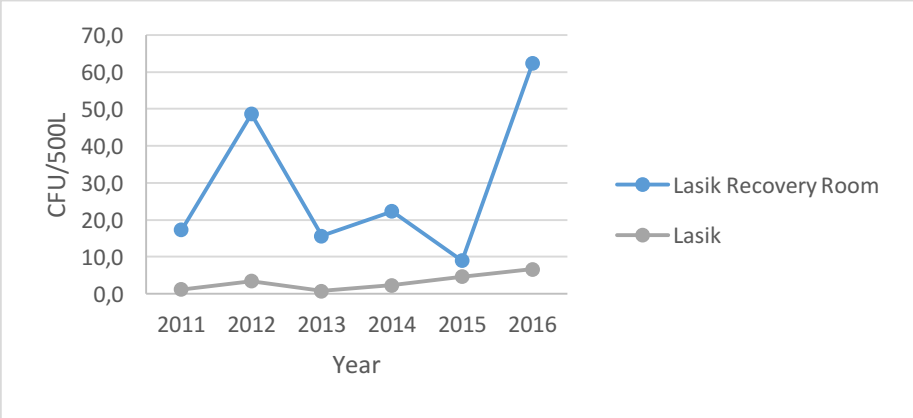

Figure 3.3 – TYMC averages (expressed in CFU/500L) of the air samples collected in the Lasik room and the Lasik recovery room, for the years 2011-2016. Sabouraud Chloramphenicol Agar medium and the Impaction Method were used.

There was a wide variation in air microbial contamination in the Lasik recovery room in the years 2011-2016, as may be observed from the TAMC and TYMC averages (see Figure 3.2 and Figure 3.**3**). The average TAMC ranged from 29 CFU/500L to 94 CFU/500L and the average TYMC ranged from 9 CFU/500L to 62 CFU/500L. In the Lasik room no significant variation was observed, particularly with respect to the average TYMC. The highest average TAMC (25,25 CFU/500L) occurred in 2013.

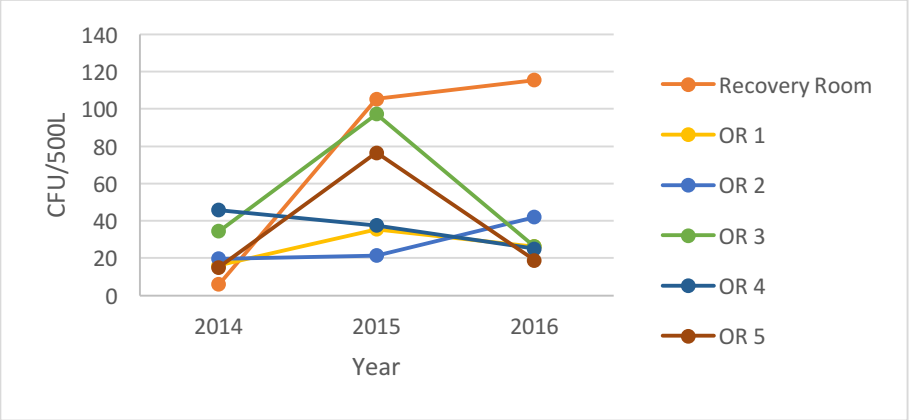

Figure 3.4 – TAMC averages (expressed in CFU/500L) of the air samples collected in each general OR and in the general recovery room, for the years 2014-2016. Trypto-Casein Soy Agar medium and the Impaction Method were used

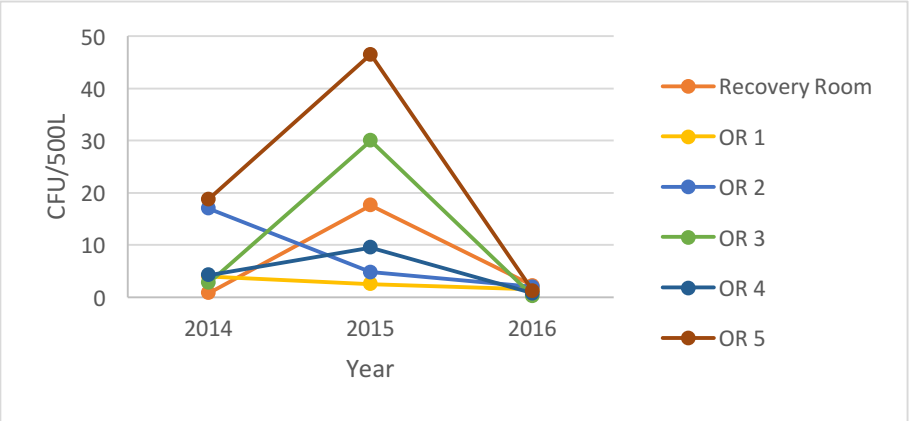

Figure 3.5 – TYMC averages (expressed in CFU/500L) of the air samples collected in each general OR and in the general recovery room room, for the years 2014-2016. Sabouraud Chloramphenicol Agar medium and the Impaction Method were used.

With respect to the general ORs and general recovery room, it may be noted that there was a higher air microbial contamination level in 2015 in OR3, OR5 and in the general recovery room. In 2016 a higher average TAMC (115,5 CFU/500L) was also observed in the Lasik recovery room. Variations in the air microbial contamination levels were not significant in the remaining ORs.

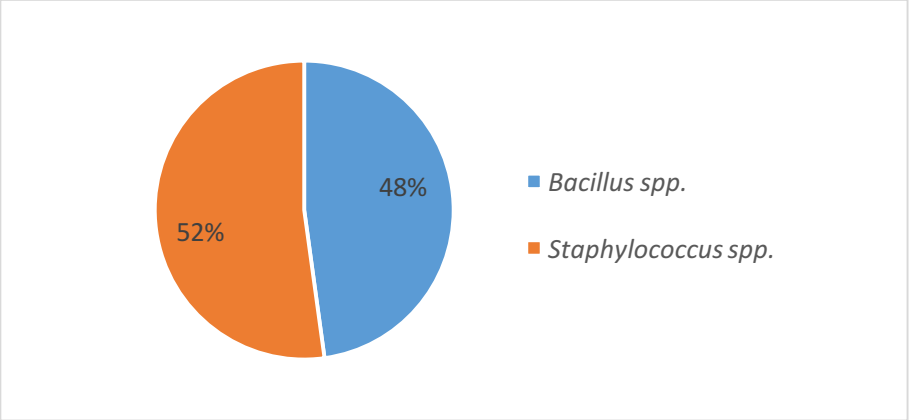

Figure 3.6 – Distribution of bacteria isolated from the air samples collected in all ORs and recovery rooms. Trypto-Casein Soy Agar medium and the Impaction Method were used

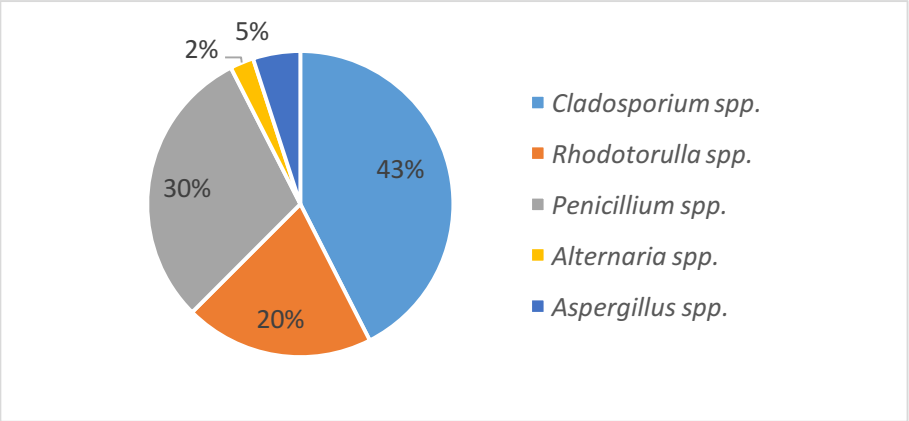

Figure 3.7 - Distribution of fungi and yeast isolated from air samples collected in all ORs and recovery rooms. Sabouraud Chloramphenicol Agar medium and the Impaction Method were used.

With regard to the distribution of microbial species in the surveyed operating and recovery room sites, *Staphylococcus* spp. were the predominant species of the bacterial population in the air samples (52%). The remaining 48% corresponded to *Bacillus* spp. (Figure 3.6). The *Staphylococcus* species identified were *Staphylococcus epidermidis* (50%) and *Staphylococcus cohnii* (50%).

Let us note that *Staphylococcus epidermidis* is an opportunistic human pathogen and is an important cause of nosocomial infections (41). It is the most frequently encountered member

of the coagulase-negative staphylococci on human skin and mucous membrane microflora and presents unique problems in the diagnosis and treatment of infections involving biofilm formation on implanted biomaterials (42,43).

Stphylococcus cohnii is a coagulase-negative staphylococci and is considered as normal flora. However, it has been isolated from urinary tract infections and surgical prostheses but its relation with staghorn stones is still not clear (44).

Bacillus is a spore forming organisms that can survive for long periods of time and can cause serious medical problems (45). Although it is not recognized as a major human pathogen, with recent advances in medical technology and an increased number of immunosuppressed patients, it has become increasingly recognized as an opportunistic pathogen in the hospitalized patient.

With regard to the characterization of indoor air fungi, the prevalent species were *Cladosporium* (43%) and *Penicillium* (30%). Nevertheless, other fungi such as Aspergillus spp. (5%) and Alternaria spp. (2%) were also isolated from the air (see Figure 3.7). All these fungal species are strongly associated with allergic respiratory diseases, especially asthma (46). The yeast species *Rhodotorulla* was also isolated (20%).

A two-year survey on airborne fungal load in a Badajoz (Spain) hospital also indicated the five most frequent groups of fungi to be *Cladosporium,* yeasts, *Alternaria*, *Penicillium* and *Aspergillus* (47).

These studies, including other previous studies (23,48) support our findings concerning the prevalence and persistence of certain bacterial and fungal species in the hospital environment.

To date, there are no published international guidelines establishing limits of acceptable microbial contamination levels for hospital environments such as ORs and recovery rooms.

Many countries have established their standards according to the International Standards Organization (ISO) 14644 – Cleanrooms and associated controlled environments, based on the particle count method. There, it is proposed that ORs (without HEPA filters) should meet the requirements of a cleanroom of ISO Class 6 or 7. The particle count method was proposed to determine the effectiveness of the filters in the ventilation systems as well as to establish the level of biological contamination (19). However, in some studies (30,49) there was no

correlation between the number of airborne particles and the number of CFUs, suggesting that there is no reason to replace microbiological sampling with particle counting for routine evaluation of microbiological contamination in conventionally ventilated ORs.

According to the guidelines for Good Manufacturing Practice, 4 grades can be distinguished for the manufacture of sterile medicinal products (Grades A, B,C and D). For Grade B (at rest) the airborne particle classification is ISO 5. For Grade C (at rest & in operation) the airborne particle classification is ISO 7 and ISO 8 respectively. The "at-rest" state is the condition where the installation is installed and operating, complete with production equipment but with no operating personnel present. The "in operation" state is the condition where the installation is functioning in the defined operating mode with the specified number of personnel working. Recommended limits for microbial contamination of clean areas during operation is 10 CFU/m^3 for Grade B and 100 CFU/ m^3 for Grade C (50). In our study, only 3% of all the air samples collected in the six years in the ORs revealed a contamination level above 50 CFU/500L.

Some countries have established their own standards concerning allowed airborne microbial contamination in ORs. For example, in the United Kingdom, the Health Technical Memorandum 03-01 (51) recommended that aerobic cultures on nonselective media should not exceed ten bacterial and/or fungal CFU/ m^3. In France, the microbiological limits are also 10 CFU/ m^3 for operating rooms that should meet the requirements of a cleanroom of ISO 6 or 7 (52).

With regard to Portugal, a legislation published in 2006 (D.L Nº78/2006) (53), established a maximum reference concentration of 500 CFU/500L for bacteria and fungi as a requirement for indoor air quality of buildings. More recently, in 2013, a new legislation (D.L. Nº353-A/2013) (54) was published concerning indoor air quality, which established that the total indoor bacterial concentration should be less than the outdoor concentration, plus 350 CFU/ m^3. With regard to fungi, limits are based on the degree of danger inherent to the different species. For example, for non-toxin-producing common species, such as *Cladosporium* spp., *Penicillium* spp., *Aspergillus* spp. and *Alternaria* spp., the limit concentration is ≤ 500 CFU/ m^3. However, it should be emphasized that this legislation is not specific for hospital environments, where a high degree of cleanliness is required, but for buildings in general (shopping centres, schools, gymnasiums, etc.). Clean rooms, such as ORs, must have the most stringent standards because the microbiological contamination levels of indoor air should obviously be lower than those of outdoor air.

Comparing our results with the limit (10 CFU/m^3) proposed by both the French and United Kingdom guidelines for empty ORs, 53,0% were above this value (5 CFU/500L). However, it should be noted that in our study, the sampler (MAS-100) was not activated remotely and the test persons remained in the room during the sampling process, while in the United Kingdom guidelines, the OR should be empty and the active air sampler activated remotely.

3.2 Microbiological Evaluation of Surfaces

A total of 980 surface samples were collected from 2011 to 2016, 490 for the Sabouraud Chloramphenicol Agar media and another 490 for the TSA medium. In 48% of these samples there was no microbial growth.

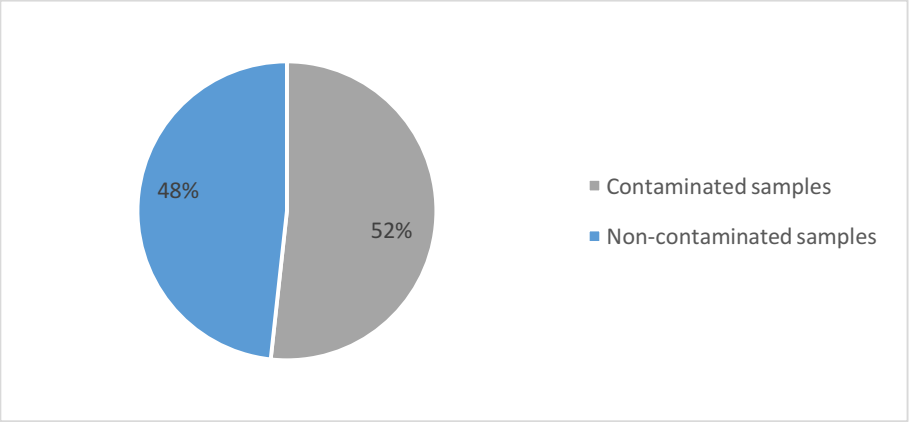

Figure 3.8 – Percentage of surface samples collected for TSA and Sabouraud Chloramphenicol Agar media with microbial growth and percentage without microbial growth

During the first three years (2011-2013) of our investigation, a total of 208 samples from a wide range of inanimate surfaces within the Lasik room and Lasik recovery room were collected and analyzed. As from 2014, when the study was extended to include the five general ORs and the general recovery room, a total of 772 microbiological samples were taken. The sampling sites included operating room tables, surgical lights, anaesthetic trolleys, anaesthetist's chair, light switches, computer keyboards, support desks, stethoscopes and doors. Contact plates were used for regular surfaces, and the Swab Test was used for irregular or hard-to-reach surfaces.

It is interesting to note that the values for TAMC are almost always higher than those for TYMC, as was the case for most of the air samples collected from the various critical points.

Table 3.2 – Percentage of the surface samples collected from 2014 to 2016 in each room that revealed limits above 300 CFU/plate.

	2014		2015		2016		2014-2016	
	Average TAMC (CFU/plate)	**Average TYMC** (CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** (CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** (CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** (CFU/plate)
Lasik Recovery Room	0,0%	0,0%	0,0%	0,0%	12,5%	0%	5,0%	0,0%
General Recovery Room	0,0%	0,0%	0,0%	0,0%	0,0%	0%	0%	0,0%
Lasik Room	0,0%	0,0%	0,0%	0,0%	28,6%	19,0%	10,3%	6,9%
OR1	0,0%	0,0%	0,0%	0,0%	6,7%	0%	2,0%	0,0%
OR2	0,0%	0,0%	0,0%	0,0%	0,0%	0%	0%	0,0%
OR3	0,0%	0,0%	0,0%	0,0%	0,0%	0%	0%	0,0%
OR4	5,6%	0,0%	0,0%	0,0%	7,1%	0%	2,0%	0,0%
OR5	11,1%	0,0%	0,0%	0,0%	0,0%	0%	3,9%	0,0%

The results from the surface samples were almost all below 300 CFU/500L. Taking only into consideration the samples collected in the years 2014-2016, only 2-10% of the surface samples from the Lasik room, the Lasik recovery room and three of the general ORs (OR1, OR4 and OR5) showed concentrations above this value (uncountable number of colonies). Of all the surface samples collected in the various ORs and recovery rooms, only 7% of those collected in the Lasik room revealed TYMC above 300 CFU/plate (see Table 3.2). The sampling sites where this was observed were the printer stand, cornea stand, computer, objectives (ocular), equipment remote controls, calculator, Corneo equipment of the Lasik room; OR1 anaesthesia cart, surgical table and arcadis (OR4); static microscopes, door and wall switches (OR5); right arm of chair (Lasik recovery room). Contamination peaks were observed in those sites which are in direct contact with the patient or health professionals, in line with similar studies carried out in Europe (31) and the USA (55).

According to the Global Guidelines for the Prevention of Surgical Site Infections (WHO) (16), high hand-touch surfaces require special attention and more frequent cleaning. After

thorough cleaning, the use of appropriate disinfectants to decontaminate these surfaces should be used.

Table 3.3 – TAMC and TYMC averages (expressed in CFU/plate) of the surface samples collected in each OR and each recovery room, for the years 2014 to 2016. The Contact Plate Method and the Swab Test were used. The Trypto-Casein Soy Agar and the Sabouraud Chloramphenicol Agar media were used for the isolation of bacteria, and yeast and fungi, respectively.

	2014		2015		2016		2014-2016	
	Average TAMC (CFU/plate)	**Average TYMC** (CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** (CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** ((CFU/plate)	**Average TAMC** (CFU/plate)	**Average TYMC** (CFU/plate)
Lasik Recovery Room	7,2	1,3	19,7	2,5	18,1	0,4	15,0	1,4
General Recovery Room	17,2	2,5	32,0	6,0	66,2	1,6	38,5	3,4
Lasik Room	4,0	0,9	3,5	0,8	59,8	7,7	22,4	3,1
OR1	12,8	1,5	23,8	0,7	8,2	0,2	15,0	0,8
OR2	13,9	3,7	13,4	0,3	10,2	0,1	12,5	1,4
OR3	8,4	3,1	11,0	0,1	18,0	0,8	12,5	1,3
OR4	19,6	0,8	5,0	0,4	11,0	0,4	11,9	0,5
OR5	11,0	2,0	2,6	0,3	6,1	0,5	6,6	0,9

Ignoring the samples where contamination peaks were verified, the average TAMC and average TYMC for the surface samples collected in the Lasik room and in the Lasik recovery room were 5,5 CFU/plate and 0,7 CFU/ plate, respectively. With respect to the surface samples collected in the general ORs and general recovery room, the average TAMC was 8,0 CFU/ plate and the average TYMC was 0,4 CFU/ plate.

The highest TAMC average (38,5 CFU/ plate) and highest TYMC average (3,4 CFU/ plate) for the surface samples collected in each room in the three years were observed in the general recovery room (see Table 3.3).

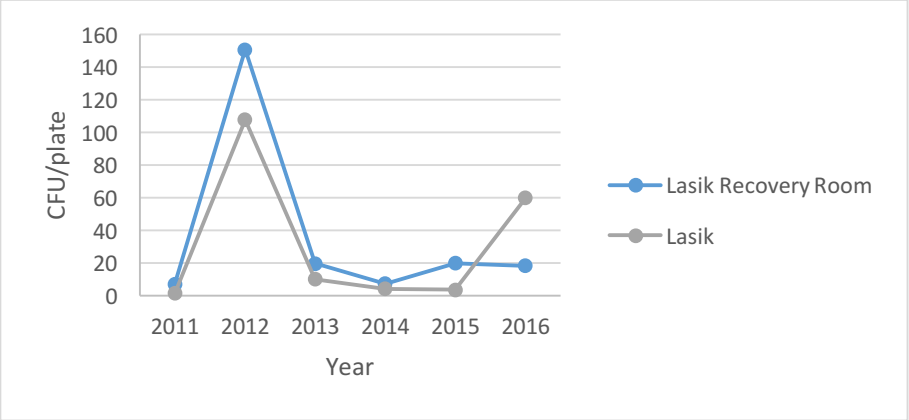

Figure 3.9 – TAMC averages (expressed in CFU/plate) of the surface samples collected in the Lasik room and in the Lasik recovery room, for the years 2011-2016. Trypto-Casein Soy Agar medium and the Contact Plate Method or Swab Test were used.

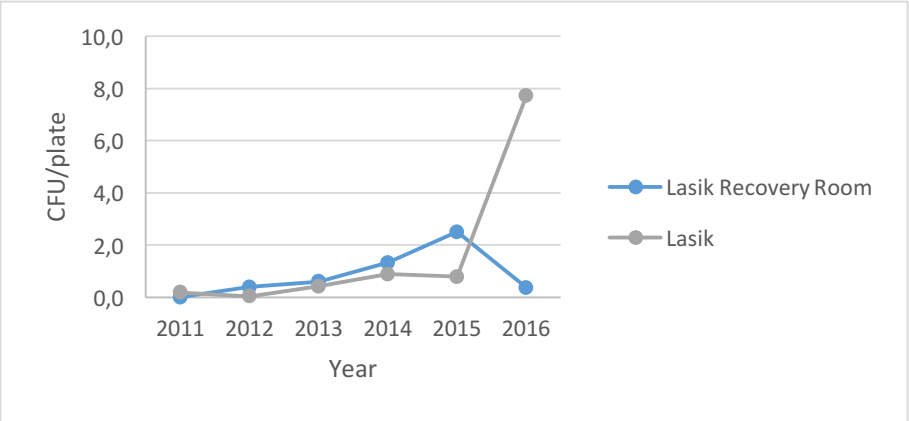

Figure 3.10 – TYMC averages (expressed in CFU/plate) of the surface samples collected in the Lasik room and in the Lasik recovery room, respectively, for the years 2011- 2016. Sabouraud Chloramphenicol Agar medium and the Contact Plate Method or Swab Test were used.

There was a wide variation in the levels of bacterial surface contamination recorded in the six years, both in the Lasik OR and in the Lasik recovery room. The highest level of contamination in both these rooms occurred in 2013 (see Figure 3.9). With respect to fungal and yeast contamination, levels were generally low over the six years, except in the last year when a peak occurred in the Lasik room (see Figure 3.10).

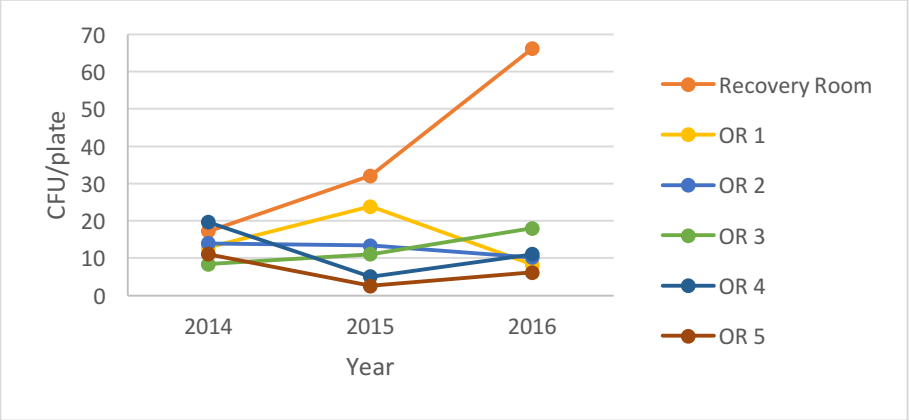

Figure 3.11 – TAMC averages (expressed in CFU/plate) of the surface samples collected in each general OR and in the general recovery room, for the years 2014-2016. Trypto-Casein Soy Agar medium and the Contact Plate Method or Swab Test were used.

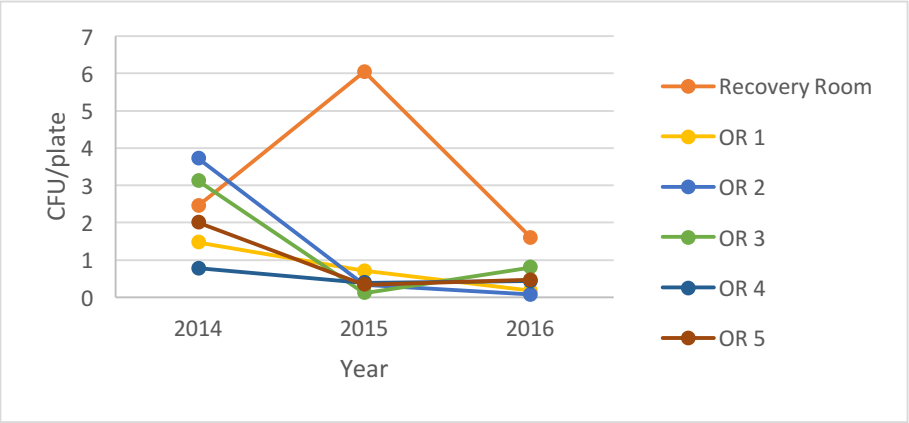

Figure 3.12 – TYMC averages (expressed in CFU/plate) of the surface samples collected in each general OR and in the general recovery room, for the years 2014-2016. Sabouraud Chloramphenicol Agar medium and the Contact Plate Method or Swab Test were used.

For all five general ORs, no significant variation in the TAMC average was observed in the three years. Only in the general recovery room was there a progressive increase in the bacterial contamination level (Figure 3.11).

As for fungal and yeast contamination, there was a reduction in the average TYMC in the general operating rooms after the first year of study. A contamination peak occurred in 2015 in the general recovery room (Figure 3.12).

According to the guidelines for Good Manufacturing Practice, previously mentioned, the recommended limits for microbial contamination of clean areas during operation are 5 CFU/plate for Grade B and 25 CFU/plate for Grade C (50). In our study, only 11% of all the surface samples collected in the six years revealed a contamination level above 25 CFU/plate.

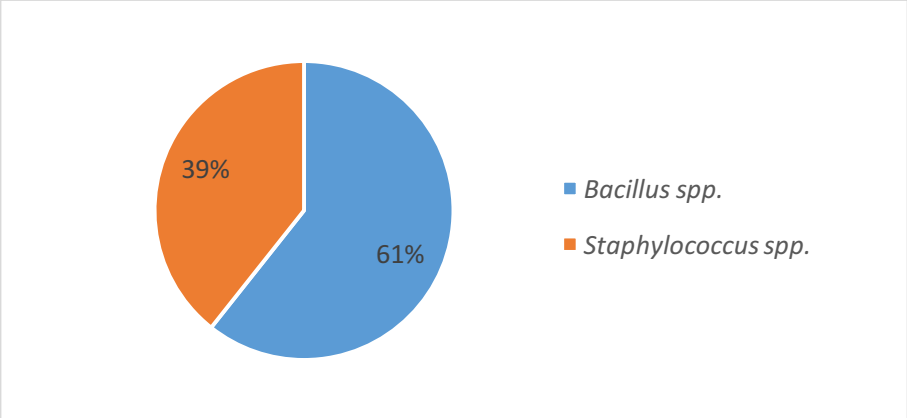

Figure 3.13 - Distribution of bacteria isolated from the surface samples in all ORs and recovery rooms. Trypto-Casein Soy Agar medium and the Contact Plate Method or Swab Test were used.

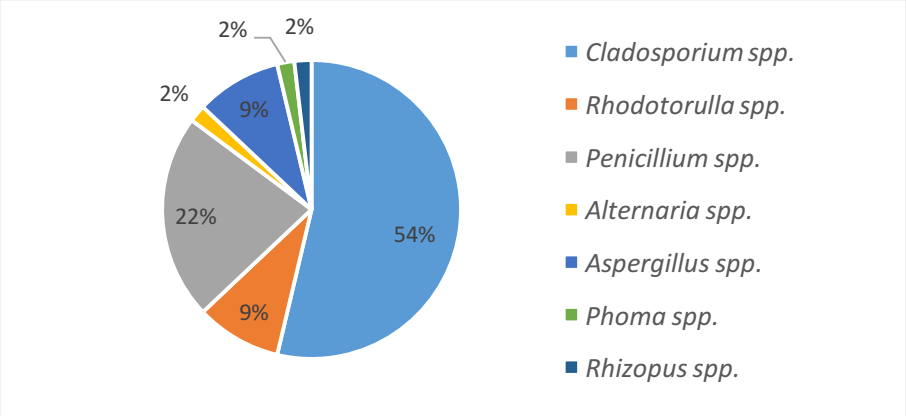

Figure 3.14 – Distribution of fungi and yeast isolated from the surface samples in all ORs and recovery rooms. Sabouraud Chloramphenicol Agar medium and the Contact Plate Method or Swab Test were used.

With regard to the bacteria isolated from the surface samples collected in the ORs and recovery rooms, it was found that *Bacillus spp*. was the predominant bacterial species in the ORs and recovery rooms (61%), followed by *Staphylococcus spp.* (39%) (Figure 3.13). The *Staphylococcus* species identified were *Staphylococcus epidermidis* (54%) and *Staphylococcus cohnii* (46%). The predominant fungal species found in the samples collected in all the rooms was *Cladosporium spp.* (54%), followed by *Penicilium spp.* (22%), *Aspergillus spp*. (9%), *Alternaria spp.* (2%), *Phoma spp.* (2%) and *Rhizopus spp.* (2%). The remaining 9% corresponded to the yeast *Rhodotorulla spp.* (Figure 3.14). The *Aspergillus* species most frequenlty isolated was *Aspergillus niger* (78%).

It is interesting to note that in several other studies (1,20,38,39,56) concerning the microbiological assessment of OR surfaces, a predominance of the bacterial species *Bacillus* and *Staphylococcus* was also observed.

3.3 Monitoring for *Legionella pneumophilla*

Monitoring for *Legionella pneumophila* was performed in 2015 and 2016. Condensed water samples and swabs were taken from the ducts of the five general OR and recovery room air conditioners. This bacteria was not detected in any of the 12 condensed water samples, neither in any of the 12 swab samples collected in these years.

It is important to note that the International Organization for Standardization (ISO 11731:2017) recommends, for the microbial monitoring of cooling towers, the no detection of *Legionella pneumophila* as acceptable. The same recommendation is found in the Portuguese legislation (D.L. Nº353-A/2013).

4 Conclusion

The quality of the operating room environment affects the health and safety of patients and of all who work therein.

It is known that microbial contamination during a surgical procedure is a precursor to surgical site infection which can result in morbidity and mortality. In particular, superficial surgical site infections are often associated with environmental factors such as air and surface contamination by yeast, fungi and bacteria.

In this study, the levels of air and inanimate surface contamination in the operating and recovery rooms were evaluated and the critical points of contamination were identified. In addition, monitoring for the presence of *Legionella pneumophila* in the ducts of the air conditioners of the operating rooms was also performed. This bacteria can persist for long periods of time in water and biofilms commonly found in air conditioning equipments. Legionnaires' disease can be acquired by inhalation of aerosols containing legionella and so it is essential to guarantee the absence of this bacteria in the hospital environment.

The air samples for this study were collected using the impaction method and the Merck™ MAS 100 equipment, while the surface samples were collected using contact plates or the Swab Test. After the respective incubation times, the Total Aerobic Bacteria Count (TAMC) and the Total Yeast and Fungi Count (TYMC) were performed with a manual colony counter and the results were expressed as colony-forming units (CFU)/500L for the air samples and as CFU/plate for the surface samples. The suspected colonies were identified.

With respect to monitoring for the presence of *Legionella pneumophila* in the ducts of the operating room air conditioners, condensed water from the air conditioners and swab samples were collected.

The results obtained revealed that in 11% of the 230 air samples collected from 2011 to 2016 there was no microbial growth, while 48% of the 980 surface samples collected in the same period did not reveal microbial growth.

An effective reduction in the concentration of microorganisms from outdoor to indoor was observed in the general ORs and in the general recovery room, as might be expected.

In general, the microbial contamination of both air and surfaces was higher in the recovery rooms than in the ORs.

In the several sampling sites, the TAMC was almost always higher than the TYMC.

The sampling sites showing higher microbial contamination were those in direct contact with the patient or health professionals. These included equipment controls, calculator, anaesthesia cart, surgical table, static microscopes and switches.

The bacteria isolated from the samples collected in the ORs were Staphylococcus spp. and Bacillus spp. and the fungi isolated *were Cladosporium* spp., *Penicilium* spp., *Aspergillus* spp., *Alternaria* spp., *Phoma* spp. and *Rhizopus* spp. Yeast *Rhodotorulla* spp. was also identified.

Comparing our results with the recommended limits for microbial contamination of clean areas during operation for Grade C proposed by the guidelines for Good Manufacturing Practice, only 3% of all air samples and 11% of all surface samples collected in the six years in the ORs revealed a contamination level above the limits (100 CFU/m^3 and 25 CFU/plate, respectively).

Legionella pneumophila was not detected in any of the samples collected in 2015 and 2016.

The contamination peaks observed could be associated with inadequate hygienization and disinfection procedures, changes in environmental conditions (temperature and humidity), lack of effectiveness of the filters in the ventilation system, or contamination by operators during sampling.

A periodic review of the microbiological quality of the air and surfaces (which permits the detection of critical contamination points), associated with cleaning/disinfection plans, contributes to the quality control of these rooms and thus to the prevention of surgical site infections and other nosocomial infections.

This was the first time that an environmental monitoring program was carried out in this hospital and it is intended to continue to perform this evaluation on an annual basis so that the data collected can be analysed to provide trend analysis and to monitor hygiene efficacy over time. It would, however, be desirable to increase the frequency of monitorization as well as to determine the microbial quality of the air, both during and after surgery, in addition to evaluating the surface contamination after surgery, before and after the cleaning and disinfection process.

The aim is also to target lower maximum contamination levels (such as those set in the French and United Kingdom legislations), in order to guarantee higher standards of quality in operating rooms and a subsequent reduction in the surgical site infection rate.

Microbial monitoring in ORs has been a subject of interest and debate over the past years. At present, no generally accepted sampling methods and threshold values are available. Studies have shown that different culture techniques used for environmental sampling of bacterial pathogens can influence the reported level of environmental contamination. Standardized methods of environmental sampling would allow the implementation of guidelines for acceptable levels of environmental contamination (air and surfaces), and thus facilitate research by enabling meaningful comparisons to be made between research studies.

Unfortunately, to date, there is no international standard for allowed microbial contamination in operating rooms, but several countries have adopted their own standards. In Portugal, the only existing legislation (D.L. Nº353-A/2013) concerns indoor air quality which is not specific for hospital environments, where a high degree of cleanliness is required. It is of utmost importance that operating rooms have the most stringent standards.

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Appendix

A1. Microbial assessment of air in general ORs, general recovery room, Lasik room and Lasik recovery room in the several years of study

Table 4 Microbiological assessment of air in the Lasik room between 2011 and 2016

	2011		2012		2013		2014		2015		2016	
	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)
Air adjacent to operating table	3	1	9	3	34	0	5	3	10	1	8	7
Air adjacent to air duct	4	1	11	6	25	0	8	2	11	7	27	4
Air at front of door	2	2	4	1	32	1	6	2	31	6	22	9
Air adjacent to Statim bench	2	1	3	4	10	2	—	—	—	—	—	—

Table 5 Microbiological assessment of air in general operating room 1 (OR1) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)
Air at patient entrance door	0	0	28	5	11	1
Air in operating table area	3	1	9	1	8	1
Air at personnel entrance door	3	0	26	4	3	1
Air in front of room 1 (outside)	58	15	79	0	83	3

Table 6 Microbiological assessment of air in general operating room 2 (OR2) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)
Air at patient entrance door	74	83	35	9	47	0
Air adjacent to operating table legs	4	0	14	4	37	1

Air at personnel entrance door	0	0	19	4	19	2
Air adjacent to 3 stored equipments	10	2	26	2	—	—
Air next to window	10	0	13	5	—	—
Air in front of room 2 (outside)	—	—	—	—	65	5

Table 7 Microbiological assessment of air in general operating room 3 (OR3) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)
Air at patient entrance door	8	0	24	28	9	0
Air at end of room facing operating table	3	0	104	33	18	0
Air at personnel entrance door	5	0	20	37	10	0
Air in front of room 3 (outside)	122	11	241	22	68	1

Table 8 Microbiological assessment of air in general operating room 4 (OR4) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)
Air at patient entrance door	3	3	27	9	10	1
Air at end of room facing the window	14	1	35	10	34	0
Air at end of room facing operating table	7	1	25	11	6	0
Air in front of room 4 (outside)	159	12	63	8	50	2

Table 9 Microbiological assessment of air in general operating room 5 (OR5) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)	**TAMC** (CFU/500L)	**TYMC** (CFU/500L)
Air at patient entrance door	9	0	29	57	14	0
Air at end of room facing operating table	3	0	43	5	15	1
Air at end of room facing personnel door	2	0	15	31	11	1
Air in front of room 5 (outside)	46	75	219	93	35	3

Table 10 – Microbiological assessment of air in Lasik recovery room between 2014 and 2016

	2011		2012		2013		2014		2015		2016	
	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)
Air next to equipment	37	22	115	8	43	19	47	20	23	10	94	58
Air at entrance door	28	15	97	72	25	18	73	24	39	11	54	65
Air adjacent to doctor' s bench	34	15	70	66	19	10	70	23	56	6	59	64

Table 11 – Microbiological assessment of air in general recovery room between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)	TAMC (CFU/500L)	TYMC (CFU/500L)
Air at entrance door	9	0	95	8	108	1
Air between beds (facing exit)	8	0	97	18	34	3
Air at exit door	8	2	228	39	111	1
Air in administrative area (reception)	1	2	47	17	—	—
Air between beds (facing entrance)	4	0	60	6	—	—
Air at exit door (outside)	—	—	—	—	209	4

A2. Microbial assessment of surfaces in general ORs, general recovery room, Lasik room and Lasik recovery room in the several years of study

Table 12 Microbiological assessment of surfaces in Lasik room between 2014- 2016

	2011		2012		2013		2014		2015		2016	
	TAMC (CFU/plate)	TYMC (CFU/plate)	TAMC (CFU/plate)	TYMC (CFU/plate)	TAMC (CFU/plate)	TYMC (CFU/plate)	TAMC (CFU/plate)	TYMC (CFU/plate)	TAMC (CFU/plate)	TYMC (CFU/plate)	TAMC (CFU/plate)	TYMC (CFU/plate)
Air conditioner control	0	0	1	0	5	6	2	0	1	1	0	0
Telephone	0	0	0	0	1	0	0	0	0	1	0	0
Printer stand	1	0	6	0	7	0	0	1	28	0	>300	>300
Round button next to door	0	0	2	0	0	0	0	0	0	0	1	1
Corneo stands	0	1	5	0	13	0	1	1	1	0	>300	>300
Computer	8	1	300	0	0	0	0	0	3	8	>300	>300
Schwind	—	—	300	0	2	0	10	0	0	0	32	0
Statim	>300	0	0	0	5	0	0	0	1	1	1	1
Printer	0	0	300	0	12	0	1	0	0	0	47	4
Operating table handles	0	0	300	0	12	0	11	0	2	0	281	1
Objectives (ocular)	10	0	32	0	1	1	23	0	0	0	>300	0
Operating table	3	0	34	0	10	0	2	0	11	0	61	0
Bausch e Lomb - bench	0	0	300	0	9	1	2	1	1	1	0	0
Schwind - buttons	7	0	300	0	100	0	1	0	3	1	129	129
Video	0	1	25	1	0	0	0	0	1	0	0	0
Equipment controls and calculator	1	2	300	0	26	1	19	12	8	2	>300	>300
Ball-point pen	0	0	32	0	0	0	0	1	2	0	160	0
Statim - bench	2	0	300	0	0	0	0	0	0	0	2	3
FEMTO LDV Z6	—	—	—	—	—	—	—	—	5	0	0	0
Corneo	0	0	—	—	0	0	—	—	—	—	—	—
Yellow container outside lid	0	0	2	0	20	0	—	—	—	—	—	—
Air conditioner control	0	0	0	0	8	0	—	—	—	—	—	—
Telephone	0	0	0	0	6	0	—	—	—	—	—	—
Printer stand	0	0	1	0	0	0	—	—	—	—	>300	0
Round button next to door	0	0	0	0	3	1	—	—	—	—	0	0
Glass shelves in front of door	—	—	—	—	—	—	—	—	—	—	—	—
Aspirec AC1-R	0	0	43	0	—	—	—	—	—	—	—	—
Masterguard	0	0	—	—	—	—	—	—	—	—	—	—
Lamp	0	0	—	—	—	—	—	—	—	—	—	—

Table 13 Microbiological assessment of surfaces in general operating room 1 (OR1) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Cupboard glass doors	12	13	5	0	51	0
Surgeon's chair	46	1	53	2	22	0
X-ray observation panel	1	0	7	0	0	0
Support tables	0	0	1	0	1	0
Anaesthesia carts	4	0	14	3	>300	0
Cupboard stainless steel doors	6	0	2	1	0	0
Computer keyboard	5	0	4	0	0	0
Operating table	6	2	2	0	0	0
Leica microscope	60	3	204	3	13	0
Thermo-hygrometer	1	1	1	0	—	—
Shelves	1	0	2	—	2	2
Equipment	2	2	88	1	7	1
Personnel entrance door	4	2	8	0	3	0
Patient entrance door	12	0	9	0	1	0
Switches	19	0	0	1	—	—
Exit door soiled material	2	0	3	0	14	0
Datex Ohmega-Oxigenometer	37	1	—	—	—	—
Drager Fabius Tiro	—	—	2	1	1	0

Table 14 Microbiological assessment of surfaces in general operating room 2 (OR2) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Cupboard glass doors	3	0	1	0	0	0
Personnel entrance door	5	0	4	4	7	0
Anaesthesia cart	8	2	0	0	—	—
Electric scalpel	0	4	3	0	1	0
Intercom	18	0	11	0	0	0
Pantofes	12	9	9	0	0	0
Computador keyboard	0	0	55	0	6	1
Computer mouse	58	6	10	0	1	0
Anaesthesia stool	7	19	19	1	21	0
Serum supports	0	0	33	0	7	0
Ventilator above monitor	1	1	29	0	0	0
Ventilator connections	0	0	34	1	19	0
Sevorane vapor 2000	15	1	5	0	8	0
Mobile equipament	56	19	4	0	54	0
Air outlet grid	67	6	24	0	29	0
Stainless steel door	0	0	0	0	0	0
Ventilator below Monitor	0	0	0	0	—	—
Operating table	1	0	0	0	—	—

Table 15 Microbiological assessment of surfaces in general operating room 3 (OR3) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Personnel entrance door	4	0	3	0	0	0
Operating table	4	0	0	0	17	0
Patient entrance door	0	0	0	0	—	—
Anaesthesia cart	0	4	14	1	9	3
Surgeon/Anaesthetist chair	50	0	1	0	16	0
Switches and exit sensors	20	0	1	0	95	1
Cupboard glass doors	0	0	11	0	25	0
Ventilator cart	11	0	41	1	3	0
Vaporizer	2	0	3	0	32	2
Behind ventilator (pipes)	2	0	4	0	0	0
Mobile equipment	6	0	—	—	15	0
Electric scalpel	6	0	23	0	19	0
Intercom	28	49	72	0	3	0
Pantofes	0	0	0	0	34	1
X-ray Negatoscope	0	0	1	0	1	5
Stethoscope	10	0	7	0	1	0
Support tables	0	0	0	0	—	—
Waste products/ ventilator tube	—	—	6	0	—	—

Table 16 Microbiological assessment of surfaces in general operating room 4 (OR4) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Personnel entrance door	3	0	2	1	18	1
Operating table	>300	0	2	0	0	0
Ventilator	0	0	1	1	1	0
Sevorane doser	0	0	3	0	25	0
Stryker	1	0	0	2	8	0
Computador keyboard	0	0	50	1	4	0
Patient entrance door	4	0	0	0	1	1
Glass cupboards	5	0	14	0	3	1
Stainless steel cupboards	1	1	13	1	24	0
Window	0	0	0	0	—	—
Anaesthesia cart	14	3	1	0	0	0
Surgeon/ Anaesthetist chair	3	1	4	1	3	0
Switches and exit sensors	11	1	0	0	49	0
Arcadis	0	4	0	0	>300	2
Mobile benches	—	—	0	0	—	—
Heart rate monitor	26	0	0	0	7	1
Equipment	1	0	0	0	—	—
Mobile microscope	262	4	—	—	—	—

Stethoscope	3	0	—	—	—	—
Stainless steel door adjacent to glass cupboard	—	—	0	0	—	—

Table 17 Microbiological assessment of surfaces in general operating room 5 (OR5) between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Anaesthesia cart	43	19	1	0	12	2
Vaporizor	7	2	1	5	0	0
Personnel door	14	0	4	0	1	0
Cables anaesthetic gases	66	0	4	0	1	0
Intercom	1	0	6	0	2	0
Eq. Aliseo Buttons	0	0	1	0	1	0
Serum bench	0	0	1	0	13	0
Glass cupboards	3	1	1	0	1	0
Stainless steel cupboards	29	0	1	1	0	0
Computer mouse	0	0	0	0	15	1
Surgeon/ Anaesthetist chair	2	8	2	0	10	0
Computer keyboard	9	0	18	0	0	0
Static microscopes	>300	6	1	0	9	1
Pantofes	0	0	0	0	13	2
Wall and door switches	>300	0	3	0	14	1
Patient entrance door	1	0	1	0	—	—
Operating table	1	0	1	0	—	—
Ventilator	0	0	0	0	—	—

Table 18 Microbiological assessment of surfaces in Lasik recovery room between 2014 and 2016

	2011		2012		2013		2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
Observation device	1	0	300	1	131	0	4	0	13	4	0	0
Right chair back	0	0	300	0	4	0	1	1	1	0	104	0
Door handle	1	0	300	0	4	0	1	0	1	0	5	0
Surgeon´s chair	44	0	2	0	2	0	3	4	11	0	3	0
Bench for dressing material	5	0	300	1	4	2	2	2	80	9	2	3
Equipment in area of patient observation	9	0	300	1	1	0	32	1	—	—	6	0

Right chair arm	4	0	4	0	47	3	—	—	12	2	>300	0
Surgeon's bench	2	0	0	0	0	0	—	—	—	—	7	0
Switches	3	0	0	1	2	0	—	—	—	—	—	—
Left chair back	1	0	1	0	0	1	—	—	—	—	—	—

Table 19 – Microbiological assessment of surfaces in general recovery room between 2014 and 2016

	2014		2015		2016	
	TAMC (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)	**TAMC** (CFU/plate)	**TYMC** (CFU/plate)
1st bed handle (feet zone)	33	12	55	4	46	0
Sink bench	92	4	18	12	132	0
Computer bench	0	8	123	9	30	0
Computer keyboard	0	0	119	45	167	0
Chair in administrative area	17	0	44	49	63	0
Telephone	34	0	64	7	118	2
Anaesthesia cart/ventilator	20	11	41	0	84	2
Sink tap	5	0	37	0	50	1
Cupboard handles	0	0	9	2	—	—
Bed headboard	0	3	30	0	76	7
Light switches	0	0	8	0	—	—
Serum supports	0	0	2	0	4	3
Portable blood pressure monitors	50	5	98	2	2	0
Suspended monitor	2	1	0	0	—	—
Wooden cupboard	22	0	7	0	106	2
Handle wooden drawers	0	0	9	0	—	—
Heating chamber	7	2	13	1	2	5
Glove cupboard glass door	63	0	1	0	25	0
Sensors (opening door)	33	0	19	0	88	2
Cupboard storeroom entry	1	1	1	0	—	—
Transport table for small material	0	0	3	0	—	—
Mobile work bench	0	7	4	2	—	—
Cupboard handles	0	0	9	2	—	—

A3. Poster presented at the *Congresso Nacional dos Farmacêuticos 2017*

Microbiological Control of Air and Surfaces in Operating and Recovery Rooms of a Lisbon Hospital

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INTRODUCTION

Microbiological assessment of operating rooms (ORs) is crucial for maintaining an aseptic environment and preventing surgical site infections. Studies carried out in Europe and the USA provide evidence of the importance of environmental microbiological control in the prevention of these infections[1,2] .

The Quality Management Department of the Ordem Terceira Hospital, in collaboration with the Microbiological Quality Control Laboratory of FFUL, initiated, in 2011, an annual study concerning the microbiological evaluation of air and surfaces in the **ophthalmology OR (Lasik)** and **recovery room**. As from the year 2014, the study was extended to **five general ORs** and corresponding recovery room.

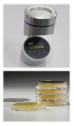
As a complement of the study, **monitoring of *Legionella pneumophila*** in the ducts of the OR air conditioners was also undertaken as from 2015.

AIM

- To identify the critical contamination points within ORs and recovery rooms.
- To verify whether the hygienization and disinfection processes implemented were in fact effective and if the health professionals were implementing good practice strategies.

METHODS

- The **Impaction Method** using Merck® MAS 100 equipment was used for collecting indoor air samples.
- The **Contact Plate Method** and the **Swab Test** were used for surface sampling.
- For all samples, the Total Aerobic Bacteria Count (TAMC) and the Total Yeast and Fungi Count (TYMC) were performed.



- For the investigation of *Legionella pneumophila,* condensed water from the air conditioners and the Swab Test were used (ISO 11731 and 11731-2:2004).

RESULTS

- Surface samples collected in the Lasik room and recovery room revealed mean TAMC and TYMC values of 5,5 CFU (Colony Forming Units)/m² and 0,7 CFU/m², respectively, while those collected in the general ORs and corresponding recovery room revealed mean TAMC and TYMC values of 8,0 CFU/m² and 0,4 CFU/m², respectively.

- Air samples from the Lasik room and recovery room revealed mean TAMC and TYMC values of 29,1 CFU/m³ and 4,6 CFU/m³, respectively, and those collected in the general ORs and corresponding recovery room revealed mean TAMC and TYMC values of 35,7 CFU/m³ and 3,2 CFU/m³, respectively.

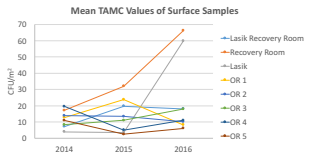


Figure 1: Mean TAMC values of surface samples collected in each OR and each recovery room in 2014, 2015 and 2016. Trypto-Casein Soy Agar medium and the Contact Plate Method or Swab Test were used.

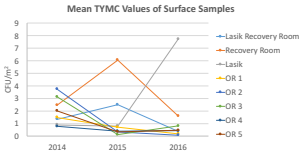


Figure 2: Mean TYMC values of surface samples collected in each OR and each recovery room in 2014, 2015 and 2016. Sabouraud Chloramphenicol Agar medium and the Contact Plate Method or Swab Test were used.

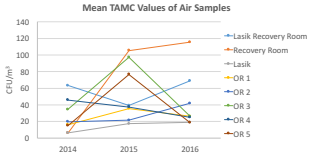


Figure 3: Mean TAMC values of air samples collected in each OR and each recovery room in 2014, 2015 and 2016. Trypto-Casein Soy Agar medium and the Impaction Method were used.

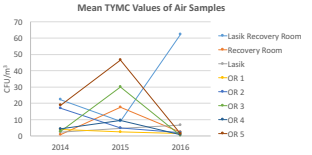


Figure 4: Mean TYMC values of air samples collected in each OR and each recovery room in 2014, 2015 and 2016. Sabouraud Chloramphenicol Agar medium and the Impaction Method were used.

- *Legionella pneumophila* **was not present** in any of the samples collected.

CONCLUSION

- The results from the samples collected from multiple points within the ORs and recovery rooms during the several years of our study were almost all within the limits specified in the existing legislation (D.L. Nº353-A/2013). In fact, only 2-5% of the surface samples collected in the Lasik room and recovery room and in three of the general ORs revealed TAMC>500 CFU/m². With respect to fungi, of all the surface samples collected in the various ORs and recovery rooms, only 7% of those collected in the Lasik room revealed TYMC above the limit established.

- A periodic review of the microbiological quality of the air and surfaces, associated with cleaning/disinfection plans, contributes to the quality control of these rooms, the detection of critical contamination points and the prevention of surgical site infections.

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