Universidade de Aveiro
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Carolina de Matos Santos Coimbra Machado

Espécies prevalentes em infeções associadas a dispositivos médicos de acesso vascular

Prevalent species in vascular access medical device-associated infections

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Microbiologia, realizada sob a orientação científica da Professora Doutora Sónia Cristina das Neves Ferreira, Professora Auxiliar Convidada do Departamento de Ciências Médicas da Universidade de Aveiro.

“Live as if you were to die tomorrow, learn as if you were to live forever”.

Mahatma Gandhi

o júri

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palavras-chave

Dispositivos médicos de acesso vascular, cateteres, infeções da corrente sanguínea, infeções nosocomiais, epidemiologia, *Staphylococcus* spp.

resumo

Os cateteres venosos centrais (CVCs) e os cateteres venosos periféricos (CVPs) quebram a barreira da pele, abrindo uma porta a microrganismos potencialmente causadores de infeções da corrente sanguínea (ICS). Este estudo teve como objetivos, inferir sobre a epidemiologia de espécies associadas ao uso de CVCs e o desenho de um protocolo para a vigilância da colonização bacteriana de CVPs no Centro Hospitalar do Baixo Vouga.
Foi feito o rolamento de pontas de cateter através da técnica de Maki, identificação de microrganismos através de MALDI-TOF MS e o teste de suscetibilidade a antimicrobianos pelo método de difusão em disco.
A maioria dos doentes deste estudo são homens com idade superior a 60 anos. 39% das pontas de CVC analisadas, testaram positivo à presença de microrganismos, dos quais a maioria eram bactérias de Gram-positivo e apenas 15% do total de isolados foram considerados causadores de ICS associada ao uso de CVC. Das pontas de CVP analisadas apenas uma pequena porção teve um resultado positivo para a presença de microrganismos, sendo *Staphylococcus epidermidis* o microrganismo mais frequente. Observou-se ainda a presença de espécies de *S. epidermidis* e *S. aureus* resistentes à meticilina (MRSE/MRSA).
A presença de microrganismos, ainda que pertencentes ao microbiota da pele humana, em pontas de cateter é, por si só, um risco para o desenvolvimento de ICS associadas ao uso de cateter.

keywords

Vascular access medical devices, catheters, bloodstream infections, nosocomial infections, epidemiology, *Staphylococcus* spp.

abstract

Central venous Catheters (CVCs) and Peripheral venous Catheters (PVCs) break the skin barriers, opening a door to microorganisms that potentially cause bloodstream infections (BSIs).This study aimed to infer about the epidemiology of species associated with the use of CVCs and the design of a protocol for the surveillance of PVC tip bacterial colonization at Centro Hospitalar do Baixo Vouga.

The rolling of catheter tips was performed using the Maki technique, microorganisms were identified using MALDI-TOF MS and the antimicrobial susceptibility test by disk diffusion method was performed.

The majority of patients in this study were men over 60 years old. 39% of the analysed CVC tips tested positive for the presence of microorganisms, of which most were Gram-positive bacteria and only 15% of the total isolated microorganisms were considered the source of a BSI-associated with CVC use. Of the analysed PVC tips, only a small portion had a positive result for the presence of microorganisms, with *Staphylococcus epidermidis* being the most frequent one. Methicillin-resistant *S. epidermidis* and *S. aureus* (MRSE/MRSA) were also observed.

The presence of microorganisms even though they belong to the human skin microbiota, in catheter tips is, in itself, a risk for the development of BSIs associated with catheter use.

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Abbreviations

AST – Antimicrobial Susceptibility Testing

BSI – Blood Stream Infection

CDC – Centers for Disease Control and Prevention

CLABSI – Central Line-Associated Bloodstream Infection

CoNS – Coagulase-negative *Staphylococci*

CRBSI – Catheter-Related Blood Stream Infection

CVC – Central Venous Catheter

GCL-PPCIRA – *Grupo Coordenador Local* of the Program for the Prevention and Control of Infections and Resistance to Antimicrobials

HAI – Healthcare-Associated Infection

ICU – Intensive Care Unit

INICC – International Nosocomial Infection Control Consortium

MALDI-TOF MS – Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

MRSA/MRSE – Methicillin Resistant *Staphylococcus aureus* / *Staphylococcus epidermidis*

MSSA/MSSE – Methicillin Susceptible *Staphylococcus aureus* / *Staphylococcus epidermidis*

PICC – Peripherally Inserted Central Catheter

PIVC – Peripheral Intravenous Catheter

PVC – Peripheral Venous Catheter

SEM – Scanning Electron Microscopy

WHO – World Health Organization

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Chapter I – Introduction

1. Background

Central vascular access devices are used, among many medical uses, to facilitate administration of drugs, such as chemotherapy, long-term intravenous antibiotics and to do frequent bloodstream draws. However, there is an high risk for developing medical complications, namely, catheter-related bloodstream infections (CRBSIs), which increases morbidity, length of stay, hospital costs and mortality [1, 2, 3].

These type of bloodstream infections (BSIs) are characterized by the entry and consequent multiplication of microorganisms in the circulatory system. BSIs are diseases commonly associated with hospital settings and the groups of microorganisms responsible for these infections are Gram-positive and Gram-negative bacteria and fungi [4].

BSIs can be considered preventable, leading to the request for public information in infection rates, these CRBSI rates, etiology and antimicrobial resistance of associated pathogens over time, must be scrupulously evaluated, in order to empower key prevention strategies [5].

Epidemiology studies the distribution and determinants of health status or associated events, such as host, causing agent and environment, in specific populations and the application of that study to the control of health problems like infectious diseases.

2. Microbiology

Microorganisms, usually single-celled beings, are found in all types of habitats and are clustered in different groups, such as the bacteria group and the fungi group. Many of these microorganisms can be valuable in the environment, in bioremediation, agriculture, the food industry, the chemical industry, energy production, biotechnology and the pharmaceutical industry. However, some microorganisms are known as pathogenic, that is, disease-causing agents.

2.1. Bacteria

Bacteria are usually spherical, rod or spiral shaped single-celled organisms, as shown in figure 1. They do not have a cell nucleus or the membrane-enclosed intracellular structures. These microorganisms are capable, not only of absorbing nutrients from the environment, but also producing

them through photosynthesis or other synthetic processes. They are present in almost any type of environment, from our body to the most remote and extreme environments [6, 7]

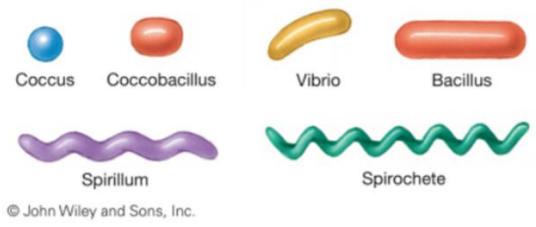


Figure 1 - Common bacterial shapes [6]

Gram staining is a method that allows differentiating bacteria with different cell wall structures from the stains they acquire after treatment with specific chemical agents. As illustrated in figure 2, this method is based on the cell wall ability of Gram-positive bacteria to retain the crystal violet dye in the cytoplasm during an ethanol-acetone treatment while the cell wall of Gram-negative bacteria does not have this ability. Gram staining is one of the most important staining methods used in microbiology and clinical analysis laboratories and is almost always the first step in the characterization of bacterial samples. The technique is of great clinical importance since most of the infection-associated bacteria are quickly observed and characterized as Gram-positive or Gram-negative in smears of pus or organic fluids, for instance. This information allows the clinician to monitor the infection until culture data is available. It is possible to analyse several smears per slide, which facilitates the comparison of clinical samples, in addition these slides can be perpetual and preserved for documentation [6, 7].

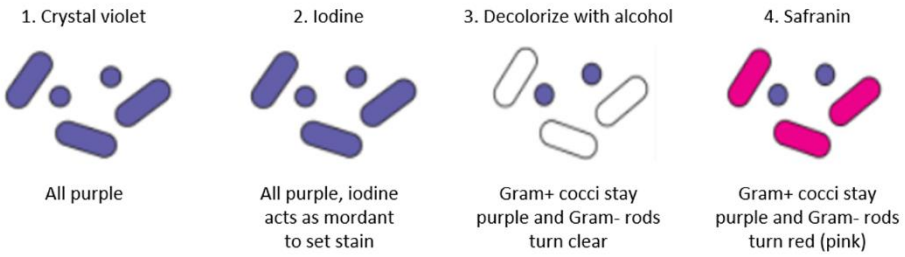


Figure 2 - Simplified main steps of the Gram staining technique [6]

The cell wall of Gram-negative bacteria, unlike the Gram-positive one, is essentially composed by a thin layer of peptidoglycan, lipoproteins, an outer membrane and lipopolysaccharides, as shown on the right side of figure 3. Peptidoglycan is responsible for the most rigid form of bacterial cells and for cytoplasm protection against differences in osmotic pressure. Lipoproteins are accountable for stabilizing the outer membrane and fixing it to the peptidoglycan layer. The outer membrane is held responsible for preventing the loss of periplasmic proteins and preventing the access of hydrolytic enzymes and certain antibiotics to the peptidoglycan molecules. Finally, lipopolysaccharides participate in the pathogenic mechanisms of the bacterial cell and determine the biological effects that result in the amplification of inflammatory reactions [8].

Unlike Gram-negative bacteria, Gram-positive bacteria have a relatively simpler cell wall, as shown on the left side of figure 3. The Gram-positive bacteria cell wall is mostly constituted by a thick layer of peptidoglycan, teichoic acid and lipoteichoic acid. As in the case of Gram-negative bacteria, peptidoglycan plays an important role in the maintenance of the cell structure and protection from the osmotic pressure exerted by the cytoplasm. Teichoic acid participates in the regulation of cytolysins and the adherence to hosted cells. Lipoteichoic acid is responsible for the pathogenic mechanisms, inducing inflammation and contributing to serious infections [9].

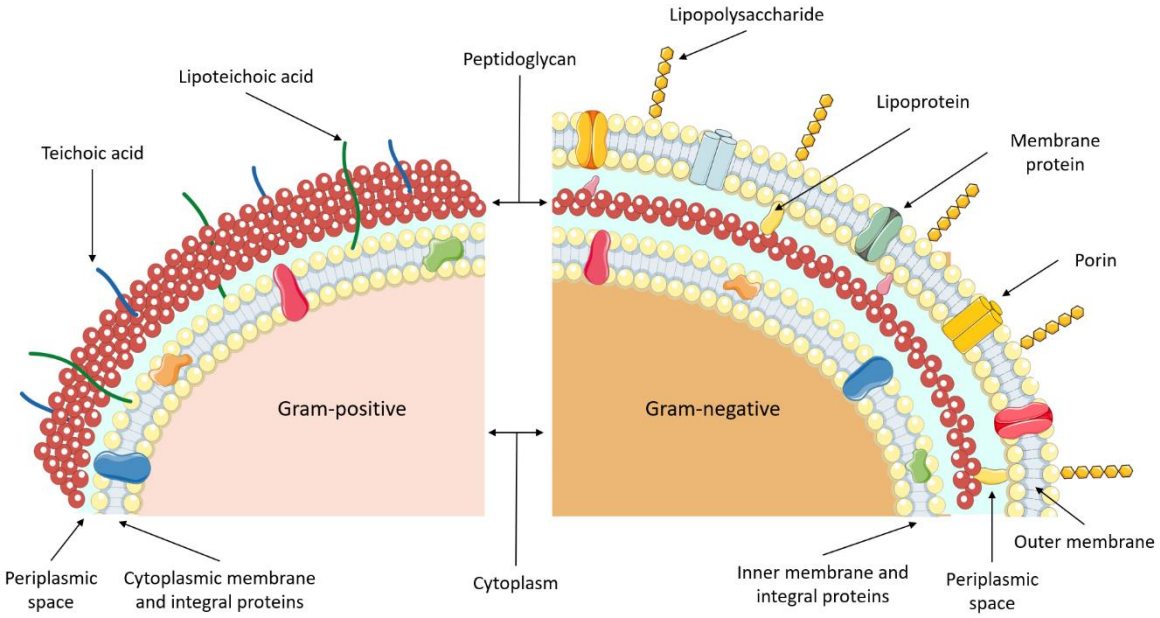


Figure 3 – Main differences in Gram-positive and Gram-negative cell wall structure

2.2. Fungi

Fungi, eukaryotic microorganisms, generally have biphasic life cycles, with a vegetative and a reproductive phase. They can be divided into two large groups. Molds, colonies of long, filamentous cells, visible to the naked eye and yeasts, single-celled organisms. There are fungi species that have the particularity of being dimorphic, that is, they are able to alternate between mold, when grown at room temperature and yeast, when gown at body temperature, being pathogenic [7].

Other than bacteria, fungal parasites can also origin nosocomial infections, acting as opportunistic microorganisms in patients with a compromised immune system. *Aspergillus* spp, *Candida albicans*, usually found in the human large intestine and *Cryptococcus neoformans* are some of these pathogens [7, 10].

2.3. Skin Microbiota

Our skin, the membrane that forms the outer surface of our body is constantly colonized by different groups of microorganisms. We call these the resident microbiota, composed of bacteria, fungi and viruses. Within the bacteria group, the Gram-positive Actinobacteria and Firmicutes phyla and the Gram-negative Bacteroides and Proteobacteria phyla belong to our skin microbiota. Most of these microorganisms are not only harmless to our health, but beneficial, being responsible for creating a protective barrier, preventing the penetration of pathogenic microorganisms. It is through our secretions and skin nutrients that these microorganisms can survive and grow. However, as we know, the skin on our hands is quite different, for example, from the skin on our genital area, so the species present in each skin sites also vary, as well as the variety of microorganisms present in every single site, which the amount and variance of them is not constant and may change several times. Changes in the skin microbiota may be associated with several factors such as administration of antibiotics and chemotherapy [6, 7, 11, 12].

When it is necessary to break the skin barrier and access the bloodstream, for example to take blood samples or to insert a catheter and administer intravenous medication, there is a risk of transporting the microorganism present in the skin into the bloodstream. While present in their ecological niche, our skin, these microorganisms do not cause us disease, but when they enter the bloodstream, they can cause severe infections, becoming pathogenic. In order to reduce contamination of the bloodstream, it is necessary to adopt and keep best practices on disinfecting the skin before, during and after handling [13].

3. Nosocomial Infections

Bacteria, such as coagulase negative *staphylococci*, coryneforms, are the most common cause of nosocomial infections acting as opportunistic when our immune system is down [13].

To induce disease, pathogens must break through the host barriers and have access to target tissues to grow. The main steps of the infection process are shown in figure 4. The entry of microorganisms may occur through coughing or sneezing, through an open wound, the presence in urine or feces, bites of insects or bloodstream draws, among others. The adherence of microorganisms to the host's epithelial cells may be a tissue- or host-specific process. Many bacteria have surface macromolecules that bind to the host's surface receptors (polysaccharides), may have fimbriae and "pili" and may also have antigenic colonization factors. Fungi, on the other hand, express different molecules on the surface that are responsible for adhesion [7].

Despite some toxin-producing microorganisms, which do not need access to tissues, after penetration through the host's epithelium, the process of colonization and growth begins, however, the initial inoculum it is not enough to cause damage. After multiplication, the microorganism can go through the lymphatic vessels or reach the bloodstream and be transported to other parts of the body. Bacteria can produce toxins, enzymes that degrade tissues, cytolysins, IgA1-proteases, antiphagocytic factors, they can have intracellular pathogenicity and even antigenic heterogeneity. Fungi may have mechanisms that facilitate their multiplication in the host, produce proteinases, phospholipases and lipases, alter their morphology, have antigenic heterogeneity, molecular mimicry, intracellular pathogenicity, also have a capsule and produce mycotoxins [7].

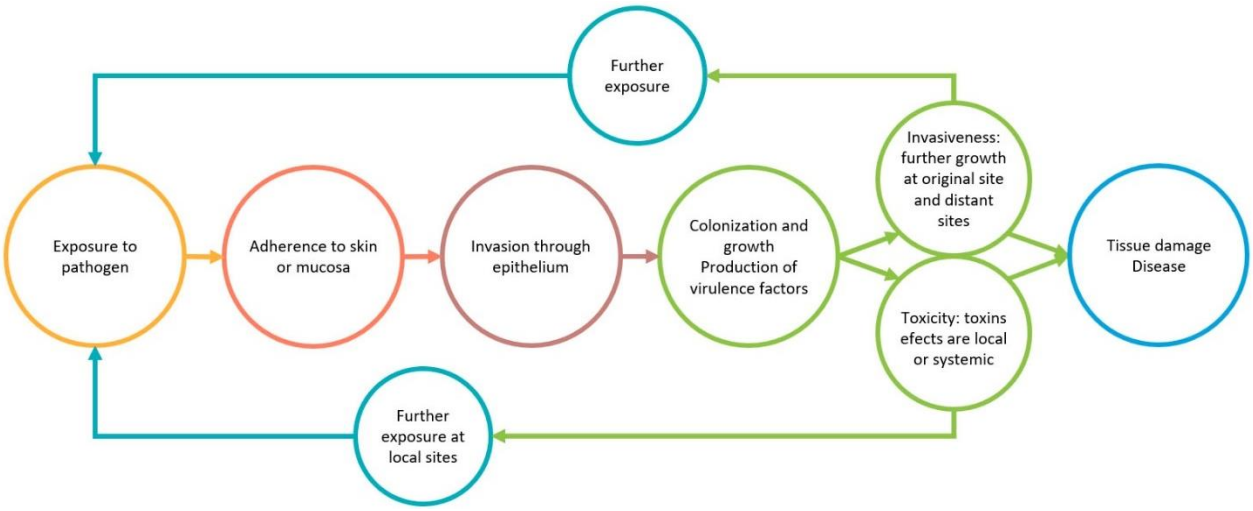


Figure 4 - Infection process main steps [6, 7]

In the infection-fighting process, one of the major contributors to reducing the need for prolonged antibiotic administration is our immune system. Generally, without antibiotic administration, the infection process is acute. While the immune system is not activated, microbial growth is exponential and, as the number of cells in the immune system increases, the microorganisms are eliminated, mainly by effector cells. After decreasing the number of microbial cells, effector cells also undergo a decline, some of which differentiate into cells with immune memory. This way, in the next infection caused by the same pathogen, high levels of memory cells will provide a faster and more effective immune response [14].

The innate immune system (IIS) response is expected to be sufficient against infections caused by low-density pathogenic microorganisms, whereas infections caused by high-density or high-virulent microorganisms may overload the IIS, activating the adaptive immune system response [12].

A nosocomial infection or healthcare associated infection (HAI) stands for an infection acquired or developed in hospital settings, meaning the patient wasn´t infected at the admission to the hospital, becoming infected afterwards, during the stay and even after discharge [15].

This type of infection is more frequent in intensive care units (ICUs) than in other healthcare units, with long periods of hospitalization and the severity of the disease being among the primary plausible causes [16].

These infections can be related to invasive devices such as catheters, commonly used in modern healthcare facilities. The most frequent infections are central line-associated bloodstream infections (CLABSIs), catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia [16, 10].

3.1. Nosocomial Infections Sources

In this type of infection the transmission process can occur during the treatment through direct contact between patient and health care professionals and even through direct contact with environmental sources, where pathogens may be present, including surfaces, medical equipment and devices [10]. This makes the lack of hand hygiene on the part of both patients and health professionals a strong source of transmission, with health professionals becoming one of the main vehicles of transmission [17].

Nosocomial infections can be acquired by patients after touching a contaminated surface, by eating contaminated food while receiving medical assistance or even through vascular access medical devices. The first and third case scenarios are due to the formation of biofilms, structured consortia of cells involved in a self-produced extracellular matrix, becoming resistant to cleaning and disinfection. The developmental cycle, shown in figure 5, is composed of an initial adhesion phase, where the microorganisms begin to attach freely to a surface followed by another irreversible adhesion phase, as the microorganisms begin to attach and multiply, forming a microcolony. The maturation stages occur in which formation of microcolonies protected by extracellular polymeric substances take place. In a last stage, dispersion, the microorganisms separate from the biofilm and disperse [18].

The formation of biofilms in medical vascular access devices, the major problem with this microbial dispersion, is the subsequent presence of pathogenic microorganisms in the bloodstream, which can cause acute infection [19].

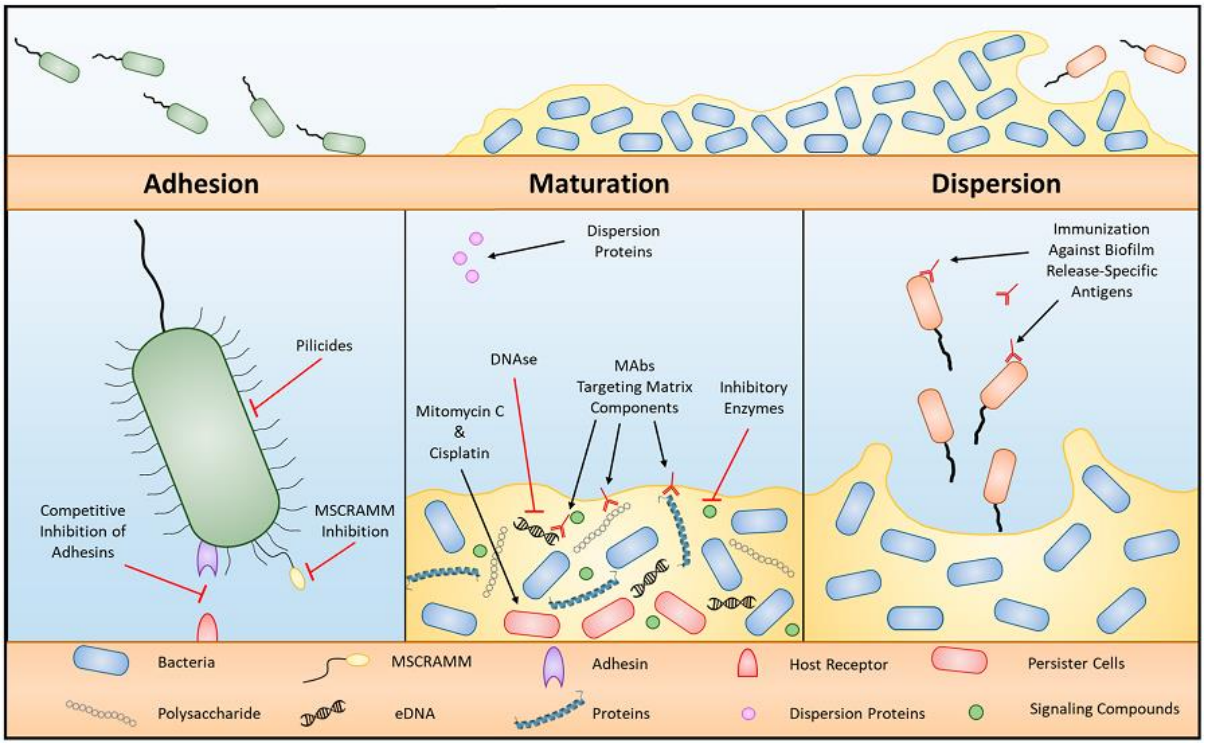


Figure 5 – Main steps of bacterial biofilm process[20]

3.2. Risk Factors and Target Population

There are umpteen risk factors related to nosocomial infections, which can be divided into risk factors associated with: the hospital environment, the patient and health professionals.

In developing countries hospitals, there is lack of hygiene conditions, inadequate waste disposal, poor infrastructure, insufficient equipment, understaffing, overcrowding, absence of local and national guidelines and policies, in fact, several studies have shown that the socioeconomic level of these countries is direct and inversely related to the risk of infections. However, as claimed by the World Health Organization (WHO), nosocomial infections still occur in 7% of high-income countries [21]. Regarding the risk factors associated with the patient and their susceptibility factors, such as immunosuppressed patients, prolonged hospitalization in the ICU, prolonged administration of antibiotics, prolonged use of medical devices, age and other underlying patient conditions are related the occurrence of infections. Concerning to health professionals, an unawareness leading to improper use of injection techniques, poor knowledge of basic injection control measures, inappropriate use of invasive devices, like catheters, lack of control policies, insufficient application of standard and isolation precautions [3, 10, 22, 23].

Here it is important to reinforce the idea of the high risk of infection that the use of a central vascular catheters (CVC) entails. Since the mere fact that a CVC already poses a major risk factor, added to the fact that the patients need this type of medical devices for the application of vasopressor infusions, extracorporeal support or parenteral nutrition further increases the risk of infection [24].

When it comes to nosocomial infections, the most affected people are patients in the ICUs, burn victims, undergoing organ transplant and neonates [10]. Moreover, since the most affected patients are those with a weakened immune system and those who most frequently visit hospital units, the elderly thus become one of the major risk groups [23].

3.3. Infection Indicators

After the entry in the host's circulatory system, the presence of pathogenic microorganisms activates the host's immune system, which leads to the production of a variety of immune cells, such as cytokines/chemokines, that can be used as infection markers for the rapid diagnosis of BSIs. These immune cells can even be considered infection markers on the differentiation of BSIs cause by Gram-positive bacteria, Gram-negative bacteria or fungi [4]. Still concerning the immune system cells, since they play an important role in the infection fighting process, neutrophils also considered as a potential infection markers [25]. Fever or the presence of bacterial cells in the blood are the most significant signs of infection [7].

3.4. Nosocomial Pathogens and Epidemiologic Data

In nosocomial infection-associated microorganisms, there are many differences when it comes to the most prevalent ones, perhaps since nosocomial infections themselves are very different. For example, in a study carried out at a University Hospital's ICU in Morocco, *Enterobacteriaceae* family was the most isolated group of microorganisms (30%), followed by *Staphylococcus* spp. (24%) and *Pseudomonas aeruginosa* (10%), but the most isolated microorganism was *Acinetobacter baumannii* (31%) [16]. *Acinetobacter* spp. has been one of the most relevant nosocomial pathogen, representing about 80% of ICU infections and even accounts for 7% of medical device-associated infections [10, 17, 26, 27]. In fact multi-drug resistant *A. baumannii* infections represent about 12,000 *A. baumannii* infections every year in USA, with 500 deaths associated [17].

A study carried out at the ICU of a teaching hospital in India, concluded that 75% of the microorganisms associated with nosocomial infections were Gram-negative bacteria, the most prevalent being *P. aeruginosa*, followed by *Escherichia coli* and *Klebsiella pneumoniae*, 15% were fungi of the *Candida* genus and 10% were Gram-positive bacteria, with coagulase-negative *Staphylocci* (CoNS) only representing 2,5% of the microorganisms causing nosocomial infections [28].

On the other hand, for CLABSIs, a specific case of nosocomial infections, the values are different, as concluded in a study at a Japanese acute care hospital, where about 49% of CLABSIs were caused by Gram-positive cocci and only 13% by Gram-negative bacilli, *Candida* spp. was responsible for about 39% of CLABSIs [29]. In addition, *Candida* spp. is a major cause of CVC-associated candidaemia [30].

A study with the objective of evaluating the interaction between *C. albicans*, methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) growing in biofilms of one and two microbial species, concluded that these three species are capable of both types of biofilms formation. In fact, in this study, *C. albicans* showed greater growth both in single and dual biofilms, when compared with exclusively MRSA or MSSA biofilm formation. The figure 6 shows the result obtained using the scanning electron microscopy (SEM) technique, where the preferential adherence of MRSA and MSSA to the hyphae of *C. albicans* is observed when forming dual biofilms [31].

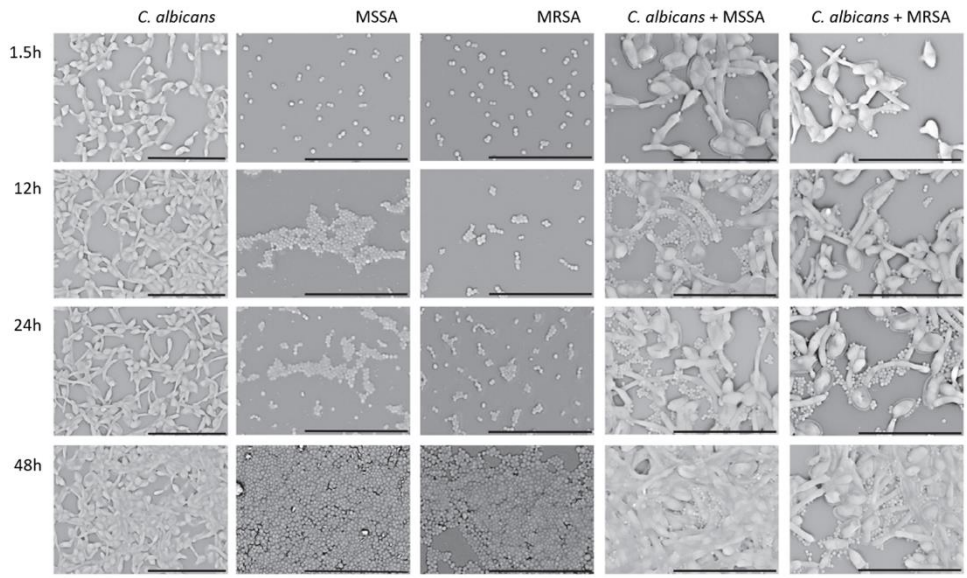


Figure 6 - SEM of adhered single and dual species (1.5h) and biofilms (12h, 24h and 48h) of *C. albicans*, MSSA and MRSA in RPMI at 37° C.
The bar in images corresponds to 20 µm for *C. albicans* (magnification of x2500) and 20 µm for bacteria and mixed growth (magnification of x5000) [31]

MRSA is one of the main causes of nosocomial infections high incidence rates, worldwide [10]. Another study with the objective of assessing nasal colonization of *S. aureus*, including MRSA in 47 portuguese nursing students, over time, found that almost half of the students had nasal MSSA at the time of admission to the course and none had nasal MRSA. During training, more than 80% of students were colonized by *S. aureus* at least once, of which five were MRSA, of these, three wee colonized by the clone that predominantly circulates in national hospitals (ST22-IVh). Thus, they inferred that the community of portuguese nurses is one of the most important groups of *S. aureus* carriers, being of great importance to continue to invest in education and training on infection control strategies [32].

Taking into consideration that *S. aureus* (MSSA and MRSA) is known to be one of the most important nosocomial pathogens, the concern with community-associated MSSA (CA-MSSA) and MRSA (CA-MRSA) cases of infection is still important. It was with this in mind that a study was developed to assess the origin of MRSA in the portuguese community. They found that only about 11% of the MRSA isolates in the community were CA-MRSA clones. In other words, most MRSA cases in the community ware hospital-associated MRSA clones. They assumed, therefore, that CA-MRSA cases are highly likely to be related to spread through the hospital [33].

3.5. Repercussions

Among other complications, nosocomial infections are interrelated with increased hospital stays, long term disability, spiral antimicrobial resistance, addiction in socio-economic disturbance and increased mortality rates [10].

In uncontrolled cases, infections such as BSIs may develop into sepsis or septic shock and, in extreme cases, leading to death [4].

3.6. Antimicrobial Therapy

Antimicrobials are drugs whose objective is to treat infectious diseases caused by pathogenic microorganisms [10]. The national health service estimates that about 60% of portuguese people think antibiotics, as in antibacterial, act against virus and 50% believe that they treat colds and the flu [34].

It is partly due to the misuse of antimicrobials that many microorganisms, such as bacteria, have been developing resistance mechanisms, which in turn, affect the treatment of infections, making it ineffective. This phenomenon is especially worrying in a hospital environment, namely the use of antibiotics to prevent post-surgical infections [14]. Among the many factors that contribute to the development of antibiotic resistance, self-medication, incorrect dosage and prolonged use can be highlighted. This development of resistance occurs not only for causes related to use in humans, but also in animals, namely in the veterinary treatments [10].

According to WHO, antibiotic resistance is one of the biggest threats to global public health today, leading to longer hospital stays, higher medical costs and increased mortality. Denote that without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised [35].

The exponential need for antibiotics and its accumulation in the environment, namely hospital settings, induced a worldwide crisis of antibiotic-resistant bacteria. Antibiotic resistance occurs by transferring antimicrobial-resistance genes between taxa and through lateral gene transfer, an microorganism acquired resistance mechanisms in response for the enormous amount of antimicrobial compounds present in the hospital environment [15].

A study carried out by the International Nosocomial Infection Control Consortium (INICC), involving 50 countries, for 5 years, reported higher rates of antimicrobial resistance of *Klebsiella*

pneumoniae, *Pseudomonas* spp., *Escherichia coli* and *Acinetobacter baumannii* in blood cultures of ICU patients, than the rates reported by the Centres for Disease Control and Prevention National Healthcare Safety Network (CDC-NHSN). The resistance rates of *Staphylococcus aureus* and *Enterococcus faecalis* have been reported as highly elevated by both INICC and CDC-NHSN [3].

In order to obtain the best performance on the infection control measures implemented by the respective health authorities, it is necessary to make a joint effort in the local epidemiological study, either for each country, as a region or hospital infrastructure [36].

3.7. Infection Control Strategies

To prevent its spread, nosocomial infections need to be prevented early on. It may be necessary to develop new diagnostic techniques at the same time as the constant updating of knowledge regarding the development of antibiotic resistance [10]. In fact, despite all the progress related to the prevention of HAIs, some bacteria continue to manage to overcome this progress, through the development of resistance mechanisms [18].

As stated by WHO, health care professionals must perform constant hand hygiene, either before, during or after contact with patients, and preference should be given to alcoholic solutions (60 to 95% alcohol) over soaps, as they are more effective against bacteria and fungi, but especially multidrug-resistant bacteria [37]. Another way to prevent the spread of drug-resistant microorganisms, protecting the heath of patients, health workers and the public in general, is to ensure the safe management of healthcare wastes [38].

On the other hand, health care methodologies may need to be refined. An infection prevention study, using a mandatory electronic communication tool (MECT) to reduce complications and costs associated with Peripherally Inserted Central Catheters (PICCs), has shown that the use of MECT improves clinical quality and achieves zero premature PICC removal and zero CLABSIs. This result is quite important since PICCs when removed prematurely due to suspected unconfirmed infection can lead to reinsertions increasing complications [39].

Another way to prevent HAIs outbreaks is by constantly monitoring the rates of occurrence. That's why the INICC has an online surveillance system, one of the objectives of which is to create a global network in order to reduce HAIs and consequent mortality, bacterial resistance, length of stay and associated costs [3, 40].

In the United States of America (USA), the CDC aims to track infections and antibiotic resistance in the USA and has a Healthcare-Associated Infections – Community Interface (HAIC). The HAIC activities include tracking infections inside and outside of healthcare settings for invasive MRSA and MSSA infections, multi-site Gram-negative surveillance initiative, *Candida* spp. BSIs and even tracking the burden and types of HAIs and antimicrobial drugs used in healthcare settings [41].

In Portugal, we have the Ricardo Jorge Institute whose objective is to carry out epidemiological studies in order to understand and enlighten about the frequency, distribution and etiological factors and their consequences on the national population [42]. Together with the "Direção-Geral da Saúde" (DGS), a guideline was developed whose main goal is to reduce mortality and morbidity directly associated with resistance to antimicrobials [43, 44].

4. Vascular Access Medical Devices

A medical device is an instrument that can be used alone or in combination for diagnostic and/or therapeutic purposes. With the intent of saving lives or improve therapeutic outcomes, catheters, implantable medical devices for vascular access, are commonly used as part of modern medicine. However, they are often associated with severe cases of infections, representing one of the most common healthcare-associated infections [45].

Catheters can be placed in the jugular, subclavian and femoral veins and even in the arms and hands for peripheral access. Depending on the function of the catheter and the needs of the patient, the length of catheter placement may vary from days to weeks and may even be permanent, some patient may also have multiple vascular access devices. Since microorganisms can adhere to the surface of the device and colonize it, there is an augmentation of morbidity, mortality, length of stay and hospital costs [1, 45].

5. Scope

With the increasing antibiotic resistance concern, this work aims to infer about the epidemiology of catheter-associated bacteria at "Centro Hospitalar do Baixo Vouga", develop an experimental protocol to analyse the presence of microorganisms in the PVC tips and develop communication materials with the previous results.

This dissertation is organized into six main chapters, the first one with a general introduction, the second one with the materials and methods used in the study, the third one, divided in two subchapters with the results, discussion and interpretations of the study, the fourth one about scientific communication, the fifth one with the overall conclusions and the last one with all the references used to write the paperwork.

Within the third chapter, in the first subchapter we intend to evaluate the epidemiology of catheter-associated bacteria in the Centro Hospitalar do Baixo Vouga, through demographic data, including patient age and gender, medical ward and sample specimen. In the second subchapter we developed a protocol to evaluate the presence of bacteria in the PVC tips. Finally, in the fourth chapter the results of the first subchapter are presented aiming to address the health professionals but also general population. In this chapter, a pedagogic material was developed and the message passed to the target public.

Chapter II – Material and Methods

1. Central Hospital Characterization

The present work was performed in the sections of Microbiology and Molecular Biology of the Clinical Pathology Department, at "Centro Hospitalar do Baixo Vouga, E. P. E. – Hospital Infante D. Pedro" (CHBV - HIP), which incorporates the Cardiology, General Surgery, Gynaecology, Infectiology, ICU, Internal Medicine, Medicine I, II and III, Obstetrics, Orthopaedics, Paediatrics, Pneumology and Urology wards. The Emergency Department of this hospital is also composed by a series of medicinal specialities, where Clinical Pathology is included. This hospital provides differentiated healthcare and its area of influence covers nine municipalities in the Aveiro's district: Águeda, Albergaria-a-Velha, Aveiro, Estarreja, Ílhavo, Murtosa, Oliveira do Bairro, Sever do Vouga and Vagos.

2. Clinical data collection

The data for the first subchapter of this study was collected in the APPOLO 3 – Laboratory Information Management System | © 2006 Confidentia, Lda. For the year of 2019, in a total of 170 CVC tip samples, information was collected on patient type, sex and age, the hospital admission date and ward, the CVC insertion date, insetion reason, anatomic insertion site and insertion length, CVC tip sample collection date, the tube code, respective CVC tip bearing culture colony count and isolated microorganisms, if the presence of microorganisms was associated with infection or contamination, if the CVC was removed, if antibiotherapy was administrated and which antibiotic was used, as for infection cases, what was the infection-associated outcome, the hospitalization length and outcome. Data regarding clinical risk factors, such as personal background and diagnosis were collected from the patient's medical records. For the data treatment the software Microsoft Office Excel was used.

3. Study population

For the second subchapter of this study a total of 24 Peripheral Venous Catheter (PVC) tips were collected by trained nurses, in the Cardiology ward at CHBV, into sterile flasks and transported to the Clinical Pathology Laboratory for microbiological analysis. The patient information was coded information collected on bed code, admission date, admission motive, age, sex, PVC, insertion date, dressing, disinfectant, anatomical insertion site, removal date, removal motive and catheter tip bearing culture result. Some observations on medication and body temperature were further indicated.

4. Sampling and microbiological analysis

All sample collection procedures in which contact with the patient was required were performed by the hospital nurses, as already done routinely.

Of the microbiological analysis performed the CHBV-HIP's microbiology laboratory, the most important for this study are blood cultures and catheter tip bearing cultures.

Blood samples were taken when there was an indication of infection and was suspected that the agent may be in the bloodstream. Before blood draw, it is necessary to disinfect the skin with an antiseptic, using concentric movements from the inside to the outside. After the skin dried the blood is harvested by aseptic sting in a peripheral vein. The volume should be 10 mL for adults and 2 mL for children, respecting the volume indicated on the blood culture bottle and no air must be let into the bottle. The samples must be sent immediately to the laboratory or if there is a delay, they must be kept in a 37° C incubator.

Since any type of bacteria can grow in this biological product, the culture medium to be used must allow the growth of Gram-positive cocci, Gram-negative bacilli, Gram-negative coco-bacilli and fungi. When the blood culture is positive, it is inoculated in PVX agar plates (BioMérieux, Marcy L'Étoile, France) and incubated at 37° C, for 24 hours.

As for the catheter tips, after removing the dressing, the catheter is removed and 1 to 2 cm of the distal end is cut with a sterile scissors into a sterile container and sent immediately for laboratory analysis. The catheter tips are cultured semiquantitatively, being rolled on PVX agar plates (BioMérieux, Marcy L'Étoile, France) and incubated at 37° C, for 24 hours.

5. Bacterial identification using VITEK®2

VITEK®2 (BioMérieux, Marcy L'Étoile, France) is an automated detection system commonly used in microbial identification within an 18 to 24-hour period. The process occurs through the inoculation and incubation of identification cards, submitted to an optic system combining a multichannel fluorometer and photometer that registers alterations in fluorescence, turbidity and colour.

The identification is made from a fresh and pure culture, of which a 0,55 to 0,65 McFarland suspension is made for further inoculation through a vacuum system into the identification cards and

incubation at 35,5 ± 1° C, the cards are read every 15 minutes. The identification cards are composed of sundry wells with biochemicals, a control well with a growth control agent and they´re specific for Gram-negative bacilli (fermenters or non-fermenters), Gram-positive cocci and ex-non-sporulated bacilli, yeasts and cocci formed by Gram-positive spores. The results are crossed with a database and the identification is obtained with a degree of similarity of the metabolic test.

6. Bacterial identification using MALDI-TOF MS

The method matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (BioMérieux, Marcy L'Étoile, France) has been increasingly validated in the bacterial identification, as it provides fast and reliable results.

MS is an analytical technique that allows the identification and quantification of molecules based on their movement through an electric magnetic field, analysing the ratio between the analyte mass and its charge (m/z), resulting in an intensity graph as a function of the m/z ratio, a mass spectrum.

MALDI is an ionization technique, in which a matrix absorbs energy form ultraviolet (UV) lasers to create ions from large molecules with minimal fragmentation. The m/z ratio can then be determined by the TOF of the ions that the detector measures, to calculate its mass.

The MS analysis can be performed in a few minutes, however a preliminary cultivation step is necessary. In most cases, the samples are cultured and isolated and a single colony is selected to undergo analysis. As a matrix, for the absorption of laser energy, organic acids, such as α-cyano-4-hydroxybenzoic acid (HCCA) and 2,5-dihydroxybenzoic acid, are generally used for microbiological analysis. HCCA is most often used.

The procedure consists of three fundamental steps: ionization of the sample, separation of the ions and finally the detection of ions. In an initial phase, the sample is ionized with the matrix, in a stainless stell 96 well plate. After the samples are dried and crystallized in each well, the plate is inserted into the spectrometer, where it is bombarded with brief pulses of UV laser, usually at a 337 nm wavelength. The laser light energy is absorbed by the matrix molecules that transfer it to the sample molecules, resulting in the molecules 'smooth desorption and ionization, forming a "cloud" of ions in the gas phase. Once the ions are separated, their mass can now be analysed by the TOF mass spectrometer. This separation is carried out in a vacuum-controlled tube to avoid collision with air molecules, where the necessary flight time, between the laser signal and the moment when the ions

reach the detector, is measured. The size of the ions is inversely proportional to the flight time. Thus, the final product is a spectrum that associated the m/z ration and the signal strength and this profile is compared with a database.

7. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing (AST) is indicated for microorganisms that contribute to an infectious process and justify antimicrobial therapy. It is also considered to be fundamental to the type of information that must be obtained before deciding what type of clinical treatment should be applied in case of infection.

The VITEK®2 TSA is based on the determination of minimal inhibitory concentrations (MICs) using different concentrations of antibiotics and is therefore determined from the lowest concentration at which inhibition of bacterial growth occurs. The inoculum performed for the microorganism identification is sufficient to the antibiogram. All cards have a control well, only with microbiological culture medium. Each AST card has 64 microwells with different antibiotics, at various concentrations. The apparatus monitors the microbiological growth in every single well, over 18 hours. It is based on the light intensity measured by the optical reader that the microbiological growth is quantified. At the end of the run, according to the EUCAST guidelines, the result on sensitive, intermediate or resistant strain and its respective MIC is obtained.

The MALDI-TOF MS technique has shown high potential in responding to the need for rapid identification of bacterial strains that cause nosocomial infections. Thus, a good approach to the knowledge of resistance patterns and clonal dissemination pathways. Although optimization measures are necessary so that this method can be applied in the clinical routine, there are already principles for the detection of AST to be developed and studied, such as the detection of biomarkers of antibiotic resistant strains. Detection of the degradation of antibiotics by MS, detection of stable isotopic amino acids, among others, is also carried out.

Chapter III – Results

Subchapter I – Prevalence of Central Vascular Catheter-Associated Species in Centro Hospitalar do Baixo Vouga – Hospital Infante D. Pedro

1. Context

The following topic is dedicated to CVCs, millimetre diameter tubes, placed in the jugular, subclavian or femoral veins, often necessary when treating patients. However, these devices are associated with the risk of complications, including those resulting in BSIs, which can result in worse patient outcome or even death. Studies have shown both the added healthcare costs and risk of death of CVC-associated BSIs. Initiatives to reduce CVC-associated BSIs have shown substantial clinical and economic effects [46].

In Portugal, the epidemiological surveillance system of the Program for the Prevention and Control of Infections and Resistance to Antimicrobials (PPCIRA) monitors the incidence of CVC-associated BSIs through two programs, the Hospital Acquired Infection (HAI)-ICU and the Nosocomial Bloodstream Infection (INCS) [47]. By crossing their data and other obtained in APPOLO 3, it was possible to achieve the results exhibited in this subchapter.

This subchapter was developed with the objective of giving an insight of the situation of the CHBV-HIP regarding the use of CVCs, together with the study already carried out annually by the "*Grupo Coordenador Local*" (GCL)-PPCIRA of the CHBV-HIP. A study on the CVC-associated BSIs in the non-ICUs obtained the result of 1.2 per 10000 bed-days of CVC- associated BSIs occurred in non-ICU settings, as oppose to 1.5 per 10000 bed-days of CVC-associated BSIs. Emphasizing the need to watch out for the non-ICU CVC-associated BSIs [48]. This is exactly why this study researched all medical wards of this hospital and not just the ICU.

Among others, the characterization of the population, the use of CVCs themselves, the rate of CVC-associated infections and which were the most prevalent microorganisms were some of the aspects studied.

2. Patient Demographics

2.1. Patient Age and Sex

The total of 170 catheter tips that were sent to laboratory for analysis, actually corresponded to 143 patients, since due to the insertion of multiple catheters, some patients have more than one sample. Among the 143 patients, 57 (40%) were female and 86 (60%) were male (figure 7).

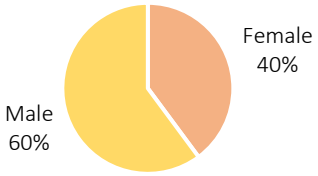


Figure 7 - Patient distribution according to gender

The patient age distribution is shown in figure 8, were it is clear that most of, both male and female, patient's age concentrates between 50 and 75 years old. The average patients age was 62 years old. Thus, an average age very is close to the age considered to define an older population as a risk group. The average female patients age was 65 years old and 60 years old for male patients, with a male patient being just 3 weeks old.

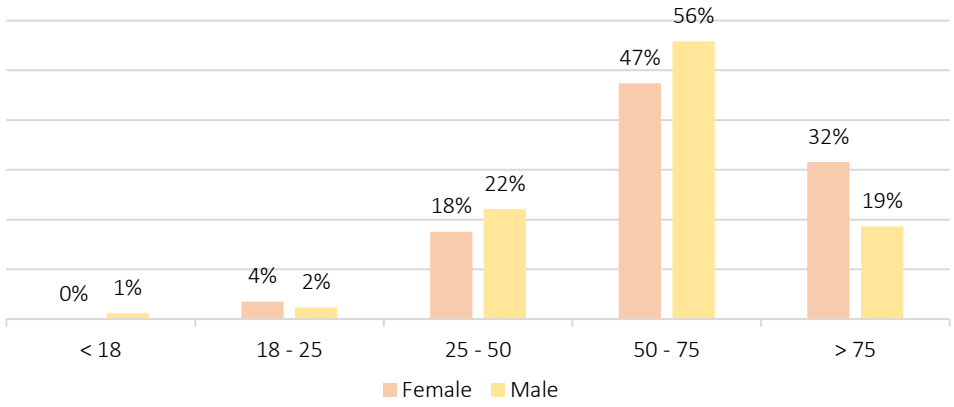


Figure 8 – Patient age distribution according to gender

2.2. Patients' Medical Wards Admissions

In the table 1, the patients were divided according to the medical wards in which they were hospitalized, namely Cardiology, Emergency, ICU, Medicine II and III, Neonatal-ICU, Neurology, Orthopedics and Surgery. Patients who had been transferred from another hospital were also included.

The medical ward with most patients is the ICU, with a total of 84 patients (59%), known to have the higher rate of device use [49], followed by the Surgery ward with 29 patients (20%). The result obtained for the ICU was expected since the patients admitted to this ward are highly dependent and/or ill. Most of the times they are in induced coma and therefore all medications and fluids are given this way.

It should be noted that the total number of patients in table 1 does not correspond to the actual total number of patients, this is explained by the fact that there was a patient from another hospital who was later admitted to the Medicine II ward. Another patient who was hospitalized in the ICU was later transferred to the Cardiology ward.

Table 1 - Patient distribution according to the medical ward

Medical Ward	Total Patients	
	N	%
Another Hospital Transfer	3	2%
CHUC[1]	2	1%
CHUC[1] (Cardiology)	2	1%
Cardiology	6	4%
Emergency	2	1%
Observation Room	1	1%
Cardiology	1	1%
Intensive Unit Care	84	59%
Medicine II	4	3%
Medicine III	1	1%
Neonatal Intensive Unit Care	1	1%
Neurology	2	1%
Orthopedics	7	5%
Surgery	29	20%
Total	145	

[1]*Centro Hospitalar Universitário de Coimbra*

2.3. Patients' Medical Background and Diagnosis

Among this population included in this study, the most common comorbidity was the cardiovascular diseases (63%), especially arterial hypertension (50%), followed by diabetes mellitus (24%), addiction diseases (14%), obesity (13%) and oncological diseases (12%). Ten per cent of the patients did not have any type of comorbidity.

Table 2 shows the distribution of female and male patients, according to the diagnosis that was assigned to them on the date of admission. The list of different diagnoses is very extensive, nonetheless it was possible to observe that pneumonia and septic shock with abdominal source were the most prevalent diagnosis that led more patients being hospitalized (both 11%), followed by trauma (9%) and septic shock (7%).

Table 2 – Diagnosis assigned to the patients at the moment of admission

Diagnosis	Patients					
	Female		Male		Total	
	N	%	N	%	N	%
Acute kidney disease/injury	2	3%	-	-	2	1%
Acute myocardial infarction	2	3%	-	-	2	1%
Cardio-respiratory arrest	-	-	1	1%	1	1%
Cardiovascular disfunction/surgery	3	5%	2	2%	5	3%
Diabetic ketoacidosis	1	2%	-	-	1	1%
Encephalopathy	-	-	2	2%	2	1%
Fever	2	3%	-	-	2	1%
Gastrointestinal disfunction	1	2%	-	-	1	1%
Gastrointestinal hemorrhagy	-	-	1	1%	1	1%
Gastrointestinal surgery	-	-	3	3%	3	2%
Genetic disease	1	2%	-	-	1	1%
Hypovolemic shock	3	5%	5	6%	8	5%
Inectious disease	-	-	1	1%	1	1%
AIDS	-	-	1	1%	1	1%
Diabetic foot infection	1	2%	-	-	1	1%
Nervous sustem infection	-	-	1	1%	1	1%
Pneumonia	5	8%	12	14%	17	11%
Surgical site infection	-	-	1	1%	1	1%
Intestinal obstruction	2	3%	2	2%	4	3%
Myxedematous coma	1	2%	-	-	1	1%
Neurological disease	1	2%	1	1%	2	1%
Oncological disease/surgery	-	-	4	5%	4	3%
Osteomyelitis	2	3%	2	2%	4	3%
Pancreatitis	2	3%	5	6%	7	5%
Peritonitis	1	2%	2	2%	3	2%
Pulmonary thromboembolism	1	2%	2	2%	3	2%
Respiratory disfuncion	2	3%	1	1%	3	2%
Sepsis	1	2%	5	6%	6	4%
Septic shock	5	8%	6	7%	11	7%
Septic shock - abdominal source	10	16%	7	8%	17	11%
Septic shock - respiratory source	3	5%	5	6%	8	5%
Septic shock - urinary source	3	5%	1	1%	4	3%
Shock	1	2%	1	1%	2	1%
Surgical complication	-	-	1	1%	1	1%
Trauma	4	7%	10	11%	14	9%
Voluntary drug poisoning	1	2%	1	1%	2	1%
Unknown diagnosis	-	-	2	2%	2	1%
Total	61		88		149	

Once again, it should be noted that the values referring to the total number of patients, do not correspond to the actual total value of patients, because some patients have been assigned more than one type of diagnosis.

Although this population has a low percentage of an already existent pulmonary disease, it is necessary to emphasize that this condition is taken into account with regard to the risk factors for the BSI development [49].

The percentage obtained regarding trauma was the second highest and it is important to mention that this percentage, nonetheless, related to trauma, belongs to polytraumatized patients who were admitted to the ICU.

2.4. Patients' Hospitalization Length and Outcome

Table 3, shows patients were separated according to the total time they were hospitalized, less than a week, between a week and 15 days, between 15 days and a month, from one to two months, from two to three months, from three to four months and more than four months.

It is possible to observe that 35% of patients were hospitalized between 15 days and one month, 27% between one week and 15 days and 24% from one to two months. The average number of days of hospitalization was 28 days, which may be considered a long time. One must bear in mind that such long time increases the possibility of occurrence of nosocomial infections.

Table 3 - Hospitalization Length

Hospitalization Length	Total Patients	
	N	%
< 1 week	8	6%
1 week - 15 days	38	27%
15 day - 1 month	50	35%
1 month - 2 months	34	24%
2 months - 3 months	10	7%
3 months - 4 months	2	1%
> 4 months	1	1%
Mean (days)	28	

In table 4, patients are separated according to the result of their hospitalization, whether they were discharged, died, were transferred to another hospital or, in the case of only one patient, it was not possible to access information about the hospitalization outcome. It is possible to observe that the majority of the patients (81% of patients) were discharged.

Table 4 - Hospitalization Outcome

Hospitalization Outcome	Total Patients	
	N	%
Discharge	116	81%
Death	19	13%
Transferred to another hospital	7	5%
Unknown	1	1%

3. Central Vascular Catheters

3.1. CVC Anatomical Insertion Sites

From all 170 catheter tips sent to analysis, only one was from a Peripheral Insertion Central Catheter, which was from the 3-week-old male patient. As shown in figure 9, among the remain 169 catheter tips, 33 (19%) were inserted in one of the femoral veins, 32 (19%) in one of the jugular veins, 103 (61%) in one of the subclavian veins and 1 (1%) in an unknown anatomic insertion site.

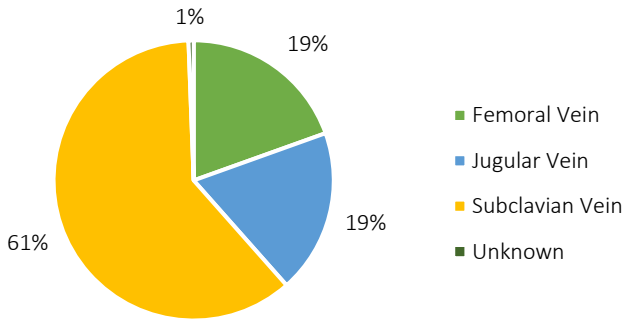


Figure 9 - Distribution of the different anatomic catheter insertion sites

The results obtained in this study are in accordance with several studies that have been demonstrating that the subclavian vein is the best option in terms of CRBSIs avoidance. Therefore, this

anatomical insertion site must be chosen first, followed by the jugular vein and finally the femoral vein [49, 50]. For example, in patients undergoing allogeneic hematopoietic cell transplantation, CVCs are usually placed in the subclavian or jugular veins. However, some studies do not show the subclavian vein as the best insertion site, in fact, local inflammation and fever seems to be risk factors in this anatomic insertion site [51].

Among the 143 patients studied, 23 (16%) had more than one CVC inserted during this study, from which 14 (60%) had two or more CVCs inserted simultaneously.

Table 5 shows patients who had, simultaneously, a catheter in one of the femoral veins and another in one of the subclavian veins (43%), in one if the femoral veins and another in one of the jugular veins (21%), one in one of the subclavian veins and another in one of the jugular veins (14%), in both subclavian veins (14%) and, finally, in both subclavian veins and in one of the femoral veins (7%).

Table 5 - Percentage of patients with two or more CVCs simultaneously inserted

Simultaneous multiple CVCs[1]	N	%
Femoral and subclavian veins	6	43%
Femoral and jugular veins	3	21%
Subclavian and jugular veins	2	14%
Both subclavian veins	2	14%
Both subclavian and femoral veins	1	7%
Total	14	

[1]Central Vascular Catheters

The use of multiple CVCs is considered as a risk factor for the development of CVC-associated BSIs [49]. Therefore, it is important to look at the results obtained in order to understand if it is possible to improve procedures and/or if in our hospital the infections are related with the insertion of multiple CVCs.

3.2. CVCs' Insertion Length

Table 6 shows a relationship between the CVCs anatomical insertion sites and their insertion length. In general, 46% of the CVCs were inserted for less than a week, 37% were inserted from one week to fifteen days, 14% from fifteen days to one month, 2% more than one month and 1% of CVCs were inserted for an unknown period. The mean of days that CVCs were inserted was ten days.

Most of CVCs inserted in one of the femoral veins were inserted during less than a week (58%), as well as those inserted in one of the jugular veins and in one of the subclavian veins (50% and 41%, respectively). The only CVC whose anatomical insertion site is unknown was inserted for two days. 27% of CVCs inserted in one of the femoral veins were inserted between one week to fifteen days, as well as most CVCs inserted in one of the jugular and subclavian veins (44% and 38%, respectively). 15% of CVCs inserted in one of the femoral veins were inserted between fifteen days and one month, as well as 17% of those inserted in one of the subclavian veins and only 3% of those inserted in one of the jugular veins. There were no CVCs inserted in one of the femoral veins lasting more than one month, On the other hand, there were about 3% both of those inserted in one of the jugular and subclavian veins that lasted more than one month. There were also no CVCs inserted in one of the femoral veins neither jugular veins of unknown duration, but there were about 1% of those inserted in one of the subclavian veins.

CVCs inserted in one of the femoral veins lasted nine days average, those inserted in one of the jugular veins eight days average and the subclavian veins eleven days average.

Table 6 - Relationship between the CVC anatomic insertion site and insertion length

	Catheter Insertion Site									
	FCVC[1]		JCVC[2]		SCVC[3]		UCVC[4]		Total CVCs[5]	
Insertion Lenght	N	%	N	%	N	%	N	%	N	%
< 1 week	19	58%	16	50%	42	41%	1	100%	78	46%
1 week to 15 days	9	27%	14	44%	39	38%	-	-	62	37%
15 days to 1 month	5	15%	1	3%	17	17%	-	-	23	14%
> 1 month	-	-	1	3%	3	3%	-	-	4	2%
Unknown	-	-	-	-	2	2%	-	-	2	1%
Total	33		32		103		1		169	
Mean (days)	9		8		11		2		10	

[1]Femoral vein inserted central vascular catheter
[2]Jugular vein inserted central vascular catheter
[3]Subclavian vein inserted central vascular catheter
[4]Central vascular catheter whose anatomical insertion site is unknown
[5]Central vascular catheter

Most CVCs that were inserted less than a week were from the ICU (62%) and from the surgery ward (21%). The same happened with the CVCs that lasted between one week and fifteen days, with 69% being from the ICU and 15% from the surgery ward those that lasted between fifteen days and one month with 70% from the ICU and 13% from the surgery ward. The orthopedics ward was the only one with CVCs lasting more than one month.

The ward with the highest average CVC length was orthopedics with 27 days, followed by the neurology ward with 17 days, then medicine II with 15 days and the CHUC's cardiology ward with 12 days. Only afterwards, the medical wards with the largest number of CVC insertions, the ICU with 10 days and surgery with 9 days. The remaining wards had the average lengths below the general average of 10 days.

Contrary to what would be expected in the ICU, where the most critical patients are admitted, with longer hospitalization periods and with a higher CVC insertion rate, it is the orthopedics ward that is responsible for CVCs with longer insertion periods. The average duration of CVCs in the orthopedics ward was 27 days, since there were four cases in which CVCs were inserted for 34, 35, 51 and 53 days. These were cases of osteomyelitis that required CVCs for prolonged antibiotic administration, which contributed to the high average CVC insertion period.

In the ICU most catheters were inserted for, even relatively, short periods of time (10 days mean) which may contribute to a reduced rate of CVC tip contamination and CVC-associated BSI. This is explained by the fact that at the ICU, stricter protocols are implemented for the CVC monitoring, CVCs are exchanged more frequently that in other services with less critical patients. Where these CVCs were really necessary, they could have been replaced by PVCs, with less associated risk of infection. Studies have shown that by avoiding the short-term use CVCs, catheter-associated BSI are prevented [52].

4. Microbiological Results of the CVC Tip Cultures

Among 170 catheter tips sent for laboratory analysis, only 66 (39%) shown bacterial growth. As shown in figure 10, regarding the results of microbial colony count of catheter tip bearing cultures, 102 (60%) catheter tips had a result below 15 UFC, which was reported as negative result since no action is taken regarding the colonies that appeared and 2 (1%) a negative result. In this last case there was no growth observed.

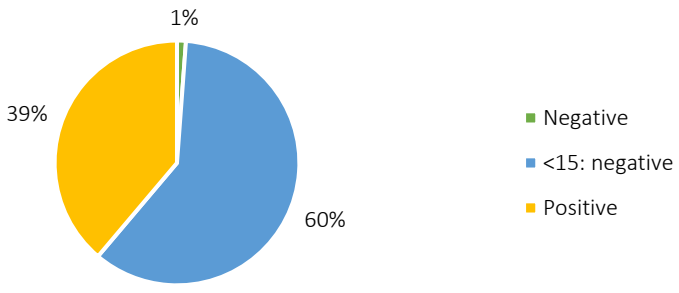


Figure 10 - Catheter tip bearing culture results

Table 8 shows the list of all isolated and identified microorganisms from the catheter tip where growth was seen. Note that the total number of isolates does not correspond to the total number of positive cultures because there were cultures in which more than one species were isolated.

Of the 66 positive cultures, 9% of yeasts of the genus *Candida* were isolated, 66% of Gram-positive bacteria, including several species of coagulase-negative *Staphylococci* (CoNS) and 25% of Gram-negative bacteria.

Table 7 - Isolated microorganisms from positive catheter tip bearing cultures

Microorganisms	N	%
Yeast	**7**	**9%**
Candida albicans	5	
Candida parapsilosis	2	
Gram-positive bacteria	**52**	**66%**
Enterococcus faecalis	3	
CoNS[1]	**49**	
Staphylococcus capitis	1	
Staphylococcus epidermidis	34	
Staphylococcus haemolyticus	5	
Staphylococcus hominis	6	
Staphylococcus simulans	1	
Staphylococcus warneri	2	
Gram-negative bacteria	**20**	**25%**
Enterobacter aerogenes	5	
Escherichia coli	3	
Klebsiella pneumoniae	4	
Proteus mirabilis	3	
Pseudomonas aeruginosa	3	
Serratia marcescens	1	
Stenotrophomonas maltophilia	1	
Total Isolates	**79**	

[1] Coagulase-negative *Staphylococci*

In figure 11 it is possible to observe the percentual difference of the isolated yeast species, with *Candida albicans* being isolated 71% of the time and *Candida parapsilosis* 29%.

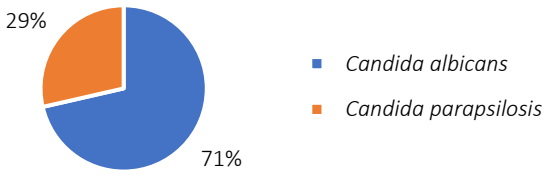


Figure 11 - Isolated yeast

As for the isolated Gram-negative bacteria, represented in figure 12, of the 20 isolates, 25% were *Enterobacter aerogenes*, 20% *Klebsiella pneumoniae*, 15% *Escherichia coli*, 15% *Proteus mirabilis*, 15% *Pseudomonas aeruginosa*, 5% Serratia marcescens and 5% *Stenotrophomonas maltophilia*.

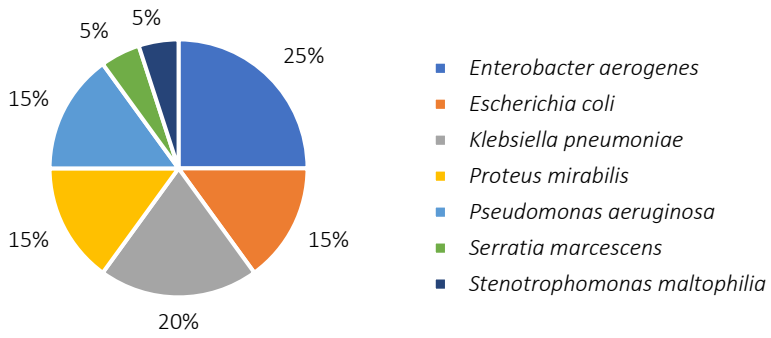


Figure 12 - Isolated Gram-negative bacteria

Within the group of Gram-positive bacteria, 94% were coagulase-negative *Staphylococci* (CoNS), of which the highest percentage (70%) of the isolated microorganisms were *Staphylococcus epidermidis*, followed by 12% *S. hominis*, 10% *S. haemolyticus*, 4% *S. warneri*, 2% *S. capitis* and 2% *S. simulans*, as shown in figure 13, relative to the percentage of CoNS Gram-positive bacteria.

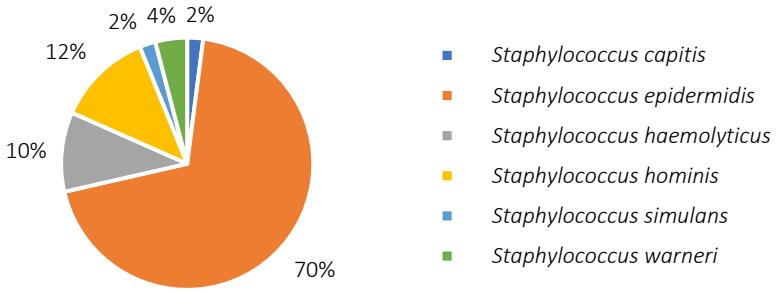


Figure 13 - Isolated Gram-positive coagulase-negative bacteria

As can be seen in table 9, concerning to the relationship between isolated microorganisms and the different CVC anatomical insertion sites, *C. albicans,* apart from *S. epidermidis,* was isolated from all anatomical insertion sites, including the tips of catheters which the anatomical insertion site is unknown. Within the group of Gram-positive bacteria, it should be noted that most of the CoNS bacteria were isolated from catheter tips inserted in one of the subclavian and jugular veins, with *S. epidermidis*, the most prevalent microorganism, being isolated in 23% of the tips of catheters inserted in one of the

subclavian veins, 11% of the tips of catheters inserted in one of the jugular veins and 8% of the tips of catheters inserted in one of the femoral veins. The bulk of Gram-negative bacteria was isolated from tips of catheters inserted in one of the femoral veins.

Table 8 - Relationship between the isolated microorganisms and the different anatomic insertion sites

Microorganisms		Catheter Insertion Site							
		FCVC[2]		JCVC[3]		SCVC[4]		UCVC[5]	
		N	%	N	%	N	%	N	%
Yeast									
Candida albicans		1	1%	1	1%	2	3%	1	1%
Candida parapsilosis		-	-	-	-	2	3%	-	-
Gram-positive bacteria								-	
Enterococcus faecalis		3	4%	-	-	-	-	-	-
CoNS[1]									
Staphylococcus capitis		1	1%	-	-	-	-	-	-
Staphylococcus epidermidis		6	8%	9	11%	18	23%	1	1%
Staphylococcus haemolyticus		-	-	2	3%	3	4%	-	-
Staphylococcus hominis		2	3%	1	1%	3	4%	-	-
Staphylococcus simulans		1	1%	-	-	-	-	-	-
Staphylococcus warneri		-	-	-	-	2	3%	-	-
Gram-negative bacteria									
Enterobacter aerogenes		2	3%	2	3%	1	1%	-	-
Escherichia coli		3	4%	-	-	-	-	-	-
Klebsiella pneumoniae		2	3%	-	-	2	3%	-	-
Proteus mirabilis		3	4%	-	-	-	-	-	-
Pseudomonas aeruginosa		1	1%	2	3%	-	-	-	-
Serratia marcescens		1	1%	-	-	-	-	-	-
Stenotrophomonas maltophilia		1	1%	-	-	-	-	-	-
Total Isolates	79	27		17		33		2	
Total CVCs[6]	66	19		17		29		1	

[1] Coagulase-negative Staphylococci
[2] Femoral vein inserted central vascular catheter
[3] Jugular vein inserted central vascular catheter
[4] Subclavian vein inserted central vascular catheter
[5] Central vascular catheter whose anatomical insertion site is unknown
[6] Central vascular catheters

These results are in line with studies carried out on the human skin microbiota, which shown that the microorganisms that are part of our skin, directly affect the occurrence of CRBSIs, namely the most prevalent here, *Staphylococcus epidermidis* [53].

With regard to the microorganisms found in the subclavian vein inserted CVC tips and the jugular vein inserted CVC tips, which are found in oily skin sites such as the neck and upper chest, these results agree with those that have been described as the microorganisms typically present in these type of area. The same happens with the microorganisms found in the femoral vein inserted CVC tips, a vein located near the groin area, a moist area. [7, 54, 55, 56, 51, 57, 52].

4.1. CVC-Associated Infections

Table 10 shows the number of catheter-associated infections and the number of contaminations and their associated microorganisms. Of the positive, only 10 (15%) were considered catheter-associated infections, the remaining 56 (85%) were considered catheter tip contaminations. These embody 33% of the total catheter tips and the 10 catheter-associated infections symbolize only 6% of the analysed catheter tips.

Regarding the microorganisms, of 79 isolates, only 14 (18%) were considered infection responsible microorganisms, while the remaining 65 (82%) were considered contaminants. *Candida parapsilosis*, *Staphylococcus capitis*, *S. haemolyticus*, *S. hominis*, *S. simulans*, *S. warneri*, *Serratia marcescens* and *Stenotrophomonas maltophilia* were only considered catheter tip contaminants, whereas *Klebsiella pneumoniae* was always considered a catheter-associated infection microorganism and *Staphylococcus epidermidis,* responsible for 42% of catheter tip contaminations, was also considered a microorganism that caused 1 % of the catheter-associated infections.

Table 9 - Isolated microorganisms and the number of catheter-associated infections/contaminations

Microorganisms		Infection		Contamination	
		N	%	N	%
Yeast (Fungi)					
Candida albicans		1	1%	4	5%
Candida parapsilosis		-	-	2	3%
Gram-positive bacteria (cocci)					
Enterococcus faecalis		2	3%	1	1%
CoNS[1]					
Staphylococcus capitis		-	-	1	1%
Staphylococcus epidermidis		1	1%	33	42%
Staphylococcus haemolyticus		-	-	5	6%
Staphylococcus hominis		-	-	6	8%
Staphylococcus simulans		-	-	1	1%
Staphylococcus warneri		-	-	2	3%
Gram-negative bacteria (bacilli)					
Enterobacter aerogenes		1	1%	4	5%
Escherichia coli		1	1%	2	3%
Klebsiella pneumoniae		4	5%	-	-
Proteus mirabilis		2	3%	1	1%
Pseudomonas aeruginosa		2	3%	1	1%
Serratia marcescens		-	-	1	1%
Stenotrophomonas maltophilia		-	-	1	1%
Total Isolates	79	14		65	
Total CVCs[2]	66	10		56	

[1]Coagulase-negative *Staphylococci* [2]Central Vascular Catheters

After an online CVC-associated BSI surveillance program in ICUs in England was implemented, with the data for one year, it was found that 96,1% of the blood cultures performed in adult, paediatric and neonatal ICU were positive. 56% of positive adult blood cultures corresponded to BSIs with the majority being CVC-associated BSIs and, of these, 2.3 per 1000 ICU-CVC-days were ICU-associated CVC-BSI. Regarding the microorganisms responsible for the positive blood cultures, 38% were skin commensals and of these 3% caused a BSI. Of the total positive blood cultures, 12,1% were associated with polymicrobial infections. Comparing the microorganisms responsible for the contaminations, 40,1% were CoNS and 9,3% *E. coli*. However the numbers as if inverted in relation to the ones that caused ICU-associated BSI, 8,9% of CoNS and 11,6% *E. coli* [58].

Another study about the incidence, risk factors and health care costs of CLABSIs, observed that the most prevalent microorganisms involved in CLABSIs were, in descendent order, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *S. haemolyticus*, *Escherichia coli*, among others [49].

A study on CVC-related BSIs in trauma patients found to be CoNS species, the most prevalent microorganisms in this type of infection (36,2%), followed by *S. aureus* (15,5%), *Enterococcus faecalis* (3.4%), *Acinetobacter baumannii* (8,6%), *Serratia marcescens* (3,4%), *Klebsiella pneumonia* (3,4%), *Candida parapsilosis* (15,5%), *C. albicans* (10,3%), *C. pelliculosa* (1,7%) and *C. intermedia* (1,7%) [50].

In table 11 was compared the CVC anatomical insertion site, the CVC insertion length and the existence of CVC-associated infection or contamination of the CVC tip.

In general, of the 18 CVCs inserted during less than a week, in 35% of the cases the existence of a contamination was considered and in only 1% of the cases it was considered an CVC-associated infection. Of the 62 CVCs inserted between one week and fifteen days, in 32% of the cases the existence of a contamination was considered and in 8% of the cases it was considered an CVC-associated infection. Of the 23 CVCs inserted for fifteen days to one month, in 30% of the cases the existence of a contamination was considered and in 13% of the cases it was considered an CVC-associated infection. Of the 4 CVCs inserted during more than one month, in 50% of the cases the existence of a contamination was considered and in 25% of the cases it was considered an CVC-associated infection. There were no contaminations neither CVC-associated infections in the two cases of CVCs inserted for an unknown period. Regarding the insertion length of the CVCs with contamination, these were inserted for an average of 11 days, while 15 days was the average inserted length for the CVC-associated infection cases.

As for CVCs inserted in one of the femoral veins (FCVCs), for less than a week, 42% were contaminated and 5% were considered cases of FCVC-associated infections. Of the 9 FCVCs inserted

between one week and fifteen days, 33% was the percentage both for cases of contamination and for those with an FCVC-associated infection. Of the 5 FCVCs inserted between fifteen days and one month, 40% was the percentage for both cases of contamination and for those with an FCVC-associated infection. In average terms, the cases of contamination, had the FCVCs inserted for 8 days, while the cases of FCVC-associated infection had the catheter inserted for 12 days.

Concerning the CVCs inserted in one of the jugular veins (JCVCs), of the 16 inserted during less than a week, there were contaminations in 56% of the JCVC tips and there were no cases of JCVC-associated infections. Of the 14 JCVCs inserted between one week and fifteen days, contamination was found in 43% of them and JCVC-associated infections in 7% of the cases. There was no contaminations or JCVC-associated infections in those inserted for fifteen days t one month and the only JCVC inserted for more than one month was contaminated. The cases of JCVC contamination had an average insertion length of 11 days and the only case of JCVC-associated infection had it inserted for 13 days.

Of the CVCs inserted in one of the subclavian veins (SCVCs), for less than a week, there were only cases of contamination (21%), Of the 39 SCVCs inserted during a week to fifteen days, there were 28% of contamination cases and 3% of SCVC-associated infection cases. Of the 17 SCVCs inserted for fifteen days to one month, there were 29% of contamination cases and 6% of SCVC-associated infection cases. Of the SCVCs inserted for more than a month, there were 33% of both contamination cases and SCVC-associated infection cases. On average, the SCVC contamination cases had them inserted for 13 days and 22 days was the average time for SCVC-associated infections.

The only case of a CVC with an unknown anatomical insertion site was contaminated and had an insertion length of 2 days.

As shown in figure 9, the subclavian vein was the anatomic insertion site of choice for the CVC insertion, in this study, which is in agreement with the fact that the insertion in this anatomical site is considered as a protective factor for BSIs [49]. However, when the time factor is added, in this study the appearance of SCVC-associated BSIs is verified, when inserted for more than a week. Moreover, ICU patients generally required prolonged treatment, which results in long hospital stays. Time it self, is already a risk factor, due to the prolonged exposure to resistant microorganisms common in hospital settings [59].

Table 10 - Relationship between the CVC anatomic insertion site-associated infection/contamination and the CVC insertion length

Catheter-Associated Infection or Contamination	Catheter Insertion Length											
	< 1 week		1 week to 15 days		15 days to 1 month		> 1 month		Unknown		Total CVCs[5]	Mean (days)
	N	%	N	%	N	%	N	%	N	%		
FCVC[1]	**19**	**58%**	**9**	**27%**	**5**	**15%**	**-**	**-**	**-**	**-**	**33**	**9**
Infection	1	5%	3	33%	2	40%	-	-	-	-	6	12
Contamination	8	42%	3	33%	2	40%	-	-	-	-	13	8
JCVC[2]	**16**	**50%**	**14**	**44%**	**1**	**3%**	**1**	**3%**	**-**	**-**	**32**	**8**
Infection	-	-	1	7%	-	-	-	-	-	-	1	13
Contamination	9	56%	6	43%	-	-	1	100%	-	-	16	11
SCVC[3]	**42**	**41%**	**39**	**38%**	**17**	**17%**	**3**	**3%**	**2**	**2%**	**103**	**11**
Infection	-	-	1	3%	1	6%	1	33%	-	-	3	22
Contamination	9	21%	11	28%	5	29%	1	33%	-	-	26	13
UCVC[4]	**1**	**100%**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**	**2**
Infection	-	-	-	-	-	-	-	-	-	-	-	-
Contamination	1	100%	-	-	-	-	-	-	-	-	1	2
Total CVCs[5]	**78**	**46%**	**62**	**37%**	**23**	**14%**	**4**	**2%**	**2**	**1%**	**169**	**10**
Infection	1	1%	5	8%	3	13%	1	25%	-	-	10	15
Contamination	27	35%	20	32%	7	30%	2	50%	-	-	56	11

[1]Femoral vein inserted central vascular catheter
[2]Jugular vein inserted central vascular catheter
[3]Subclavian vein inserted central vascular catheter
[4]Central vascular catheter whose anatomical insertion site is unknown
[5]Central vascular catheter

5. Interpretation

With these results it is possible to conclude that, regarding BSIs associated with the use of CVCs, the scenario in this hospital is not of great concern. The rate of BSIs associated with CVCs is relatively low, and even in cases of infection, none of them was caused by, for example, *S. aureus*, a common pathogen frequently involved in CRBSIs. The mortality rate is relatively low and the discharge rate is relatively high.

From these results it appears that many CVC tips are being sent to the laboratory unnecessarily. In fact, the GCL-PPCIRA of this hospital is already working in order to reduce the number of CVC tips that are sent for analysis, reinforcing the idea of sending only the CVC tips of cases where a BSI is suspected.

On the other hand, since most of the positive CVC tip culture results correspond to contamination by microorganisms belonging to the human skin microbiota, it is necessary to assess whether the skin disinfection process is being carried out correctly. Whether when inserting the CVCs, changing the dressings or removing the CVCs. And if not, how can this procedure be improved, in order to obtain even better CVC tip contamination rates.

Chapter III – Results

Subchapter II – Development of a Peripheral Venous Catheter Bacterial Colonization Surveillance Protocol

1. Context

In this topic, the focus is on PVCs, small flexible tubes, normally inserted into the blood vessels of the hands and arms, for administration of fluids such as saline, antibiotics, blood draws or transfusions. This medical device is considered to be the most used in the hospital environment, when the patients' medical condition requires the need of short-term vascular access [60, 61].

The figure 14 shows an example of a PVC inserted in the superficial dorsal vein, on the back of the left hand, for pre-operatory saline administration.

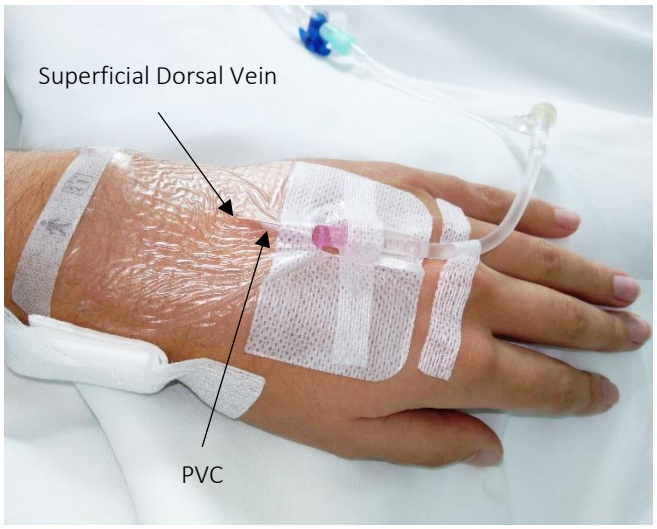


Figure 14 – Peripheral Venous Catheter

Most of the studies on CRBSIs, focus a lot on CVCs, while PVCs are also an integral part of these infections, with few studies on the subject. Although the use of PVCs is short-term, there are cases of bloodstream infections associated with them that require intensive and prolonged care and may even cause death [62].

In CHBV-HIP there is no surveillance protocol on infections associated with PVCs other than the 4-day removal protocol, so it was in this sense that this topic was developed. The objective was to design a laboratory protocol (attachment A) for the surveillance of PVC colonization, to assess the presence of bacteria on the PVC tips.

2. PVC Colonization Surveillance Protocol Design

The main objective of creating this protocol was to learn about the number of PVCs colonized by bacteria. The study timeframe was two weeks and the two services involved were the Cardiology and Clinical Pathology's laboratory, namely, the Microbiology section at CHBV-HIP. To each sample was completed a form with relevant aspects related to the sample and the patient. In summary, after the samples were collected, they were subjected to microbiological analysis, identification and antimicrobial susceptibility test of the positive cultures.

2.1. PVC Removal

The collection of 24 PVC tips was performed by the Cardiology ward nurses when removing the catheters. After removing the dressing, the skin was disinfected and the catheter was removed and placed in a sterile container.

A sample form (attachment B) was matched for each catheter tip. This form (table 12.A), maintaining anonymity, included information about the patient, namely the bed code, the date and reason for admission, age and sex, the type of venous access, data of insertion and type of dressing.

Table 11.A - Sample Form, Patient Information

<table>
<tr><th colspan="3">Patient</th></tr>
<tr><td colspan="2">Bed Code</td><td rowspan="2">Admission Motive
o Cardiological
o Endocrine
o Fever
o Gastrointestinal
o Haematological
o Hepatological
o Infectious
o Neurological
o Orthopaedic
o Pneumological
o Psychiatric
o Respiratory
o Shock
o Surgical
o Urological
o Trauma
o Other:_____</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td colspan="2">Admission Date</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td>Age</td><td>Sex</td></tr>
<tr><td></td><td>o Female
o Male</td></tr>
<tr><td>Venous Access</td><td>Insertion Date</td></tr>
<tr><td>o Artherial Line
o CVC
o PVC</td><td></td></tr>
<tr><td colspan="2">Dressing</td></tr>
<tr><td colspan="2"></td></tr>
</table>

Information about the sample was also indicated (table12.B), such as the disinfectant, the anatomical insertion site, the date and reason for removal and other relevant information, such as medication and body temperature.

Table 12.B - Sample Form, Sample Information

Sample			
Disinfectant	Removal Date	Tip Code	Result
Anatomical Site	Removal Motive		

Later in the laboratory, a code was assigned to each catheter tip and the result obtained through microbiological analysis was indicated.

Figure 15 corresponds to the patients studied, 7 women and 10 men, of which one man had three catheters and five men had two catheters, forming a total of 17 patients and 24 catheters. In the women's group, one was in her 40s, another in her 60s, three in their 70s and two in their 80s. Regarding men, one was in his 20s, another in his 30s, another in his 50s, two in their 60s, two in their 70s and three in their 80s.

Figure 15 - Patients Age and Sex

Regarding PVCs, in table 13 there is the list of the anatomical insertion sites and respective number of catheters. Two catheters were inserted in the right forearm, six in the right hand, one in the left fist, eight in the left forearm, of which one in the inside and another in the outside of the left forearm and seven in the left hand.

Table 13 - PVCs Anatomical Insertion Sites

Anatomical Insertion Site	N
Right Forearm	2
Right Hand	6
Left Fist	1
Left Forearm	6
Inside Left Forearm	1
Outside Left Forearm	1
Left Hand	7
Total Peripheral Vascular Catheters	**24**

The figure 16 is related to the dressing used to hold and protect the catheters (left side) and which antiseptic was used in the catheter removal procedure (right side). The IV-3000 dressing was used 20 of the catheters, Opsite dressing in 3 and the white hypoallergenic dressing in one of the catheters. For disinfection of the skin at catheter removal, Softasept was used in 11 of the cases, but in 13 of the cases the disinfectant was not mentioned.

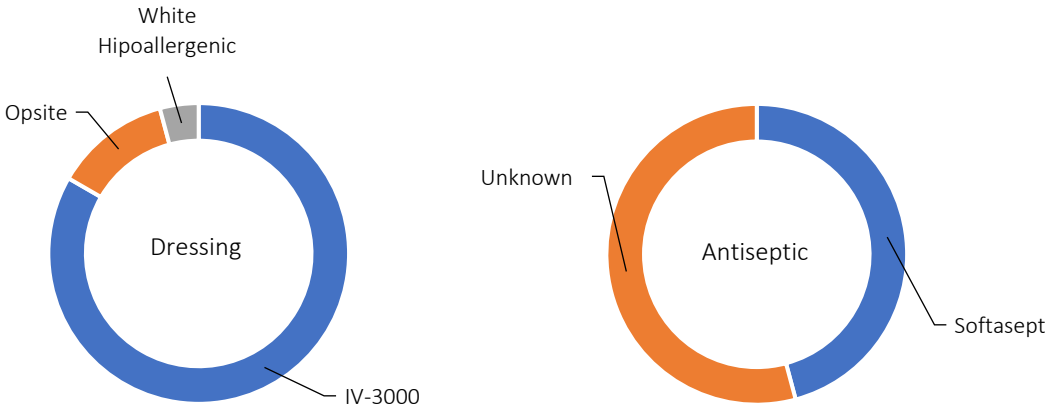


Figure 16 - Dressing and Antiseptic Used in the PCVs Care

The table 14 lists the reasons for catheter removal and the number of catheters that were removed for each reason. Fourteen catheters were removed according to the 4-days use protocol, four were removed due to rash in the insertion site, of which two of the patients also experienced pain and in one catheter there was infiltration, five of the catheters were removed because the patients were discharged and one of the catheters was removed due to medical indication.

Table 14 - List of PVC Removal Motive

Removal Motive	N
4 Day Protocol	14
Rash	1
Rash and Infiltration	1
Rash and Pain	2
Discharge	5
Medical Indication	1
Total Peripheral Venous Catheters	**24**

This study population is in accordance with several other studies on this subject, mostly men, over 65 years old, with PVCs inserted in the arms and hands [63, 64].

2.2. Sample Reception and Processing

After removing the catheter and filling out the sample form, they were sent to the laboratory for microbiological analysis. Figure 17 shows how the PVC samples arrived at the laboratory. To perform the microbiological analysis, approximately 2 cm of the catheter tip was cut with the aid of sterile blade (figure 18).

Figure 17 - PVC in a Sterile Sample Container

Figure 18 - Sterile Blade

Finally, with a loop, the tip was rolled on a PVX agar plate (figure 19), for about 30 seconds, after which the plate was incubated at 37° C for 24-48 hours.

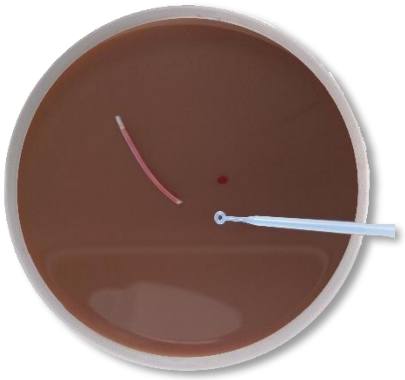


Figure 19 - PVC Tip Bearing Culture Technique

Of the 24 PVC tips sent to the laboratory, only 18 were submited to microbiological analysis, since 6 of the tips were sent more than 24 hours after catheter removal.

Depending on the bacterial growth, after 24 to 48 hours semi-quantitative colony counts were performed, according to the Maki technique [65]. Cultures in which no bacterial growth was found were considered negative and discarded. Cultures with bacterial growth below 15 CFU, as the one shown in figure 20, were also considered negative. Cultures with bacterial growth above 15 CFU were considered positive and macroscopically different colonies were isolated on new PVX plates and incubated at 37° C.

Figure 20 - Negative <15 CFU PVC Tip Bearing Culture

As shown in figure 21 , after 24 to 48 hours of incubation at 37° C, 8 negative and 5 negative <15 CFU were verified, of which 3 followed for identification, together with 11 positive cultures >15 CFU.

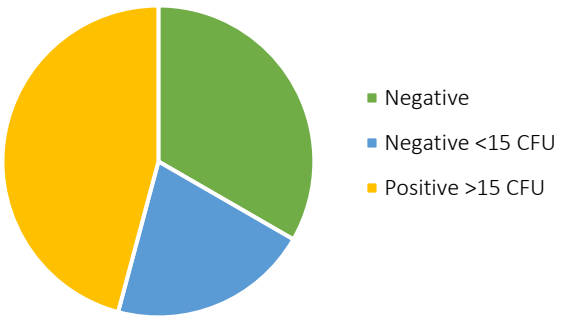


Figure 21 - Catheter Tip Bearing Culture Results

The majority of the PVC tips cultures had a negative result, being in accordance with another study that also took place in a Cardiology ward, with about 11% of infectious complications associated with PVC use [66].

2.4. Bacterial Identification

After 24 hours, the colonies were identified using MALDI-TOF MS. In the impossibility of identifying bacterial colonies within 48 hours after isolation, each colony can alternatively be picked with a loop into a cryopreservation medium tube (figure 22) and stored at -80° C, until identification.

Figure 22 - Cryopreservation Tubes

Of the eighteen cultures, fourteen *Staphylococcus* spp. bacteria were identified. As shown in figure 23, one *S. aureus*, one *S. capitis*, one *S. caprae*, five *S. epidermidis*, two *S. haemolyticus* and four *S. hominis* were identified. In five cultures more than one bacterium was present, one of the cultures had colonies of *S. hominis*, *S. epidermidis* and *S. haemolyticus*, in another culture colonies of *S.*

haemolyticus and *S. epidermidis* were isolated, in two cultures *S. hominis* and *S. epidermidis* were isolated and in one of the negative <15 CFU cultures, *S. capitis* and *S. caprae* were isolated.

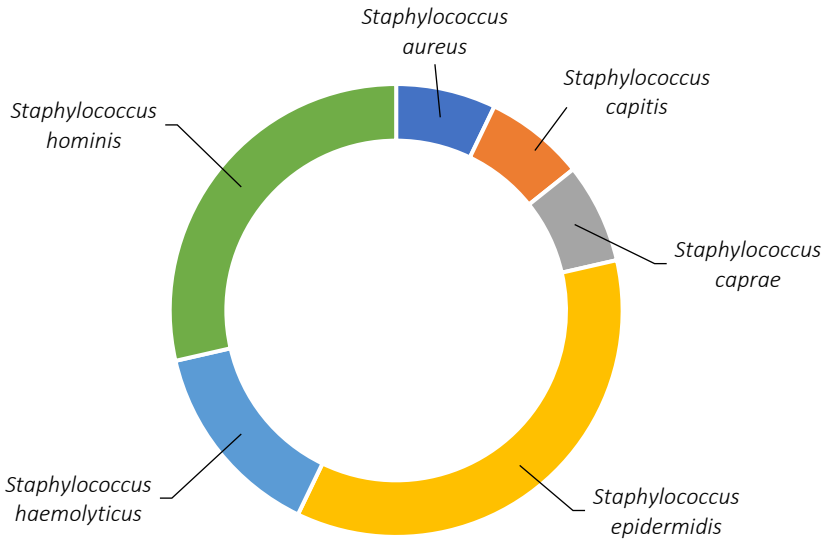


Figure 23 - Isolated Microorganisms

These results are in line with many studies on PVC-associated BSIs, where the most common microorganisms responsible for this type of infection are Gram-positive cocci, namely *S. epidermidis* [63, 64].

Figure 24 shows two positive >15 CFU cultures, *S. epidermidis* after 48 hours of incubation in figure 24.A and *S. aureus* after 24 hours of incubation in figure 24.B. I t should be noted that colonies with different sizes can be observed in figure 24.A, however, after identification by MALDI-TOF MS, it was found that they are the same species, which can be explained by the fact that they presented different growth phases.

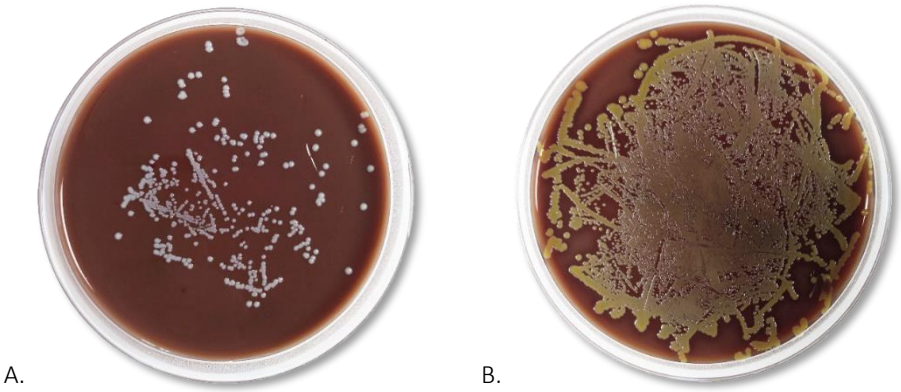


Figure 24 - Positive >15 CFU PVC Tip Bearing Cultures

As mentioned before *S. epidermidis* is one of the most commonly isolated microorganism when it comes to PVC-associated BSIs. *S. aureus* also have been described as one of the most common microorganism responsible for CRBSIs. In fact, a study demonstrated that S. aureus was about 2.5 times more common for PVC-associated bacteraemia than for CVC-associated bacteraemia [67].

Figure 25.A shows a positive >15 CFU PVC tip bearing culture with 24 hour of incubation, where two macroscopically different bacterial species can be observed. From this culture, one of the largest colonies was isolated, having been identified as *S. hominis* (figure 25.A1) and one of the smallest colonies, having been identified as *S. epidermidis* (figure 25.A2) after 24 hours of incubation.

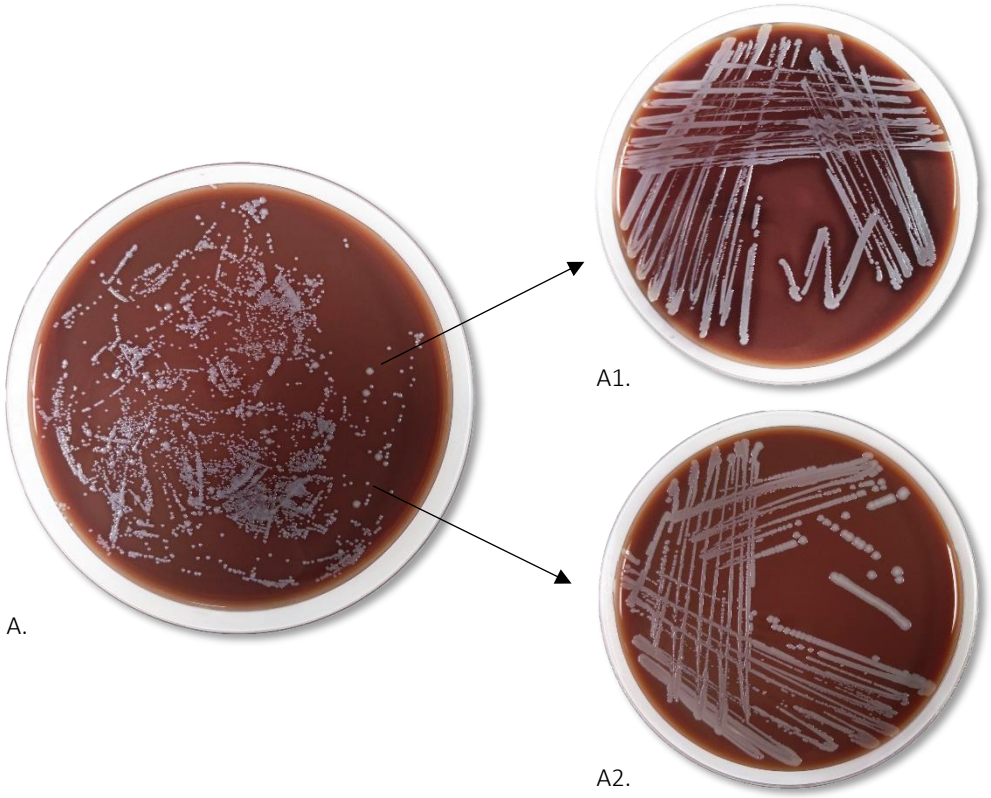


Figure 25 - Positive >15 CFU Cultures

Figure 26.A is a positive >15 CFU PCV tip bearing culture where three macroscopically different colonies are observed. One of the yellow colonies, despite not having a CFU count over fifteen, was isolated for suspected of being *S. aureus*, however after 24 hours of incubation it was identified by MALDI-TOF MS as *S. hominis* (figure 26.A1).

One of the largest white colonies (figure 26.A2) was identified as *S. haemolyticus* and one of the smallest colonies (figure 26.A3) was identified as *S. epidermidis* after 24 hours of incubation.

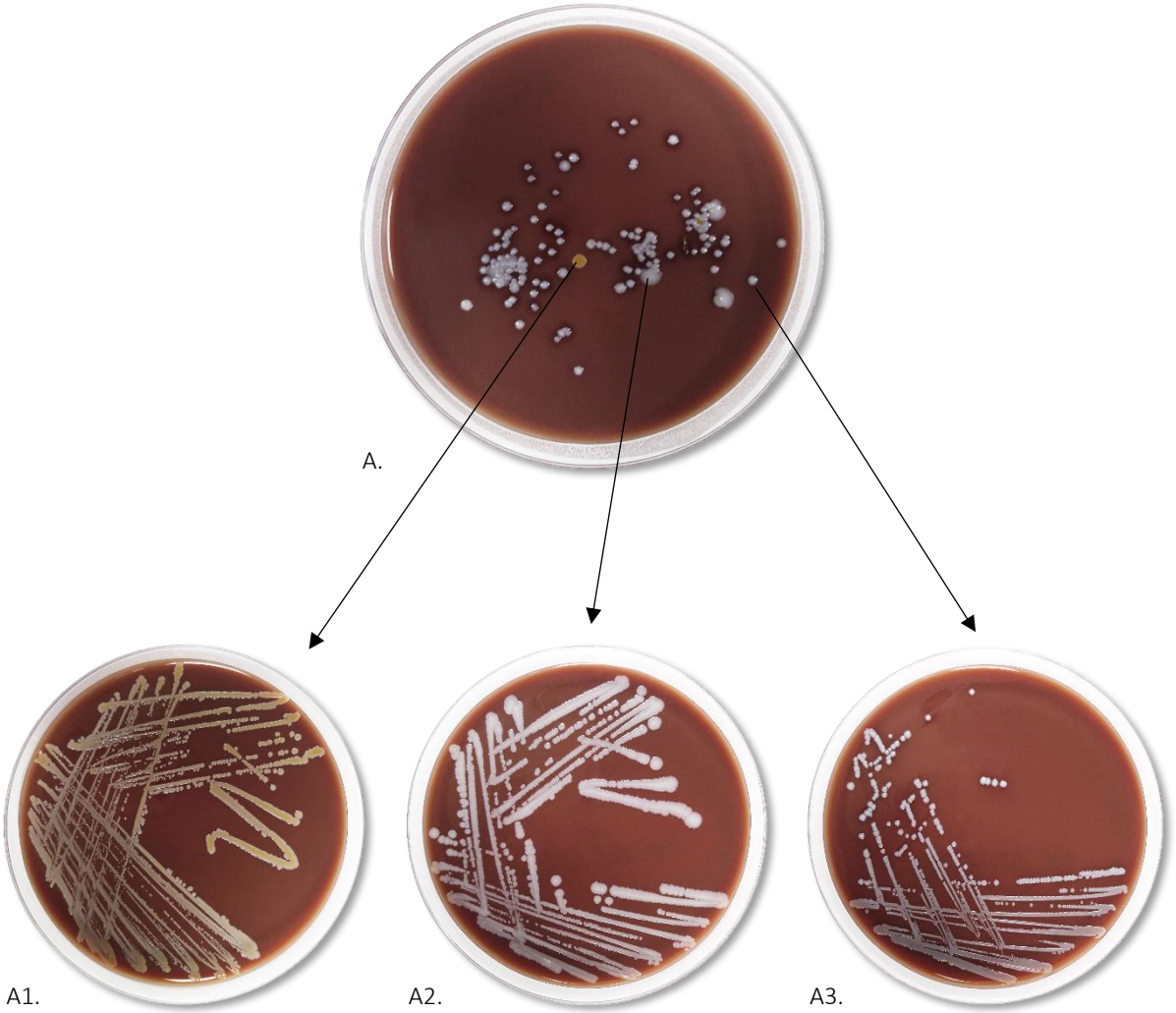


Figure 26 - Positive >15 CFU Cultures

2.5. Antimicrobial Susceptibility Testing: Disk Diffusion Method

For the antimicrobial susceptibility test (AST) of the identified bacterial species, the disk diffusion method was performed. For that, each colony from the pure and fresh cultures were touched with a swab and dipped in a NaCl 0,9% matrix. After obtaining a suspension with a minimum density of 0.5 McFarland (figure 27), inoculation was done in Muller Hinton (MHE) agar plates, as shown in figure 28.

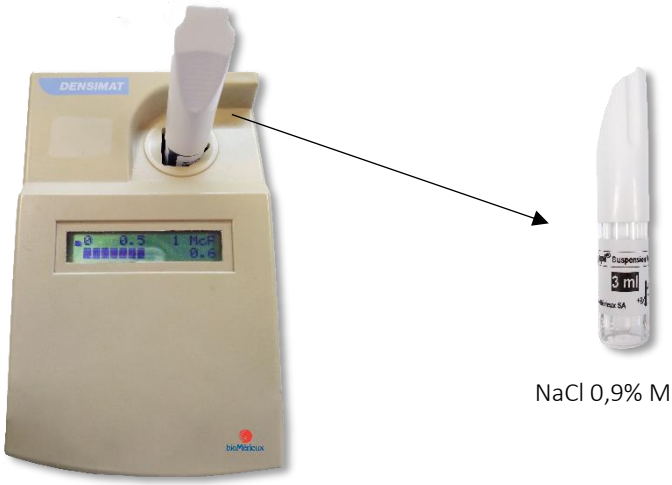


Figure 27 – Densitometer

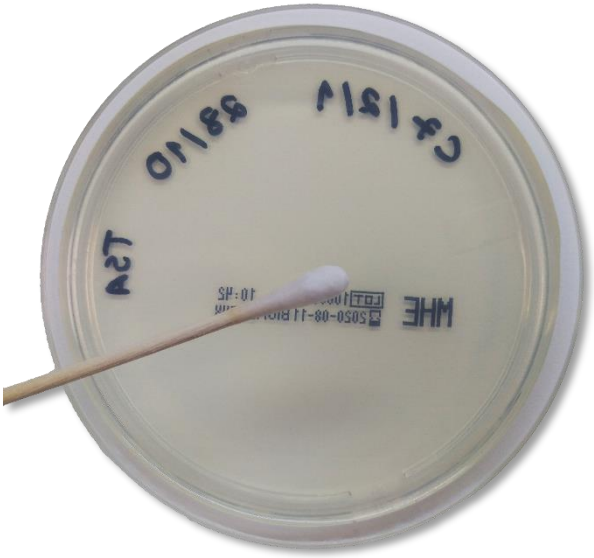


Figure 28 – Muller Hinton Agar Plate

Then, with the help of tweezers, an amoxicillin + clavulanic acid (AMC) and a cefoxitin (FOX) disk (figure 29) were placed on the medium surface and the plates were incubated a 37° C. After 24 hours, inhibition halos were measured (mm) and the species were phenotypically classified as sensitive or resistant.

Figure 29 - Amoxicillin + Clavulanic Acid (on the left) and Cefoxitin (on the right) Disks

These were the antibiotics chosen for AST, since AMC is one of the most widely used antibiotics clinically and the worrying increase in AMC resistance has been reported. Due to the prevalence of *S. epidermidis* and the existence of *S. aureus*, it was felt the need to evaluate the resistance of the species to Methicillin, but as this substance is very unstable *in vitro*, it is customary to infer about Methicillin resistance using Cefoxitin in the disk diffusion method [68, 69, 70, 71].

According to the Breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2020, if the growth inhibition halo is greater than 25 mm in diameter, the bacterium is classified as sensitive to AMC and FOX, but if the growth inhibition halo has less than 25 mm in diameter, the bacterium is categorised as resistant to both AMC and FOX.

In this study, a total of 10 *Staphylococcus* species were subjected to the AMC susceptibility test, with the result of 5 resistant and 5 AMC sensitive species, as shown in figure 30. For the FOX susceptibility test, of 11 *Staphylococcus* species, 5 showed sensitivity and 6, of which *S. aureus*, were considered resistant, as illustrated in figure 31.

Figure 310 - AMC Susceptibility Testing Results

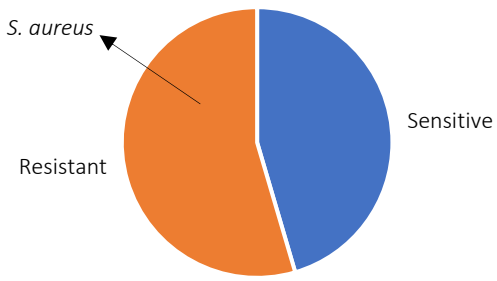


Figure 301 - FOX Susceptibility Testing Results

The following are some examples of AMC and FOX susceptibility tests.

Figure 32 shows an MHE agar plate with a pure culture of *S. epidermidis*, an AMC disk and another FOX disk. After 24 hours of incubation, there was observed bacterial growth inhibition around the AMC disk, with a diameter of 15 mm and the absence of bacterial growth inhibition around the FOX disk. Thus, according to the EUCAST guidelines, this strain of *S. epidermidis* was considered resistant to FOX, therefore MRSE.

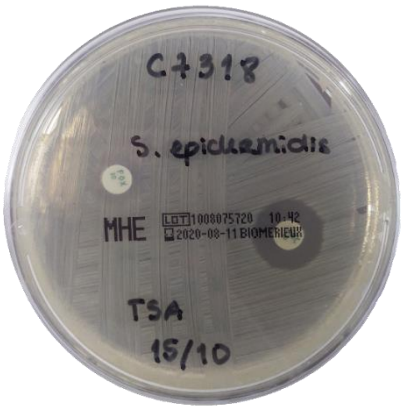


Figure 32 – MHE Agar Plate inoculated with *S. epidermidis* and an AMC Disk and a FOX Disk

Figure 33 shows an MHE agar plate inoculated with a pure culture of *S. aureus* and a FOX disk. After 24 hours of incubation, a 17 mm growth inhibition halo was observed around the disk. Thus, according to the EUCAST guidelines, this strains of *S. aureus* was considered resistant to Fox and therefore MRSA.

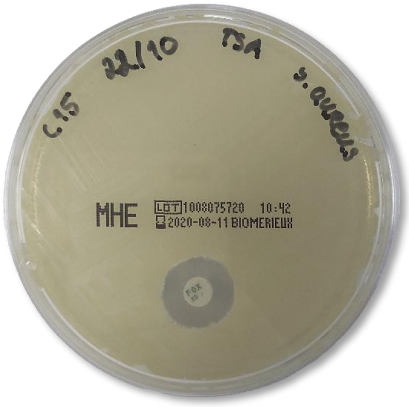


Figure 33 - MHE Agar Plate Inoculated with *S. aureus* and a Fox Disk

Despite the only *S. aureus* isolate of this study being MRSA, without complementary information about the blood culture results, it is impossible to infer if this was a case of PVC-associated BSI.

However, it should be taken into account that studies have shown that the majority of S. aureus that caused PVC-associated BSIs were MSSA [64, 72].

Figure 34 shows a MHE agar plate inoculated with a pure culture of *S. hominis*, an AMC disk and a FOX disk. After 24 hours of incubation, a 36 mm bacterial growth inhibition halo was observed around the AMC disk and another 35 mm halo around the FOX disk. This *S. hominis* strain was then considered sensitive to both AMC and FOX, according to the EUCAST guidelines.

Figure 34 - MHE Agar Plate Inoculated with *S. hominis*, an AMC disk and a FOX disk

Figure 35 shows a MHE agar plate inoculated with a fresh *S. haemolyticus* culture, an AMC disk and a FOX disk. After 24 hours of incubation, a 40 mm bacterial growth inhibition halo was measured around the AMC disk and another 34 mm halo around the FOX disk. This strain of *S. haemolyticus* was considered sensitive to both AMC and FOX, according to the EUCAST guidelines.

Figure 35 - MHE Agar Plate inoculated with *S. haemolyticus*, an AMC Disk and a FOX disk

Figure 36 shows a MHE agar plate inoculated with a pure culture of *S. epidermidis*, an AMC disk and a FOX disk. After 24 hours of incubation, a 28 mm bacterial growth inhibition halo was measured around the AMC disk and another 32 mm halo around the FOX disk. This strain of *S. epidermidis* was then considered sensitive to both AMC and FOX, according to the EUCAST guidelines and therefore a MSSE strain.

Figure 36 - MHE Agar Plate inoculated with *S. epidermidis*, an AMC Disk and a FOX Disk

6. Interpretation

These results are only part of an initial phase of a study with high potential. Due to the small number of samples, it is difficult to compare results with other studies that are much more in-depth.

In this sense, with a considerable number of samples for statistical analysis, the next steps would be as follows. Cross check the results of PVC tip cultures with the respective blood cultures and skin swabs at the anatomical insertion site. This way, it would be possible to perceive the effectiveness of the disinfection procedure of the anatomical insertion site, in the various stages of PVC usage, insertion, dressing change and removal. It would also be possible to see if, in BSI associated with PVC usage cases, the microorganism causing the infection would be the same clone as the one that would be present on the anatomic insertion site skin.

Chapter IV – Scientific Communication

1. Context

The evolution of Science depends on the interactions within the scientific community, whether through the creation of scientific ideas, the testing of those ideas or the communication of results obtained in scientific journals, conferences, etc. But are there magazines and conferences sufficient for health professionals to have sufficiently updated information in their daily working lives?

Science communication is a process of communication between peers, not just a result, but an interactive process in which knowledge is communicated, used and developed in a community. In communication, the exchange of information is made possible, promoting the exchange of ideas between individuals. In the specific case of scientific communication, the production, sharing and application of information are part of this process, from the moment the researcher has an idea, until he/she shares his/her results.

Vascular access medical devices are used, among many medical applications, to facilitate the administrations of drugs, such as chemotherapy, long-terms intravenous antimicrobial therapy, and frequent blood draws. However, the risk of developing medical complications is high, such as bloodstream infections related to the use of a catheter, a common medical device, which increases morbidity, length of stay, hospital costs and mortality.

Thus, as a risk factor for infections, catheters should be closely monitored for their development. A common practice for detecting infections associated with catheter use is cultural examination of the catheter tip.

Bearing in mind the growing concern about the antibiotic resistance, this study was carried out with the objective of inferring about the epidemiology of bacteria and fungi associated with catheter use at the CHBV, assessing antibiotic resistance of the most prevalent species, in vitro, and develop teaching materials to raise awareness to the subject. This was carried out in the sections of Microbiology and Molecular Biology of the Laboratory of Clinical Pathology of the CHBV, where data related to cases in which a catheter was inserted during the year of 2019 were analysed. It was possible to verify that the overwhelming majority of the results were negative, that is, no microorganisms were present in the catheter tip. On the other hand, of the few positive results, most of the microorganisms found were skin contaminants.

One of the conclusions that can be drawn with these results is related to the fact that an unnecessary amount of catheter tips is being sent for analysis. If it was previously considered good

medical practice to send all catheter tips for analysis, today it may only be necessary to send the catheter tips for cases where an infections is suspected. This change in medical practice can translate into a reduction in costs associated with this analysis, as well as in the profitability of professionals working time.

The communication of these results is of great importance, since the medical community, having a very high degree of training, requires constant renewal of information regarding the reality observed in the hospital laboratory. Thus, a flyer (attachment C) was prepared in which, in a simple way, the medical community of this hospital is informed about the outdated methodology they have been practicing.

2. Flyer Content

With the title "Prevalence of Species Associated with the use of Central Vascular Catheters at Hospital Infante D. Pedro", the front page of the Flyer has a short introduction to the theme, referring to the objective of the study. The study population, the percentages for each CVC anatomical insertion site, the main reason for insertion and which are the main medical services where the insertion was performed are presented.

The back page shows the percentages of the catheter tip culture results, which microorganisms were found and the respective percentages and which the CVC-associated infection and catheter tip contamination rates are.

Also, on the back page of the Flyer, there is a QR code whose scan leads to the Association for World Innovation in Science and Health Education (AWISHE) website where, in addition to all the information that gave rise to this Flyer, there is a discussion chat to the topic.

3. Involvement

It is very importance that researchers communicate the results of their studies in the form of an article to the scientific community. These are submitted to various stages of evaluation and acceptance, so that the information is published with the vest quality and highlighted in the best scientific journals. But these magazines are often not available to the general public.

It was in this concept that we sought to not only distribute the Flyer to the medical community of this hospital, but also to extend this information to the general population, through the AWISHE website, a free access website.

Moreover, the design of this Flyer was published as a Poster (attachment D) at the Online Congress SciComPt2020. SciComPt is the Portugal's network of Science and Technology Communication professionals, whose main objectives are to: promote Science Communication in all its aspects; promote exchanges between Science Communication professionals in Portugal and between them and their international counterparts; promote informed citizen participation in all matters involving Science, Technology and Innovation; act as a representative and spokesperson for its associates; promote the training of Science Communication professionals; support the investigation of Science Communication [73].

Chapter V – Conclusions

The results obtained in this study show that, although the majority of microorganisms found in the CVC and PVC tips are part of the human skin microbiota, the threat of resistance to antimicrobials does not allow the risk of ignoring them as a potential pathogenic source of BSIs.

This is especially important, for example, in cases where a very common microorganism, found on our skin has been developing resistance to antimicrobials, such as MRSE.

This study has the most diverse limitations, they are as follows. The number of samples is reduced, making statistical data processing impossible. Although an initial study was carried out on the theme CVCs, covering all hospital wards, when the laboratory analysis of PVCs was conducted, it was only possible to study the Cardiology ward. And yet, this laboratory analysis was very superficial.

In a next phase, it would be interesting to evaluate the consequence of the presence of microorganisms in the catheter tips, even after their removal.

In subchapter II, only the test of the peripheral venous catheter bacterial colonization surveillance designed protocol was carried out, in the next phase, the implementation of same protocol should be proceeded, evaluating its impact.

In relation to science communication, it will be of great value, to assess the impact of the distribution of the Flyer. In the medical community of this hospital, would be important to assess what was their feedback and what would they change in their medical practice after the knowledge acquired with this communication tools.

Chapter VI – Bibliographic References

1. Lutwick L, Al-maani AS, Mehtar S, et al. Managing and preventing vascular catheter infections : A position paper of the international society for infectious diseases. *Int J Infect Dis*. 2019;84:22-29. doi:10.1016/j.ijid.2019.04.014
2. Bianco G, Boattini M, Iannaccone M, Sidoti F, Cavallo R, Costa C. Detection of antibiotic resistance genes from blood cultures : performance assessment and potential impact on antibiotic therapy management. *J Hosp Infect*. 2019;102(4):465-469. doi:10.1016/j.jhin.2019.03.007
3. Rosenthal VD, Al-abdely HM, El-kholy AA, et al. International Nosocomial Infection Control Consortium report , data summary of 50 countries for 2010-2015 : Device-associated module. *Am J Infect Control*. 2016;44:1495-1504. doi:10.1016/j.ajic.2016.08.007
4. Li X, Yuan X, Wang C. The clinical value of IL-3, IL-4, IL-12p70, IL17A, IFN-ɣ, MIP-1β, NLR, P-selectin, and TNF-α in differentiating bloodstream infections caused by gram-negative, gram-positive bacteria and fungi in hospitalized patients. *Medicine (Baltimore)*. 2019;98(38).
5. Worth LJ, Spelman T, Bull AL, Brett JA, Richards MJ. Central line-associated bloodstream infections in Australian intensive care units: Time-trends in infection rates, etiology, and antimicrobial resistance using a comprehensive Victorian surveillance program, 2009-2013. *Am J Infect Control*. 2015;43(8):848-852. doi:10.1016/j.ajic.2015.03.036
6. Black JG, Black LJ. *Microbiology: Principles and Explorations*. 10th ed. John Wiley & Sons; 2018.
7. Pommerville JC. *Fundamentals of Microbiology*. 10th ed. Jones & Bartlett Learning; 2014.
8. Rojas ER, Billings G, Odermatt PD, et al. The outer membrane is an essential load-bearing element in Gram-negative bacteria. *Nature*. 2018.
9. Fernández-Rojas B, Vázquez-Cervantes GI, Pedraza-Chaverri J, Gutiérrez-Venegas G. Lipoteichoic acid reduces antioxidant enzymes in H9c2 cells. *Toxicol Reports*. 2020;7(December 2019):101-108. doi:10.1016/j.toxrep.2019.12.007
10. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pac J Trop Biomed*. 2017;7(5):478-482. doi:10.1016/j.apjtb.2017.01.019
11. Hall MD, Routtu J, Ebert D. Dissecting the genetic architecture of a stepwise infection process. *Mol Ecol*. 2019:3942-3957. doi:10.1111/mec.15166
12. Coleman M, Elkins C, Gutting B, Mongodin E, Solano-aguilar G, Walls I. Microbiota and Dose Response : Evolving Paradigm of Health Triangle. *Risk Anal*. 2018;38(10). doi:10.1111/risa.13121
13. Lamy B, Sundqvist M, Idelevich EA. Bloodstream infections – Standard and progress in pathogen diagnostics. *Clin Microbiol Infect*. 2020;26(2):142-150. doi:10.1016/j.cmi.2019.11.017

14. Gjini E, Brito PH. Integrating Antimicrobial Therapy with Host Immunity to Fight Drug-Resistant Infections : Classical vs . Adaptive Treatment. *PLoS Comput Biol*. 2016;12(4):1-35. doi:10.1371/journal.pcbi.1004857
15. Lax S, Gilbert JA. Hospital-associated microbiota and implications for nosocomial infections. *Trends Mol Med*. 2015;21(7):427-432. doi:10.1016/j.molmed.2015.03.005
16. El mekes A, Zahlane K, Ait said L, Ouafi AT, Barakate M. The clinical and epidemiological risk factors of infections due to multi-drug resistant bacteria in an adult intensive care unit of University Hospital Center in Marrakesh-Morocco. *J Infect Public Health*. 2019. doi:10.1016/j.jiph.2019.08.012
17. Nasr P. Genetics, epidemiology, and clinical manifestations of multidrug-resistant Acinetobacter baumannii. *J Hosp Infect*. 2019;104:4-11. doi:10.1016/j.jhin.2019.09.021
18. Bonilla-gameros L, Chevallier P, Sarkissian A, Mantovani D. Silver-based antibacterial strategies for healthcare-associated infections: Processes, challenges, and regulations. An integrated review. *Nanomedicine Nanotechnology, Biol Med*. 2019;xx(xxxx):0-13. doi:10.1016/j.nano.2019.102142
19. Zhang L, Keogh S, Rickard CM. Reducing the risk of infection associated with vascular access devices through nanotechnology: A perspective. *Int J Nanomedicine*. 2013;8:4453-4466. doi:10.2147/IJN.S50312
20. Beitelshees M, Hill A, Jones CH, Pfeifer BA. Phenotypic variation during biofilm formation: Implications for anti-biofilm therapeutic design. *Materials (Basel)*. 2018;11(7):1-18. doi:10.3390/ma11071086
21. Patient Safety. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/patient-safety. Published 2019. Accessed October 30, 2019.
22. Denstaedt SJ, Singer BH, Standiford TJ. Sepsis and nosocomial infection: Patient characteristics, mechanisms, and modulation. *Front Immunol*. 2018;9(2446). doi:10.3389/fimmu.2018.02446
23. Liu X, Zou N, Zhu D, Wang D. Influencing factors analysis and modeling of hospital-acquired infection in elderly patients. *J Comb Optim*. 2019;37(1):248-270. doi:10.1007/s10878-017-0222-1
24. Wong SW, Gantner D, McGloughlin S, et al. The influence of intensive care unit-acquired central line-associated bloodstream infection on in-hospital mortality: A single-center risk-adjusted analysis. *Am J Infect Control*. 2016;44(5):587-592. doi:10.1016/j.ajic.2015.12.008
25. Shao S, Cong H, Wang M-Y, Liu P. The diagnostic roles of neutrophil in bloodstream infections. *Immunobiology*. 2019;(April). doi:10.1016/j.imbio.2019.10.007
26. Chen C, Wang Y, Kuo S, Shih F, Chen T, How C. Community-acquired bloodstream infections

caused by Acinetobacter baumannii : A matched case e control study. *J Microbiol Immunol Infect*. 2018;51(325):629-635. doi:10.1016/j.jmii.2017.02.004

27. Shefali G, Shrikara P. M, Ashok B, Shrikala B. Microbiology of Non-Tunnelled Catheter-Related Infections. *J Clin Diagnostic Res*. 2016;10(2016 Jul):24-29. doi:10.7860/JCDR/2016/19058.8155
28. Dasgupta S, Das S, Chawan NS, Hazra A. Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. *Indian J Crit Care Med*. 2015;19(1):14-20. doi:10.4103/0972-5229.148633
29. Matsui Y, Shimatani M, Kuzuhara K, et al. Three-year prospective, observational study of central line-associated bloodstream infections in a 600-bed Japanese acute care hospital. *Am J Infect Control*. 2015;43(5):494-498. doi:10.1016/j.ajic.2015.01.011
30. Lee YM, Kim DY, Kim YJ, Park KH, Lee MS. Clinical impacts of delayed central venous catheter removal according to the severity of comorbidities in patients with candidaemia. *J Hosp Infect*. 2019;103:420-427. doi:10.1016/j.jhin.2019.08.018
31. Zago CE, Silva S, Sanitá PV, et al. Dynamics of Biofilm Formation and the Interaction between Candida albicans and Methicillin-Susceptible (MSSA) and -Resistant Staphylococcus aureus (MRSA). *PLoS One*. 2015:1-16. doi:10.1371/journal.pone.0123206
32. Lencastre D, Aires-de-sousa M. Carriage of Staphylococcus aureus among Portuguese nursing students : A longitudinal cohort study over four years of education. *PLoS One*. 2017:1-10.
33. Tavares A, Miragaia M, Rolo J, Coelho C, Lencastre H De. High prevalence of hospital-associated methicillin-resistant Staphylococcus aureus in the community in Portugal : evidence for the blurring of community – hospital boundaries. *Eur J Clin Microbiol Infect Dis*. 2013:1269-1283. doi:10.1007/s10096-013-1872-2
34. Dia Europeu dos Antibióticos. Serviço Nacional de Saúde. https://www.sns.gov.pt/noticias/2016/11/18/dia-europeu-dos-antibioticos/. Published 2017. Accessed November 9, 2019.
35. Antibiotic Resistance. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Published 2019. Accessed October 24, 2019.
36. Leal HF, Azevedo J, Evelyn G, et al. Bloodstream infections caused by multidrug-resistant gram-negative bacteria : epidemiological , clinical and microbiological features. *BMC Infect Dis*. 2019;19(609):1-12.
37. Suner A, Oruc OE, Buke C, Ozkaya HD, Kitapcioglu G. Evaluation of infectious diseases and clinical microbiology specialists' preferences for hand hygiene: Analysis using the multi-attribute utility theory and the analytic hierarchy process methods. *BMC Med Inform Decis Mak*. 2017;17(129):1-11. doi:10.1186/s12911-017-0528-z

38. Health-care Waste. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/health-care-waste. Published 2019. Accessed October 30, 2019.
39. Rn SJK, Rn EA, Rubin ZA, Words K. A novel infection prevention approach : Leveraging a mandatory electronic communication tool to decrease peripherally inserted central catheter infections , complications , and cost. *AJIC Am J Infect Control*. 2016;44(11):1335-1345. doi:10.1016/j.ajic.2016.03.023
40. Institutional Goals. International Nosocomial Infection Control Consortium. http://www.inicc.org/. Published 2013. Accessed January 23, 2020.
41. Healthcare-Associated Infections - Community Interface (HAIC). Centers for Disease Control and Prevention. https://www.cdc.gov/hai/eip/. Published 2016. Accessed January 23, 2020.
42. Epidemiologia. Instituto Nacional de Saúde Doutor Ricardo Jorge. http://www.insa.min-saude.pt/category/areas-de-atuacao/epidemiologia/. Published 2017. Accessed January 7, 2020.
43. Direção Geral da Saúde. *Vigilância Epidemiológica Das Resistências Aos Antimicrobianos*. Portugal; 2013:1-9.
44. Vigilância Epidemiológica das Resistências aos Antimicrobianos. Instituto Nacional da Saúde Doutor Ricardo Jorge. http://www.insa.min-saude.pt/category/areas-de-atuacao/doencas-infeciosas/vigilancia-epidemiologica-das-resistencias-aos-antimicrobianos/. Published 2017.
45. Neoh KG, Li M, Kang E-T, Ghiong E, Tambyah PA. Surface modification strategies for combating catheter-related complications : recent advances and challenges. *J Mater Chem B*. 2017;5:2045-2067. doi:10.1039/C6TB03280J
46. Brunelli SM, Turenne W, Sibbel S, Hunt A, Pfaffle A. Clinical and economic burden of bloodstream infections in critical care patients with central venous catheters. *J Crit Care*. 2016;35:69-74. doi:10.1016/j.jcrc.2016.04.035
47. Direção Geral da Saúde. *"Feixe de Intervenções" de Prevenção de Infeção Relacionada Com Cateter Venoso Central*. Portugal; 2015:1-17.
48. Aminzadeh Z, Simpson P, Athan E. Central venous catheter associated blood stream infections (CVC-BSIs) in the non-intensive care settings: Epidemiology, microbiology and outcomes. *Infect Dis Heal*. 2019;24(4):222-228. doi:10.1016/j.idh.2019.07.003
49. Baier C, Linke L, Eder M, et al. Incidence, risk factors and healthcare costs of central line-associated nosocomial bloodstream infections in hematologic and oncologic patients. *PLoS One*. 2020;15(1):1-12. doi:10.1371/journal.pone.0227772
50. Youn SH, Lee JCJ, Kim Y, Moon J, Choi Y, Jung K. Central Venous Catheter-Related Infection in Severe Trauma Patients. *World J Surg*. 2015;39(10):2400-2406. doi:10.1007/s00268-015-3137-

y

51. Heidenreich D, Hansen E, Kreil S, et al. Influence of the Insertion Site on Central Venous Catheter-Related Complications in Patients Undergoing Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant*. 2020;26(6):1189-1194. doi:10.1016/j.bbmt.2020.02.007
52. Tirumandas M, Gendlina I, Figueredo J, et al. Analysis of catheter utilization, central line associated bloodstream infections, and costs associated with an inpatient critical care-driven vascular access model. *Am J Infect Control*. 2020;000:6-9. doi:10.1016/j.ajic.2020.10.006
53. Carre Y, Moal B, Germain C, et al. Randomized study of antiseptic application technique in healthy volunteers before vascular access insertion (TApAS trial). *J Infect*. 2020;81(4):532-539. doi:10.1016/j.jinf.2020.08.022
54. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018;16(3):143-155. doi:10.1038/nrmicro.2017.157
55. Bartlett JG. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. *Infect Dis Clin Pract*. 2004;12(6):376. doi:10.1097/01.idc.0000144912.27311.19
56. Pérez-Granda MJ, Guembe M, Cruces R, Barrio JM, Bouza E. Assessment of central venous catheter colonization using surveillance culture of withdrawn connectors and insertion site skin. *Crit Care*. 2016;20(1):1-7. doi:10.1186/s13054-016-1201-0
57. Bell J, Goyal M, Long S, et al. Anatomic Site-Specific Complication Rates for Central Venous Catheter Insertions. *J Intensive Care Med*. 2020;35(9):869-874. doi:10.1177/0885066618795126
58. Gerver SM, Mihalkova M, Bion JF, et al. Surveillance of bloodstream infections in intensive care units in England, May 2016–April 2017: epidemiology and ecology. *J Hosp Infect*. 2020;106(1):1-9. doi:10.1016/j.jhin.2020.05.010
59. Sahutoglu S, Savran Y, Comert B. Risk factors for resistant gram negative infections in intensive care unit. *J Crit Intensive Care*. 2020;11(1):21-27. doi:10.37678/dcybd.2020.2302
60. Oliveira VS. Staphylococcus spp. present in peripheral intravenous catheters, their virulence factors and antibiotic resistance. 2019.
61. Choudhury MA, Sidjabat HE, Zowawi HM, et al. Skin colonization at peripheral intravenous catheter insertion sites increases the risk of catheter colonization and infection. *Am J Infect Control*. 2019;47(12):1484-1488. doi:10.1016/j.ajic.2019.06.002
62. Sato A, Nakamura I, Fujita H, et al. Peripheral venous catheter-related bloodstream infection is associated with severe complications and potential death: A retrospective observational study.

BMC Infect Dis. 2017;17(1):1-6. doi:10.1186/s12879-017-2536-0

63. Tsuboi M, Hayakawa K, Mezaki K, et al. Comparison of the epidemiology and microbiology of peripheral line– and central line–associated bloodstream infections. *Am J Infect Control*. 2019;47(2):208-210. doi:10.1016/j.ajic.2018.08.016
64. Guembe M, Pérez-Granda MJ, Capdevila JA, et al. Nationwide study on peripheral-venous-catheter-associated-bloodstream infections in internal medicine departments. *J Hosp Infect*. 2017;97(3):260-266. doi:10.1016/j.jhin.2017.07.008
65. Maki DG, Weise CE, Sarafin HW. A Semiquantitative Culture Method for Identifying Intravenous-Catheter-Related Infection. *N Engl J Med*. 1977;(296):1305-1309. doi:10.1056/NEJM197706092962301
66. Ghali H, Ben Rejeb O, Bouafia N, et al. Incidence and risk factors of peripheral venous catheter-related adverse events in cardiology department of a Tunisian university hospital: A prospective observational study. *Ann Cardiol Angeiol (Paris)*. 2019;68(4):207-214. doi:10.1016/j.ancard.2018.08.025
67. Ruiz-Giardin JM, Ochoa Chamorro I, Velázquez Riós L, et al. Blood stream infections associated with central and peripheral venous catheters. *BMC Infect Dis*. 2019;19(1):1-10. doi:10.1186/s12879-019-4505-2
68. Fusier I, Parent de Curzon O, Touratier S, et al. Amoxicillin–clavulanic acid prescriptions at the Greater Paris University Hospitals (AP–HP). *Med Mal Infect*. 2017;47(1):42-49. doi:10.1016/j.medmal.2016.09.003
69. Holmbom M, Möller V, Nilsson LE, et al. Low incidence of antibiotic-resistant bacteria in south-east Sweden: An epidemiologic study on 9268 cases of bloodstream infection. *PLoS One*. 2020;15(3):1-17. doi:10.1371/journal.pone.0230501
70. Pomorska-Wesołowska M, Chmielarczyk A, Chlebowicz M, et al. Virulence and antimicrobial resistance of Staphylococcus aureus isolated from bloodstream infections and pneumonia in Southern Poland. *J Glob Antimicrob Resist*. 2017;11:100-104. doi:10.1016/j.jgar.2017.07.009
71. Kot B, Wierzchowska K, Piechota M, Gruzewska A. Antimicrobial Resistance Patterns in Methicillin-Resistant Staphylococcus aureus from Patients Hospitalized during 2015-2017 in Hospitals in Poland. *Med Princ Pract*. 2020;29(1):61-68. doi:10.1159/000501788
72. Saliba P, Hornero A, Cuervo G, et al. Interventions to decrease short-term peripheral venous catheter-related bloodstream infections: impact on incidence and mortality. *J Hosp Infect*. 2018;100(3):e178-e186. doi:10.1016/j.jhin.2018.06.010
73. SciComPt - Regulamento Interno. http://scicom.pt/index.php/scicompt/regulamento-interno/. Published 2020. Accessed May 20, 2020.

Attachments

Attachment A - Peripheral Venous Catheter Bacterial Colonization Surveillance Protocol

1. Peripheral Venous Catheter Removal

This step must be done by trained nurses at PVC removal, following the already implemented catheter removal protocol

a. Place the PVC tip in a sterile container, duly identified;
b. Fill the sample form;
c. Send the sample and form to the laboratory immediately.

2. Sample Reception and Processing

a. Cut, approximately, 2 cm of the PVC tip distal end, with a sterile blade/scissors;
b. Roll the PVC tip on a PVX agar plate, in all directions, for about 30 seconds, with a loop;
c. Discard the PVC tip;
d. Incubate the PVX agar plate for 24 to 48 hours, at 37° C.

3. Bacterial Colony Count

a. Perform the semi-quantitative bacterial colony count, according to the Maki technique;
b. Discard the negative colony count result plates;
c. Isolate the positive colony count result on another PVX agar plate;
d. Incubate the PVX agar plate for 24 hours, at 37° C.

4. Bacteria Identification using MALDI-TOF MS

a. Prick the colony to identify with a sterile toothpick into one of the wells of the stainless steel 96 well plate;
b. Put 1 µL of the matrix solution ad let it dry;
c. Place the multi well plate in the MALDI-TOF MS apparatus and run the analysis.

5. Antimicrobial Susceptibility Testing

a. Prepare a 0.5 McFarland bacterial suspension;
b. Inoculate in a Muller-Hinton agar plate;
c. Place the chosen antibiotics in the medium surface, with a sterile tweezers;

d. Incubate the Muller-Hinton agar plate for 24 hours, at 37° C.

e. Measure the bacterial growth inhibition halos.

Attachment B – Peripheral Venous Catheter Sample Form

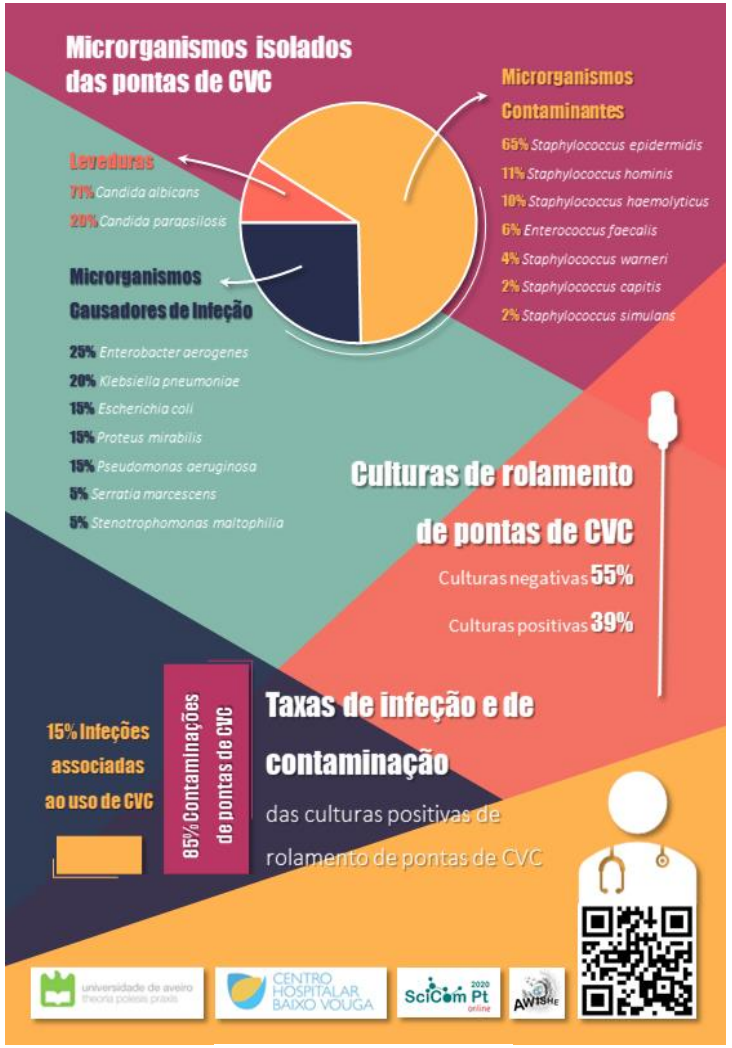
<table>
<tr><th colspan="3">Patient</th></tr>
<tr><td colspan="2">Bed Code</td><td>Admission Motive</td></tr>
<tr><td colspan="2"></td><td rowspan="11">o Cardiological
o Endocrine
o Fever
o Gastrointestinal
o Haematological
o Hepatological
o Infectious
o Neurological
o Orthopaedic
o Pneumological
o Psychiatric
o Respiratory
o Shock
o Surgical
o Urological
o Trauma
o Other:_____</td></tr>
<tr><td colspan="2">Admission Date</td></tr>
<tr><td colspan="2"></td></tr>
<tr><td>Age</td><td>Sex</td></tr>
<tr><td></td><td>o Female
o Male</td></tr>
<tr><td>Venous Access</td><td>Insertion Date</td></tr>
<tr><td>o Artherial Line
o CVC
o PVC</td><td></td></tr>
<tr><td colspan="2">Dressing</td></tr>
<tr><td colspan="2"></td></tr>
</table>

<table>
<tr><th colspan="4">Sample</th></tr>
<tr><td>Disinfectant</td><td>Removal Date</td><td>Tip Code</td><td>Result</td></tr>
<tr><td></td><td></td><td rowspan="3"></td><td rowspan="3"></td></tr>
<tr><td>Anatomical Site</td><td>Removal Motive</td></tr>
<tr><td></td><td></td></tr>
</table>

Observations

Attachment C – Flyer

Front Page



Back Page

Attachment D – Scientific Poster

COMUNICAÇÃO DE CIÊNCIA: INVESTIGADOR (IN)FORMA MÉDICO

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Prevalência de espécies associadas ao uso de dispositivos médicos de acesso vascular

Foi realizado um estudo com o objetivo de inferir acerca da epidemiologia das bactérias associadas ao uso de catéteres vasculares centrais no CHBV, através da análise de dados relativos às pontas de catéter enviadas para análise laboratorial, no ano de 2019.

Os catéteres vasculares centrais (CVCs) são catéteres intravasculares que terminam no ou perto do coração num dos grandes vasos. São utilizados regularmente com objetivo de auxílio no processo de tratamento, para efetuar perfusões, colheitas de sangue ou para monitorização hemodinâmica. Ocorrendo com frequência, infeções a eles associadas são indesejáveis.

Desafio e Grupo-alvo

A comunicação dos resultados deste estudo é importante, uma vez que a comunidade médica, mesmo com elevadíssimo grau de formação, requer constante renovação da informação relativa à realidade que se observa no laboratório hospitalar.

Sendo assim, este Flyer destina-se a toda a equipa médica do CHBV, dos serviços médicos responsáveis pela colocação de catéteres.

Conceito e Implementação

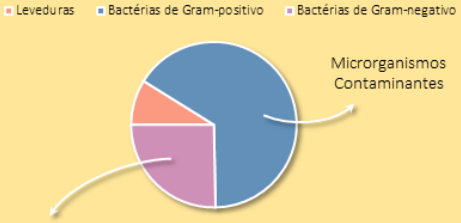
Foi elaborado um Flyer onde, de forma simples, se informa a comunidade médica deste hospital para a possível desatualizada prática médica. Este seria colocado em pontos estratégicos da instalação hospitalar, com o objetivo de chamar à atenção da comunidade médica.

Conteúdo do Flyer

Caracterização da amostra:

- População em estudo - maioritariamente homens, em média com 62 anos;
- Internados sobretudo nos serviços de Medicina Intensiva e Cirurgia;
- Principal motivo de colocação de catéter: instabilidade hemodinâmica.

Microrganismos isolados das pontas de catéter:



Microrganismos Causadores de Infeção:

- *Klebsiella pneumoniae*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*

Sustentabilidade

Sendo o Flyer um meio de comunicação relativamente pequeno, onde se torna impraticável colocar toda a informação obtida durante uma investigação, surgiu a ideia de expandir o acesso a essa informação.

Através do *scan* do código QR presente no Flyer, os interessados podem encontrar, na página da AWISHE, todos os resultados obtidos durante o estudo, bem como as conclusões retiradas e ainda a possibilidade de participação num fórum de discussão.