TURKISH REPUBLIC OF NORTH CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCE INSTITUTE

EVALUATION OF BLOOD CULTURE RESULTS BETWEEN 2016 and 2020 AT NEAR EAST UNIVERSITY HOSPITAL, MICROBIOLGY LABORATORY

LINA ALMASOODI

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

MASTER OF SCIENCE THESIS

SUPERVISOR

Prof. Dr. NEDİM ÇAKIR, MD

NICOSIA

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The Directorate of Health Sciences Institute,

This study has been accepted by the Thesis Committee in Medical and Clinical Microbiology Program as a Master of Science Thesis.

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STATEMENT (DECLARATION)

Hereby declare that the work in this thesis entitled **"EVALUATION OF BLOOD CULTURE RESULTS BETWEEN 2016-2020 AT NEAR EAST UNIVERSITY HOSPITAL, MICROBIOLGY LABORATORY"** is product of my own research efforts under the supervision of Prof. Dr. Nedim ÇAKIR. I had no unethical behaviour in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behaviour of breeching patent rights and copyright infringement during the study and writing of this thesis.

Name, Last Name: **Lina Almasoodi**

Signature:

Date:

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ABSTRACT

Lina Almasoodi: Evaluation of Blood Culture Results Between 2016 and 2020 at Near East University Hospital, Microbiolgy Laboratory. Near East University, Institute of Health Sciences, Medical Microbiology and Clinical Microbiology Program, M.Sc. Thesis, Nicosia, 2021.

Objective: Blood culture is the most reliable method for diagnosis in cases such as sepsis and bacteremia. It was aimed to identify microorganisms isolated from blood cultures sent to medical microbiology laboratory from various clinics between January 2016 and December 2020, and to determine their resistance profiles against antibiotics.

Method: Blood cultures were studied with BACTEC 9120 automation system. Bacterial colonies were obtained by using routine media upon blood culture signaling. Identification of microorganisms was done with the BD Phoenix Automated Microbiology Identification System and Vitex.

Results: It was determined that the most common Gr (-) bacteria was *E coli* (26.8%), while *Morganella morganii* and *Citrobacter spp*. were the bacteria that grew the least (0.3% and 0.5%, respectively). *P aeruginosa* and *A baumannii* was found 8.4% and 13.88% respectively. When the frequency of ESBL from Gr (-) bacteria in blood cultures was examined, 96 (33.4%) out of 287 cultures were found to be ESBL (+) while 191 (66.6%) were ESLB (-). A total of 939 Gr (+) bacteria were detected in blood culture samples, 83.9% of which were CoNS. *S. aures* and *E. feacalis* was found 8.4% and 4.6%. *S. aureus* resistant to methicillin was detected in 30.3%.

Conclusion: The type of bacteria isolated from blood cultures and their antibiotic susceptibility varies depending on different reasons. For this reason, determining the microorganisms and antibiotic susceptibility isolated from blood cultures at regular intervals in every hospital is both a guide to the clinician in empirical treatment and it is important in determining antibiotic usage policies.

Keywords: Bloodstream infections, blood culture, antibiotic, antimicrobial resistance

ÖZET

Lina Almasoodi: 2016 ve 2020 Yıllari Arasında Yakın Doğu Üniversitesi Hastanesi, Mikrobiyoloji Laborutuarında Kan Kültürü Sonuçlarının Değerlendirilmesi. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2021.

Amaç: Kan kültürü, sepsis ve bakteremi gibi durumlarda tanı için en güvenilir yöntemdir. Ocak 2016-Aralık 2020 tarihleri arasında çeşitli kliniklerden tıbbi mikrobiyoloji laboratuvarına gönderilen kan kültürlerinden izole edilen mikroorganizmaların belirlenmesi ve antibiyotiklere karşı direnç profillerinin belirlenmesi amaçlanmıştır.

Yöntem: Kan kültürleri, BACTEC 9120 otomasyon sistemi ile çalışılmıştır. Bakteriyel koloniler, kan kültürü sinyali üzerine rutin ortam kullanılarak elde edildi. Mikroorganizmaların tanımlanması, BD Phoenix Automated Microbiology Identification System ve Vitex ile yapılmıştır.

Bulgular: En sık görülen Gr (-) bakterinin E. coli (%26,8) olduğu belirlenirken, Morganella morganii ve Citrobacter spp. (Sırasıyla %0,3 ve %0,5) en az büyüyen bakteriler olduğu belirlendi P aeruginosa ve A baumannii sırasıyla %8,4 ve %13,88 bulundu. Kan kültürlerinde Gr (-) bakterilerden ESBL görülme sıklığı incelendiğinde 287 kültürden 96'sı (%33,4) ESBL (+), 191'i (%66,6) ESLB (-) bulundu. Kan kültürü örneklerinde %83,9'u CoNS olmak üzere toplam 939 Gr (+) bakteri tespit edildi. *S aures* ve E Feacalis %8,4 ve %4,6 olarak bulundu. Metisiline dirençli S aureus %30,3 oranında tespit edildi.

Sonuç: Kan kültürlerinden izole edilen bakterilerin türü ve antibiyotik duyarlılıkları farklı nedenlere bağlı olarak değişmektedir. Bu nedenle her hastanede düzenli aralıklarla kan kültürlerinden izole edilen mikroorganizma ve antibiyotik duyarlılığının belirlenmesi, hem ampirik tedavide klinisyene yol gösterici hem de antibiyotik kullanım politikalarının belirlenmesinde önemlidir.

Anahtar kelimeler: kan akım enfeksiyonları, kan kültürü, antibiyotik, antimikrobiyal direnç

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ABBREVIATIONS

A. baumannii: Acinetobacter baumannii

ABIM: American Board of Internal Medicine

ACP: The American College of Physicians

AST: Antibiotic susceptibility test

BC: Blood culture

C. albicans: Candida albicans

CDC: Centers for disease control and prevention

CFU: Colony forming units

CLSI: Clinical and Laboratory Standards Institute

CNS: coagulase negative staphylococci

CO_2: Carbon dioxide

CoNS: coagulase negative *Staphylococci*

CSI: Circulatory system infections

DM: Diabetes mellitus

E. coli: Escherichia coli

EMB: Eosin methylene blue agar

False (+): False-positive

Gr (-): Gram-negative

Gr (+): Gram-positive

ICU: Intensive care units

ID: Identification

IL-: Interleukin-

Klebsiella spp*: Klebsiella* species

MALDI-TOF MS: Matrix-assisted laser desorption ionization-time of flight mass spectrometry

MRSA: Methicillin resistant *Staphylococcus aureus*

NASA: National Aeronautics and Space Administration

P. aureginosa: Pseudomonas aureginosa

PCR: Polymerase Chain Reaction

PGE2: Prostaglandin E2

PNA-FISH: Protein-nucleic acid fluorescence in situ hybridization

Pseudomonas spp.: Pseudomona species

S aureus: Staphylococcus aureus

S bovis: Streptococcus bovis

SDA: Sabouraud dextrose agar

SIRS: Systemic inflammatory response syndrome

SPS: Sodium polyethylene sulfonate

SSC: Surviving Sepsis Campaign

TNF-α: Tumor necrosis factor alpha

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CHAPTER ONE

1. INTRODUCTION

Circulatory system infections (CSI) continue to be major causes of morbidity and mortality despite antimicrobial and supportive treatments. Therefore, early diagnosis and appropriate treatment of bloodstream infections are clinically important. Blood cultures (BC) can detect microbial etiology in suspected infection cases. It also plays a role in guiding treatment as described (Tabriz et al, 2004, Mylotte and Tayara, 2000). However, difficulties are sometimes encountered in the interpretation and treatment of CSI. The blood collection method is important to avoid misinterpretation of microorganisms in the skin. False-positive (+) BC cause errors in clinical interpretation, inappropriate use of antibiotics, additional laboratory tests, long-term hospitalization, and an increase in costs (Trautner et al, 2002; Kim et al, 2000).

The spectrum of microorganisms that cause bloodstream infections is quite wide. Staphylococcus aureus (*S aureus*), voagulase negative Staphylococci *(*CoNS*), Escherichia coli* (E *coli*), other *enterobacteriaceae* family members, *Pseudomonas aureginosa (P aureginosa), Acinetobacter baumanii (A baumannii) and Candida albicans* (*C albicans*) are the most common infectious agents.

The increase in the elderly age group in the society, the prolongation of the life expectancy of those with chronic diseases, the widespread use of immunosuppressive drugs, the increase in invasive interventions for diagnosis or treatment are among the factors that increase the incidence of sepsis. Nosocomial sepsis is more common in hospitals with high bed capacity, intensive care units (ICU) and frequent invasive procedures (Siddharth et al, 2020). The primary focus of infection in sepsis is the urinary system, genital system, respiratory system, skin and soft tissue, abdominal and intravenous catheters. While respiratory and urinary systems are the most common

entrance gates in sepsis developing outside the hospital, intravascular catheter and urinary catheter procedures are the first in nosocomial sepsis. In ICUs, nosocomial pneumonia comes to the fore as the primary infection focus (Didier et al, 1997).

The diversity of microorganisms and the increase in resistance rates cause problems in treatment and these infections can progress with high mortality. Therefore, selection of the appropriate antibiotic is important in blood circulation infections. It is important to evaluate microbiological data in selecting the most effective antibiotic possible in empirical treatment.

1.1. Aim of the Research

In this study, BC samples from the Near East University (NEU), Faculty of Medicine Hospital between 01 January 2016 and 31 December 2020 were evaluated retrospectively. It was aimed to reveal the distribution of the agents grown in blood cultures, determination of antibiotic susceptibilities and the change over the years.

CHAPTER TWO

2. GENERAL INFORMATION

2.1. History of Blood Culture

Blood culture methods used in the past were made with intensive labor in the laboratory. In the 1800's, leeches absorbed blood treatment methods were used to remove microorganisms from the blood. In a microbiology textbook dated 1911, it was suggested to make cultivation by preparing culture media at the bedside in the period when there were no methods to properly deliver the blood taken from the patient to the laboratory. The blood sample placed in molten broth agar was then poured into a petri dish and cultured (Glen, 2016). The method in which blood samples were collected using glucose broth containing anticoagulant in glass vacuum tubes was started to be used. In the article published by Pulvertaft on BCs in 1930; The blood / broth ratio was expressed as 1 in 5. This rate is still used today (Pulvertaft, 1930). From the 1940s to the 1980s, a great deal of research was conducted on various broth formulations to cover all microorganisms that could be produced in BC (Glen, 2016). In 1947, Castaneda invented a biphasic culture bottle containing both broth and sloping agar for the identification of brucella species. The biphasic culture bottle produced by Castenada has pioneered the methods used today (Ombelet et al, 2019).

"The emergence of the modern blood culture set" protocol published by Scott in 1951; suggested that the blood sample be cultivated in two sterile broths sealed with rubber for aerobes and anaerobes. The aerobic BC medium contained trypticase soy broth and an agar gradient, while the anaerobic BC medium contained thioglycolate broth (Glen, 2016). Automatic BC systems were first used in the 1970s. The first was the Bactec system manufactured by Johnston Laboratories, whose new name is Becton Dickinson. The working method of the Bactec system used culture broths containing nutrients labeled with radioactive isotopes (Murray and Masur, 2012). It is applied by monitoring the radioactive carbon dioxide (CO_2) produced by the microorganisms that feed on these substrates and the concentration of the bacteria grown in the sample. Before the Bactec method can be applied to BCs, by NASA it was proposed to detect life on Mars (Ryan and Murray, 1993). A major problem with the first BACTEC

systems was that they produced radioactive waste that had to be disposed of specifically. This is why in 1984 a new generation of BACTEC instruments using spectrophotometers to detect CO_2 was launched. The BacT / ALERT system, which was approved in 1991, was suitable for indirectly detecting CO_2 production by measuring the drop in the pH of the environment. One disadvantage in BACTEC systems that were first used was eliminated in the BacT / ALERT system. BacT / ALERT did not require the insertion of a needle into the BC medium for sampling; this reduced the frequency of contamination. The new BacT / ALERT system has made it the first system that enables continuous monitoring of BCs (Ryan and Murray, 1993). In 1992, this non-invasive measurement method was adopted by the BACTEC 9000 series, which uses fluorescent indicators to detect changes in pH (Chamberland, 2017). Difco ESP, the first model of the VersaTREK system that detects CO_2 production by measuring pressure changes, was also first approved in 1992 (Ryan and Murray, 1993). In an international survey study, 56% of the laboratories use Abbott's products as a BC system (Chapin and Lauderdale, 1996). The history of BC procedures is summarized in Figure 2.1.

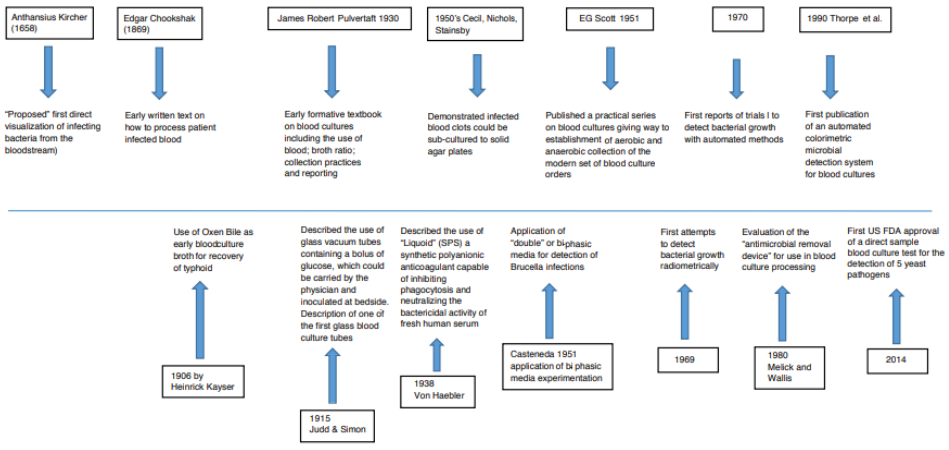

Figure 2.1. History of blood culture procedures (Hansen 2016).

2.2. Bacteremia and Septicemia

Bacteremia, fungemia, and viremia are terms used to describe that bacteria, fungi, and viruses each circulate throughout the vascular system. Signs and symptoms may be present, but are not the same in every patient. It may be subclinical in some patient groups. Patients with sepsis may not show a fever. Elderly and immunocompromised patients are atypical and sepsis should be CoNSidered even in the absence of fever. Fever, also known as pyrexia, is an increase in body temperature. Fever is one of the most common conditions in wards, emergency and ICUs. Detailed anamnesis, physical examination, medical history, drugs, antibiotics being used are helpful in determining the characteristics of the infection.

Blood cultures are used by attending clinicians to understand the cause of fever. For early targeted therapy, concerned clinicians should request BCs immediately (Dewitt et al, 2017). The presence of infection requiring antimicrobial administration is important when evaluating a patient with fever. The patient's clinical condition, medical history, profit-loss ratio, and the reliability of the applicants are important in deciding the use of BC. BCs are the gold standard in the diagnosis of bacteremia (Choi et al, 2019).

Blood cultures are often taken when fever, leukocytosis, septic shock, endocarditis is suspected or at the beginning of antibiotherapy in elderly patients and in immunocompromised patients (Dewitt et al, 2017). However, the result of BC takes time and false positive (+) results occur due to contamination. False (+) results cause unnecessary antimicrobial drug administration, increased healthcare costs, and increased development of antibiotic resistance (Choi et al, 2019).

False (+) causes an increase in costs and a prolonged hospital stay. In addition, 22.4% of clinicians continue empirical antibiotic therapy despite the culture results (Roque et al, 2012). Positive BCs are useful in the follow-up of antibiotherapy in sepsis. However, their excessive use may increase costs, and their use after antibiotherapy may lead to a decrease in sensitivity (Choi et al, 2019). In addition, the use of new molecular techniques such as genetic amplification and mass spectrometry as other diagnostic methods in the detection of bacteremia pathogens has increased in

recent years. Although the limit the use of BCs as the gold standard in the diagnosis of bacteremia, the high cost prevents their widespread use in patients.

2.3. Blood Culture Indications

Blood culture indications are varied and not standardized. BC is taken at the first admission in most patients who are admitted to the hospital with suspected infection or infection diagnosis. Distinctive symptoms and signs better guide the clinician to take a BC. Fever is a common symptom of bacteremia. Fever is the most common cause and indication for BC (Mylotte and Tayara, 2000).

The optimal use of BCs is in the diagnosis of sepsis. Nearly half of the patients with severe sepsis encounter bacteremia when diagnosed. In approximately 1/3 of bacteremias, the source cannot be determined (Ntusi et al, 2010).

2.4. Fever

Fever, also known as pyrexia, is an increase in body temperature. Normal body temperature ranges from 36.1 °C to 37.8 °C. Fever occurs at high values above this normal range. The CDC (Centers for disease control and prevention) defines fever as an internal body temperature higher than 37.8 ° C without taking antipyretic drugs. However, 38.0 °C is also widely used as a value for fire. Generally, an internal temperature of 38.3 °C is an indicator of fire (Biros and Blurn, 2018).

Fever is caused by pyrogens, which are endogenous and exogenous substances (Figure 2.2.). Endogenous pyrogens include cytokines released from leukocytes in response to infectious, inflammatory and neoplastic processes. Exogenous pyrogens include bacterial and viral products and toxins. Toxins cause the release of endogenous pyrogens in an immune response. These are interleukin-1 (IL-1), IL-6, tumor necrosis factor, interferon. Fever is the result of endogenous pyrogens and high prostaglandin E2 (PGE2) level. Staphylococcus aureus enterotoxin and group A and B streptococcal-associated super antigens are exogenous pyrogens (Biros and Blurn, 2018).

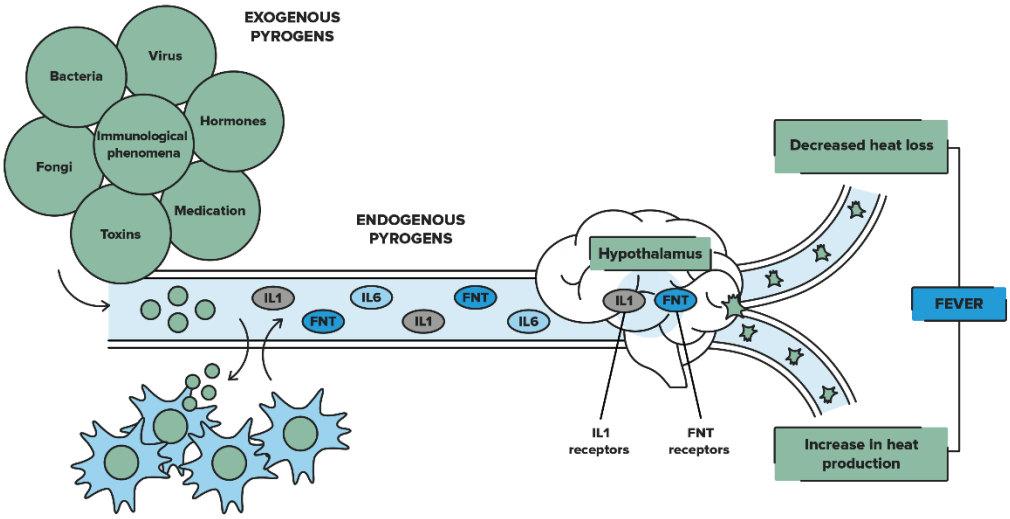

Figure 2.2. Fever is caused by pyrogens, which are endogenous and exogenous substances. https://www.clinicbarcelona.org/en/assistance/be-healthy/fever/causes access date. 17.03.2021

Fever creates a physiological burden on the patient with increased fever, increased oxygen CoNSumption, metabolic demand, protein degradation, and gluconeogenesis. Age, malnutrition, immunosuppression and chronic diseases affect the response to fever. Fever is the cause of benign disease in young adults and its mortality is less than 1%. Important in this age group is to recognize meningococcemia, meningitis, or methicillin-resistant Staphylococcus aureus infection and septic condition (Biros and Blurn, 2018).

Among the patients with fever, the group over 65 years of age or those with chronic diseases CoNStitute the high-risk group for serious disease. Hospitalization of the high- risk group is 70-90% and its one-month mortality is 7-9%. The most common cause of fever in these patients is infection. 80% of the infections are seen in the respiratory system, urinary system, skin and soft tissue. Mortality and morbidity rates are higher in the geriatric population (Biros and Blurn, 2018).

Fever is not seen in bacteremia in 25% of patients. Body temperature is detected between 36.2 °C and 38.3 °C in 50% of patients with bacteremia over the age of 65, while fever is found below 37.3 °C in at least 13%. In patients with normal

fever, the incidence of positive BC detection is high if more than 6% band form is detected in peripheral smear and urinary system catheter is present (Lee and Dean, 2010).

A febrile response to infection may not be observed in patients with immunocompromised, cancer, cirrhosis, diabetes mellitus (DM), use of systemic corticosteroids, and organ transplantation. Sepsis-related mortality in afebrile patients increases due to the delay in the onset of antibiotics (Talan and Yealy, 2019).

Fever is a common condition in emergency departments. Among patients hospitalized in the services, it is encountered in 5% of adults and 15% in older ages (Lin and Boehm, 2013; Dewitt et al, 2017). The differential diagnoses of the patient with fever in the emergency department are extensive. Most of the important causes are due to infection. Sepsis, pneumonia, urinary system infections, intra-abdominal infections, meningitis and hospital infections are common in patients who are seen in emergency services or services due to fever. In acute febrile illness, it should be evaluated whether the patient's condition is stable or not. Mental status change, respiratory distress, and cardiovascular instability are important signs and symptoms. Quick and effective treatment is required (Biros and Blurn, 2018).

Clinicians use BCs for fever etiology (Sturmann et al., 1996). When evaluating a febrile patient, the presence of an infection requiring the use of antibiotics is important. A detailed history, physical examination, medical history, medications, and antibiotics used can help determine the characteristics of the infection (Dewitt et al, 2017).

In neutropenic patients, the incidence of sepsis has increased due to the impaired immune system and its results are worse. In the neutropenic patient, fever should suggest infection, and all patients should have BCs and empirical antibiotics administered (Puskarich 2016).

Early empirical antibiotherapy should be applied to patients. Antibiotic choice depends on the etiology of fever, neutropenia and accompanying conditions such as end stage kidney disease. Antiviral and antifungal therapies are also indicated in

patients with acute fever in the presence of immunosuppression (Biros and Blurn, 2018).

2.5. Bacteremia

True bacteremia is the growth of a known pathogen in single BC or the growth of common skin pathogens (such as *coagulase-negative Staphylococcus species, Diphteroids, Bacillus species, Propionibacterium species*, or *Micrococci*) in 2 BCs (Takeshima et al, 2016). 200,000 bacteremia attacks are seen every year in America. Its incidence is 10/1000 hospital admissions. The mortality of bacteremia is between 14-37%. Mortality increases by 35%, especially in critical care patients. Bacteremia ranks 10th in deaths in America (Coburn et al, 2012).

Early diagnosis and appropriate treatment of bacteremia is clinically important. Bacteremia is a major cause of morbidity and mortality, despite advances in antibiotherapy and supportive treatments (Mylotte and Tayara, 2000). Clinicians routinely take BCs before starting empirical antibiotherapy in patients with suspected bacteremia, but BC should not cause delay in antibiotherapy (Puskarich and Jones, 2016).

Bacteremia includes endocarditis, catheter-associated bacteremia, primary bacteremia, and occurs secondary to focal infections such as pneumonia, abscess, osteomyelitis, or urinary tract infection. Intravenous catheters are the most common cause of bacteremia (Coburn et al, 2012).

In general, bacteremia is transient, intermittent and continuous (Figure 2.3.). Transient bacteremia usually occurs after mechanical or surgical intervention into infected tissue. Intermittent bacteremia is seen in abscesses that have not been drained or localized infections such as pneumonia, urinary tract infection and central nervous system infection. Persistent bacteremia is seen in intravascular infections, infective endocarditis, septic thrombophlebitis, or mycotic aneurysm (Mylotte and Tayara, 2000).

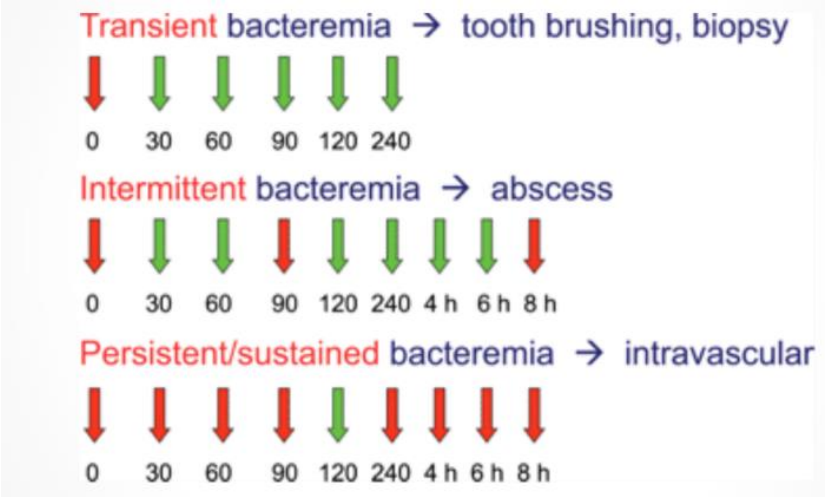

Figure 2.3. Classification of bacteremias (Seifert, 2009).

Different estimation methods are used to evaluate risk factors in bacteremia (Chase et al, 2012; Coburn et al, 2012; Eliakim-Raz et al, 2015). However, these models are limited by the heterogeneity of sepsis in different hospitals and populations (Choi et al, 2019). BC is the only way to detect bacteremia. There have been great advances in BC technologies in the last 20 years. With these developments, the detection and classification of microorganisms that cause bacteremia has been accelerated (Mylotte and Tayara, 2000). For optimal recognition of bacteremia in adults, it is the standard recommendation to take BC from 2–4 at intervals of more than 24 h (Dargere et al, 2014). BC is generally not recommended for patients who are planned to be discharged, for uncomplicated infections or if the culture result will not cause a change in treatment. However, in the case of 'severe sepsis' or 'septic shock', the clinician should definitely take BC before the initiation of antibiotherapy. Positive BCs are useful for monitoring antibiotherapy in sepsis (Choi et al, 2019).

Blood culture is often used in patients with fever, leukocytosis, focal infections, sepsis, endocarditis, or in emergency room patients or other patients when required prior to parenteral antibiotics (Coburn et al, 2012). BC should be included in the diagnosis of clinically important infectious diseases. When occult bacteremia caused by *S aureus* is missed, clinical deterioration is inevitable and cannot be diagnosed without the use of BC (Dewitt et al, 2017). It is very important for clinicians to differentiate patients at high risk of bacteremia. Because ideal BC collection should be applied in the emergency room in these patients before the initial antibiotherapy.

Immunocompromised patients (DM, cirrhosis) are more prone to bacteremia, and BCs should be obtained even if there is no vital impairment or the need for intensive care (Choi et al, 2019).

Bacteremia, when bacteria enter the bloodstream, temporary, intermittent or it can be continuous. Transient bacteremia often occurs when organisms that are part of normal flora enter the bloodstream due to minimal trauma to the membranes (e.g., brushing teeth, strain during bowel movements or medical procedures) (Lefrock et al, 1973). Intermittent bacteremia is caused by the periodic spread of bacteria into the blood, from infections of body cavities such as peritonitis or septic arthritis, from extravascular abscesses to the body. Persistent bacteremia usually occurs from intravascular sources such as infection infected endothelium (bacterial endocarditis or aneurysm) or infected circulation (arteriovenous fistulas). However, the source of the organism may not be determined in one-third of bacteremia (Koneman, 2006).

The organism profiles responsible for bacteremia have changed in recent years. Gram-negative [Gr (-)] organisms such as *E coli* and *P aeruginosa* were the most frequently isolated agents in bacteremia in 1960-1970; In 1980-1990, most bacteremia started to occur with Gr (+) organisms such as *S aureus*, CoNS and *Enterococcus*. While methicillin resistant Staphylococcus aureus (MRSA) bacteremia was rare in the 1970s, MRSA is the causative agent in more than 40% of patients who develop *S. aureus* bacteremia today (Karchmer and Boyer, 2008). Gram (-) bacteria that synthesize extended spectrum β-lactamase (ESBL) and Vancomycin-resistant Enterococci (VRE) are more frequently isolated (Chen and Hsueh, 2012). While Streptococci, *S aureus* and *E coli* are common agents in community acquired sepsis, the agents encountered in hospital acquired sepsis are Pseudomonas spp , Klebsiella spp., *E coli, S aureus and* Enterococci. In recent years, *Acinetobacter* species have also started to increase in BCs (Pien et al, 2010) (Figure 2.4.).

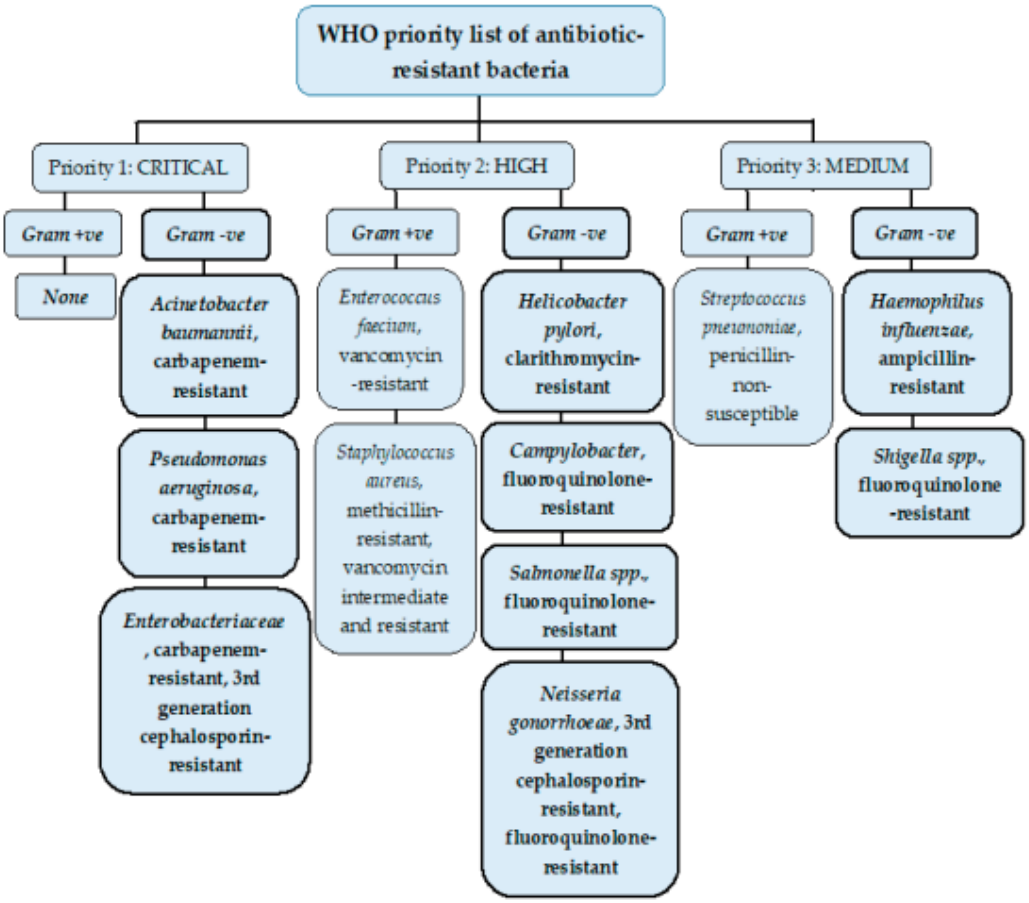

Figure 2.4. List of priority pathogens according to antibiotic resistance (Pien et al, 2010)

Bacteremia can be from the source of infection in the organ or tissue (secondary bacteremia). But often, the primary focus is unclear (primary bacteremia). In this case, the transient bacteremia may not be effectively cleared by the host defense mechanism. For example, as a result of *S aureus* being colonized in the nose can be a source of systemic infection. The factors that trigger the spread from the nostrils are not fully known, but the spread to the skin and the resulting skin infections or infections that develop with intravascular spread can be seen (Piper et al, 2001).

A variety of bacteria, both gram-negative and Gr (+), can be found in the bloodstream. Over the past decades, changes have occurred in microorganisms reproducing in BC. While the number of anaerobic isolates decreased over time, the number of fungi and coagulase-negative staphylococcal isolates increased (Weinstein et al, 1997). Containing Gr (-) nonfermented bacteria for unknown reasons bacteremias

are polyclonal compared to bacteremias including other Gr (-) bacteria (Wendt and Grunwald, 2001).

Some specific microbes are of particular clinical significance. Clostridium septicum is often ssociated with neoplastic diseases, particularly colon carcinoma, and can result in distant metastatic abscesses. Similarly, bacteremic *Streptococcus bovis* (*S bovis*) is associated with colonic diseases, including endocarditis and colon carcinoma. Rarely, Clostridium perfringens bacteremia can result in sudden dramatic hemolysis, which can be fatal very quickly; hemolysis may occur due to clostridial toxins, but why fatal hemolysis occurs only in very small clostridial bacteremias is not fully known (Tsai et al, 1989).

2.6. Sepsis

The annual incidence of severe sepsis ranges from 300-1000 / 100,000 people. Patients over $5x10^5$ present to the emergency department every year with suspected severe sepsis and it is the largest group of all inpatients with sepsis (Puskarich, 2016) (24). Sepsis is one of the most common causes of mortality and morbidity in hospitalized patients. Its mortality varies between 17.8% and 35%. Sepsis treatment accounts for 5.2% of hospital fees (Esposito et al, 2018).

Sepsis syndrome is the host response to infection. The causative agents and the activated inflammatory pathways of the host cause load on the body's defense and lead to the deterioration of homeostasis. Tachycardia, tachypnea, fever and immune system activation are the main symptoms. If this is not corrected, it will result in cellular damage, tissue damage, shock, multi-organ failure or death (Figure 2.5.; Shapiro and Jones, 2018).

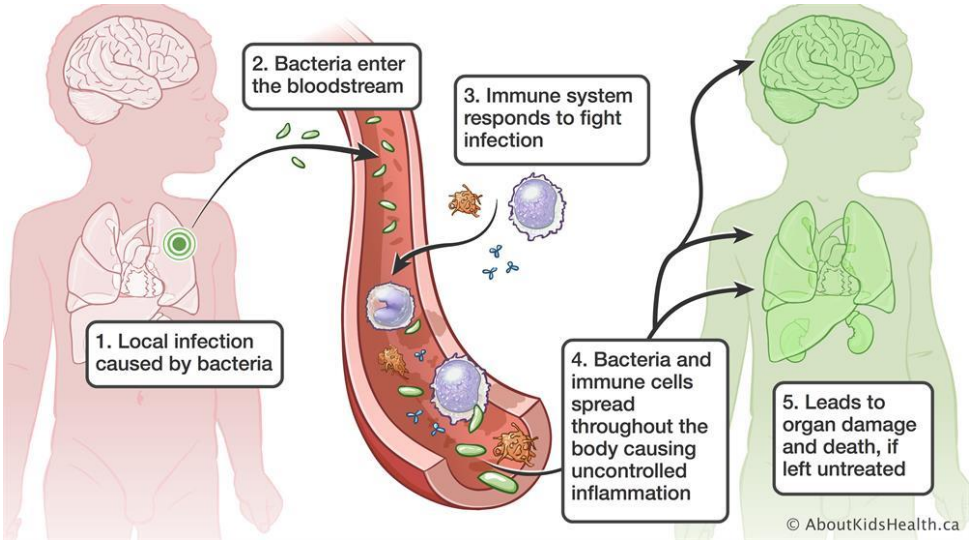

Figure 2.5. Development of sepsis.
(https://www.aboutkidshealth.ca/Article?contentid=2316&language=English)

Sepsis is a clinical diagnosis and is based on suspicion of infection or confirmation of infection, systemic inflammation, and evidence of new organ dysfunction and / or tissue hypoperfusion (Puskarich and Jones, 2016). The host's first response is the migration of inflammatory cells, neutrophils and macrophages to the site of infection.

Inflammation and coagulation pathways work with the interaction of the host and pathogen. Cytokines, chemokines, interleukins (IL-1, IL-6 and IL-8) and tumor necrosis factor alpha (TNF-α) are involved in the inflammatory process. If the result of the inflammatory response is sufficient, the infection is controlled. If the response is insufficient or excessive, it will be permanent; shock, organ failure, and death are seen (Shapiro and Jones, 2018).

The general condition of the patient is important in the development and progression of sepsis. Systemic infections are more prone to develop in elderly people and those with multiple comorbidities. Chemotherapy-induced neutropenia, AIDS and steroid addiction predispose to sepsis. The risk of systemic infection and sepsis increases with the increased use of devices such as intravascular catheters, prosthetic valves, and endotracheal tubes (Shapiro and Jones, 2018). Patients with sepsis may not

show a fever. Elderly and immunocompromised patients are atypical and sepsis should be CoNSidered even in the absence of fever (Dewitt et al, 2017) (14).

In order to be called systemic inflammatory response syndrome (SIRS), two or more of the following criteria must be met.

Tachycardia (> 90 bpm),

Tachypnea (> 20 breaths / min or PCO_2 < 32 mmHg),

Hyperthermia (> 38 °C) or hypothermia (< 35 °C),

High leukocyte count (> 12,000dl) or low leukocyte count (<4,000 dl) or band form (> 10%).

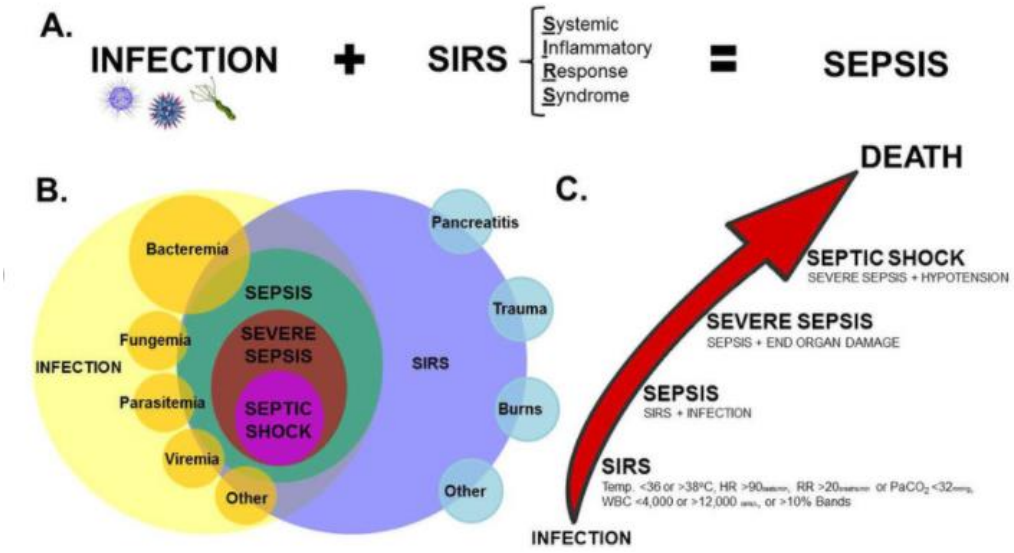

Figure 2.6. View and Definition of SIRS, Sepsis, Severe Sepsis, and Septic Shock (Delano and Ward, 2016).

2.6.1. Sepsis etiology

Sepsis is a combination of infection and SIRS. Sepsis can be detected as severe sepsis and organ dysfunction. Septic shock is the manifestation of sepsis and hypotension with systolic blood pressure below 90 mmHg, unresponsive to fluid therapy (Shapiro and Jones, 2018). SIRS can be caused by many non-infectious causes and when fever is not accompanied, it may not suggest infection to clinicians. Therefore, treatment delays and increased mortality often occur (Stoneking et al, 2013). The SIRS criteria are sensitive but nonspecific and do not indicate an increased risk of mortality (Shapiro and Jones, 2018). The mortality of patients with SIRS is proportional to the number of criteria met. Whether patients have a positive BC or not does not affect mortality rates (Puskarich and Jones, 2016).

When the cause of sepsis is acute bacterial pneumonia, the most common attachments are Gr (-) basils, *S pneumoniae*, *S aureus*, and *Legionella pneumophila*. When the cause of sepsis is acute pyelonephritis, Gr (-) enteric bacteria or Enterococci are bacteria detected in blood culture frequently (Puskarich and Jones, 2016). The most common skin and soft tissue infection that causes sepsis syndrome is cellulitis, and *S aureus* and *Streptococcus pyogenes* are the causative agents. Necrotizing soft tissue infections are more common in immunocompromised patients, diabetic patients, or those with vascular circulatory disorders. The most common primary causes of bacteremia in the ambulatory patient are *S aureus, S pneumoniae* and *Neisseria meningitides, P aeruginosa* and other Gr (-) bacteria cause bacteremia and endocarditis in intravenous drug addicts. Secondary bacteremia may occur from implanted medical devices (intraperitoneal or intravascular dialysis catheters, chemotherapy ports, peripherally located central catheters, ventriculoperitoneal shunts, and pacemaker / defibrillators). Acute bacterial meningitis is a severe but rare cause of septic shock. Community-acquired meningitis is usually the cause of *S pneumoniae* or *N. meningitidis* (Puskarich and Jones, 2016).

2.6.2. Diagnosis of sepsis

Testing BC in suspected sepsis is the gold Standard (Armstrong-Briley et al, 2015). In sepsis, at least 2 separate sets of BC samples should be taken from different peripheral venous line (Puskarich and Jones, 2016). Up to 30–50% of patients with

severe sepsis or septic shock have a positive BC (Armstrong-Briley et al, 2015). BC is positive in less than 10% of outpatients and emergency room patients. When evaluating positive BC results, the most common pathogens and contaminants as well as the patient's medical history, immune response, and general condition should be CoNSidered (Puskarich, 2016).

While Gr (+) organisms CoNStitute 25-50% of infections, Gr (-) organisms CoNStitute 30-60% and fungi CoNStitute 2-10% (Shapiro 2018) (37). Gr (+) bacteria are the main pathogen in sepsis, except for surgical indications. With the increase in antimicrobial resistance, MRSA, *vancomycin resistant* Enterococci *(*VRE*),* and other multidrug resistant organisms are more common (Puskarich 2016) (24). The distributions vary with patient factors such as the patient's immunity, age, hospitalization history, and the presence of a catheter (Shapiro and Jones, 2018). Incidence of fungi, especially in immunocompromised patients show (Puskarich and Jones, 2016).

Corynebacterium spp, Bacillus spp, *Propionibacterium* acnes are CoNSidered contamination. Viridans group Streptococci, Enterococci and voagulase negative Staphylococci (the most common microorganism in BC) are CoNSidered pathogens (Puskarich and Jones, 2016).

Signs and symptoms may be hidden in patients with bacteremia. Therefore, when applying early targeted therapy, clinicians should behave aggressively when requesting BC and antibiotherapy should be started early (Lin and Boehm, 2013). Untreated bacteremia causes the development of sepsis and septic shock (Shapiro and Jones, 2018). Rapid diagnosis is required in septic shock. Resuscitation is applied and antibiotherapy is given at the beginning of the treatment. Severe sepsis has a 20% mortality. It can reach 50% in septic shock (Puskarich and Jones, 2016). Early administration of empirical antibiotherapy is associated with low mortality (Shapiro and Jones, 2018).

2.6.3. Treatment of sepsis

The most important part of initial treatment and stabilization in severe sepsis is early diagnosis, early correction of hemodynamic disorders and early infection control.

Resuscitation should be done early and aggressively (Puskarich and Jones, 2016). Treatment should not be delayed in patients with sepsis or severe infection (Shallcross et al, 2016). With early targeted therapy reducing sepsis-related mortality, new guidelines have been developed for the management of sepsis (Esposito et al, 2018).

Surviving Sepsis Campaign's (SSC) international sepsis and septic shock management 2016 guide was developed and 3 and 6 h paths were combined. Thus, "1 hour pathway" was obtained in 2018. It is aimed to start resuscitation and treatment immediately. The 'point 0' or 'time to see' are defined as triage moments in the emergency room. Treatment includes lactate measurement, BC, fluid and antibiotic administration, and vasopressor therapy against dangerous hypotension (Levy et al, 2018). According to the SSC, antibiotics should be administered within 1 h of the diagnosis of severe sepsis and / or within the first 3 h of triage (Puskarich and Jones, 2016).

Sepsis is the most expensive cause of hospitalization. Due to the high mortality and morbidity associated with sepsis, rapid and accurate diagnosis is very important for the treatment of the patient. The first test to be performed in this is "blood culture" Taking a BC before antibiotic administration is one of the SSC recommendations (Stoneking et al., 2013). BC taking is an important part of the pathways. It is important in the detection of etiological bacteria and initiation of the appropriate antibiotic (Mariani et al, 2018).

When sepsis is suspected, empirical antibiotherapy is administered without waiting for isolation of the pathogen (Armstrong-Briley et al, 2015; Shallcross et al, 2016; Levy et al, 2018). The initiation of antibiotics should not be delayed in order to obtain a BC (Puskarich and Jones, 2016). The clinical decision of reviewing antimicrobial therapy depends on microbiological diagnosis (Shallcross et al, 2016).

There is a time frame for each patient during which the infection and the patient can be successfully treated. This is called the "therapeutic range". When the microorganism in the blood is detected with BC within the therapeutic range, effective treatment is obtained and the patient's survival is ensured. The optimal approach is early initiation of broad-spectrum antibiotics with evaluation of clinical and microbiological outcomes (NHS UK, 2018).

Antibiotics work best in the first hour. The relationship between antibiotics and mortality is greatest in patients with septic shock or hypotensive sepsis. Sepsis mortality is higher during the preantibiotic period (Talan and Yealy, 2019). Broad-spectrum antibiotics should be given immediately in severe sepsis. Combined antibiotherapy is more effective. Antifungals and antivirals should be added in immunodeficiency (Puskarich and Jones, 2016). Empirical antibiotherapy should be narrowed when the pathogen is detected and its sensitivity is understood, or discontinued if the patient is judged to be free of infection (Levy et al, 2018).

2.7. Endocarditis

The most common intravascular infection is endocarditis, which is an infection of the endothelial layer of the heart (Figure 2.7.). Although almost all organisms develop endocarditis whenever possible, most of the microorganisms that cause the infection are Gr (+). The most important of these are viridans Streptococci and *Staphylococcus aureus* found in the oral cavity. Patients with endocardial impairment and dental procedures are most at risk (Bayer, 1993).

The endocardial injury was most commonly caused by rheumatic fever. Congenital or developmental anomalies such as a bicuspid aortic valve or mitral valve prolapse have gained more importance as the incidence of this disease has decreased. Fibrin-thrombocyte thrombosis, which occurs on the eroded endocardium surface, acts as an adhesion site for bacteria that are temporarily circulating in all humans (e.g., after brushing our teeth). Certain agents, especially streptococci and enterococci, show an improved ability to cause these thromboses (Eliakim-Raz et al, 2015). Thrombosis and associated bacteria form vegetations that can be observed by radiographic or echographic techniques.

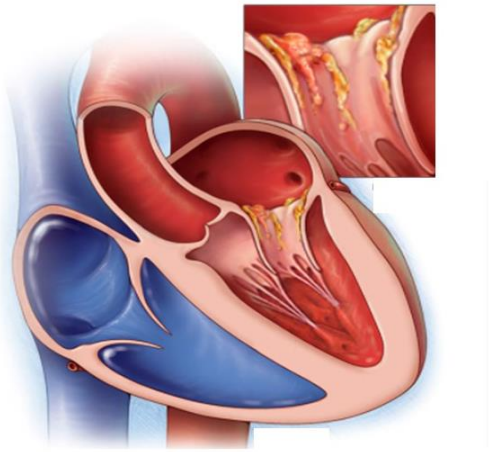

Figure 2.7. Endocarditis
https://www.mayoclinic.org/diseases-conditions/endocarditis/symptoms-causes/syc-20352576 (Accession date: 17 March 2021).

Although Gr (+) bacteria are the most common etiological cause of endocarditis, they can also infect with some Gr (-) bacteria and some fungi. Gr (-) bacteria belonging to the HACEK group (*Haemophilus aphrophilus, Actinobacillus actinomycetemcomitans, Eikenella corrodens, Kingella kingae*) particularly infect possible heart valves. It is noteworthy that the fungi and some of the Gr (-) bacteria mentioned above tend to break off and move to distant areas along with the bloodstream, forming large vegetations (septic embolism). Most cases of endocarditis involve the left side of the heart, which is the high pressure in the system. However, if bacteria are injected directly into the venous system, as is the case with intravenous injection of narcotic drugs, it may result in left-sided endocarditis, often caused by *P aeruginosa* (Dargere et al, 2014).

It is very difficult or impossible to isolate the etiologic factor in a minority of patients with signs and symptoms of endocarditis (culture-negative endocarditis). The diseases that cause marantic endocarditis may be responsible, but certain microbes cannot currently be isolated using conventional culture methods. Notable among these are *Chlamydia pneumoniae, Coxiella burnettii* (Q fever), Bartonella spp., and Legionella spp. could be found.

The most serious complications of endocarditis are cardiac valve rupture resulting in heart failure and metastatic disease due to embolization of infected vegetation fragments. Sudden cardiac decompensation, which may require surgical emergencies, is a particular problem in endocarditis caused by *S aureus.* Renal failure and paralysis may occur, respectively, as a result of septic embolism in the kidney or brain.

2.8. Catheter-Associated Bacteremia

2.8.1. Epidemiology

Catheter-associated bacteremia is a major cause of nosocomial bacteremia (Figure 2.8.).

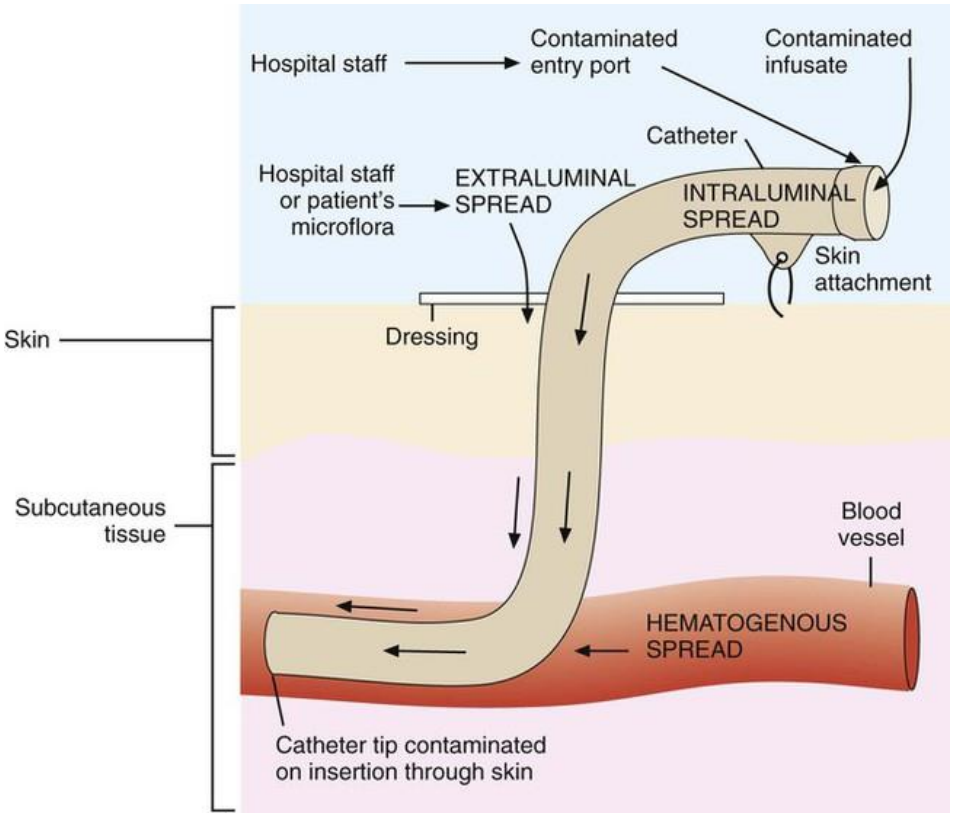

Figure 2.8. Bacteremia associated with an intravenous catheter, one of the possible ways microorganisms gain access to the bloodstream. https://clinicalgate.com/bloodstream-infections/. (Acession date: 14 march 2021).)

More than 400,000 catheter-associated bacteremia occurs annually in the United States, with a mortality of 4–10%. Catheter-related bacteremia is diagnosed by rolling the catheter on medium (Chatzinikolaou et al, 2004). Catheter infection should be CoNSidered when BCs from catheter cultures become positive before 2 h compared to venous BCs (Opota et al, 2015; IDSA and Surviving Sepsis Guideline). The catheter lumen can become colonized over time, leading to bacteremia. Colonization can be demonstrated with BCs taken from the catheter before true bacteremia occurs (Mermel, 2019; Beutz et al, 2003).

2.8.2. Diagnosis

Generally, there is no evidence of infection at the catheter insertion site, microorganisms are often normal flora elements and BC contaminants. Catheter-associated bacteremia is often diagnosed with the recovery of sepsis unresponsive to antimicrobial therapy by detecting the same microorganism from the blood and the purulent catheter site, or by removal of the catheter. In catheter-associated infection, the bacterial load of blood taken from the catheter is greater and has a shorter positivity time than peripheral intake (NHS UK, 2018). Newly placed intravenous catheters can be used for BC specimen. There is a marked increase in the rate of contamination in chronically found catheters.

2.9. Characteristics of Blood Culture Medium

Blood cultures are the main method to understand the etiology of bacteremia. Because they are very sensitive and easy to work with (Opota et al, 2015). The clinical benefit of BCs is based on their ability to detect pathogens that cannot be reliably sampled from other body fluids and to guide the duration or intensity of antibiotic therapy (Figure 2.9; Stalnikowicz and Block, 2001). Visualization of blood culture progress is given in Figure 2.10.

Figure 2.9. Blood culture media. https://www.bd.com/en-us/offerings/capabilities/microbiology-solutions/blood-culture/blood-culture-media. (Accesion date: 10 March 2021).

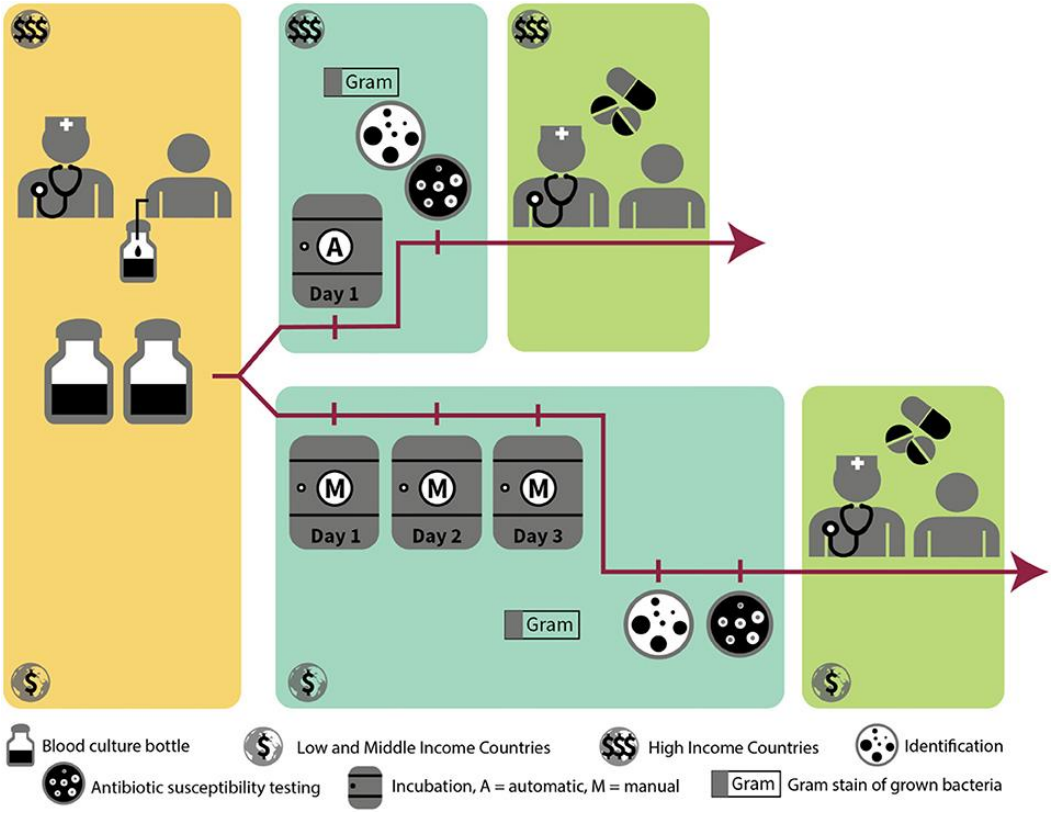

Figure 2.10. Workflow of grown blood cultures setting (Ombelet et al., 2019).

In the clinic, bacteremia suspects the patient's chills or fever. In this case, taking BC is a guide to determine the agent. In the absence of fever, BC can be obtained from patients with local infections (such as pneumonia, meningitis, osteomyelitis), patients with renal failure and unexplained leukocytosis, patients with the impaired immune system, or patients with unexplained pulmonary, renal, and hepatic dysfunction, and unexplained hemodynamic disorders. It is suggested that in almost all cases without antibiotic treatment, three BCs taken at 30 min intervals are sufficient to obtain the organism. When necessary, cultures can be taken from different areas for 5 min. Extreme care should be taken when collecting blood samples to avoid accidental contamination of the culture medium with organisms from the skin or the environment. According to the standards published by the American Society of Microbiology, the BC contamination rate should not exceed 3 %.

In adults, it is optimal to obtain 20 ml of blood per culture set (2 bottles) to increase the yield of true positive cultures. Normally, 2 vials (1 aerobic vials and 1 anaerobic vial or 2 aerobic vials) are inoculated in adults (Buehler, 2016; Mushtaq et al, 2019). The sensitivity of BC is related to the sample size. Standard bottles receive 10 ml of blood. A blood sampling for adults usually contains 20 ml of blood for inoculation in two bottles (1 aerobic bottle and 1 anaerobic bottle). Taking 2-4 BCs before antibacterial therapy detects 80-96% of the causative agent of bacteremia. Detection of bacteremia depends on the bacterial or fungal concentration and the collected blood volume (Lamy et al, 2016). The number of pathogens present in the blood during bacteremia ranges from 1–10 colony forming units (CFU / ml) to $1x10^3$-$1x10^4$ CFU / ml (Opota et al, 2015). Standard bottles are designed for aerobic and anaerobic reproduction. BC bottles contain culture media, anticoagulants, often resin or chelators to reduce the effect of antibiotics and other toxic compounds (Kirn and Weinstein, 2013). The main media most commonly used in automated BC bottles are tryptic media and soy-casein-peptone (Khatib, 2015).

Complement, phagocytes, and antibodies are elements found in the blood. When these are present in high concentrations during incubation, they reduce the effect of BC. The optimal blood-to-medium ratio is stated as 1/5 and 1/10. Most blood culture media contain Sodium polyethylene sulfonate (SPS). SPS is a polyanionic

anticoagulant and inactivates antimicrobials such as aminoglycosides. BC volume should be less than 1% of the total blood volume (Mylotte and Tayara, 2000).

2.10. Sample Collection for Blood Culture

Blood culture is affected by many factors such as culture time, blood volume, source of blood, number of cultures, and underlying infection (Mylotte and Tayara, 2000). The patient's clinical condition, medical history, cost-compatibility ratio, and the reliability of the administering agent are important in deciding the use of BC (Choi et al, 2019).

2.10.1. Sampling

First of all, the patient identity is verified. Antisepsis is provided. In at least 2 sets, 10 ml of blood is collected for each bottle. The expiry date of the bottles must be checked. After the blood is bottled, it is gently shaken to prevent coagulation (Mylotte and Tayara, 2000). Before taking BC samples, skin antisepsis should be applied. According to today's evaluations, tincture iodine, chlorine peroxide, and chlorhexidine gluconate are superior to povidone-iodine and are applied in skin antisepsis before BC is taken. This reduces the rate of contamination and facilitates the evaluation of results for the clinician (Kirn and Weinstein, 2013). Contamination rate with appropriate antisepsis remains below the threshold value of 3% in BC samples (Lamy et al, 2016).

It is less critical to follow two sets of BCs when the clinical situation is clear. For example, contaminants are easily detected in urinary tract infections and pathogens can be isolated from other samples. In addition, if the possible CoNSequences of bacteremia are of high importance to clinicians, they themselves set the threshold for BC acquisition, for example in prosthetic devices (Stalnikowicz and Block, 2001).

By taking an appropriate amount of blood sample, optimal sensitivity for detecting bacteremia is achieved by increasing the number of venous intakes (multi-sampling strategy) or by taking large amounts of blood in a single collection (single-sampling strategy) (Lamy et al, 2016). To improve BC acquisition, strict criteria must be taken into account. Advances in antiseptic technique [decreases false (+)],

informing the clinician in a shorter time, enable the clinician to be more effective in choosing antibiotics (Roque et al, 2012).

2.10.2. Sample reception place

The peripheral venous route is the recommended BC method (Lamy 2016, Roque 2012). The American College of Physicians (ACP) guidelines do not recommend blood collection from intravascular instruments, and the Clinical and Laboratory Standards Institute (CLSI) recommendation is to evaluate positive results by taking BCs from intravenous devices with venous cultures (Kirn and Weinstein, 2013). There is a 36%, 10%, and 7% risk of contamination of the peripheral venous, arterial or central venous route, respectively (Opota et al, 2015).

2.10.3. Number of cultures

It is difficult to interpret the positivity of a single culture. If continuous bacteremia is expected, it is appropriate to obtain two BCs. When intermittent bacteremia is suspected, 3 BCs are taken. As the amount of blood taken (number of bottles) increases, the detection increases, and the false (+) rate decreases. The IDSA (Infectious Diseases Society of America) recommends repeating the BC 2-4 days after the initial positive BC in *S aureus* bacteremia, and taking BCs daily or every other day in candidemia (Mushtaq et al, 2019). In case of suspicion of acute endocarditis, it is recommended to perform three sets of BCs from 3 separate sites. By increasing the number of samples, sensitivity is achieved to be in the range of 88-99% (Myolete and Tayara, 2000). When evaluating the etiology of fever, 2-4 sets of blood are taken from different sites and, if necessary, repeated within 24-48 h (Mushtaq et al, 2019).

2.10.4. Blood culture sensitivity

Sensitivity is related to the blood volume collected. When 30 ml of blood is incubated, 10^3 CFU of pathogens are detected and bacteremia is shown in 95–99% (Lamy et al, 2016). As the number of samples increases, sensitivity increases, sensitivity is approximately 99% in 4 BCs (Sturmann et al, 1996; Dargere et al, 2014; Opota et al, 2015). Small sample collection, poor sampling method, prior antibiotic

usage, and false positivity due to BC contamination reduce BC sensitivity (Shallcross et al, 2016).

2.10.5. Time to sample

It is not recommended to take a BC before symptoms begin. BCs should not be taken from different places at the same time. BC should be taken in the absence of antibiotics, during periods of rising fever, and at 30-60 minutes intervals. It is recommended to take BCs at 6-36 h intervals in patients with intermittent bacteremias, such as infective endocarditis and *S aureus* bacteremia. If the patient is on antibiotic therapy, the BC should be taken just before the next antibiotic dose or, if clinically appropriate, the antibiotic should be discontinued, and re-cultured 48 h later (Lamy et al, 2016).

2.10.6. Sample collection

Today, many manual and automated systems are available for clinical use. Fully automated systems for BC are the most preferred and reliable methods (NHS UK, 2018). Blood samples coming to the laboratory are taken into the incubation protocol is continuously monitored BC devices (Kirn and Weinstein, 2013).

Processes affecting BC results: Transport time (from sampling to delivery of the laboratory), evaluation time of the laboratory, time of detection (the period from evaluation to a positive result), typing of the positive result, reporting the result (NHS UK, 2018). Culture samples must be delivered to the laboratory within 30 min to 2 h. Ambient temperature should not exceed 35-37 °C (Mylotte and Tayara, 2000). When BC bottles are kept at 4 °C or room temperature for over 24 h and at 37 °C for more than 12 h, pathogens detection will be delayed. These specimens should not be stored in a refrigerator or frozen. Isolates associated with true bacteremia should be stored in the laboratory by serial subculture. Thus, if necessary, additional tests can be performed and stored for a long time in frozen archives to enable the investigation of recurrent bacteremia in eligible patients (Kirn and Weinstein, 2013).

2.10.7. Evaluation of results

Blood culture is a diagnostic test that is affected by personal factors and clinical judgment. Along with clinical signs and symptoms, many parameters are helpful in detecting positive BC: Number of positive bottles, number of positive BC sets, the proportion of positive cultures, sample site (catheter, peripheral venous path), and time to become positive, different positive time between samples taken from different sample areas. (Opota et al, 2015). Attention should be paid to the percentage of positive BCs, the percentage of contaminated BCs, the percentage of BC samples showing optimal filling, the average time from the collection of BCs to their delivery to the laboratory, and the average time to Gram staining of positive BCs (Buehler, 2016).

The rate of positivity is approximately 1/3 in applications due to reasons such as localization of the infection, for example not being taken at the right time, insufficient blood supply, or antibiotic treatment of the patient (Opota et al, 2015). When BC is positive, the first step is gram staining. This is essential for showing bacteria or fungi. If there is growth, the phenotype first indicates the etiology of the infection (Buehler, 2016).

Some protocols have been developed for rapid diagnosis and antibiotic susceptibility testing from bottles with growth with Gram staining. Among these, direct identification with commercial biochemical panels, detection of some bacteria-specific enzymes such as coagulase, direct identification with antibody tests/probe hybridization, microorganism identification with protein-nucleic acid fluorescence in situ hybridization (PNA-FISH) can be made. Polymerase Chain Reaction (PCR) tests and the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), which has contributed greatly to routine microbiology in recent years. Amplification methods do not yet seem suitable for routine use due to trained staff, special equipment, and (Opota et al, 2015). The clinical significance of positive BC has been studied for the past 30 years. These studies helped to identify the most common microorganisms, identify sources of infection, and determine mortality factors in true bacteremia (Pien et al, 2010).

Positive Culture Results: The standard incubation period is 7 days. Microorganisms frequently seen in routine microbiology laboratories are detected during this period. A longer incubation time is required in case of dimorphic fungi and Legionella, Brucella, Bartonella or Nocardia. In mycobacteria, the BC incubation period is 4 weeks (Kirn and Weinstein, 2013). When contamination is excluded, the agent detected and determined in the blood culture is probably present in the blood at the time of sampling and this is called bacteremia or fungemia. Bacteremia or fungemia can be temporary or permanent. When the majority or all of the BC sets taken by the peripheral venous route are positive for the same microorganism, it is highly likely that true bacteremia occurs, regardless of the identity of the bacteria (Kirn and Weinstein, 2013). In transient bacteremia, a positive BC is obtained once because the microorganism is present in the blood for a short time (≤ 30 min). It usually occurs with contaminated mucosa or invasive respiratory, gastrointestinal, and urogenital intervention. Detection of many positivity in bottles taken from different times is an indicator of permanent bacteremia or fungemia. Persistently positive BCs are encountered in the presence of endovascular infections such as endocarditis (Opota et al, 2015).

2.11. Blood Culture Isolates

The most important in distinguishing true bacteremia from contamination is typing (Hall and Lymann, 2006; Novak-Weekley, 2006). *S aureus, S pneumonia, Enterobacteriaceae, P aeruginosa, and C albicans* are always indicative of true bacteremia. Viridans group Streptococci, voagulase negative Staphylococci *(*CoNS*)* and Enterococci are involved in true bacteremia 38%, 15% and 78%, respectively (Hall and Lyman, 2006; Kirn and Weinstein, 2013). Most routine manual and automatic BC systems allow the growth of yeasts such as candida. However, if there is a high suspicion of fungemia but routine BCs are negative, alternative methods should be CoNS considered. For example, lysis centrifugation is one of the preferred methods (Kirn and Weistein, 2013).

2.12. Contaminants

It is the isolation of a microorganism that is not pathogenic for the patient, that is not found in the patient's blood when blood is taken, and that contaminates the BC during sample collection or processing. Contamination should be below 3% (Roque et al, 2012). 6-21% of true bacteremias in high-risk group patients are polymicrobial (Hall and Lyman, 2006). Approximately half of all positive BCs are contaminated (Mylotte and Tayara, 2000). There are different sources of contamination: patient skin, tools used to take samples and fill the bottle, sampling hygiene, or environmental conditions. Bacterial concentration in the skin is between 10^3-10^6 CFU / ml in the forearm and groin area. 80% of the skin flora is temporary and superficial. The deep layer (20%) CoNSisting of sebaceous glands and hair follicles is permanent, unlike the skin flora. The vast majority of the skin flora is Gr (+) and Gr (-) aerobes and can be removed with disinfection and antisepsis (Lee and Dean, 2010).

In contaminant microorganisms such as CoNS, *Corynebacterium*, and *micrococci*, true bacteremia is differentiated according to the number of positive BCs (Kirn and Weinstein, 2013; Lamy et al, 2016). CoNS is the most common BC contaminant; It is 70-80% of all contaminants (Hall and Lyman, 2006). It is also an important cause of bacteremia in patients with implanted devices and catheters (Kirn and Weinstein, 2013). Corynebacterium spp., Bacillus spp. *Bacillus anthracis*, *Propionibacterium acnes*, Micrococci species, Viridans group Streptococci, Enterococci, show significant patient contamination (Hall, 2006).

Contamination is not always easily demonstrated. Factors causing contamination in immunocompromised patients or foreign body implantation (prosthesis, heart valves, catheters) may be the real factors. Therefore, repeated BCs and additional clinical evaluations are required to distinguish true positive BC results from false (+) culture results (Sturmann et al,1996).

Many criteria are used to distinguish between contamination and true bacteremia and to make a positive result clinically significant. These are organism typing, number of positive sets, number of positive bottles in the set, place of culture, quality of growth, and clinical and laboratory data (Hall and Lyman, 2006; NHS UK,

2018). False-(+) BCs result in unnecessary use of antibiotics, excessive laboratory testing, and increased length of hospital stay, additional hospital fees, and unnecessary treatment (Dargere et al, 2014; Shapiro and Jones, 2018). Many methods have been developed to reduce contamination of BCs; Peripheral venous collection protocols, antiseptic preparations, and trained phlebotomist, BC kits for BC collection.

2.13. Blood Culture and Mortality

Determining the microorganisms that grow as soon as possible and showing whether they are causative or contaminant, and directing the treatment correctly by performing antibiotic susceptibility tests of the microorganism that is CoNSidered to be the agent are very important in reducing mortality and morbidity. Early diagnosis and treatment of patients with severe sepsis is important. Prognostic factors of BCs studied late are important for early targeted therapy. Prognostic factors of BCs are important for early targeted therapy. Because late diagnosis and deterioration in clinical condition worsen patient outcome. Early diagnosis and appropriate antibiotherapy reduce the hospital mortality rate due to septic shock from 80% to 20-30%. The clinician should immediately evaluate bacteremic patients with BC results. Delay in treatment of bacteremia is fatal (Stoneking et al, 2013).

2.14. Blood Culture and Cost-Compatibility

Over the past few years, high standards of care have increased, avoiding unnecessary and repetitive testing. In 2012, American Board of Internal Medicine (ABIM) introduced the "deliberate selection campaign" and decisions were made to reduce medical waste and excessive use (Linsenmeyer et al, 2016). In this campaign, deciding on which patient it is appropriate to take a BC has been highlighted. Studies evaluating risk factors for bacteremia have led to the development of many classification systems (Mylotte and Tayara, 2010). The routine use of BC in the ward by anticipating infection in patients is a pragmatic approach. Changing the clinical approach to the standard, evidence-based approach instead of the clinician's decision

may result in a significant reduction in the number of cultures were taken and patient fees. Additionally, high-risk patients can be identified who would benefit from early aggressive therapy (Shapiro and Jones, 2018). Approximately 22.4% of clinicians continue empirical antibiotic therapy despite the culture results (Rogue et al, 2012).

False-(+) BC results result in a 20% increase in hospital charges. Unnecessary BC intake causes expenditure on health resources and waste of health workers' time (Rogue et al, 2012; Pawlowicz and Jones, 2015). With false (+) BCs, hospitalization may prolong 5.4 days (Rogue et al, 2012; Armstrong-Briley et al, 2015). Many studies state that improvement in contamination rate is achieved by training of employees, experienced phlebotomists, preference of peripheral venous route instead of blood sampling from the catheter, application of appropriate collection techniques, replacement of skin antiseptic solutions, standardization or sterilization of collection kits, and compliance with laboratory protocols that limit the study of possible contaminated cultures (Posillico et al, 2018).

Costs can be reduced and unnecessary antibiotic treatments can be avoided by identifying patients with a low likelihood of bacteremia for whom BC is unnecessary (Chase et al, 2012; Eliakim-Raz et al, 2015). The lack of limited use of BCs creates a significant financial burden, wastes healthcare professionals' time, causes unnecessary injections in patients, and poses a risk to healthcare professionals.

2.15. Antimicrobial Resistance

The prevalence of antibiotic-resistant pathogens in the hospital and the community and the identification of risk factors for infections caused by them are helpful in choosing antibiotics (Chase et al, 2012). Hospitalized, long-term care patients and those receiving close-term antibiotics may not be treated with empirical antibiotic treatment. Comorbidities of these patients also affect the effective use of antibiotics and patient outcomes. When prescribing empirical antibiotics, the drug history should be reviewed. The most important is "not to start antibiotics, but to start the right antibiotic early" (Talan and Yealy, 2019). Empirical antibiotic treatment is selected based on clinical and epidemiological data and is started immediately after

BC sampling. However, especially with the increase of microorganisms with the development of multidrug resistance, its suitability cannot be guaranteed until the microbiological result is known (Opota et al, 2015).

Blood cultures should be taken accurately and on time to monitor antibiotic therapy against Gr (-) bacteria, MRSA, VRE, and other resistant organisms in which multi-drug resistance develops, and to reduce the use of broad-spectrum antibiotics. With the development of automated, continuous monitoring BC systems, pathogens (*S aureus*, *Gr (-)* Bacil, Streptococcus) were detected earlier and better typed (NHS UK, 2018). Despite the criteria that distinguish contamination and true positive blood cultures, many clinicians treat patients with broad-spectrum intravenous antibiotics, even if BCs result in contamination (Pawlowicz et al, 2015). The economic and clinical effects of antibiotic resistance are increasing, and the focus is on antibiotic management to reduce inappropriate antibiotic prescription. The decision to prescribe antibiotics should be taken in conjunction with a clinical evaluation, which includes but is not limited to the BC result, with extensive diagnosis (Shallcross et al, 2016).

CHAPTER THREE

3. MATERIAL AND METHOD

3.1. Patient Groups and Ethics

This study was carried out with BC samples taken from patients who were followed up between January 1, 2016 and December 31, 2020 at the Near East University Hospital. All samples sent to the microbiology laboratory were evaluated retrospectively. Ethics committee approval was obtained from Near East University Faculty of Medicine Ethics Committee with NEU-2021/88 dated 25.02.2021.

3.2. Collection of Blood Cultures

Blood cultures received in sets in accordance with the transport rules were included in the study. Among the blood samples received in sets, more than one BC sets belonging to the same patient were also evaluated. No intervention was made while taking BC from the services. Samples coming to the microbiology laboratory were evaluated in the routine workflow.

3.3. Preparation of Hemocultures Samples

Blood culture sets sent to the microbiology laboratory were recorded and placed in the BD BACTEC 9120 (Becton Dickinson, USA) device (Figure 3.1.). Hemoculture samples were evaluated in Bactec 9120 (Becton Dickinson USA) automated BC systems, which detect growth by signaling. Hemoculture samples were incubated for seven days, and kept for 21 days for samples with a pre-diagnosis of brucellosis and infective endocarditis.

Figure 3.1. BD BACTEC 9120 device (Becton Dickinson, USA) where blood culture sets are placed

In the seven-day incubation period, bottles with positive signals were removed from the device by recording their positivity times. After wiping the plastic caps with alcohol, 1-2 ml of blood-broth mixture was aspirated from the bottle with the help of a sterile syringe. A portion of the blood sample was taken and smear was prepared for Gram staining in a class 2 biosafety cabinet, and the remaining amount was subcultured to 5% sheep blood agar, EMB agar and Sabouraud dextrose agar and incubated overnight at 35 ° C in an aerobic environment. In addition, samples taken from anaerobe culture bottles with positive signals were added to 5% sheep blood agar, EMB agar and incubated in a jar with the gas package at 35 ° C for 48-72 h. Microorganisms grown after 24 h were identified by conventional methods. Unidentified microorganisms were identified with the BD Phoenix (Becton-Dickinson) and Vitek (Biomerieux, France) bacteria identification system.

At the end of the incubation period, the bottles that did not receive a reproductive signal were terminated as "negative" by the device, and 1-2 ml of blood-

broth mixture was taken from the bottles under aseptic conditions, 5% sheep blood agar subcultures were made in a class 2 biosafety cabinet and simultaneous Gram smear preparations were examined.

3.4. Media Used for Subcultures

3.4.1. Preparation of blood agar (Oxoid / UK)

The powder medium was dissolved in distilled water to a concentration of 40.0 g / l. It was sterilized in autoclave at 121 °C for 15 min. At the exit of the autoclave, it was cooled to 45-50 °C, 7% defibrinated sheep blood was added and mixed, and 15-20 ml was poured into petri dishes.

3.4.2. Preparation of eosin methylene blue agar (EMB) (Oxoid / UK)

The powder medium was dissolved in distilled water to a concentration of 37.5 g / l. It was sterilized in autoclave at 121 °C for 15 min. At the autoclave outlet, it was cooled to 45-50 °C and 15-20 ml was poured into petri dishes.

3.4.3. Preparation of sabouraud dextrose agar (SDA) (BD / USA)

The powder medium was dissolved in distilled water to a concentration of 65 g / l. It was sterilized in autoclave at 121 °C for 15 min. At the autoclave outlet, it was cooled to 45-50 °C and 15-20 ml was poured into petri dishes.

3.5. Identification of Bacteria and Antibiogram Tests

Traditional tests, bacteria identification and antibiotic susceptibility test panels of Phoenix (Becton Dickinson, USA) and Vitek (Biomerieux, France) automated systems were used for the identification of bacteria grown.

3.5.1. Gram stain

Smear was made from all positive signaling, non-growth flasks and colonies in subcultures. Air-dried smears were determined by passing through the burner flame two or three times and the following dyeing procedure was applied;

Crystal violet dropwise, 30 s. it was waited and the preparation was washed with water. Lugol dropped, 30 s. it was waited and washed with water. The acetone is decolorized with alcohol and washed with water. Safranin was dropped dropwise, left for 30 s and rinsed with water. It was dried by pressing lightly between the blotter paper and examined by dropping immersion oil with an objective of 100 magnification.

3.5.2. In vitro coagulase test

In vitro coagulase test (Plasmatec, UK) was performed on colonies whose gram-stained preparations and colony morphologies were compatible with staphylococci and whose catalase test was positive. From the staphylococcus colonies to be examined in a sterile and capped test tube containing 0.5 mL of plasma, they were taken with 3-4 loops and homogenized. The coagulase test was evaluated as positive when there was a loose fibrin network formation or complete coagulation in the tube

3.5.3. Catalase test

Catalase test (Merck, Germany) was carried out on colonies with Gr (+) cocci in gram-stained preparations and producing Gr (+) coc in their subcultures. 3-4 pure colonies grown in solid media were transferred to a clean slide with a loop and a drop of 3% H_2O_2 was dropped on it. Foaming interpreted as a positive catalase test.

3.5.4. Oxidase

Colonies with gram smears and colony morphology compatible with Gr (-) bacteria were tested for oxidase (Oxoid, UK). The oxidase test is based on the demonstration of cytochrome oxidase enzyme production. Some bacteria have cytochrome oxidase or indophenol oxidase, an iron-containing hemoprotein. These enzymes catalyze electron transport from donor compounds such as NADH to an

electron acceptor (usually oxygen). The colonies to be examined from fresh culture were taken with 3-4 loops and rubbed on blotter paper and 1-2 drops of oxidase reagent (p-phenylene diamine dihydrochloride) were dropped on it. It was observed whether the color turned blue-purple within 10 s. In the event of a blue-purple color, the test was interpreted as positive.

3.5.5. Phoenix and Vitek bacteria identification panels

In the Phoenix system (BD, USA), bacteria identification (ID) and antibiotic susceptibility tests (AST) are located on the same panel. The panels contain 136 microwells. 51 of these wells are for identification purposes. Identification wells contain dried biochemical substrates and 2 fluorescent control wells. AST includes 84 wells containing lyophilized antimicrobial agent and 1 growth control well. Incubation is done at 35 °C in the device. During the incubation period, the device monitors the panels periodically. Biochemical reactions or turbidity that occur in the wells are automatically tested every 20 minutes and data for that agent is collected in the processor of the system. After the reactions are completed, the bacteria are defined at the genus and species level by comparing them with the information in the database. NMIC / ID-99 panel was used for the identification of Gr (-) bacteria and antibiotic sensitivity, and the PMIC / ID-101 panel was used for Gr (+) bacteria.

In the Vitek 2 system (Biomerieux, France), there are four different colorimetric cards to identify Gr (-) fermentative and non-fermentative bacilli, Gr (+) cocci and bacilli, yeasts, and Gr (+) spore bacilli. Reagent cards each have 64 wells. For bacterial identification, a sufficient number of colonies from pure culture was taken and 0.50 McFarland value was set in 3 ml sterile saline. Suspension tube and ID card were placed in the cassette. For the antibiogram, an AST tube containing 3 ml of sterile saline was added from the ID tube, and 145 µl for Gr (-) bacteria, 280 µl for Gr (+) bacteria, and the AST card was placed in the cassette.

3.6. Evaluation of Results

Growth and growth times of isolates grown in aerobic and anaerobic BC bottles were compared. The bacteria found in the blood samples were evaluated in the light of the clinical information of the patients in the LIS system and other laboratory data.

- Real Positivity; If the patient's clinical and laboratory findings were compatible with bacteremia, a single agent not belonging to the skin flora had grown, CNS growth was detected in two or more BC bottles, or if bacteremia was CoNSidered clinically and laboratory, the bacteria detected in a single culture were CoNSidered as pathogen.
- Contamination; If the clinical and laboratory findings of the patient do not support bacteremia, if CNS was isolated from a single BC bottle or two different CNS strains with different antibiotic patterns were isolated from more than one bottle taken within 24 h without clinical and laboratory findings supporting bacteremia, if three or more different bacterial growth was detected evaluated as contaminant.
- False (+); Although the culture antibiogram device gives a positive signal, no growth was detected in subcultures and no microorganisms were observed in Gram-stained preparations.
- False (-); Detection of bacterial growth in subcultures made after the 7-day incubation of the samples in the culture antibiogram device and the "negative" signal of the device and the detection of microorganisms in the Gram-stained preparations.
- True (-); After 7 days of incubation in the BACTEC 9240 device, no bacterial growth was detected in subcultures made from bottles with negative signals and no microorganisms were observed in Gram-stained preparations.

Clinical branch and demographic information were obtained from the files of the patients. The presence or absence of growth in the BC results of the patients whose BC was taken and if there was growth, the type of the causative microorganism was reported. Microorganisms grown in BCs were detected. The distribution of BCs with reproduction is classified as internal sciences, surgical sciences and intensive care unit. Changes in reproduction rates in BCs by years were examined.

3.7. Statistical Analysis

Statistical analyses were carried out through the Statistical Package for the Social Sciences (version 22.0, SPSS Inc., Chicago, IL, USA) program. As descriptive statistics; Number (n) and percentage (%) were used in the evaluation of categorical variables. Statistical power analysis of the sample number was done by Student's t-test. Presence of microorganisms (fungal, Gr (+) and Gr (-) bacterial growth) according to blood culture results; Correlations between sex, age and year were analyzed using the Pearson chi-square test. $p < 0.05$ was CoNSidered significant.

CHAPTER FOUR

4. RESULTS

4.1. Demographic Data

The number of blood cultures coming to the microbiology laboratory between 2016 and 2020 was 7866. Distribution of blood culture samples by gender was found as 3276 (41.6%) for women and 4590 (58.4%) for men (Figure 4.1.).

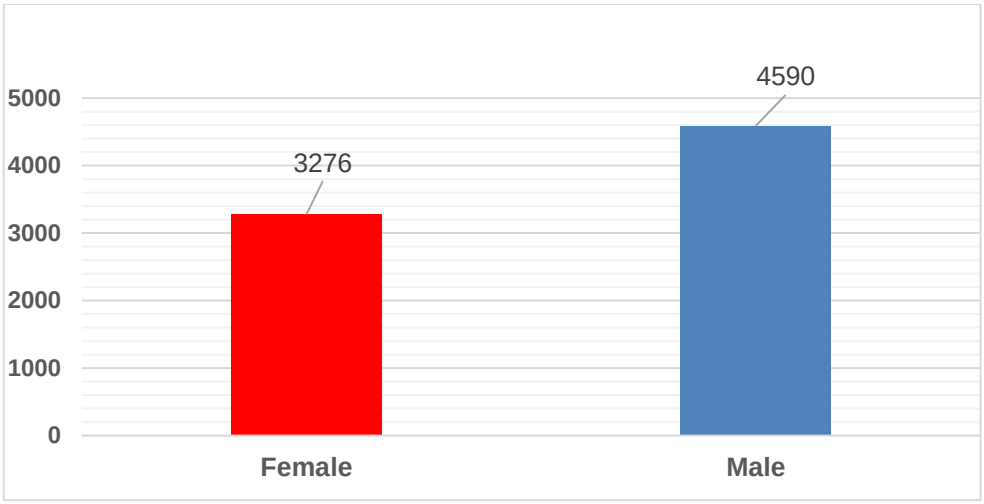

Figure 4.1. Distribution of patients who applied to the NEU Hospital between January 1, 2016 and December 31, 2020 and whose blood culture samples were sent to the microbiology laboratory by gender.

When the age distribution of the patients is analysed; The number of patients in the range of < 20, 21-40, 40-60 and over (>) 60 years, respectively 819 (10.5 %), 732 (9.3 %), 1317 (16.7 %) and 4998 (63.5 %) (Figure 4.2.).

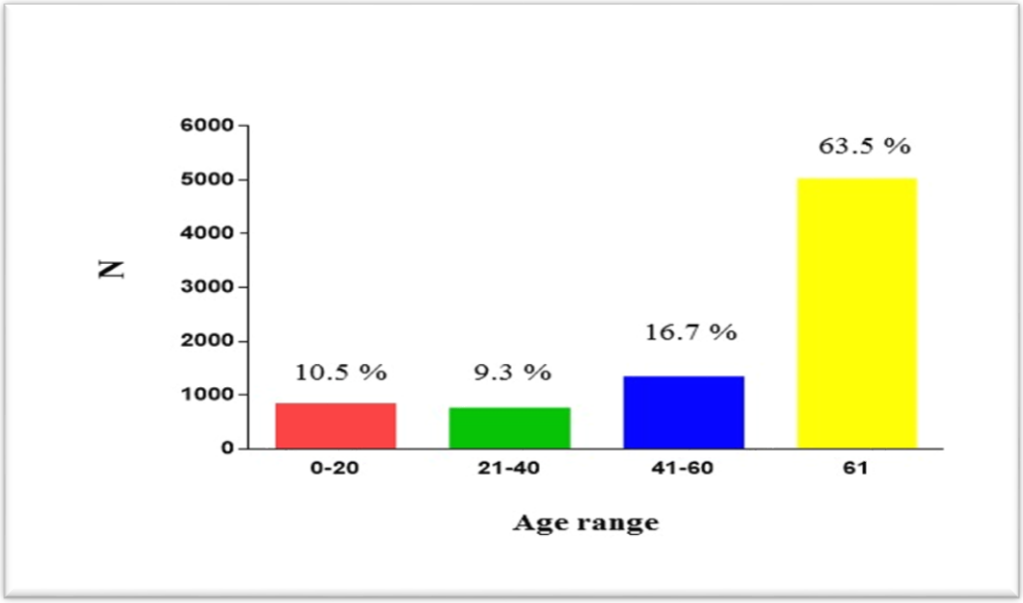

Figure 4.2. Distribution of patients who applied to the NEU Hospital between January 1, 2016 and December 31, 2020 and whose blood culture samples were sent to the microbiology laboratory by age.

4.2. Blood Culture Results

While growth rates were not detected in 6531 (83 %) blood culture samples followed in the microbiology laboratory, it was observed that there was growth in 1335 (17 %) samples (Figure 4.3.).

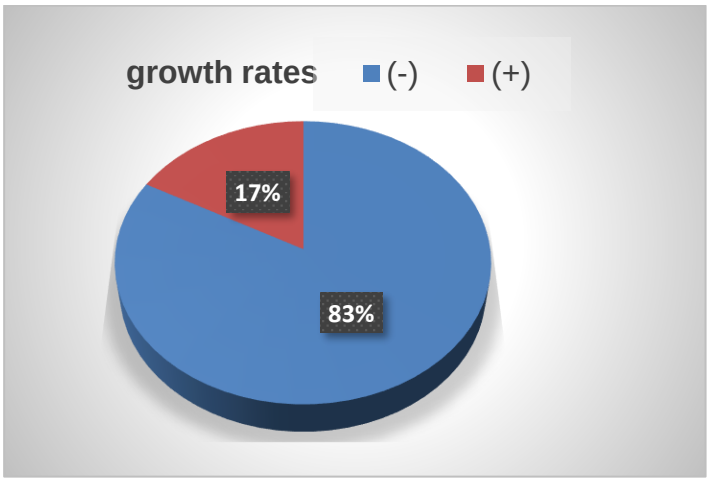

Figure 4.3. Growth rates of microorganisms grown in blood culture.

When the presence of microorganisms in blood culture samples examined in the microbiology laboratory was examined by years, it was determined that the most samples were evaluated in 2017 (n = 1825, 23.2%) and the least samples was evaluated in 2016 (n = 1176, 15%; Table 4.1.).

Table 4.1. Presence of microorganisms by years in blood culture samples examined in the microbiology laboratory.

			2016	2017	2018	2019	2020
Growth	-	**n**	990	1545	1429	1275	1292
		%	84.2	84.7	84.0	78.6	83.8
	+	**n**	186	280	272	347	250
		%	15.8	15.3	16.0	21.4	16.2
Total		**n**	1176	1825	1701	1622	1542
		%	100	100	100	100	100

The distribution of blood culture samples sent to the microbiology laboratory according to the type of microorganism is given in Table 4.2.

Table 4.2. Distribution of blood culture samples according to the type of microorganism they contain.

Microorganism type	N	%
A baumannii	33	2.5
Achromobacter spp.	6	0.4
Burkholderia cepacia	8	0.6
Candida spp.	4	0.3
Cedecea neteri	4	0.3
Citrobacter spp.	2	0.1
CoNS	788	59.0
E coli	105	7.9
E faecalis	43	3.2
Enterobacter aerogenes	12	0.9
Enterobacter cloacae	22	1.6
Enterococcus faecium	16	1.2
Klebsiella oxytoca	9	0.7
Klebsiella pneumoniae	82	6.1
Micrococcus spp.	2	0.1
Morganella morganii	1	0.1
P aeruginosa	54	4.0
Proteus mirabilis	13	1.0
S aureus	79	5.9
S marcescens	37	2.8
Stenotrophomonas maltophilia	4	0.3
Streptococcus spp.	11	0.8
Total	1335	100.0

Distribution of Gr (-) bacteria according to blood culture results is given in Table 4.3. According to our results, it was determined that the most common Gr (-) bacteria was *E coli* (n=105, 26.8%), while *morganella morganii* and Citrobacter spp. were the bacteria that grew the least (n=1, 0.3% and n= 2, 0.5% respectively).

Table 4.3. Distribution of gram-negative bacteria according to blood culture results.

Gr (-)	N	%
Enterobacter cloacae	22	5.6
Stenotrophomonas maltophilia	4	1.0
Klebsiella pneumonia	82	20.9
Citrobacter spp.	2	.5
Burkholderia cepacia	8	2.0
Acinetobacter baumanii	33	8.4
Proteus mirabilis	13	3.3
Morganella morganii	1	0.3
Enterobacter aerogenes	12	3.1
Klebsiella oxytoca	9	2.3
Achromobacter spp.	6	1.5
Cedecea neteri	4	1.0
Pseudomonas aeruginosa	54	13.8
Escherichia coli	105	26.8
Serratia marcescens	37	9.4
Total	392	100.0

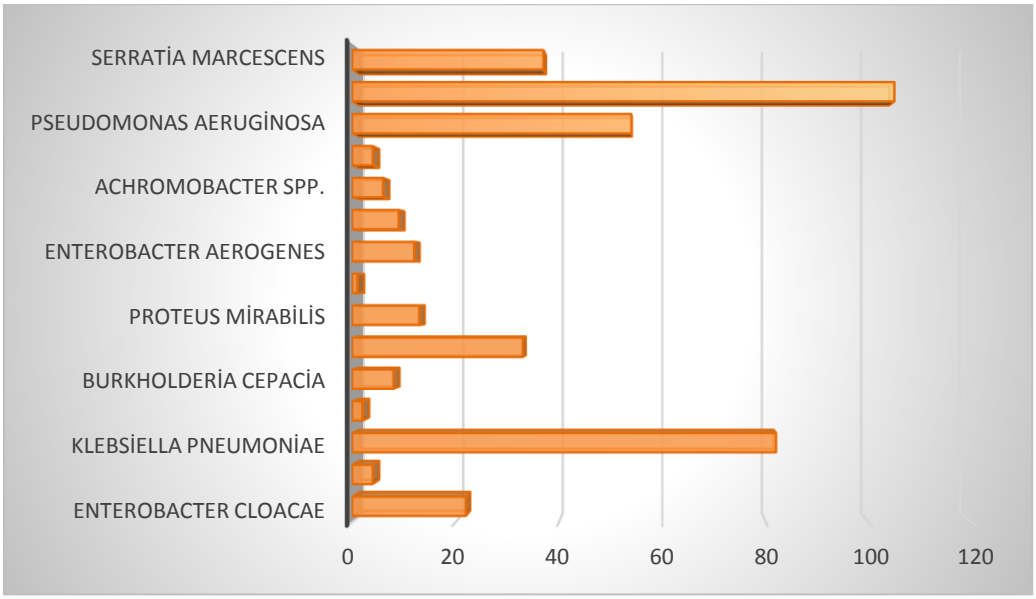

Figure 4.4. Presence gram-negative bacteria according to blood culture results.

Distribution of Gr (-) enteric bacteria according to blood culture results is given in Table 4.4. A total of 287 Gr (-) enteric bacteria were detected in blood culture samples, of which 36.6% were found to be *E coli*.

Table 4.4. Distribution of gram-negative enteric bacteria according to blood culture results.

Gr (-) Enteric	**N**	**%**
Enterobacter cloacae	22	7.7
Klebsiella pneumonia	82	28.6
Citrobacter spp.	2	0.7
Proteus mirabilis	13	4.5
Morganella morganii	1	0.3
Enterobacter aerogenes	12	4.2
Klebsiella oxytoca	9	3.1
Cedecea neteri	4	1.4
Escherichia coli	105	36.6
Serratia marcescens	37	12.9
Total	287	100.0

Distribution of Gr (+) bacteria according to blood culture results is given in Table 4.5. A total of 939 Gr (+) bacteria were detected in blood culture samples, of which 83.9% were found to be CoNS.

Table 4.5. Distribution of gram-positive bacteria according to blood culture results.

Gr (+)	N	%
S aureus	79	8.4
Micrococcus spp.	2	0.2
E faecium	16	1.7
Streptococcus *spp.*	11	1.2
CoNS	788	83.9
E faecalis	43	4.6
Total	939	100.0

The growth rates of blood culture samples sent to the microbiology laboratory by years are given in Figure 4.5.

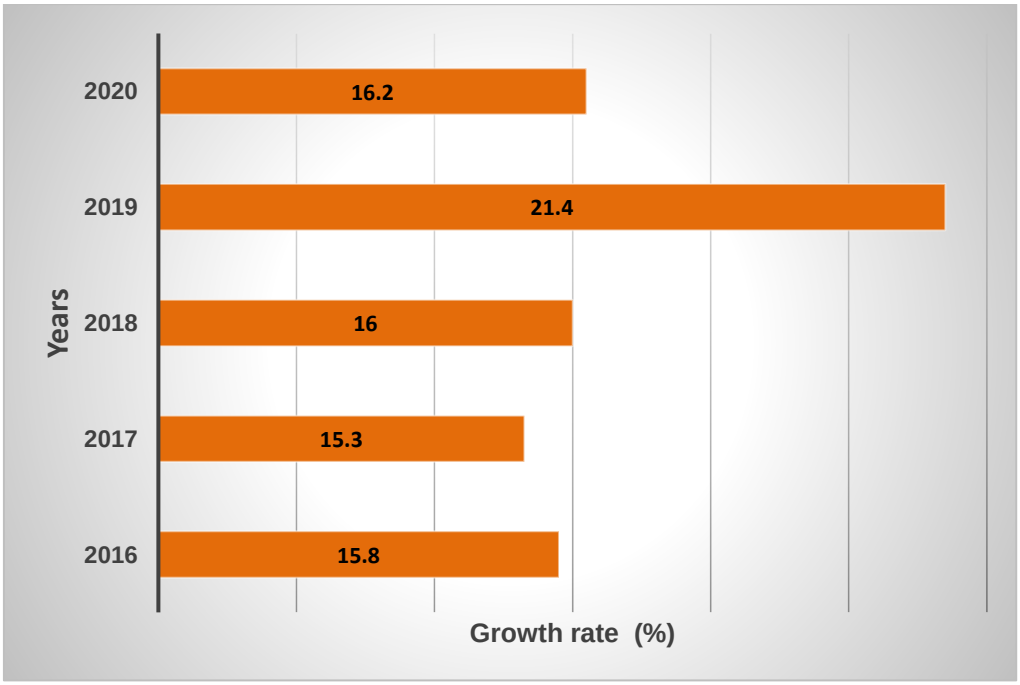

Figure 4.5. Growth rates in blood culture samples by years

When the reproduction rates in blood culture samples were examined by years, it was determined that there was a significant increase in 2019 ($p < 0.05$).

The distribution of blood culture samples sent to the microbiology laboratory by clinic is given in Table 4.6.

Table 4.6. Distribution of blood culture samples sent to the microbiology laboratory according to the clinics

Department	N	%
Emergency	116	1.5
Neurology	237	3.0
Oncology	360	4.6
Orthopedics and Traumatology	53	0.7
Anesthesia and Reanimation	904	11.5
Urology	61	0.8
Obstetrics and Gynecology	28	0.4
Dermatology	5	0.1
Gastroenterology	65	0.8
Infectious Diseases	1117	14.2
General Surgery	74	0.9
Plastic Surgery	3	0.0
Nephrology	6	0.1
Otolaryngology	3	0.0
Cardiology	1826	23.2
Chest Diseases and Allergy	1027	13.1
Child Health and Diseases	569	7.2
Internal Medicine	816	10.4
Geriatrics	116	1.5
Dialysis	63	0.8
Neurosurgery	417	5.3
Total	7866	100,0

It has been determined that the top three departments that send the most blood culture samples to the microbiology laboratory are cardiology (n=1826, 23.2%),

infectious diseases (n=1117; 14.2%) and anesthesia and reanimation (n=904; 11.5%) respectively.

When the frequency of ESBL from Gr (-) bacteria in blood cultures was examined, 96 (33.4%) out of 287 cultures were found to be ESBL (+) while 191 (66.6%) were ESLB negative (Table 4.7.).

Table 4.7. The prevalence of ESBL, one of the Gr (-) bacteria, according to years

Years	**ESBL**		**Total**	***p***
	(+) n (%)	**(-) n (%)**		
2016	14 (14.6)	30 (15.7)	44 (15.3)	0.006
2017	29 (30.2)	37 (19.4)	66 (23)	
2018	22 (22.9)	27 (14.1)	49 (17.1)	
2019	24 (25)	55 (28.8)	79 (27.5)	
2020	7 (7.3)	42 (22)	49 (17.1)	
Total	96 (100)	191 (100)	287 (100)	

When the relationship between the prevalence of ESBL in blood cultures and the years was evaluated, it was determined that the prevalence of ESBL was highest in 2017 and the lowest in 2020. When the frequency of ESBL prevalence was evaluated by years, it was determined that the decrease in ESBL prevalence in 2020 was statistically significant compared to other years (2016-2019). It was determined that the incidence of ESBL reproducing in blood cultures decreased significantly in 2020 compared to other years (Figure 4.10).

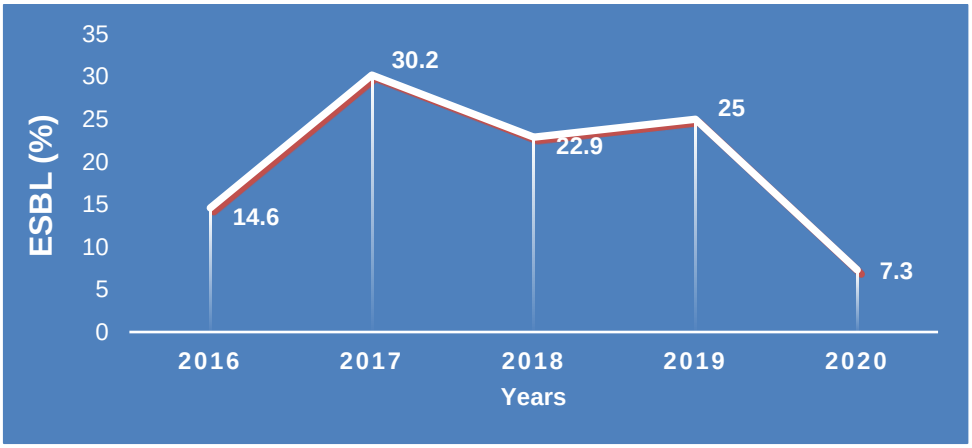

Figure 4.6. *ESBL* prevalence by years

When the relationship between the prevalence of *P aeruginosa* in blood cultures and the years was evaluated, it was determined that the prevalence of *P aeruginosa* was highest in 2018 and the lowest in 2016 and 2020 (Table 4.8.).

Table 4.8. The prevalence of *P aeruginosa* according to years

Years	***P. aeruginosa* (+)**	
	n	**(%)**
2016	6	11.1
2017	12	22.2
2018	18	33.3
2019	12	22.2
2020	6	11.1
Total	54	100

It was determined that the incidence of *P aeruginosa* reproducing in blood cultures decreased significantly in 2020 compared to 2017, 2018 and 2019 years (Figure 4.10).

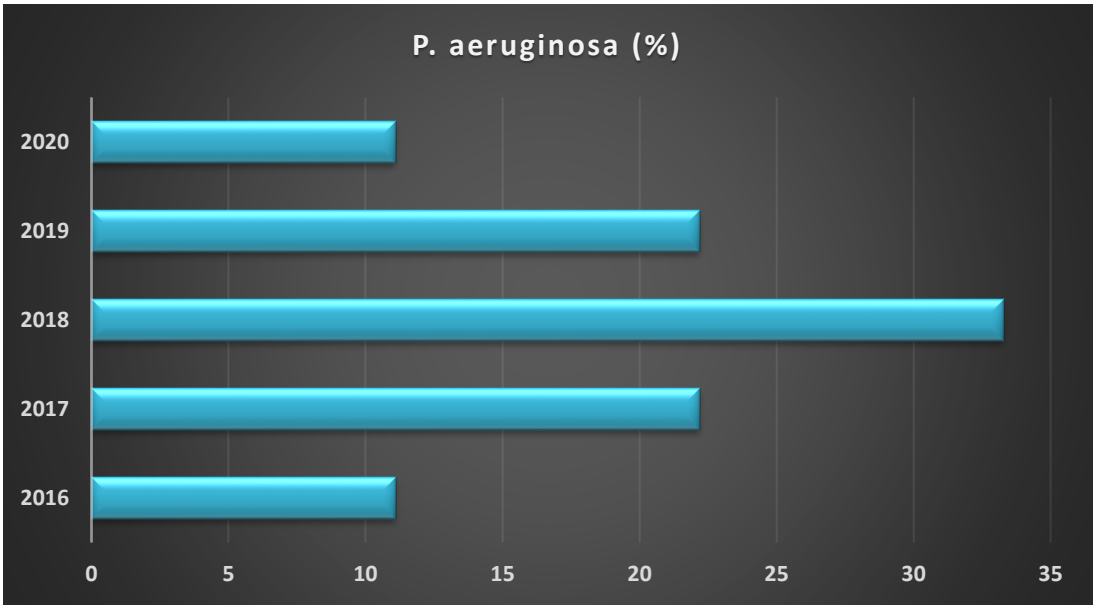

Figure 4.7. *P aeruginosa* prevalence by years

When the antimicrobial resistance profile of *P. aeruginosa* was examined, it was determined that it was most sensitive to amikacin (98.1 %) and most resistant to cefepime (36.5 %) (Figure 4.8.).

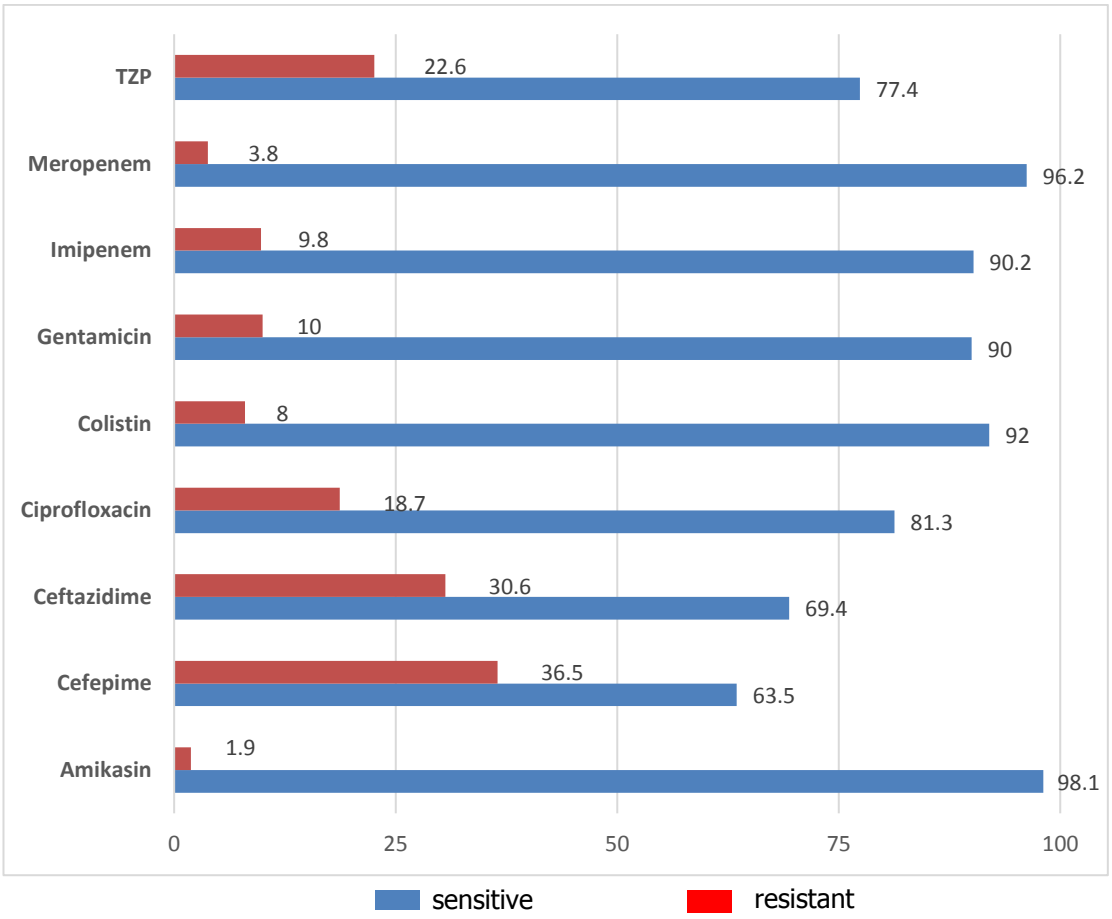

Figure 4.8. Antimicrobial resistance profile of *P aeruginosa*

When the relationship between the prevalence of *A baumannii* in blood cultures and the years was evaluated, it was determined that the prevalence of *A. baumannii* was highest in 2019 and the lowest in 2018 (Table 4.9.).

Table 4.9. The prevalence of *A baumannii* according to years

Years	***A baumannii* (+)**	
	n	**(%)**
2016	3	9.1
2017	10	30.3

2018	2	6.1
2019	12	36.4
2020	6	18.2
Total	33	100

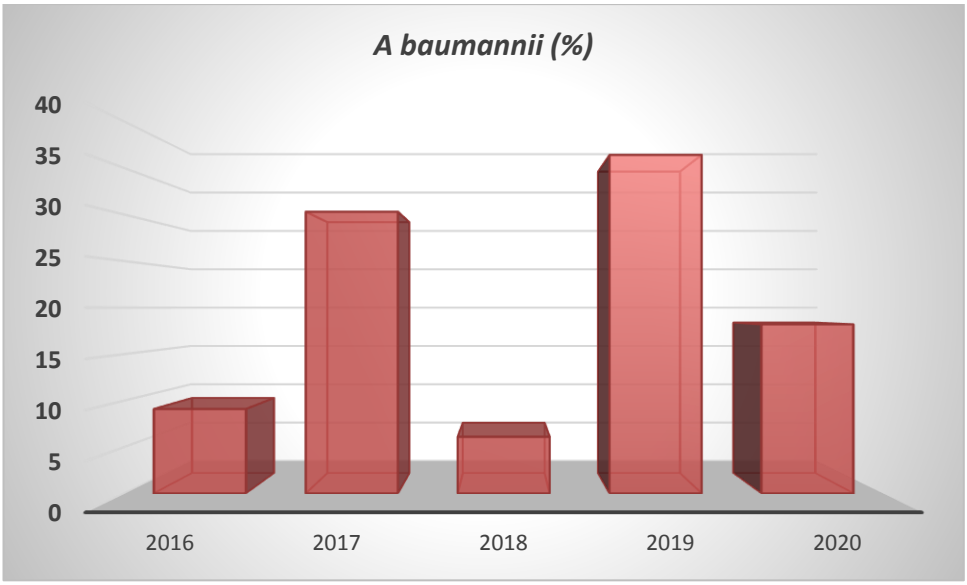

Figure 4.9. *A baumannii* prevalence by years

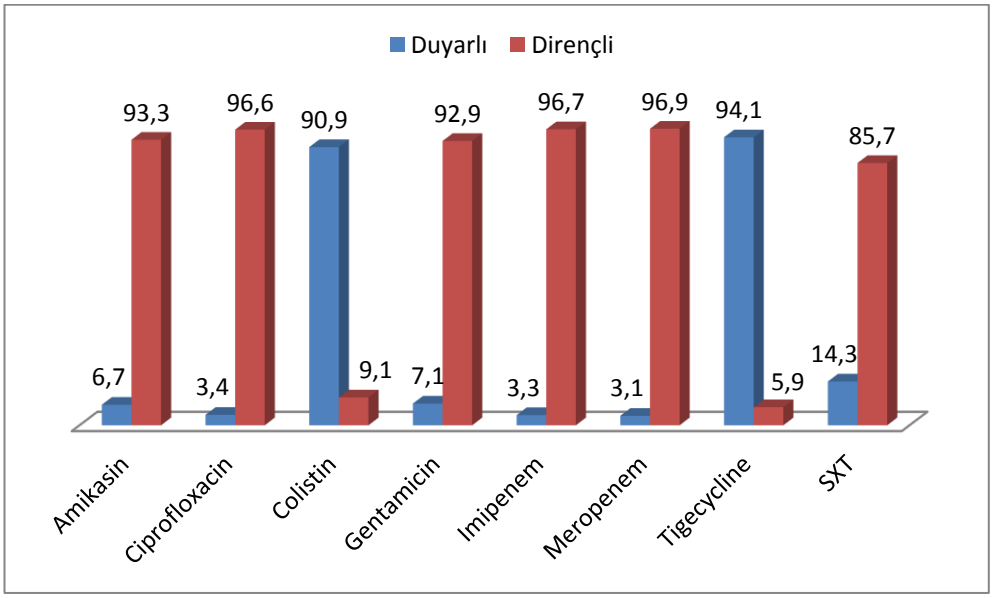

Figure 4.10. *A baumannii* antibiotic resistance pattern

The distribution of Gr (+) bacteriae in blood cultures is given in Table 4.10. It was determined that the most common Gr (+) bacteria in blood cultures were CoNS and the least grown bacteria was Micrococcus spp. (Table 4.10.).

Table 4.10. The distribution of Gr (+) bacteriae in blood cultures.

Gr (+)	**N**	**%**
S aureus	79	8.4
Micrococcus spp.	2	0.2
Enterococcus faecium	16	1.7
Streptococcus spp.	11	1.2
CoNS	788	83.9
Enterococcus faecalis	43	4.6
Total	939	100.0

When the growth rates of *S. aureus*, a Gr (+) bacterium, are compared in blood culture, it was found that it reproduced the most in 2018 and at least 2019 (Table 4.11).

Table 4.11. The prevalence of *S aureus* according to years.

Years	***S. aureus* (+)**	
	n	**(%)**
2016	18	22.8
2017	13	16.5
2018	27	34.2
2019	9	11.4
2020	12	15.2
Total	79	100

When the growth rates of MRSA, a Gr (+) bacterium, are compared in blood culture, it was found that it reproduced the most in 2018 and at least 2019 (Table 4.12).

Table 4.12. The prevalence of MRSA according to years

Years	MRSA **(+)**	
	n	**(%)**
2016	6	25
2017	5	20.8
2018	3	12.6
2019	5	20.8
2020	5	20.8
Total	24	100

Table 4.13. Microorganism growth rates in the cardiology clinic by years

			Years					
			2018	2019	2020	2016	2017	Total
Growth	**-**	**n**	367	296	311	193	369	1536
		%	87.4%	76.9%	84.1%	89.4%	84.8%	84.1%
	+	**n**	53	89	59	23	66	290
		%	12.6%	23.1%	15.9%	10.6%	15.2%	15.9%
Total		**n**	420	385	370	216	435	1826
		%	100%	100%	100%	100%	100%	100%

CHAPTER FIVE

5. DISCUSSION

Circulatory system infections are a clinical picture with high mortality and morbidity, and mortality rates decrease when early diagnosis is made and treated. With the rapid diagnosis of bacteremia and fungemia, determining the possible agent and its sensitivity to antimicrobials and arranging the necessary treatment is important in terms of survival (Obara et al, 2011).

Blood cultures are the gold standard in the diagnosis of sepsis, although results are obtained in a relatively long time (Bloos et al, 2012). For nearly 50 years, continuously monitored blood culture systems have been used in many clinical microbiology laboratories for early detection of the presence of microorganisms in the blood.

Samples are kept in the system for up to seven days as long as the device does not give a signal (Tsalik et al, 2010). Identification of pathogens from reproductive blood culture and performing antibiotic sensitivity tests takes approximately 24-72 h (Hettwer et al, 2011). Because of this delay, clinicians often make the diagnosis of sepsis according to clinical symptoms and start antibiotic treatment according to the clinical situation. According to the Gram staining results, the clinician can direct the treatment. However, broad-spectrum empirical treatment causes an increase in mortality rate and antibiotic resistance (Wolk and Dunne, 2011).

Blood cultures are positive in approximately 30-40% of circulatory system infections (Klouche and Schröder, 2008; Towns et al, 2010). It was found that the positivity rate of 306 Bcs studied within 4 months in 2015 was 79 % and the contamination rate was 21 % (Aillet et al, 2018). In one of the studies, 3,890 sets of BCs were taken from 1,962 patients in 7 months. Of these, 541 (13.9 %) were positive for BC and 3.349 (86.1 %) were negative for BC (Nannan-Panday et al, 2019). The difference in the results is thought to be caused by the differences in the populations studied and the variation in the clinical conditions and age groups of the patients. In our study, this positivity rate was found to be 17 %. The area where the study was conducted was made only according to the results of the microbiology laboratory. No intervention was made during the taking of the samples. In our study, a high rate of 83 % was obtained as a negative BC result. It suggests that patients who are requested to have a BC should be evaluated more carefully by clinicians.

In the study of Boyles et al., 2.6 % of BCs are contamination (Boyles et al, 2015). Contamination in positive BCs in other studies the rate ranges from 9–61 % (Ehrenstein et al, 2005; Leyssene et al, 2011; Carmi et al, 2015; Aillet et al, 2018; Nannan-Panday et al, 2019). In positive BCs with CoNS, only one of the two samples taken from different places from a patient is CoNSidered to be a contamination. However, if both samples are positive, the causative agent can be reported as pathogen. In studies, CoNS CoNStitutes 15 % of positive BCs and 77 % of contaminants (Carmi et al, 2015; Aillet et al, 2018). In our study, 788 (83.9 %) of 939 BCs with Gr (+) growth were evaluated as CoNS. In 91 (11.5%) of 788 CoNS BCs, samples taken from both arms were found to be positive. BCs are usually taken by nurses in the hospital. In this study, no intervention was made regarding sampling. It is unknown how long it waited to be taken to the laboratory. Our hospital has instructions for taking a BC. I anticipate that by increasing compliance with this directive, our contamination rates can be reduced. If employees are informed about taking blood cultures, better results can be obtained with more professional practices.

In studies conducted, the average age of patients with positive BCs ranges from 58–68 (Kaoet al, 2011; van Walraven and Wong, 2014; Rannikko et al, 2017).

When the age distribution of the patients is analysed; The number of patients in the range of < 20, 21-40, 40-60 and over (>) 60 years, respectively 819 (10.5 %), 732 (9.3 %), 1317 (16.7 %) and 4998 (63.5 %). Age distributions of blood culture positive and negative groups were found to be statistically significant. ($p < 0.001$).

The results of our study are similar to other studies. In the study of Lucas et al. 1139 positive BC were evaluated. CoNS and *E coli* are the most common agents at 23.4 % and 18.1 % (El Lucas, 2017). In some studies, the most common Gr (-) bacteria isolated from BCs have been reported as *E coli, Enterobacter, Pseudomonas, Acinetobacter and Klebsiella* (Aube et al, 1991, Martin, 1991). In studies, the most common factors in positive BCs are Gr (-) bacteria, especially *E coli* at rates of 45 % and 48.2 % (Ramos et al, 2004; Kao et al, 2011; van Walraven and Wong, 2014; Boyles et al, 2015; Rannikko et al, 2017). In the study of Leyssene et al. 78.5 % of positive BCs contain pathogenic bacteria and the most frequently isolated microorganism is CoNS 27.5 % (Leyssene et al, 2011). Of the 1335 microorganisms

isolated from BCs in our hospital, 392 (29.3 %) were found as Gr (-) and 943 (70.6 %) as Gr (+). In the distribution of Gr (-) bacteria, 26.8% were *E coli,* 20.9 % *Klebsiella pneumoniae* 13.8 % *P aeruginosa*, 8.4 % *A baumanii* and 5.6 % *E cloacae*. In the distribution of Gr (+) bacteria, CoNS had the highest rate at 83.9 %. As shown in Table 4.1, when all the growing microorganisms are evaluated, similar rates are found in our study.

In our study; *E. coli* (26.8%) and Klebsiella spp. (20.9%) species were the most frequently isolated *Enterobacteriaceae* members, whereas bacteria of the genus *P aeruginosa* (13.8 %) and *A baumanii* (8.4 %) were determined from nonfermentative Gr (-) rods. ESBL production varies according to geographic region, hospital type and patient characteristics. Close to the island of Cyprus in terms of similarity to the geographic area countries, Turkey, Syria, Egypt has been viewed odds on this subject. In our study, when the frequency of ESBL from Gr (-) bacteria in BCs was examined, 96 (33.4 %) out of 287 BCs were found to be ESBL (+) while 191 (66.6 %) were ESLB (-). A study conducted in Turkey between the years 2014-2017 was found in 34 % of ESBL (+) (Bayraktar, 2019). In a study conducted in Latakia, Syria between 2014-2016, ESBL (+) was found to be 26 % (Baaity et al, 2017). In a study conducted in Egypt, the ESBL (+) rate was found to be 48.9 % (Abdallah et al, 2015). When the relationship between the prevalence of ESBL in BCs and the years was evaluated, it was determined that the prevalence of ESBL was highest in 2017 and the lowest in 2020. Although the rate of ESBL positivity seems to have decreased in our hospital in 2020, it should not be forgotten that it is associated with the decrease in the number of patients hospitalized due to the COVID-19 pandemic.

In a study in which a total of 21,367 BCs were examined, it was found that there was 20.5 % reproduction, 5.9 % of positive BCs had *P. aeruginosa* and 5.7% had *A. baumannii* growth. In this study conducted between 2013-2017, no change was found in the rate of *P aeruginosa*. However, it has been shown that there is a decrease in the *A baumannii* positivity rate. (Bolukçu, and Okay, 2021). NEU hospital results showed that; it was determined that the incidence of *P aeruginosa* reproducing in BCs decreased significantly in 2020 compared to 2017, 2018 and 2019 years. When the relationship between the prevalence of *A baumannii* in blood cultures and the years was evaluated, it was determined that the prevalence of *A baumannii* was highest in

2019 and the lowest in 2018. When Bolukçu's research and our study are compared; although the incidence of *P aeruginosa* was similar, there was no similarity in the incidence of *A. baumannii*.

The most common accompanying comorbidities in positive BCs are usually DM (15–28 %) and malignancy (5–15 %) (Wildi et al, 2011; Van Walraven and Wong, 2014; Boyles et al, 2015; Carmi et al, 2015; Rannikko et al, 2017). In the study of Rannikko et al., Cardiovascular diseases are the most common comorbidity at a rate of 35 % (Rannikko et al, 2017). BC request was mostly made from cardiology. The risk of infection increases due to invasive interventions performed on patients followed up in cardiology services. 1826 (23.2 %) of 7866 BCs included in the study were requested from the cardiology clinic. The positivity rate in blood cultures from the cardiology clinic was found to be 15.9%.

Although the mortality rates due to circulatory system infections vary from center to center, it is between 12-80 % and the average is around 35 % (Erbay et al, 2003; Paolucci et al, 2010). In the study of Jessen et al., the mortality of the patients was 1 % (Jessen et al, 2015). In other studies, the mortality rate in positive BCs ranges from 1.6-20.7% (Kao et al, 2011; Van Walraven and Wong, 2014; Rannikko et al, 2017; Aillet etal, 2018). In the studies of McCaig et al., 47.5 % of the patients who requested blood culture were discharged (McCaig et al, 2007). Discharge of patients with positive blood cultures in studies rate is 75% and 86% (Ramos et al, 2004; Carmi et al, 2015). In this study, the discharge and mortality rates of patients for whom blood culture was requested were not investigated. Since the aim of the study is to evaluate the blood culture samples coming to the microbiology laboratory, it can be CoNSidered as the lack of the study.

CHAPTER SIX

As a result, the type of bacteria isolated from blood cultures and their antibiotic susceptibility varies depending on different reasons. For this reason, determining the microorganisms and antibiotic susceptibility isolated from blood cultures at regular intervals in every hospital is both a guide to the clinician in empirical treatment and it is important in determining antibiotic usage policies.

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