Universitatea Transilvania din Brașov

INTERDISCIPLINARY DOCTORAL SCHOOL

Faculty of Medicine

Ioana FLUTURU

BACTERIOLOGICAL CHARACTERIZATION OF AACOUNTY EMERGENCY CLINICAL HOSPITAL, PREMISE OF THE CONTROL OF CIRCULATING GERMS. OPPORTUNITIES AND SOLUTIONS

SUMMARY

Scientific supervisor

Prof. PhD Prof. Codruța NEMET

BRAȘOV, 2024

CONTENT

List of abbreviation

Abbreviation	Description
ABR	Antibioresistance
ATCC	American type culture collection
ICU	Anesthesia Intensive Care
CAESAR	Antimicrobial resistance surveillance in Central Asia and Europe
CDC	Center for Infection Prevention and Control
CHAFEA	Consumer, Health, Agriculture and Food Executive Agency
CLSI	Clinical Laboratory Standards Institute
CPAP	Continuous positive air pressure
CRE	Enterobacteriaceae resistant to carbapenems
DALY	Disability-adjusted life years
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Center for Disease Prevention and Control
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESBL	Beta-lactamase-secreting Enterobacteriaceae
ESKAPE	*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa* și *Enterobacter*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
GAIHN	Global Action în Healthcare Network
GLASS	Global Antimicrobial Resistance and Utilization Surveillance System
HAI	Healthcare-associated infections
ITU	Urinary tract infections
MDR	Multi drug-resistant
MRSA	Methicillin-resistant *S. aureus*
OIE	World Organization for Animal Health
OMS	World Health Organization
PDR	Pan drug-resistant
PIB	Gross domestic product
AMR	Antimicrobial resistance
ReLAVRA	Latin American Antimicrobial Resistance Surveillance Network
SEE	European Economic Area
STAG-AMR	Strategic and Technical Advisory Group on Antimicrobial Resistance
SUA	United States of America
TATFAR	Transatlantic Task Force on Antimicrobial Resistance
UE	European Union
UTI	Intensive Care Unit

UNEP	United Nations Environment Programme
VRE	Vancomycin-resistant *Enterococcus spp*
VRSA	Vancomycin-resistant *S. aureus*
WPRACSS	Western Pacific Regional Antimicrobial Use Surveillance System
XDR	Extensively drug-resistant

ABSTRACT

Knowledge of germ circulation on hospital environmental elements, patients, medical staff, and auxiliary staff is very important in a healthcare facility due to the nosocomial risks it entails.
The study was conducted on 17 categories of biological products collected from 19,360 patients during the year 2020: urine cultures, purulent secretions, tracheo-bronchial aspirates, wound secretions, blood cultures, pharyngeal swabs, sputum, nasal swabs, cervical secretions, peritoneal fluids, conjunctival secretions, pleural fluids, bile fluids, cerebrospinal fluids, ear secretions, vaginal secretions, and joint fluids.

Of these, 3,750 were bacteriologically positive as follows: 1,133 bacterial strains of *Escherichia coli*, 888 strains of *Staphylococcus spp*., 864 strains of *Klebsiella spp*., 332 strains of *Pseudomonas spp*., 201 strains of *Acinetobacter spp*., 197 strains of *Enterococcus spp*., and 135 strains of *Proteus spp*.

Based on the bacteriologically positive biological products and the identified etiological agents, we reached the following conclusions: the potential invasive germs that predominate in the hospital are – 87.47%, antibiotic-sensitive bacteria – 80.96%, nosocomial origin microorganisms – 68.07% of the total isolated.

For a realistic bacteriological characterization, we grouped the 42 departments and units of a County Emergency Clinical Hospital into 4 study groups based on their activity profile, namely: the Intensive Care Unit, Clinical Surgical Departments, Clinical Medical Departments, and Clinical Oncology, Hematology, Radiotherapy Departments.

For the analysis of resistant germs, we used the WHONET software.

In the Intensive Care Unit, the proportion of germs in descending order is as follows: *Staphylococcus spp* – 31.74%, *Klebsiella spp* – 29.26%, *Escherichia coli* – 12.41%, *Acinetobacter spp* – 11.70%, *Pseudomonas spp* – 9.40%, *Enterococcus spp* – 3.46%, and *Proteus spp* – 2.04%.
All the germs studied were identified in different percentages in the following biological products: tracheo-bronchial aspirates – 31.47%, urine cultures – 16.05%, blood cultures – 15.87%, pharyngeal swabs – 15.51%, nasal swabs – 9.40%, wound secretions – 4.88%, purulent secretions – 4.08%.
Germs with an XDR resistance phenotype from this department hold supremacy at 48.01%, followed by microorganisms with an MDR resistance phenotype at 26.30%, and those with a PDR resistance phenotype at 25.69%.
Microbiological alerts with the highest prevalence are represented by those with low priority – 48.05%, followed by microbiological alerts with medium priority at 41.18%.
Of the total germs isolated in the Intensive Care Unit, 75.09% have invasive potential, being isolated from normally sterile sites, 79.32% are of hospital origin with nosocomial potential, and 71.28% show in vitro sensitivity to the action of antibiotics.

In surgical departments, the proportion of germs in descending order is: *Escherichia coli* – 34.34%, *Staphylococcus spp* – 24.16%, *Klebsiella spp* – 16.42%, *Pseudomonas spp* – 9.03%, *Enterococcus spp* – 7.38%, *Proteus spp* – 5.59%, and *Acinetobacter spp* – 3.08%.

The germs studied were identified in descending order in the following biological products: purulent secretions – 31.83%, urine cultures – 26.74%, wound secretions – 13.19%, cervical secretions – 8.82%.

Bacteria with an MDR (Multi-Drug Resistant) phenotype occupy the first place with an isolation rate of 43.98%, followed by bacteria with an XDR (Extensively Drug-Resistant) phenotype with a proportion of 42.77%, and then microorganisms with a PDR (Pan-Drug Resistant) phenotype at 13.25%

The highest proportion of microbiological alerts, 54.44%, is held by medium-priority microbiological alerts, followed by low-priority microbiological alerts at 37.07%, and then high-priority alerts at 8.39%.

10.54% of the microorganisms identified in surgical departments originate from their habitat as colonizers, 55.95% have a nosocomial origin being isolated after a hospitalization period of over 72 hours, and 87.96% have a phenotype sensitive to antimicrobials.

In medical departments, the proportion of germs in descending order is as follows: *Escherichia coli* – 46.76%, *Klebsiella spp* – 25.83%, *Staphylococcus spp*. – 13.34%, *Pseudomonas spp* – 5.26%, *Enterococcus spp* – 4.77%, *Proteus spp* – 2.33%, and *Acinetobacter spp* – 1.71%.

All germs studied were identified in different percentages from the following biological products in descending order: urine cultures – 72.58%, sputum – 9.06%, blood cultures – 6.61%, and purulent secretions – 5.39%.

Germs with an MDR (Multi-Drug Resistant) phenotype hold the highest proportion, with 52.08%, followed by germs with an XDR (Extensively Drug-Resistant) phenotype at 38.19%, and then those with a PDR (Pan-Drug Resistant) phenotype with an isolation rate of 9.72%.

The microbiological alerts with the highest proportion in medical departments are those with medium priority at 43.57%, followed by low priority microbiological alerts with a proportion of 41.07%, and lastly, high priority microbiological alerts at 15.36%.

Germs identified in bacteriologically positive samples from patients in medical departments with an invasive character represent 96.82% of the total, 54.48% have nosocomial origin, and 82.25% are sensitive to a wide range of antimicrobial substances.

In the clinical departments of Oncology, Hematology, and Radiotherapy, the proportion of germs in descending order is as follows: *Escherichia coli* – 32.20%, *Klebsiella spp* – 22.93%, *Staphylococcus spp* – 20.49%, *Pseudomonas spp* – 13.90%, *Enterococcus spp* – 3.90%, *Proteus spp* – 3.66%, and *Acinetobacter spp* – 2.93%.

The germs studied were identified in descending order in the following biological products: urine cultures – 42.44%, wound secretions – 19.02%, sputum – 16.10%, and purulent secretions – 12.68%.

The highest proportion was recorded by bacteria with an XDR (Extensively Drug-Resistant) phenotype – 46.75%, followed by microorganisms with an MDR (Multi-Drug Resistant) phenotype – 44.16%, and then those with a PDR (Pan-Drug Resistant) phenotype – 9.09%.
The most frequent microbiological alerts are low-priority ones, with a proportion of 46.02%, followed by medium-priority alerts at 45.58%, and finally, high-priority microbiological alerts with a proportion of 8.41%.
Among the germs isolated from patient samples in this category of departments, those with invasive potential dominate at 95.85%: 72.73% have nosocomial origin, and 81.22% are sensitive to a wide range of antimicrobial substances.

Considering that from the first days of hospitalization, patients acquire microorganisms associated with the room they are admitted to and subsequently transfer some of their own microbiota to their environment, the bacteriological characterization of hospital departments can be useful for preventing nosocomial infections, improving patient care quality, hospital resource planning, developing and updating antibiotic policies, and enhancing interdepartmental communication and collaboration.

THE MOTIVATION FOR CHOOSING THE TOPIC

In this thesis, I aim to demonstrate the practical applications of medical microbiology, which go beyond simply providing results for hospital departments. It also opens the door to interdisciplinarity offered by the bacteriologist to curative colleagues, the infectious disease specialist, and the hospital epidemiologist.

I have addressed and thoroughly discussed "some bacteria," trying to show that differentiating germs into Gram-negative and Gram-positive is not just a diagnostic step for the microbiologist but is also useful for you. The Gram-positive ones can easily be transferred by medical and auxiliary staff through hands, while the Gram-negative ones can be taken from patients and transferred into the hospital environment, potentially infecting other patients, especially when cleaning and disinfection are not effective in destroying them.

I have treated the germs as both colonizers and invaders, notions useful to you since identified colonizing germs are found in their natural habitat in humans. The presence of these in their normal sites should not be regarded as an infection and should be treated accordingly only under the guidance of the infectious disease physician.

Germs identified in normally sterile sites should not be present there; their presence can certainly be treated with antibiotics, but it remains to be determined why and how they reached a normally sterile site. This marks the beginning of the long but correct path of investigating a healthcare-associated infection (HAI) within a team that includes the attending physician, infectious disease specialist, microbiologist, and epidemiologist. The germs identified in these patients pose a danger.

I can specify whether the germs are sensitive or multi-resistant to antibiotics. These characteristics are not only useful to the patients you treat but also help evaluate, along with other results, the hospital's antibiotic policy.

I can demonstrate that these germs identified in patients can have a community or nosocomial origin, highlighting possible sources of nosocomiality. If not investigated, isolated, and limited, these can lead to outbreaks of infections that are difficult to eradicate, involving significant human and material costs.

I can show you that by using the WHONET software, which analyzes the trend of the number of isolates, the resistance profiles of germs, temporal and zonal patterns, and groups of similar multi-resistant microorganisms present in a hospital ward over a short period, it provides a complete picture of how these germs are transmitted between hospital wards and detects the presence of nosocomial infection clusters. All of these allow for the prompt and efficient implementation of infection control measures, contributing to the safety and health of patients, protecting medical staff, and reducing hospital costs.

These are data, thoughts, opportunities, and solutions that I have transposed into my thesis, which you should consider. In turn, I seek to enrich my knowledge with your scientific expertise.

GENERAL PART
Chapter 1

INTRODUCTION

Just a few hours after hospitalization, the normal microbial flora of patients begins to change, acquiring bacterial strains characteristic of the hospital environment. Most patients who develop healthcare-associated infections (HAIs) either have a predisposition to infection due to minimally or maximally invasive procedures they undergo during hospitalization or have a compromised immune status in the context of their underlying disease.

Despite the opportunities currently offered to hospitalized patients, such as advanced medical technologies, modern medical equipment and devices, and innovative and effective treatments, the contemporary hospital faces significant challenges in managing microbial pollution.

Knowing the circulation of normal bacterial flora, as well as the specific flora of a certain profile of the section or compartment where the patient is temporarily hospitalized, is very important for the prompt detection of infections, their etiological diagnosis, and the early initiation of anti-infective treatment. Antibiotic therapy must be in accordance with the local epidemiological patterns of microbial susceptibility.

The ubiquitous nature of conditionally pathogenic germs allows them to implant in the receptive body of the patient, either through natural or artificial means. In such cases, medical devices such as peripheral or central intravenous catheters, intubation probes, urinary catheters, and the instruments used can play an important role.

Studying the germs circulating in the hospital environment is of major importance for ensuring the safety and health of patients and medical staff and for preventing the spread of nosocomial infections, which have represented a real challenge for hospitals throughout history.

1.1. HISTORY OF BACTERIOLOGY

Bacteria are prokaryotic microorganisms essential for the balance of ecosystems, with applications in various fields of activity such as agriculture, medicine, the food industry, and environmental protection. Their extreme adaptability is highlighted by their survival in harsh conditions, such as those in the Mariana Trench.

From air, water, and soil to the organs of the human body, the entire world is full of bacteria. In the human body, bacteria outnumber eukaryotic cells and play a vital role in the normal flora of the skin and intestines, although some are pathogenic, causing serious diseases.

The history of bacteriology begins with Antonie van Leeuwenhoek, who observed bacteria as early as 1676, followed by Christian Gottfried Ehrenberg, who introduced the term "bacterium" in 1828. Ferdinand Cohn classified bacteria into four morphological groups. Louis Pasteur and Robert Koch demonstrated the role of bacteria in diseases and fermentation, with Koch elaborating the postulates that still bear his name and are applicable today.

Over time, several scientists, such as Sir Alexander Ogston, Friedrich Julius Rosenbach, Carl Gessard, Carl Friedländer, Theodor Escherich, Martinus W. Beijerinck, Thiercelin, Paul Ehrlich, and others, discovered and characterized various pathogenic bacteria.

The introduction of penicillin in 1943, discovered by Sir Alexander Fleming in 1928, revolutionized the treatment of infections. Shortly after, in 1945, Sir Alexander Fleming warned his audience during his Nobel Prize lecture about the clinical and community impact of bacterial resistance to antibiotics. Mary Barber emphasized as early as 1946 that penicillin-resistant staphylococci outnumbered strains sensitive to the same antibiotic. Thus, antibiotic resistance quickly became a major problem in modern medicine.

1.2. EPIDEMIOLOGICAL CONTEXT OF THE CORRELATION BETWEEN THE CIRCULATION OF MICROORGANISMS IN HOSPITALS, HEALTHCARE-ASSOCIATED INFECTIONS, AND ANTIBIOTIC RESISTANCE

1.2.1. Healthcare-Associated Infections

Healthcare-associated infections can be defined as infectious diseases caused by a pathogen transmitted from an infected person to a receptive host.

In the circulating flora of a hospital, we find microorganisms originating from one or more sources of infection, most often represented by shedding patients who can easily contaminate the hospital environment, medical equipment and devices, and even medical and auxiliary staff, amplifying the diffusion of germs through various transmission routes and mechanisms. A classic model of causality of infectious diseases is represented by Snieszko's epidemiological triad from 1974, which states that such diseases result from a combination of a pathogenic or conditionally pathogenic agent, a susceptible host – the patient, and hospital environmental factors, including equipment and devices.

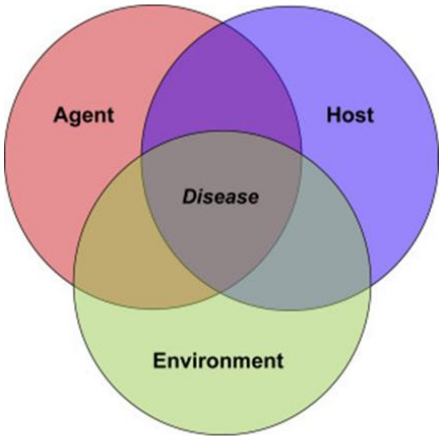

Figure no. 1.1. The epidemiologic triad model of infectious disease causation. The triad consists of an agent (pathogen), a susceptible host, and an environment (physical, social, behavioral, cultural, political, and economic factors) that brings the agent and host together, causing infection and disease in the host.
Source: van Seventer JM, Hochberg NS. Principles of Infectious Diseases: Transmission, Diagnosis, Prevention, and Control. International Encyclopedia of Public Health 2017;22-39. two: 10.1016/B978-0-12-803678-5.00516-6 [66.]

1.2.2. Etiological Agents of Healthcare-Associated Infections

The etiological structure of HAIs has significantly broadened the scope of medical microbiology, as virtually any so-called "parasitic" bacteria can act as an etiological agent for such infections. An infective dose is necessary, the involved bacteria must be present at a susceptible entry point, the duration of contact must be long enough for contamination to occur, and competing microbiocenoses should not be altered by antibiotic therapy, being replaced by hospital microbial flora.

The decline in the prevalence of so-called classical pathogens in the etiology of HAIs and the increasing role of conditionally pathogenic, opportunistic germs necessitates nuanced interpretations. The notion of a pathogenic microorganism for bacteria involved in nosocomial infections must be shifted from the species level to that of population or clone within a species. It is increasingly common for a microorganism, part of a normal biocenosis, to be reconsidered as an opportunistic, conditionally pathogenic bacterium with a definitive etiological role in HAIs.

After overcoming the barrier of natural resistance, conditionally pathogenic germs are recognized by immune effectors even in immunocompetent hosts. Ranking bacteria based on their frequency of involvement as etiological agents in HAIs is quite difficult to achieve. Data variability is influenced by the effort to control these infections, the existing information circuits, the laboratory equipment and reagents for high-performance bacteriological diagnostics, combined with possible local subjective factors.

In a hospital, the microbial environment originates from one or more sources of infection, represented by patients shedding germs, which can eventually be transmitted through various routes and mechanisms, amplifying their diffusion in the hospital environment. The distribution of bacteria based on the frequency with which they act as etiological agents of nosocomial infections may change, as has been proven, but it remains that certain bacterial genera are better represented in certain hospitals with a particular epidemiological risk profile.

Staphylococcus aureus plays an important role in the occurrence of HAIs, in most cases the source of infection being endogenous, with bacterial strains being transmitted either through direct contact or airborne. Colonization with this pathogen can be particularly dangerous for critically ill patients in intensive care units where invasive medical procedures are performed using medical equipment and devices. Contamination of these devices with Staphylococcus aureus can lead to HAIs, and the bacteria's ability to survive on surfaces for months makes it a constant source of transmission. Moreover, multi-resistant strains of Staphylococcus aureus are involved in causing serious nosocomial infections among hospitalized patients.

Other bacteria frequently found in the hospital environment are Escherichia coli and Klebsiella spp., which can cause a variety of infections, from intestinal to urinary and respiratory infections. These bacteria can be transmitted fecal-orally, through objects or food, and through the hands of staff and contaminated medical equipment and devices.

Escherichia coli often causes urinary tract infections, and non-compliance with hygiene and antisepsis rules can lead to surgical wound infections. Generally, the transmission of these bacteria in the hospital environment is facilitated by patients' daily use items, medical equipment, and even the hands of staff. Adhering to hygiene measures and adopting appropriate practices for the prevention and control of HAIs can contribute to the efficient management of the circulation of this bacterium in the hospital environment.

Klebsiella pneumoniae, a medically important bacterial species, is significantly involved in hospital-acquired urinary tract infections, pneumonia, septicemia, and soft tissue infections. In humans, Klebsiella pneumoniae is present as a saprophyte in the nasopharynx and intestinal tract. Pathogenic reservoirs for the transmission of Klebsiella spp. are considered to be the gastrointestinal tract and the hands of hospital medical and auxiliary staff. The microorganism can pollute the hospital environment, especially moist environments, which is why medical equipment such as urinary and intravenous catheters, disinfectant solutions, and especially humidifiers and nebulizers require special attention, being frequently cleaned, disinfected, and sterilized when necessary. In the hospital environment, colonization rates with Klebsiella spp. increase proportionally with the duration of patient hospitalization. The carrier rate of Klebsiella spp. strains in hospitalized patients is 77% in the stool, 19% in the pharynx, and 42% on the hands of patients. Increasingly more studies support the nosocomial colonization of this opportunistic germ as being more frequently associated with the inappropriate use of antibiotics than with factors related to the medical care provided in the hospital. Due to their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks, especially in burn, surgery, and oncology units, often involving multi-resistant bacterial strains.

Pseudomonas aeruginosa, a bacterium frequently involved in the etiology of HAIs, is considered a "scavenger bacterium" that can cause severe infections, particularly in immunocompromised patients and those hospitalized for long periods. It colonizes moist environments, equipment, and medical devices that use water during operation and is found in the composition of biofilms under whose protection antibiotic resistance of these strains can increase by over 10 times. It is sporadically identified in samples from patients in trauma, intensive care, thoracic, and cardiovascular surgery units, as well as in diabetic, neoplastic, and geriatric patients. The presence of favorable factors such as overcrowding, service malfunctions, and prolonged treatments with broad-spectrum antibiotics can be involved in the occurrence of HAI outbreaks. Often, pharyngeal, nasal, fecal, and urinary carriers of Pseudomonas spp. can be detected among medical staff.

Proteus spp. species are more frequently involved in the occurrence of HAIs in the elderly, patients with permanent or long-term urinary catheters, or those frequently undergoing invasive urological investigations. Patients with structural urinary tract abnormalities are more prone to infections with Proteus spp. strains, especially in the context of antibiotic therapies and previous hospitalizations. For Proteus spp. as well, the hospital represents an opportunity to interact, multiply, and infect susceptible hosts. Moist environments and medical equipment that use water can also be reservoirs for this type of bacteria.

Acinetobacter spp., a conditionally pathogenic bacterium, tends to cause HAI outbreaks due to its exceptional resistance to antimicrobials and its ability to survive for over 20 days on both moist and dry surfaces. The bacterium is more frequently found on inanimate objects and the hands of staff in ICUs than Staphylococcus aureus and Pseudomonas spp. The colonization of medical equipment in hospitals can serve as reservoirs for persistent, hard-to-eradicate outbreaks. The spread of *Acinetobacter spp*. in medical environments is increasingly linked to airborne transmission, alongside Pseudomonas aeruginosa. The clinical impact of *Acinetobacter spp*. is reflected in high morbidity and mortality rates, especially in critically ill patients. Despite being considered a microorganism with low virulence, it is more commonly associated with nosocomial infections in severely ill or immunocompromised patients. Patients with prolonged mechanical ventilation, extended hospital stays, or exposure to multi-resistant antibiotic strains are at higher risk of infection or colonization, increasing the likelihood of acquiring HAIs.

1.3. CONSIDERATIONS REGARDING CONTAMINATION, COLONIZATION, CARRIER STATE, BACTEREMIA, AND INFECTION

The ability of certain microorganisms to infect a susceptible patient depends on their structural and biochemical mechanisms, which manifest pathogenically through invasiveness and toxigenesis. The progression of microorganisms from contamination to colonization, carriage, bacteremia, and infection occurs over a certain period, and not always does identifying the stage at which they are diagnosed require antibiotic treatment. Therefore, it is absolutely necessary to correctly define the terms described above, which will support the therapeutic approach determined by the clinician.

Contamination - defines the presence and multiplication of germs on inert surfaces or medical equipment, hospital surfaces, or on the host's body. Nosocomial infections can be transmitted in 70% of cases by the hands of medical and auxiliary staff, being recognized as the most important route of "horizontal" contamination.

Colonization - a normal process considered the first step towards microbial infection, occurs on the surface of the host organism, at various entry points, where microorganisms multiply without causing morphological or functional alterations to tissues and organs, immune reactions, or detectable symptoms. Colonizing bacteria are usually part of the individual's commensal flora, but there is also the possibility that various medical devices may become colonized.

The importance of colonization in current practice is noteworthy, as microbial species that multiply and invade the human body cannot cause damage to the host since, at this stage, they cannot overcome the host's immune response. Currently, the specialized literature endorses a new term that many researchers support in an attempt to explain delayed healing in the absence of any evident clinical signs, defined as "critical colonization," better explained chronobiologically than clinically.

"Critical colonization," a term first used by Davies in 1996 at a scientific meeting, presented case studies supporting and defining this notion as "the multiplication of microorganisms - without invasion but interfering with wound healing." Currently, critical colonization is accepted as an intermediate situation between colonization and infection, with microbes persisting in a site for an indefinite period, continuing to destroy host structures from insignificant to significant, and the immune response no longer effectively counteracting microbial development.

Infection is suggested by:
Classical clinical signs: abscess formation, cellulitis, serous exudate with inflammation, seropurulent, hemopurulent secretions, frank pus.
Additional criteria: color changes of the wound, friable and bleeding granulation tissue, pain, undermining in the wound bed, abnormal epithelialization, fetid odor. In an infected wound, healing is halted due to microbial multiplication, tissue invasion, and evident immunological reactions serologically.
The diagnosis of infection necessarily requires identifying the microbial species for the correct practice of antibiotic therapy, which will protect the patient from the danger of developing resistant

strains, difficult to combat. The infectious process is defined as the totality of humoral, tissue, and metabolic changes that occur in the human body due to microbial aggression.

Nosocomial infection - according to the new terminologies adopted by the medical world, is redefined as a HAI possibly contracted in healthcare facilities, referring to an infectious disease that can be clinically recognized, whether microbiologically validated or not, but for which there is necessarily epidemiological evidence of contracting it during hospitalization.

This type of infection can affect both the patient through the received medical care or the hospital environment offered, and the medical and auxiliary staff due to their activities. It is important to highlight the linkage of this type of infection to the incubation period of the disease, regardless of whether symptoms appear during hospitalization or not.

1.4. HEALTHCARE-ASSOCIATED INFECTIONS AT NATIONAL AND INTERNATIONAL LEVELS

The average incidence of HAIs calculated by the National Institute of Public Health Bucharest and reported to the total number of discharged patients is 1.34% in 2021, up from 1.04% in 2020, though HAI pathology remains underestimated.

The nosocomial pathology of respiratory infections represented 36% of the total HAIs reported in 2021, twice as frequent as the 18.5% recorded in the pre-pandemic year 2019.

Healthcare-associated infectious syndromes detected in ICU wards are represented by pneumonia - 65%, septicemia - 25%, and urinary tract infections - 15%.

The etiology of HAIs in these wards is dominated by *Acinetobacter spp* - 36%, followed by Klebsiella spp - 28%, Pseudomonas spp - 18%, Enterococcus spp - 10%, Staphylococcus spp - 6%, and Escherichia coli - 2%.

The HAI pathology in the surgical wards included in the study comprises superficial wound infections - 37%, deep wound infections - 35%, and organ infections - 23%.

In the etiology of HAIs in these wards, the following are involved: Escherichia coli - 30%, Enterococcus spp - 23%, Klebsiella spp - 21%, Pseudomonas spp - 13%, Staphylococcus spp - 7%, and A*cinetobacter spp* - 6%.

The WHO, following a multicenter study conducted in ICUs, found that 51% of patients developed a HAI, which prolonged their hospitalization and increased the risk of subsequent infections or other morbidities. It is estimated that over 1.4 million patients have at least one HAI at any given time, both in economically advanced and emerging countries, entailing a significant financial burden at individual, community, and public levels.

1.5. THE ROLE OF EUROPEAN UNION AGENCIES IN THE FIELD OF ANTIMICROBIAL RESISTANCE

1.6. ANTIBIOTIC RESISTANCE

Antimicrobial resistance (AMR) is a characteristic that can be attributed, in principle, to each of the microorganisms involved in the etiology of HAIs. Resistant microorganisms to one or more antimicrobials are responsible for:

Nosocomial infections detected in hospitals;
- Infections in patients outside hospitals;
- Community-acquired infections;
- Carrier states;
- Their presence in animals;
- Isolation from food;
- Pollution of the external environment.

AMR is the natural consequence of evolution, natural selection, and genetic mutations, which may be transmissible to descendants, conferring resistance to microorganisms. The exaggerated use of drugs, especially antibiotics, exerts significant pressure on microbes, managing to amplify the speed of resistance emergence, leading to the selection of resistant microorganisms in both human and animal populations.

The WHO recognizes antimicrobial resistance as a difficult-to-manage problem that threatens human health and global food security. The European Centre for Disease Prevention and Control (ECDC) reported, based on 2022 data, that over 670,000 HAIs are due to multi-resistant bacteria, and 35,000 people die annually in the EU/EEA as a result of infections caused by drug-resistant bacteria.

The additional hospitalization costs generated by AMR in Europe are significant, estimated at approximately 1.1 billion euros annually according to an ECDC and WHO report. These costs include prolonged hospitalizations, additional medical treatments, and the use of additional resources from health systems.

In the period 2022-2023, approximately 35% of HAIs in European hospitals were attributed to antibiotic-resistant microorganisms. ECDC closely monitored these trends, highlighting the urgent need to improve infection control and antimicrobial stewardship interventions to mitigate the growing threat of AMR in healthcare facilities across Europe.

EUROPEAN CENTER FOR DISEASE PREVENTION AND CONTROL

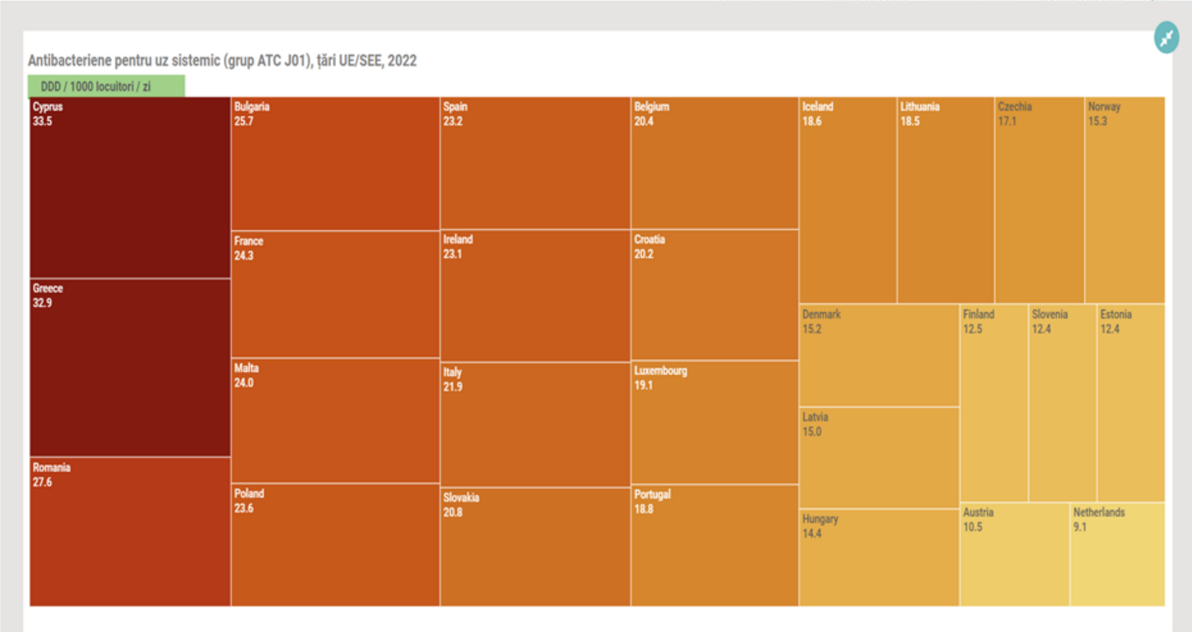

Figure no. 1.5. Antibiotic consumption in EU and EEA countries in 2022. Source: European Center for Disease Prevention and Control

The O'Neill Report published in 2016 estimated that by 2050, RAM could lead to induced losses in global GDP of up to $100 billion, with annual costs potentially increasing unpredictably and uncontrollably, similar to those recorded in the year of onset of the global financial crisis of 2008 [178].
The economic impact of AMR is profound and extends beyond health systems, affecting the global economy, labor productivity, the food industry and society as a whole. Combating AMR requires a global and multidisciplinary approach, with significant investments in prevention, education, research and the development of new treatments.

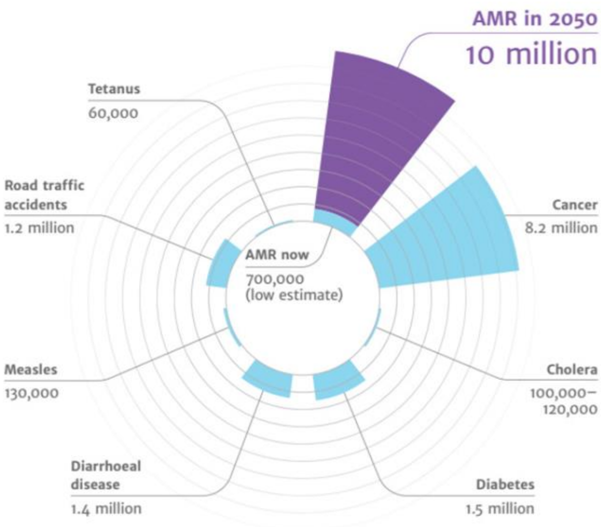

Figure no. 1.6. AMR impacts in 2016 and 2050
Source: UNEP_Annual_Report_2023.pdf/unep.org/environment-fund

Two major factors governing RAM are recognized, namely:

the uncontrolled use of antibiotics which, through environmental pressures on micro-organisms, leads to the emergence and selection of antibiotic-resistant bacteria in populations;

the possibility of the spread and cross-transmission of antibiotic-resistant micro-organisms between humans, between animals and between humans, animals and the environment.

In this context, two ways of monitoring, controlling and limiting antibiotic resistance are proposed: prudent administration of antibiotics only when they are really necessary, in the correct dosage, respecting the administration intervals specified in the package leaflet, for an adequate duration of time and by observing the Universal and Additional Precautions[181] .

Multidrug-resistance is also relevant for the bacteria responsible for HPAI, food-borne infections, tuberculosis, etc., pointing to the limited number of therapeutic options for infected patients.

Bacteria unanimously recognized as resistant to several antibiotics are:

– Methicillin-resistant *Staphylococcus aureus* (MRSA);
– Vancomycin-resistant *Enterococcus* (VRE);
– Enterobacteriaceae producing extended-spectrum beta-lactamases and carbapenemases (ESBL and CPE): *Escherichia coli and Klebsiella pneumoniae*;
– Carbapenem-resistant *Pseudomonas aeruginosa*;
– Multiresistant *Acinetobacter spp*[183] .

The ESKAPE project was proposed by a group of researchers led by Louis B. Rice in 2008 to draw attention to the challenges of infections caused by resistant bacteria and to encourage research into the development of new antibiotics.

The ESKAPE study came about as a result of a combination of negative factors favoring AMR and MDR, including: overuse of antibiotics in hospitals and in the community to treat patients, misuse and inadequate adherence to treatment guidelines and, equally important, overuse of antibiotics for prophylactic or curative purposes in the animal and agricultural sector. [185.]

The literature states that MDR bacteria are implicated in approximately 15.5% of hospital-acquired infections, with 700,000 deaths due to nosocomial infections with drug-resistant germs. Specifically, nosocomial opportunistic nosocomial ESKAPE pathogens correspond with the highest mortality risk, which has as the majority of isolates MDRs[186].

The ESKAPE group of antibiotic-resistant *Acinetobacter* and *Enterobacteriaceae* germs have been on the CDC's list as urgent threats since 2019. The other 5 germs in the group are on the same list as serious threats.

In addition, the WHO also provides a global list of priority pathogens of ABR bacteria which it classifies into 3 categories, critical, high and medium. The pathogens in the ESKAPE group are distributed as follows: 5 are included in the critical priority list and the other 2 in the high priority list.

Germs belonging to the ESKAPE group can exist in 3 different antimicrobial resistance patterns defined in the literature according to the antibiotic spectrum to which they show a lack of sensitivity, namely: multidrug-resistant - MDR, extensively drug-resistant - XDR, pan-resistant to the antibiotic classes used in the treatment of patients - PDR[190] .

Epidemiologically significant antibiotic classes were constructed for each bacterium. The proposed panels for antimicrobial susceptibility testing were created using documents and breakpoints from Clinical Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Food and Drug Administration (FDA) in the United States since 2008[192] .

Thus:
-MDR was defined as acquired non-susceptibility to at least one a g ent of three or more antimicrobial classes;
-XDR has been defined as non-susceptibility to at least one agent from all but two or fewer antimicrobial classes, i.e. bacterial isolates remain susceptible to only one or two antibiotic classes;
-PDR was defined as non-susceptibility to all agents in all antimicrobial classes.

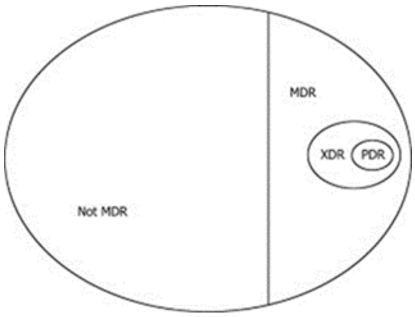

Figure 1.7. Relationship diagram between MDR, XDR and PDR

Source: Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x[192] .

Chapter 2

RETROSPECTIVE EPIDEMIOLOGICAL EPIDEMIOLOGICAL STUDY OF BACTERIA ACCORDING TO HABITAT, LEVEL OF ANTIBIOTIC SUSCEPTIBILITY AND THE BIOLOGICAL PRODUCT FROM WHICH THEY WERE IDENTIFIED - A FIRST STEP IN THE BACTERIOLOGICAL CHARACTERIZATION OF THE WARDS OF THE COUNTY EMERGENCY HOSPITAL

2.1. INTRODUCTION

Germs isolated from patients' biological products, such as blood, urine, respiratory secretions and other body fluids, provide essential information about the types of micro-organisms circulating in the hospital and their resistance to antimicrobials.

The study of germ isolates allows rapid identification of pathogens and their resistance to antibiotics, thus contributing to the application of appropriate and prompt measures to prevent and control infections.

2.2 PURPOSE OFRESEARCH

Analysis of the microorganisms according to the biological product from which they were identified and their importance in the bacteriological characterization of a County Emergency Hospital.

2.3. RESEARCH OBJECTIVES

- possible associations between biological products collected from patients at the request of the attending physician and the habitat from which they originate;
- possible associations between biologicals collected from patients and antibiogram results;
- bacteriological characterization of hospital wards by activity profiles according to habitat and antibiogram results.

2.4. MATERIAL AND METHOD

A retrospective, descriptive and analytical longitudinal longitudinal study was conducted on 17 categories of biological products collected from patients at the request of attending physicians for bacteriologic examination in 2020.

The study includes a total of 19360 samples sent for bacteriological examination, for which standardized working procedures were applied, a total of 3941 germs were isolated and identified.

For a useful interpretation of the positive results of the bacteriological examinations, the wards from which the biological products originated were grouped into 4 categories according to their profile of activity and the pathology presented by hospitalized patients, namely: ITA, Clinical wards with Surgical profile, Medical profile, Oncology-Radiotherapy - Hematology.

2.5. RESULTS

Bacteriologic diagnosis was approached from two perspectives:

one relating to the proportion of bacteriologically positive biological products in the wards surveyed;

the second considered the pathogenicity and resistance profile of bacteriologically positive samples depending on the biological product analyzed.

Of the total number of bacteriologic investigations - 19360 requested by treating physicians only 3941 are positive. We point out that in bacteriology, both for a negative and a positive result, a large number of operations are necessary, starting with cultivation, incubation of the sample, growth, isolation and identification of the germ, requiring material resources with high costs and time periods that must be respected.

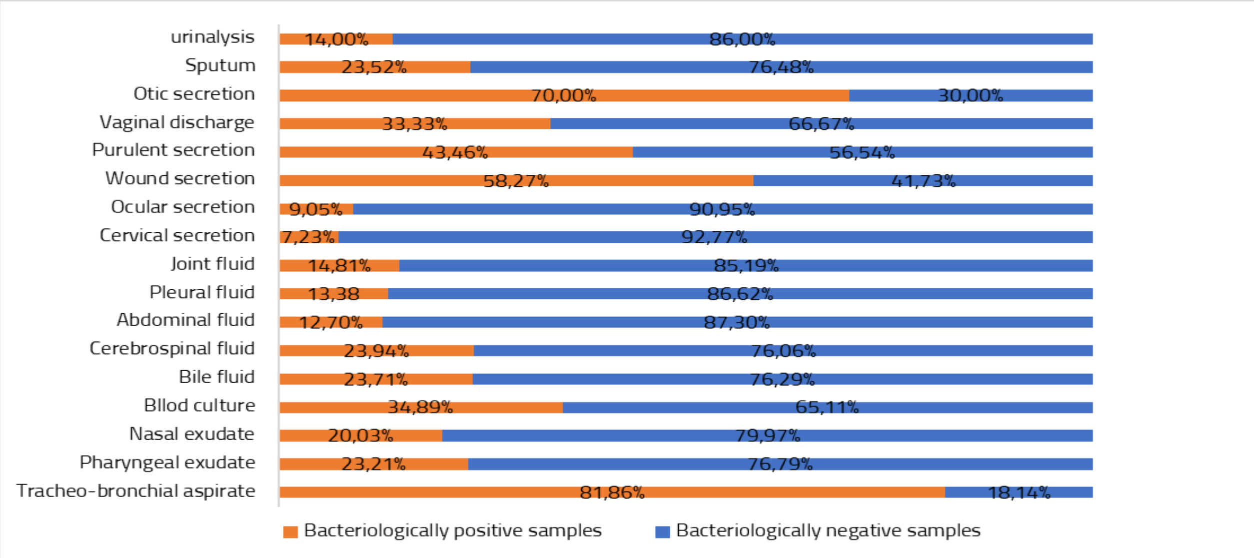

Figure no. 2.3. Percentage distribution of biological samples analyzed according to the result of bacteriological examination in hospital

The modest percentage of positive samples - 20.36% compared to negative samples - 79.64% - warns us that not enough attention is paid to the symptomatology that could guide and support a clinical suspicion of infectious diagnosis.

2.5.1. Analysis of the results in terms of the biological product examined

The laboratory physician, after performing antibiograms that represent the final point of the bacteriologic diagnosis that will benefit the attending physician in the choice of a targeted antibiotic therapy, categorizes the bacteriologically positive samples in two broad categories: with germs sensitive to the usual antimicrobials and with multidrug-resistant germs of the MDR, XDR or PDR type. We emphasize that the hospital-wide proportion of germs sensitive to common antibiotics is considerably higher - 81.88% compared to 18.12% of multidrug-resistant germs, the patient having the advantage of being able to benefit from a wider antibiotic palette.

We note the high percentage of bacteriologically positive biological products with potentially invasive germs - 86.63%, coming from normally sterile cavities and warning of an infectious pathology superimposed on the underlying disease.

In attention also remain the germs identified as colonizers - 13.37%, which from the body's natural cavities can spread endogenously to the same patient or exogenously, through staff, care maneuvers in the hospital environment and from there to other patients. The clinician may direct the therapeutic conduct in different ways: decolonizations for germs identified in natural body cavities and targeted antibiotic therapies in case of undesirable identification of invasive germs in normally sterile cavities.

For both categories of germs mentioned above, the microbiologist has another possibility, depending on the results of the antibiogram, to establish the resistance profile of these germs, either sensitive to the usual antibiotics or multi-drug resistant with major implications in the orientation of antibiotic therapy and its chances of success.

For colonizing germs the sensitivity to the usual antibiotics is 84.44%, decolonization as recommended can be used. For potentially invasive germs the sensitivity to the tested antibiotics is 81,49% antibiotic therapy is mandatory.
Products in which potentially invasive germs identified as multi-drug resistant - 18.51% - signal the difficulties that treating physicians will face in choosing antibiotic therapy.

2.5.1.1.1. Clinical Department Anesthesia Intensive Care Unit

Bacteriologically positive samples represent 37.02% of the total samples sent for bacteriologic diagnosis, which is higher than the hospital-wide percentage - 20.36%. Of these, it is important to note that the samples with germs sensitive to the usual antibiotics - 72.21% outnumber the biological products with MDR germs - 27.79% in which the antibiotic palette to be used is very low or non-existent.

Comparing the percentage of bacteriologically positive biologics with resistant germs in the ICU ward - 27.79% with that recorded for the entire hospital in 2020 - 18.12%, the difference is large. The severity of the already known cases and the difficulties of targeted antibiotic treatment on germs that are difficult to control with drugs necessarily require the opinion of the infectious diseases physician.
According to the habitat from which the biological samples were collected, those with invasive germs - 75.30% prevail over those with colonizing potential - 24.70% which draws attention to their presence in normally sterile locus, supporting the idea of at least localized if not systemic infection. Bacteriologically positive specimens with the highest proportion of colonizing germs are pharyngeal exudates 62.15% followed by nasal exudates with 37.85%. Bacteriologically positive biological products with invasive germs, in descending order are: tracheo-bronchial aspirates -42.03%, blood cultures - 21.41%, urine cultures - 21.30%, wound secretions - 6.49%, purulent secretions - 5.13%.
Further data from the study group shows that the resistance profile of bacteriologically positive biological products of germs with invasive potential - 29.95% - exceeds in percentage that of colonizing germs - 21.18%, the clinician having to treat patients with a prognosis quod ad vitam

reserved quod ad vitam and due to the reduced antibiotic palette, the infection overlapping the basic diagnosis.

2.5.1.2. Surgical Clinical Departments

In the Clinical Surgical wards of the total biological samples sent for bacteriologic diagnosis only 16.65% are positive, less than the positive samples - 20.36% confirmed throughout the hospital.

This observation has two facets: a positive one, which expresses the fact that the percentage of germs identified in the patients' specimens is low, which is favorable for them, and a negative one, which shows an overdiagnosis that can be explained by the surgeons' interest to detect as quickly as possible a possible infectious process in progress and due to the risks involved in the multitude of minimally or maximally invasive maneuvers they perform on surgical patients.

Analyzing the bacteriologically positive samples, an encouraging finding is that the germs identified as sensitive - 88.84% to the usual antibiotics are in a much higher percentage than that of resistant germs - 11.16% whose MDR profile makes the therapeutic decision of the infectious disease and surgical physician much more difficult. The wards with a surgical profile, by comparison, record higher values than the percentage values recorded for the entire hospital in 2020 for antibiotic-susceptible germs - 81.88%.

The habitat in which the germs in the harvested biological product are identified is important for surgical sections. Those isolated from natural human cavities in a percentage of 12.69% from: nasal cavity, oropharyngeal cavity, vaginal cavity, skin have a colonizing role with minimal potential for dissemination in the patient's body. The highest proportion of identified colonizing germs is found in cervical secretions - 85.34%, followed by pharyngeal exudates - 10.99%, vaginal secretions - 2.09% and nasal exudates - 1.57%.

Potentially invasive germs, in 87.31%, isolated from naturally sterile cavities, signaling to the attending physician an evolving infectious process superimposed on the underlying disease requiring targeted antibiotic treatment. Bacteriologically positive biological products identified with potentially invasive germs, in descending order are: purulent secretions - 36.38%, uro cultures - 29.91%, wound secretions - 14.08%, peritoneal fluids - 5.02% and sputum - 3.20%.

2.5.1.3. Clinical Sections with Medical profile

In Clinical wards with Medical profile, bacteriologically positive biological samples - 14.42% - report the lowest percentage compared to those recorded in wards with other activity profile: ICU - 37.02%, Oncology - Radiotherapy - Hematology - 32.44%, Surgery - 16.65% and even compared to the whole hospital 20.36%.

Although the specificity of the Clinical wards with a Medical profile is not infectious diseases, the high consumption of resources necessary for bacteriologic diagnosis, whether positive or negative, requires a better selection of hospitalized clinical cases requiring this type of medical analysis.

Only 17.16% of the biological samples collected were positive for antibiotic-resistant germs, 82.84% were sensitive to common antibiotics, and the treating physicians have several treatment options.

Also important is the place of residence of the identified microbes, which can be determined depending on the biological product harvested and the microbiologic condition of the isolated germ. Thus, biological products with germs with colonizing potential amount to 3.20% while biological samples with invasive germs found incorrectly in normally sterile sites reach 96.80% . The highest proportion of identified colonizing germs is found in pharyngeal exudates in 51.85% followed by nasal exudates - 40.74% and vaginal secretions - 3.07%.
Potentially invasive germs, in 96.80%, isolated from naturally sterile cavities, signaling to the attending physician an evolving infectious process. Bacteriologically positive biological products identified with potentially invasive germs, in descending order are: uro cultures - 73.12%, sputum - 9.41%, blood cultures - 7.82% and purulent secretions - 5.62%.
In wards with Medical profile, colonizing germs sensitive to the action of common antibiotics register comparable percentage values - 74.07% with the values of invasive sensitive germs - 83.13%, easing the burden of clinicians in the therapeutic approach.
However, for the MDR resistance profile, the therapeutic decision in both situations will be difficult.

2.5.1.4. Clinical Departments of Oncology, Hematology, Radiotherapy

In the clinical wards of Oncology, Hematology, Radiotherapy, bacteriologically positive biological samples - 32.44% report a lower percentage than that recorded in the ICU ward and higher than the other wards included in the study - 16.65% in the Clinical wards with a Surgical profile, 14.42% in the Clinical wards with a Medical profile and even higher than that recorded in the entire hospital 20.36%.

The high proportion of antimicrobial-sensitive germs - 81.88% - indicates that antibiotics are effective against bacterial infections in this category of patients and at the same time indicates good infection control practices.
For both colonizing and potentially invasive germs, similar percentages of 80.95% and 81.93% sensitivity to first-line antibiotics, respectively, are observed, drawing attention to the reduced anti-infective defense capacity faced by these patients.

2.7. CONCLUSIONS

2.7.1. The tracheo-bronchial aspirates register the highest degree of positivity out of the total number of requested samples: 81.10% in the ICU ward, 92.00% in the Clinical wards with a Surgical profile and 100% in the Clinical wards with a Medical profile, not being requested for analysis in the Oncology, Hematology and Radiotherapy wards.
2.7.2. The purulent secretions register the highest percentages in the ICU Clinical Department - 95.74%, in the Clinical Departments of Oncology, Hematology, Radiotherapy - 76.81% and only 42.30% in the Clinical Departments with a Surgical profile.
2.7.3. Wound secretions show the highest percentage of positivity in the Oncology, Hematology, Radiotherapy Clinical wards - 79.59% followed by the ICU Clinical ward - 62.64%, Medical Clinical wards - 52.38% and Surgical Clinical wards - 51.68%.

2.7.4. For blood cultures, the clinical diagnostic orientation is modest, with percentages of positivity of 48.70% in the ICU Clinical Department, 40.74% in the Oncology, Hematology, Radiotherapy Clinical Departments, 34.62% in the Surgical Clinical Departments and a modest 18.55% in the Medical Clinical Departments.

2.7.5 We note that in the hospital, in the investigated patients, potentially invasive germs predominate alarmingly - 86.63% of all positive samples. The highest percentage of positivity of invasive germs out of the total number of positive samples is found in the Clinical wards with Medical profile 96.80%, followed by Clinical wards of Oncology, Hematology, Radiotherapy with a share of 95.06%, Clinical wards with Surgical profile 87.31% and unexpectedly, 75.30% in the Clinical ward of ITA.

2.7.6. Germs with colonizing potential, out of the total number of samples collected in the hospital, are found in a percentage of 13.37%. The highest percentage of germs with colonizing potential is found in the ICU Clinical ward - 24.70%, followed by the Clinical wards with Surgical profile - 12.69%, Clinical wards of Oncology, Hematology, Radiotherapy - 4.94% and Clinical wards with Medical profile - 3.20%, with the mention that this category of colonizing germs can spread endogenously to the same patient or exogenously, through staff, care maneuvers in the hospital environment and from there to other patients.

2.7.7. The percentage of sensitive germs in the total number of positive samples per ward is as follows: the highest percentage of sensitivity to common antibiotics is recorded in the Clinical wards with a Surgical profile - 88.84%, followed by the Clinical wards with a Medical profile - 82.84%, the Clinical wards of Oncology, Hematology, Radiotherapy - 81.88% and the Clinical ward of ITA - 72.71%.

2.7.8. The percentage of resistant germs out of the total number of positive samples recorded in the hospital is 18.12%, which is a bacterium that greatly limits the therapeutic options of the attending physician. The highest percentage of resistant germs out of the total number of positive samples in each ward is presented as follows: 27.79% in the ICU ward, followed by the clinical wards of Oncology, Hematology, Radiotherapy - 18.20%, Clinical wards with Medical profile - 17.16% and 11.16% in Clinical wards with Surgical profile.

Chapter 3

ETIOLOGIC PROFILE AND RESISTANCE PHENOTYPE ANALYSIS OF POSITIVE ISOLATES FROM PATIENTS HOSPITALIZED IN A COUNTY CLINICAL EMERGENCY HOSPITAL

3.1. INTRODUCTION

The prospect of increased circulation of germs in the hospital environment leads the clinician, microbiologist, infectious disease specialist and epidemiologist to adopt common strategies to prevent episodes of infections, including nosocomial infections288 .

In this sense, the microbiology laboratory allows the characterization of the "microbial environment" in hospital wards where there are conditions for the selection of microorganisms often multiresistant to antibiotics and conditions favorable for their implantation on deficient organisms that represent the biological terrain.

Virtually any conditionally pathogenic or certifiably pathogenic bacterium can materialize as an etiologic agent if: the infectious dose is sufficiently high, the bacterium is present at the susceptible entry portal, the duration of infectious contact is long enough, the competing microbiocenosis is modified by antibiotic therapy and replaced by hospital flora292 .

3.2. RESEARCH AIM

Correlation of the bacteriologic profile of the germs identified in the wards of a County Emergency Hospital with their habitat, resistance profile and community or nosocomial origin.

3.3. RESEARCH OBJECTIVES

analysis of the germs identified according to their susceptible or resistant status and the biological product under bacteriological investigation;

Determining the community or hospital origin of germs identified on wards according to their activity profile;

the bacteriolo g ic profile of the sections studied according to the results obtained after the diagnostic steps provided by the medical laboratory;

Establishing risks and measures for infection surveillance and control.

3.4. MATERIAL AND METHOD

The retrospective longitudinal study was conducted from January 01 to December 31, 2020 on bacterial strains identified in 17 categories of biological products, requested for bacteriological testing by treating physicians and processed in the bacteriology department of the Clinical Laboratory of Medical Analysis.

3.5. RESULTS

3.5.1. In terms of germ isolates

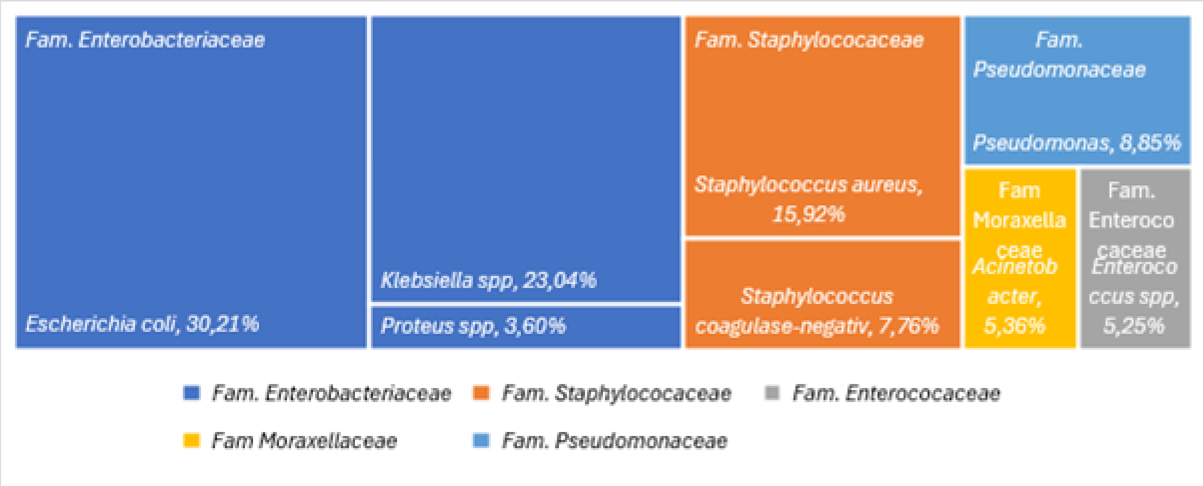

Figure no. 3.1. Distribution by Families of germs identified in the Hospital

Out of the total number of patients hospitalized in 2020 in the hospital, 3750 bacterial isolates were found to be positive, of which the Gram-positive ones are re g ăsăsesc in a percentage of 28.93% belonging to the genera: Staphylococcus spp and Enterococcus spp, the rest being negative germs G ram from the genera: *Esc h erichia coli, Klebsiella spp, Proteus spp, Pseudomonas spp, Acinetobacter spp,* in a percentage of 71.07%.

In current practice, Gram-positive germs are predominantly carried by the hands of medical and ancillary staff, and Gram-negative germs contaminate the hospital environment, persisting on various surfaces, medical devices due to non-compliance, deficiencies in cleaning and disinfection.

Strains isolated and identified from the entire hospital in 2020 by germ type and biological product in descending order are:

- In the family *Enterobacteriaceae - Escherichia coli*, out of the total number of strains - 1133, in the first three places - 65.75% of uro cultures, 8.30% of purulent secretions and 7.68% of cervical secretions. *Klebsiella spp*, of the total number of isolated strains - 864, in the first three places - 40.51% of uro cultures, 14.35% of tracheo-bronchial aspirates and 9.72% of purulent secretions. *Proteus spp*, from the total number of isolated strains - 135, in the first 3 places - 33.33% were isolated from purulent secretions, 31.11% from uro cultures, 17.78% from wound secretions.
- *Staphyloccocus spp.* - of the total 888 positive samples, in the first three places - 24.44% of purulent secretions, 17.57% of blood cultures and 12.73% of wound secretions.
- *Acinetobacter spp* - out of the total of 201 strains isolated, in the first three places - 35.82% from tracheo-bronchial aspirates, 14.93% from purulent secretions, 11.44% from wound secretions and 11.44% from blood cultures.
- *Pseudomonas spp* - of the total number of positive strains - 332 in the first three places - 23.19% in tracheo-bronchial aspirates, 21.69% in purulent secretions and 17.47% in uro cultures.
- *Enterococcus spp.* - out of the total number of identified samples - 197, in the first three places - 49.75% from uro cultures, 22.34% from purulent secretions and 11.68% from wound secretions.

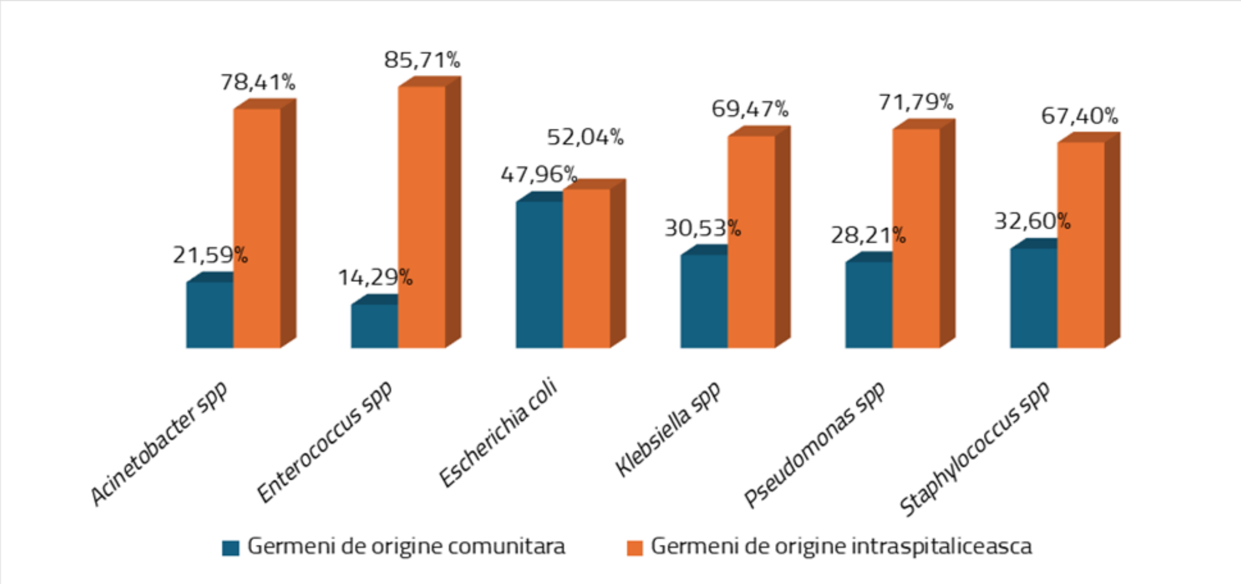

Figure no. 3.2. Community or in-hospital origin according to the time elapsed from the date of hospitalization to the date of isolation of the germs in the Hospital

According to the case definition of an HPAI as stated in the European Commission Decision (EU) 2018/945, nosocomial origin is certified for bacteriologically positive products collected after the 3rd day after the patient's admission to a hospital. 68.07% of the bacteriologically positive samples taken in the study in our hospital are of nosocomial origin.

3.5.2. Bacteriological characterization of the sections

Clinical Department Anesthesia Intensive Care Unit

The germs identified in bacteriologically positive biological samples of hospitalized patients in 2020 - 1166 isolates are represented by Gram-positive bacteria in 35.20%, namely: *Staphyloccocus spp, Enteroccocus spp*, 64.80% of which are Gram-negative germs - *Escherichia coli, Klebsiella pneumoniae spp, Proteus spp, Pseudomonas spp and Acinetobacter spp*.

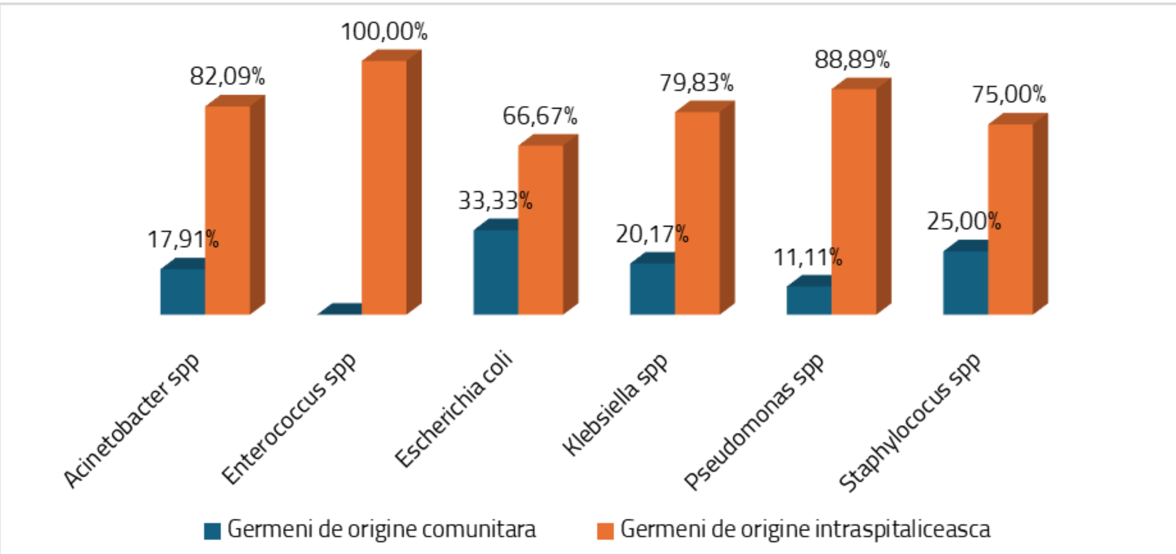

Figure no. 3.5. Community or in-hospital origin according to the time elapsed from the date of hospitalization to the date of isolation of germs in the ICU Clinical ward

Applying the same working formula as for the whole hospital in the ICU ward, for 79.32% of patients the origin of bacteriologically diagnosed infections is nosocomial.

Surgical Clinical Departments

In clinical wards with a surgical profile, 1395 strains were isolated, maintaining the same percentages that prioritize the circulating Gram-negative flora 68.46% compared to the existing Gram-positive flora -31.54%, the isolated bacteria from infected patients hospitalized, drawing attention to the way in which care techniques are practiced.

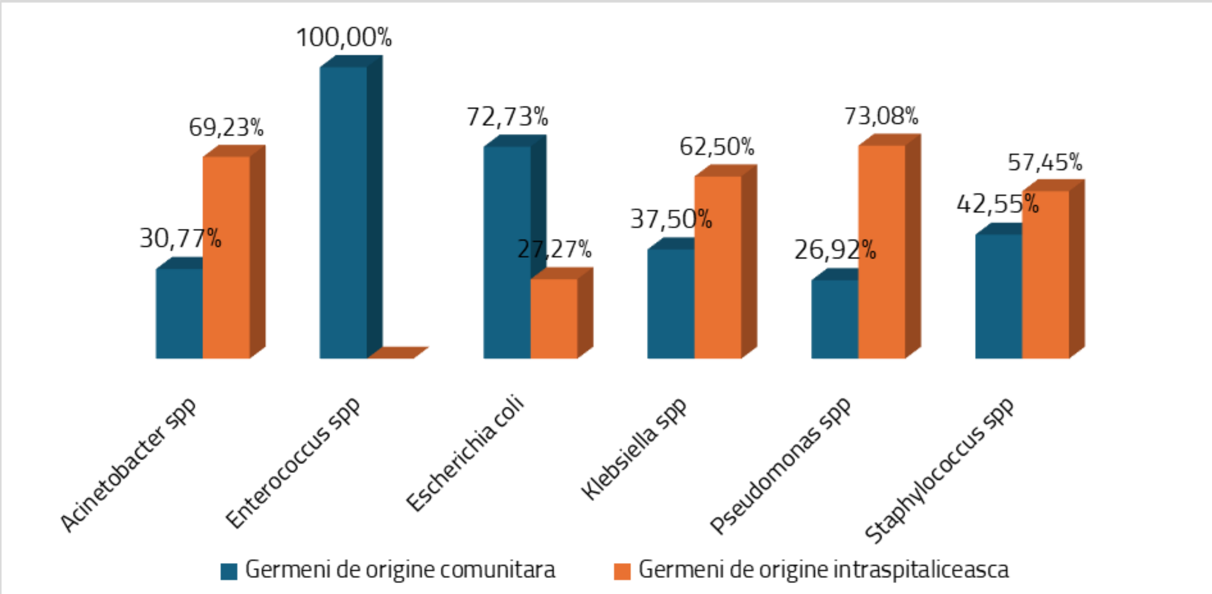

Figure no. 3.8. Community or in-hospital origin according to the time elapsed from the date of hospitalization to the date of isolation of germs in clinical wards with surgical profile

It was found that 55.95% of the bacterial strains identified in bacteriologically positive samples of patients hospitalized in these wards have nosocomial origin, according to the working methodology applied in the other wards included in the study.

Medical Clinical Departments

In the clinical wards with Medical profile, 817 bacterial strains were isolated, maintaining percentages that prioritize circulating Gram-negative flora 81.88% compared to Gram-positive flora - 18.12%, the isolated bacteria coming from hospitalized patients. The prevalence of Gram-negative flora which resists very well on multiple elements in the hospital such as various surfaces, medical devices compared to Gram-positive flora draws attention to the inefficiency of the cleaning and disinfection procedures of the hospital environment.

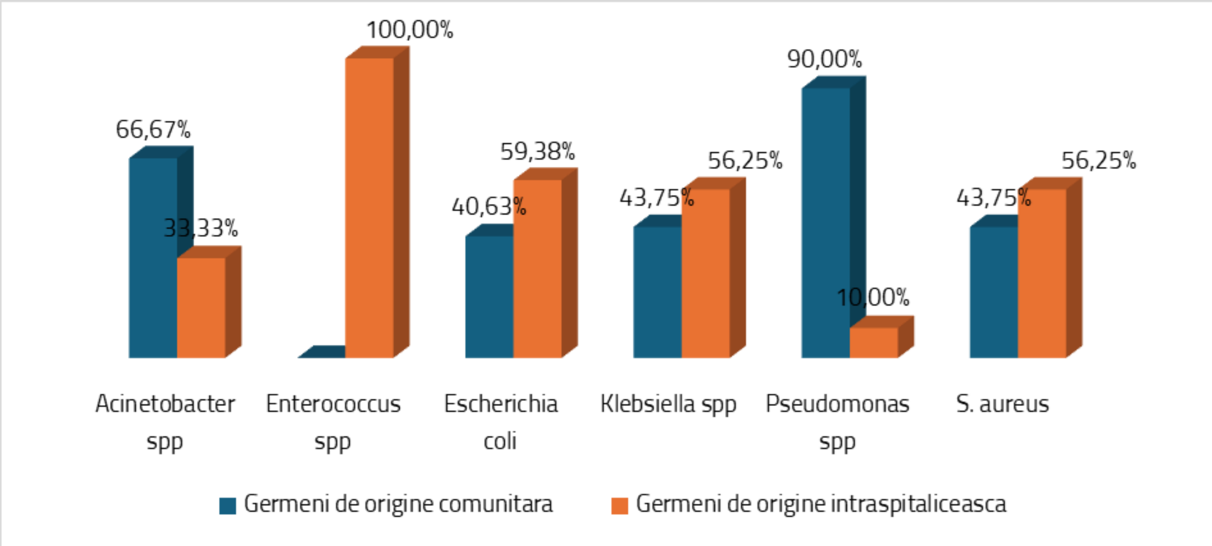

Figure no. 3.11. Community or in-hospital origin according to the time elapsed from the date of hospitalization to the date of isolation of germs by the bacteriology department in the Clinical wards with Medical profile

Consulting the databases of the Clinical wards with Medical profile, 54.48% of hospitalized patients fall into nosocomial etiology of infections detected by positive bacteriologic diagnosis.

Clinical Departments of Oncology - Hematology - Radiotherapy

In the Clinical Departments of Oncology - Hematology - Radiotherapy, 410 bacterial strains were isolated, maintaining the percentages that prioritize circulating Gram-negative flora - 75.61% compared to Gram-positive flora - 24.39%, the isolated bacteria coming from hospitalized patients. The circulating flora finds fertile ground in patients hospitalized in these wards, mostly immunocompromised due to the underlying disease and the treatments performed.

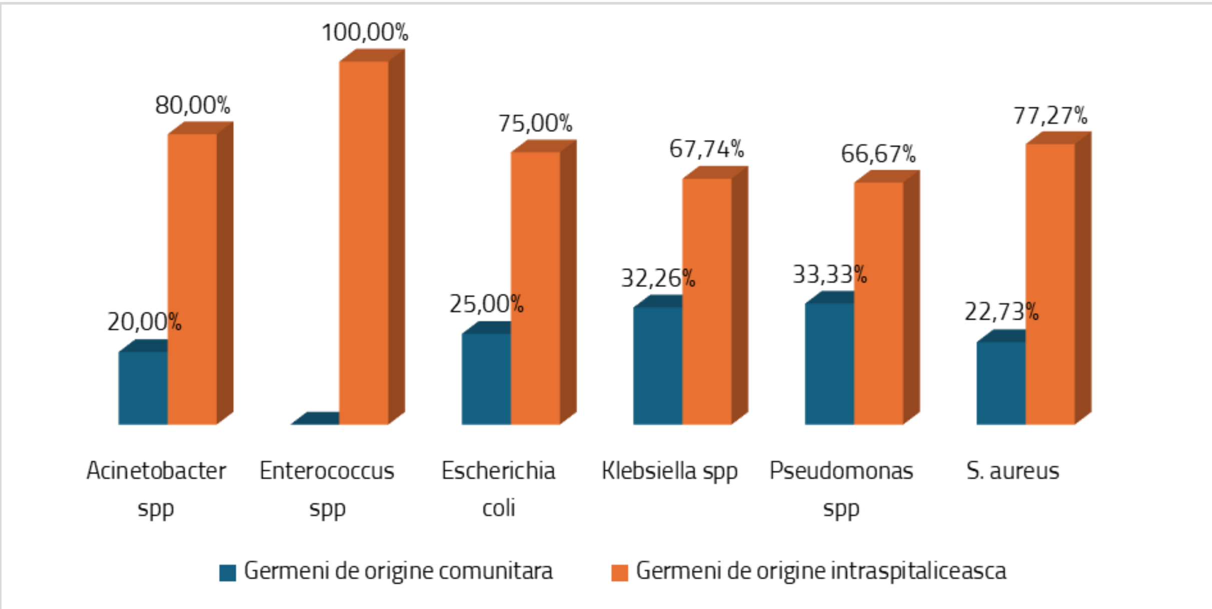

Figure 3.14. Community or in-hospital origin according to the time elapsed from the date of hospitalization to the date of isolation of germs in the clinical wards of Oncology, Hematology, Radiotherapy

From the databases made available by the Clinical Departments of Oncology, Hematology, Radiotherapy, we have
found that for 72.73% of hospitalized patients, the origin of infections found following a positive bacteriological examination was nosocomial, in accordance with the case definition specified in the European Commission Decision (EU) 2018/945 .

3.5.3 Bacteriological characterization of an Emergency County Hospital

Following the objectives of our study, we have broken down the weight of each identified germ type into high, medium and low values, in order to find a formula to describe the bacterial threats facing each ward from a dual perspective. Namely, the germs identified in patients may constitute the source of infection of the hospital environment, but at the same time, other patients may become susceptible persons, the presence of these microbes developing HAIs that may adversely influence their prognosis of the underlying disease.

Table 3.13. Share of germs according to the total number of isolations per hospital

Type of micro-organism	Section Anesthesia Intensive Care %	Sections with Surgical profile %	Sections with Medical profile %	Oncology Hematology Radiotherapy %	Total
Escherichia coli	12,36	42,28	33,72	11,65	100
Klebsiella spp	38,19	26,50	24,42	10,88	100
Proteus spp	17,04	57,78	14,07	11,11	100
Staphylococcus spp	40,32	37,95	12,27	9,46	100
Enterococcus spp	19,80	52,28	19,80	8,12	100
Acinetobacter spp	66,67	21,39	6,97	5,97	100
Pseudomonas spp	31,93	37,95	12,95	17,17	100

High incidence > 30%   Average incidence = 11,51- 29,99%   Low incidence < 11.50%

The highest frequency of strains - 5 - in the Surgical wards and - 4 - in the ICU ward, each of them registering high values, makes the above-mentioned wards the most polluted in the hospital, with a remarkable infectious risk for the rest of the patients and with a high epidemiologic risk of spreading microbes in the hospital. In these wards, more frequent bacteriological self-monitoring of the environment and the medical devices used, as well as screening of staff and patients, is necessary.

The highest frequency of strains - 5 - in the wards with Medical profile registers average values, the degree of pollution of the wards indicating an infectious risk and a moderate epidemiological risk, requiring intensification of cleaning, disinfection and bacteriological self-control measures of bacteriological surveillance at least quarterly or as necessary.

The highest frequency of strains - 5 - with low values is recorded in Oncology, Hematology and Radiotherapy wards. The degree of pollution is acceptable, the infectious risk must be carefully monitored in patients by screening them and the medical and ancillary staff serving them, taking into account their immunodeficient status.

3.7.CONCLUSIONS

1. In the County Emergency Clinical Hospital Gram-negative flora predominates in all wards as follows: in the Clinical wards with Medical profile - 81.88%, in the wards of Oncology, Hematology and Radiotherapy - 75.61%, in the Clinical wards with Surgical profile - 68.46% and in the ICU - 64.80%, which is why the cleaning and disinfection of the hospital environment and medical devices with effective biocides correctly used must become a priority.
2. In the whole hospital the germs with invasive potential have the highest share of 87.47% which denotes a large number of infected patients, the biological product collected coming from normally sterile cavities, compared to colonizing germs isolated in a percentage of 12.53%.
3. For 31.93% of hospitalized patients, the origin of the infection originated from the community environment, and for 68.07% of them the germs were isolated after 3 days of hospitalization, which encouraged us to correlate the data obtained with the case definition of HAI as mentioned in the European Commission Decision (EU) 2018/945 and to categorize them as having a possible nosocomial etiology.
4. In ATI the proportion of germs in descending order is as follows: *Staphylococcus spp* - 31,74%, *Klebsiella spp* - 29,26%, *Escherichia coli* - 12,41%, *Acinetobacter spp* - 11,70%, *Pseudomonas spp* - 9,40%, *Enterococcus spp* - 3,46% and *Proteus spp* - 2,04%.

All the germs in the study were identified in different percentages from the following biological products in decreasing order: tracheo-bronchial aspirates - 31.47%, uro cultures - 16.05%, blood cultures - 15.87%, pharyngeal exudates - 15.51%, nasal exudates - 9.40%, wound secretions - 4.88%, purulent secretions - 4.08%.

Of the total germs isolated in the ICU, 75.09% have invasive potential being isolated from normally sterile sites, 79.32% are of intraspital origin with nosocomial potential and 71.28% have in vitro susceptibility to antibiotics.

5. In the surgical wards the percentage of germs in descending order is: *Escherichia coli* - 34,34% *Staphylococcus spp* - 24,16%, *Klebsiella spp* - 16,42%, *Pseudomonas spp* - 9,03%, *Enterococcus spp* - 7,38%, *Proteus spp* - 5,59% and *Acinetobacter spp* - 3,08%.

All the germs taken in the study were identified in different percentages from the following biological products in decreasing order: purulent secretions - 31.83%, uro cultures - 26.74%, wound secretions - 13.19%, cervical secretions - 8.82%.

10.54% of the microorganisms identified in surgical wards originate from their habitat with colonizer status, 55.95% have community intraspital origin being isolated after an admission period of more than 72 hours and 87.96% have antimicrobial sensitive profile.

6. In the Medical wards the share of germs in descending order is as follows *Escherichia coli* - 46.76%, *Klebsiella spp* - 25.83%, *Staphyloccoccus spp* - 13.34%, *Pseudomonas spp* - 5.26%, *Enterococcus spp* - 4.77%, *Proteus spp* - 2.33% and *Acinetobacter spp* - 1.71%.

All the germs taken in the study were identified in different percentages from the following biological products in decreasing order: urine cultures - 72.58%, sputum - 9.06%, blood cultures - 6.61% and purulent secretions - 5.39%.

The germs identified in bacteriologically positive samples of patients in invasive medical wards represent 96.82% of the total, 54.48% are of nosocomial origin and 82.25% are sensitive to a wide range of antimicrobial substances.

7. In the Clinical Oncology, Hematology, Radiotherapy wards, the proportion of germs in descending order is as follows: *Escherichia coli* - 32,20%, *Klebsiella spp* - 22,93%, *Staphylococcus spp* - 20,49%, *Pseudomonas spp* - 13,90%, *Enterococcus spp* - 3,90%, *Proteus spp* - 3,66% and *Acinetobacter spp* - 2,93%.

All the germs taken in the study were identified in different percentages from the following biological products in decreasing order: uro cultures - 42,44%, wound secretions - 19,02%, sputum - 16,10% and purulent secretions - 12,68%.

Of the germs isolated from patient specimens in this category of wards, those with invasive potential dominate in 95.85%, 72.73% are of nosocomial origin and 81.22% are sensitive to a wide range of antimicrobial substances.

Chapter 4

OPPORTUNITIES IN MONITORING THE CIRCULATION AND RESISTANCE PHENOTYPE OF BACTERIA IN THE WARDS OF A COUNTY CLINICAL EMERGENCY HOSPITAL BY IMPLEMENTING THE WHONET COMPUTERIZED SYSTEM

4.1. INTRODUCTION

The circulation of bacteria in a hospital is a little known reality, mainly because the data provided by the microbiology department of the medical analysis laboratory is not fully utilized333 . The data from the results of bacteriologic examinations are primarily of use to treating physicians in determining the correct antibiotic therapy options, especially in the current context when bacterial resistance to antimicrobials has become a problem not only difficult to understand but also to master worldwide334 .
Knowing the circulation of germs in the hospital and the antibiotics to which they are sensitive or resistant, the hospital pharmacy will provide patients with the appropriate antimicrobial according to the indication of the infectious disease physician who makes the antibiotic policy of the hospital.
In turn, the medical epidemiologist with his team, will track the possibilities by which microbes isolated from patients can pass into the hospital environment, on surfaces, medical equipment and devices, medical and ancillary staff, and through the proposed anti-epidemic measures to limit their implementation to other patients[336] .

The Global Antimicrobial Resistance and Utilization Surveillance System (GLASS) was launched in 2015 to encourage surveillance of antimicrobial resistance (AMR), and develop strategies to limit AMR.

GLASS uses information collected and processed by the WHONET software, which is widely used globally and facilitates standardized analysis of microbiology laboratory data.
In 2017 WHO developed the Global Priority List of priority pathogens implicated in antimicrobial resistance at different levels of priority[342] .

Of great support to clinicians are the WHONET alerts on outbreaks of infections caused by resistant micro-organisms, newly emerging resistance mechanisms or significant increases in antibiotic resistance, which are critical for preventing and controlling the spread of infections[343] .
All microorganisms in our study showing multiple antibiotic resistance are on the Global Priority List of Pathogens and have different levels of microbiological alert.
The strains of Klebsiella spp, Escherichia coli, Acinetobacter spp and Pseudomonas spp that have developed resistance to carbapenems, a class of antibiotics used in the treatment of severe infections, are classified as critical priority due to severe limitations in the treatment of the infections they cause.

Strains of Staphylococcus aureus MRSA, Enterococcus VRE, Escherichia coli ESBL and Klebsiella spp ESBL are classified as high priority, have a significant increase in AMR and can cause severe infections that are difficult to treat.

4.2 PURPOSE OF THE RESEARCH

- Bacteriological characterization of the wards of a County Clinical Emergency Hospital according to the MDR, XDR and PDR resistance phenotype of the bacteria included in the study taking into account the microbiological alerts generated by these bacteria and the assessment of the risk of infections to which patients are exposed to patients from whose samples these microorganisms were isolated.

4.3. RESEARCH OBJECTIVES

- analysis of antibiotic resistance rates of the microorganisms included in the study;
- germ analysis according to MDR, XDR, and PDR resistance phenotypes;
-analysis of microbiological alerts generated by the presence of these microorganisms in samples of hospitalized patients from the clinical infectious, bacteriological and epidemiological point of view.

4.4. MATERIAL AND METHOD

The retrospective longitudinal study included the results of bacteriological examinations performed in the Microbiology Department of the Clinical Laboratory of Medical Analysis of a County Emergency Hospital between January 1 and December 31, 2020. 3750 bacterial strains were identified, isolated from 17 categories of biological products, of which 714 strains showed different antibiotic multidrug resistance profiles.

4.4.4. Data processing methods

Collected data containing bacteriological positives of biologicals worked in the laboratory from January 2020 through December 2020 were extracted and organized into a Microsoft Excel 2022 data sheet. This was then exported and analyzed using WHONET (version 5.6), a database software package for microbiology laboratory data management and analysis of antibiotic susceptibility test results.

4.5. RESULTS

4.5.1. Analysis of the antibiotic resistance profile of the micro-organisms under study

In order to achieve the proposed objectives, we used a statistical analysis program W H ONET recommended by the WHO, which generated reports with results including several items, which allowed the particularization of each tested strain. It is also worth mentioning that for each bacterial strain, the number of antibiotics recommended for testing is different but, in addition, the antibiotic palette used is also influenced by the financial resources of the executing laboratory.

The WHONET program generated box-plot diagrams considered as visual tools that express the percentage level of resistance for each strain, making it possible to interpret trends in antibiotic resistance over a given period of time.

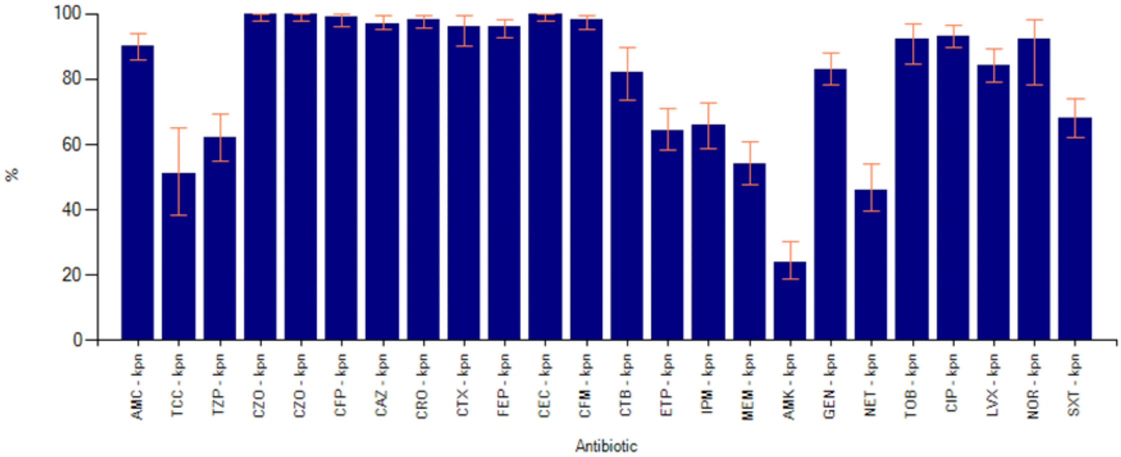

Figure no. 4.1 Box-plot plot of antibiotic resistance of Klebsiella spp. strains

For the 262 strains of multi-resistant *Klebsiella spp*, tested in different numbers against 24 antibiotics, the box-plot generated by the WHONET program expressed the percentage resistance levels of the strains that ranged from 100% to 24.26%.

The high level of resistance recorded for *Klebsiella spp*. strains limits the therapeutic options, with the use of first-line antibiotics impossible and the need to resort to reserve antibiotics. The resistance to Beta-lactam and Fluoroquinolone antibiotics present in more than 50% of the *Klebsiella spp* strains tested, requires to change the treatment initiated until the laboratory result is received and to replace it with Aminoglycosides, Carbapenems, Beta-lactam combinations with inhibitors.

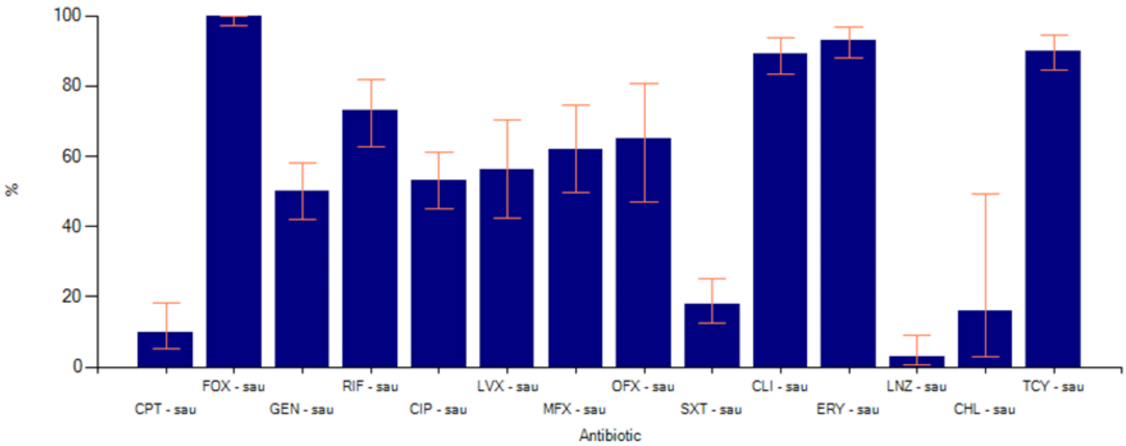

Figure no. 4.2. Box-plot plot of antibiotic resistance of Staphylococcus aureus strains

For the 181 strains of *Staphylococcus aureus* tested in different numbers against 13 antibiotics, the box-plot generated by WHONET expressed AMR levels ranging from 100% to 3.00%.

The level of AMR recorded for these strains restricts the range of antimicrobials available to treating physicians, with the treatment of MRSA infections requiring the use of second and third line antibiotics such as Vancomycin, Linezolid and Daptomycin.

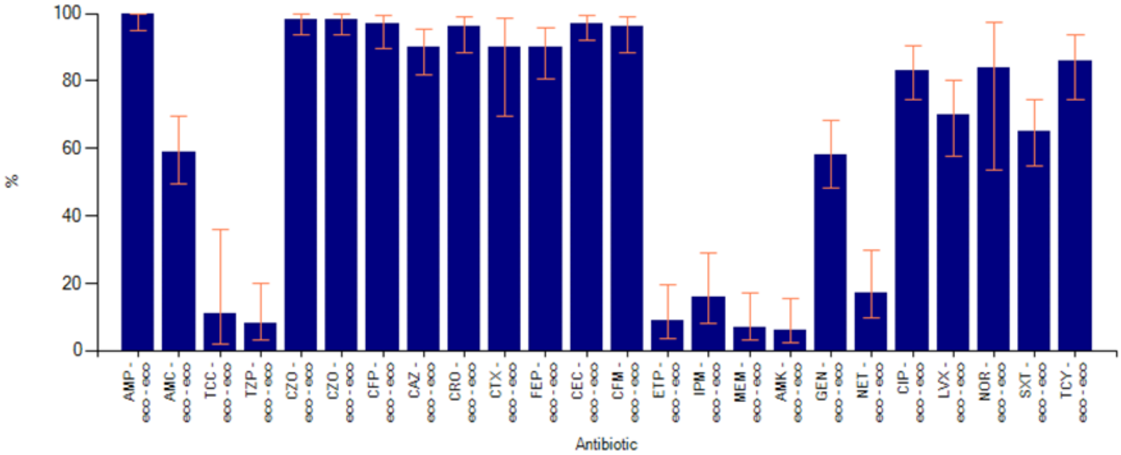

Figure no. 4.3. Box-plot of antibiotic resistance of Escherichia coli strains

The 98 strains of Escherichia coli tested in different numbers against 24 antibiotics had AMR levels ranging from 100% to 6.67%.

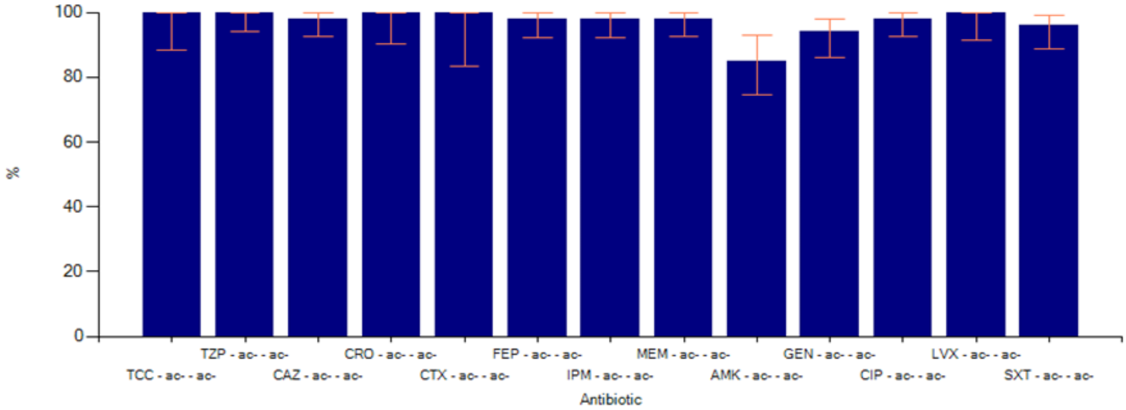

Figure no. 4.4. Box-plot plot of antibiotic resistance of Acinetobacter spp strains

For the 88 strains of *Acinetobacter spp*. tested in different numbers to the 13 antibiotics, the box-plot plot expressed percentage levels of resistance that ranged from 100% to 85.94%.

The peak level of resistance of Acinetobacter spp strains to most available antibiotics, including carbapenems, severely limits therapeutic options, necessitating the use of rescue antibiotics such as colistin or tetracycline.

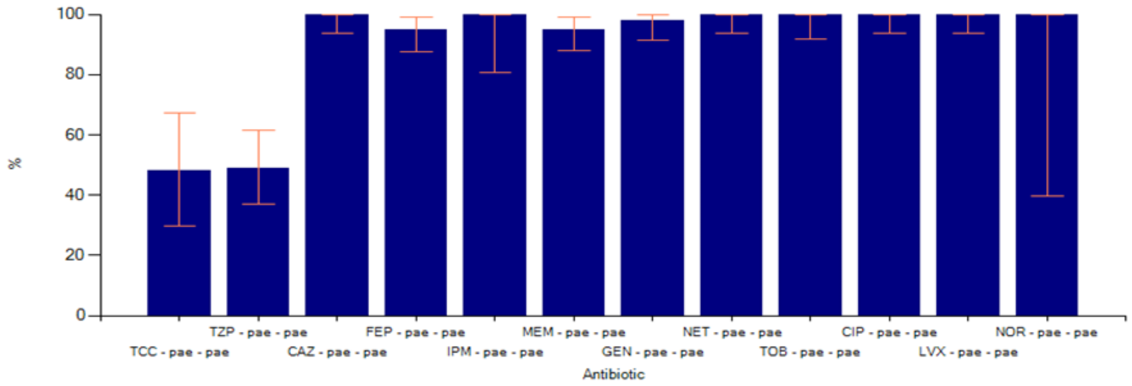

Figure no. 4.5. Box-plot plot of antibiotic resistance of *Pseudomonas spp*. strains

The 78 strains of *Pseudomonas spp*, multi-resistant *Pseudomonas spp*, tested in different numbers to 12 antibiotics, generated a box-plot that expressed resistance levels ranging from 100% to 48.28%. Such a high level of resistance forces the treating doctor to resort to back-up antibiotics such as colistin, phosphomycin or aminoglycosides.

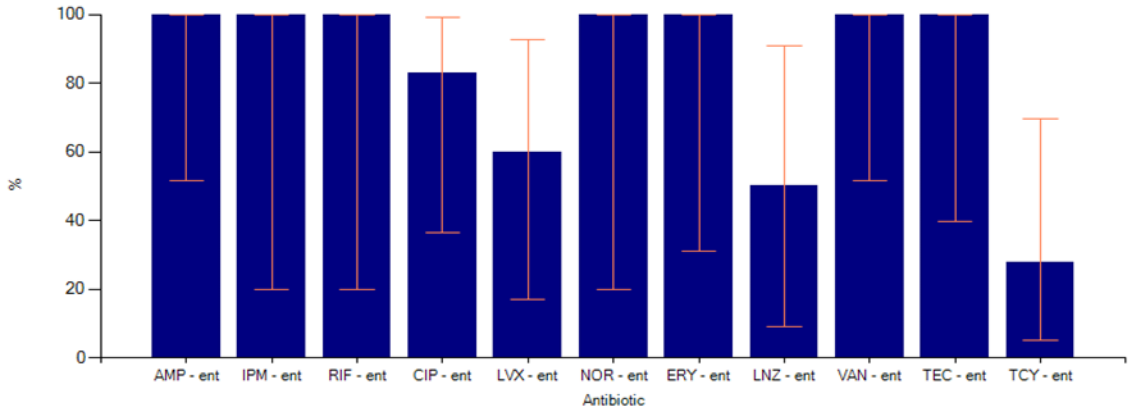

Figure no. 4.6. Box-plot plot of antibiotic resistance of *Enterococcus spp*. strains

For the 7 strains of *Enterococcus spp*. tested in different numbers against 11 antibiotics, the box-plot provided by the WHONET program expressed percentage levels of resistance ranging from 100% to 28.57%.

4.5.2. WHONET software analysis of the distribution of resistance phenotypes of bacteria isolated in the hospital and in wards

Based on the data obtained and reported, the WHONET program, following the data obtained, it was possible to create resistance profiles by categorizing the microorganisms into the three phenotypes: MDR, XDR and PDR.

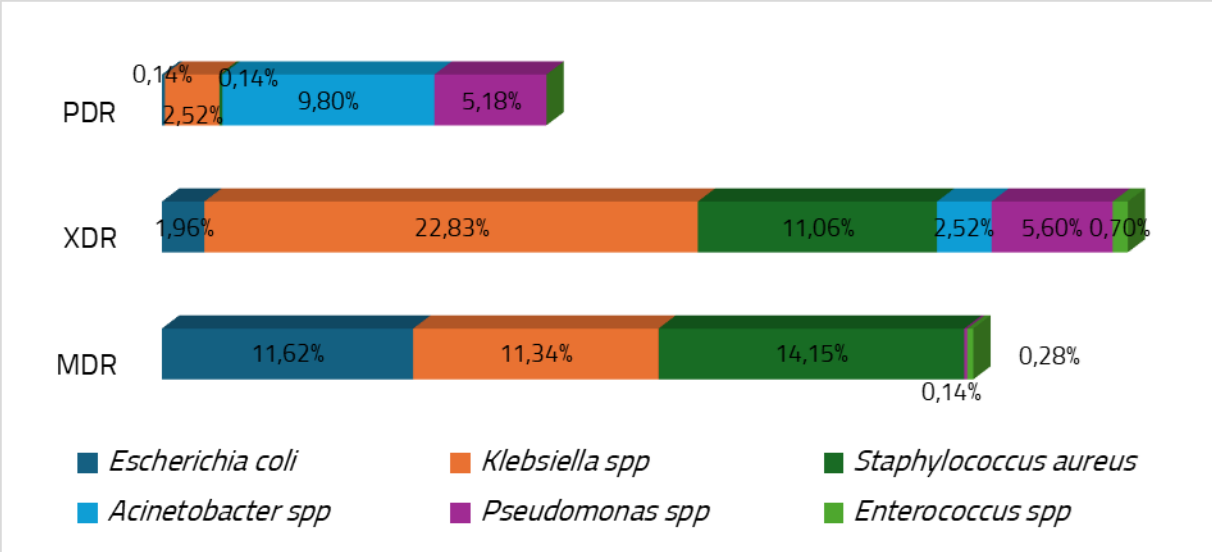

Figure no. 4.7. Distribution of bacterial resistance phenotypes in the Hospital

The presence of MDR, XDR and PDR germs in the hospital has significant epidemiologic implications that warn about the risks they pose to the hospital environment, other patients and the need to seriously implement control and prevention measures.

4.5.3 The role of microbiological alerts in rapid intervention and monitoring of bacterial resistance in a County Clinical Emergency Hospital

The microbiology alerts generated by WHONET signal the emergence of a new micro-organism or the growth and spread of antibiotic-resistant bacterial strains, which pose a risk to both patients and infection control in hospitals.

Depending on the severity and urgency of the situation, microbiological alerts are categorized into low, medium and high priority levels.

Low-priority microbiological alerts identify minor but recent changes in AMR profiles, identified sporadically, do not indicate the risk of an outbreak at the moment, but there are long-term consequences.

Medium-priority microbiology alerts indicate an increased incidence of microorganisms with AMR to essential antibiotics concomitant with early detection of their transmission between hospital wards, with the possibility of emerging resistant bacteria.

High-priority microbiological alerts include critical pathogens that pose a major threat to patients, staff and the hospital environment, signaling outbreaks of nosocomial infections caused by multi-resistant bacterial strains, requiring strict infection control measures, isolation of affected patients in specially designated wards.

Based on the microbiologic characteristics of the identified and reported bacterial strain and the clinical and epidemiologic context, the WHONET application generated for the 714 bacterial strains included in our study, a total of 1666 microbiologic alerts, with a predominance of medium priority alerts - 758, followed by low priority - 731 and high priority - 177:

Table 4.8. Microbiological alerts issued by WHONET

Type of micro-organism	Priority level	Anesthesia Intensive Care	Surgical wards	Medical sections	Oncology Hematology Radiotherapy	Hospital
Escherichia coli	Low	16	27	24	11	78
	Average	21	28	31	11	91
	Retrieved	0	5	2	1	8
	Total	37	60	57	23	177
Klebsiella spp	Low	108	44	57	29	238
	Average	40	20	24	14	98
	Retrieved	79	28	40	17	164
	Total	227	92	121	60	500
Staphylococcus spp	Low	82	41	21	19	163
	Average	79	43	18	17	157
	Retrieved	1	1	1	0	3
	Total	162	85	40	36	323
Acinetobacter spp	Low	0	0	0	0	0
	Average	62	11	2	5	80
	Retrieved	0	0	0	0	0
	Total	62	11	2	5	80
Pseudomonas spp	Low	151	42	13	44	250
	Average	103	125	43	56	327
	Retrieved	0	0	0	0	0
	Total	254	167	56	100	577
Enterococcus spp	Low	0	1	0	1	2
	Average	1	0	4	0	5
	Retrieved	0	1	0	1	2
	Total	1	2	4	2	9
Total	Low	357	155	115	104	731
	Average	306	227	122	103	758
	Retrieved	80	35	43	19	177

Type of micro-organism	Priority level	Anesthesia Intensive Care	Surgical wards	Medical sections	Oncology Hematology Radiotherapy	Hospital
	Total	743	417	280	226	1666

The highest percentage of microbiological alerts belonged to multi-resistant strains of *Pseudomonas spp* with, representing 34,63% of all alerts, followed by *Klebsiella spp* - 30,01% of alerts, *Staphylococcus aureus* - 19,39%, *Escherichia coli* - 10,62%, *Acinetobacter spp* - 4,80% and *Enterococcus spp* - 0,54%.

The highest number of microbiological alerts were issued for the ICU ward representing 44.66% of the total alerts for the entire hospital followed by the Clinical Surgical wards - 25.03%, Clinical Medical wards - 16.81% and the Oncology, Hematology, Radiotherapy wards with 13.57%.

4.6. DISCUSSIONS

The microbiological alerts issued by WHONET play an essential role in current medical practice, providing the data needed to monitor, prevent and manage infections and antimicrobial resistance. WHONET acts as a trigger that sets off a chain reaction involving multiple steps and participants in the healthcare chain.

The results of the microorganism identification tests and reported antimicrobial resistance profiles are analyzed by WHONET, which detects antimicrobial resistance abnormalities or trends exceeding predefined thresholds, automatically generating microbiological alerts.

The chain reaction triggered by WHONET brings a number of important benefits such as a prompt, rapid and coordinated response reducing the time to action, reduces the spread of multi-drug resistant germs in the hospital, improves the quality of patient care and contributes to the continuous updating of the antibiotic stewardship policy.

4.7. CONCLUSIONS

1. The bacteriologic profile of the Clinical ICU section shows notable differences between the bacterial strains isolated, with *Klebsiella spp*. with a share of - 36.73%, its presence being favored by the isolation of bacteria from tracheo-bronchial aspirates and associated with mechanical ventilation of patients, followed by *Staphylococcus aureus* - 24.60%, prevalent in pharyngeal and nasal exudates, *Acinetobacter spp* - 20.68% resistant in the moist environment provided by mechanical ventilation equipment, *Pseudomonas spp* - 11.11% - same observation as above, the last two *Escherichia coli* - 6.48*%, Enterococcus spp*. - 0,31% isolated being in uro cultures.

Germs with resistance profile XDR in this section, in percentage 48,01% hold the supremacy, followed by microorganisms with resistance phenotype MDR in percentage 26,30% and those with resistance profile PDR with 25,69%. Analyzing the prevalent bacterial strains of each resistance phenotype, we find that in the MDR phenotype, Staphylococcus aureus is in first place with 39.53%, in the XDR profile the highest share of 50.32% is held by Klebsiella spp, and in the PDR profile, Acinetobacter spp prevails with 64.29%.

The microbiological alerts with the highest prevalence are represented by those with low priority - 48.05% followed by microbiological alerts with medium priority with a share of 41.18%, the pathogen with the highest share in both categories of alerts being *Pseudomonas spp*.

2. In the clinical wards with a surgical profile, the highest share of multi-resistant germs is held by strains of Klebsiella spp - 28.57% in a lower percentage than their share in the ICU and frequently isolated from uro cultures, followed by strains of *Staphylococcus aureus* - 27.98% identified predominantly from purulent secretions, Escherichia coli - 19.64% found mainly in uro cultures, *Pseudomonas spp* - 15.48% from wound secretions, *Acinetobacter spp* - 7.74% from wound secretions, Enterococcus spp. - 0.60% of uro cultures.

Bacteria with the MDR resistance profile occupy the first place with an isolation rate of 43.98%, followed by bacteria with the XDR profile with 42.77% and then by microorganisms with the PDR profile with 13.25%. Analyzing the germs in each resistance profile, in the MDR category the first place is occupied by Escherichia coli with a percentage of 39.73%, in the XDR category Klebsiella spp. prevails with 39.44% and in the PDR profile, the supremacy is held by Pseudomonas spp. with 45.45%.

The highest share of microbiological alerts, 54.44%, is held by medium priority microbiological alerts, followed by low priority microbiological alerts with 37.07% and then by high priority with 8.39%. Also in these wards, the highest percentage of alerts is generated by *Pseudomonas spp*.

3. In the clinical wards with a medical profile, the bacteria multi-resistant to the action of antibiotics with the highest share are also represented by Klebsiella spp - 44.14% frequently isolated from uro cultures, followed by *Escherichia coli* - 22.07% found predominantly in uro cultures, Staphylococcus aureus 22.07% isolated from purulent secretions, *Pseudomonas spp* - 6.90% from uro cultures, *Enterococcus spp*. - 2.76% also from uro cultures and *Acinetobacter spp* - 2.07% from tracheo-bronchial aspirates.

Germs with an MDR resistance profile are the most prevalent, with a 52.08% share, followed by XDR germs with a 38.19% share and then PDR germs with an isolation rate of 9.72%. In the MDR germ category, Escherichia coli prevailed with a weight of 38.07%, the XDR and PDR resistance phenotypes being dominated by *Klebsiella spp* strains with weights of 69.09% and 35.71% respectively.

The microbiological alerts with the highest weight in the Medical profile wards are represented by those with medium priority in percentage of 43.57% followed by microbiological alerts with low priority with a weight of 41.07%, the last place being the microbiological alerts with high priority, in percentage of 15.36%. The micro-organism involved in the generation of microbiological alerts with the highest share of 43.21% is Klebsiella spp.

4. The multi-resistant germs identified in the Clinical Departments of Oncology, Hematology, Radiotherapy show the following isolation rates in descending order: Klebsiella spp - 40,26%, frequently isolated from uro cultures, *Staphylococcus aureus* - 28,57% identified from purulent secretions, *Escherichia coli* - 15,58% from urocultures, *Pseudomonas spp* - 7,79% frequently found in purulent secretions, *Acinetobacter spp* - 6,49%, also from purulent secretions and Enterococcus spp. - 1.30% isolated from urine cultures.

The highest share was registered by bacteria with XDR resistance profile - 46.75% followed by microorganisms with MDR resistance profile - 44.16% and then by PDR - 9.09%. In the group of MDR germs, the first place is held by *Staphylococcus aureus* in the percentage of 44.12%, in the XDR resistance phenotype the supremacy is held by *Klebsiella spp* representing 50.00% of the total, and in the category of PDR germs in these sections prevails *Acinetobacter spp* with a weight of 71.43.

In the departments of Oncology, Hematology and Radiotherapy, the most frequent microbiological alerts are the low priority ones with a share of 46.02% followed by the medium priority ones with a share of 45.58% and finally the high priority ones with a share of 8.41%. Pseudomonas spp is responsible for the highest share - 44.25% of microbiological alerts in this category of wards.

Chapter 5

FINAL CONCLUSIONS

The bacteriological characterization of clinical wards according to activity profile is as follows:

In the Anesthesia Intensive Care Unit

Staphylococcus spp frequently isolated from nasal, pharyngeal exudates, blood cultures and Klebsiella spp associated with tracheo-bronchial aspirates in mechanically ventilated patients are the bacteria with the highest proportion in the ward, followed by Acinetobacter spp and Pseudomonas spp. Invasive germs, antibiotic-susceptible strains and nosocomial bacteria predominate.
Low priority microbiological alerts represent 48.05%, medium priority alerts represent 41.18% of the total multidrug-resistant germs circulating in the ward, Pseudomonas aeruginosa being the most common pathogen incriminated in both conditions.
The ICU is a high-risk environment for bacterial infections, where bacteriologic profiling and resistance require special attention and interdisciplinary approaches to improve the quality of care of critically ill patients.

Surgical Clinical Departments

Escherichia coli is most frequently isolated from uro cultures, purulent secretions and cervical secretions and Staphylococcus spp associated with purulent secretions and wound secretions are the most prevalent germs in this category of sections, followed by Klebsiella spp and Pseudomonas spp. Invasive germs, antibiotic-susceptible bacterial strains and bacteria of nosocomial origin predominate.
Medium priority microbiologic alerts represent 54.44%, medium priority alerts represent 37.07% of the total number of multi-resistant germs circulating in the Clinical Surgical wards, Pseudomonas spp being the most frequently incriminated microorganism in both conditions.
Clinical Surgical wards constitute an environment exposed to microbial infectious risks, with a significant proportion of resistant bacteria that require constant monitoring both in terms of circulation and antibiotic resistance, concomitant with the strict implementation of infection control measures, essential for effective management of this problem.

Medical Clinical Departments

Escherichia coli is the most frequently identified microorganism with a significant presence especially in uro cultures and Klebsiella spp. associated also with uro cultures but also with sputum, followed by Staphylococcus spp. and Pseudomonas spp. Invasive germs, sensitive to most antibiotics and bacterial strains of nosocomial origin are also predominant.
Medium priority microbiological alerts represent 43.57%, low priority alerts represent 41.07%, multi-resistant Klebsiella spp being the most common bacteria responsible for generating these alerts.

The higher prevalence of invasive germs in Medical Clinical wards is associated with an increased risk of severe infections and the differences in the bacteriologic profile require specific measures for infection prevention and control.

Clinical Departments of Oncology Hematology Radiotherapy

Escherichia coli is the most common microorganism isolated from uro cultures, wound secretions and sputum and *Klebsiella spp* associated with the same types of biologicals, followed by *Pseudomonas spp* and *Staphylococcus aureus*. Invasive germs, sensitive to a wide range of antibiotics and predominantly nosocomial in origin, still predominate.

Low priority microbiological alerts represent 46.02%, closely followed by medium priority alerts with 45.58%, *Pseudomonas spp* being the main microorganism involved in the production of these alerts.

Clinical Departments of Oncology, Hematology, Radiotherapy face a bacteriologic profile dominated by antibiotic resistant germs, which requires an integrated and multidisciplinary approach for their management. The high proportion of nosocomial germs requires the adoption of effective strategies to prevent nosocomial infections.

The hospital-wide WHONET program can provide important information on the emergence of a new or particular antibiotic resistance phenotype, the transmission of identified germs between hospital wards, and detect nosocomial clusters, thus urgently prompting outbreak interventions.

The applicability of the WHONET software is very important in the hospital antibiotic policy.

Microbiologic alerts are momentary, identified on each type of bacterial strain and have maximum applicability. These alerts allow monitoring and rapid response to bacterial infections, helping to prevent the spread of infections in the hospital.

Compared to clinical guidelines that provide general recommendations for antibiotic treatment, WHONET offers originality and targeted applicability, allows specific and real-time monitoring of bacterial resistance, providing accurate and personalized data for each hospital.

The study is real, original and carried out at minimal cost, emphasizing its value and practical relevance, demonstrating that the data obtained are authentic and directly applicable in the context of the hospital studied, and the methodology used has proven its effectiveness.

CONTRIBUTIONS TO SCIENCE

1. To conduct a longitudinal, retrospective, descriptive and analytical epidemiological study including 17 categories of biological products collected from patients in 2020 and examined in the Clinical Laboratory of Medical Analysis at the request of attending physicians in order to characterize the bacteriological bacteriology of a County Clinical Emergency Hospital.

2. To perform a second retrospective longitudinal study on the bacterial strains identified in order to correlate the bacteriologic profile of the germs identified in the wards of a County Hospital with their habitat, resistance profile and community or nosocomial origin.

3. Bacteriological characterization of the wards of a County Emergency Hospital according to the MDR, XDR and PDR resistance phenotype of the bacteria included in the study taking into account the microbiological alerts generated by the WHONET program for these bacteria and the assessment of the infectious risk to which patients are exposed to from whose samples these microorganisms were isolated in the 3rd study.

USEFULNESS OF RESEARCH RESULTS

Research results could be useful for:

1. Prevention and control of nosocomial infections

Knowledge of the types of circulating germs and their resistance to antibiotics allows the hospital to implement more effective infection prevention and control measures, including procedures to limit the spread of germs, stricter hygiene and sterilization and adapted to the specificity of the microorganisms identified.

2. Personalized patient treatment

Identifying germs and their resistance to antibiotics helps doctors choose the right antibiotics to treat infections, thereby reducing the unjudicious use of antibiotics and helping to combat antimicrobial resistance.

3. Resource planning and procurement

Hospitals can use this data to plan the purchase of antibiotics and other necessary resources, making sure they have the effective drugs available for the germs identified.

4. Continuing medical educationand training of health professionals

Medical staff can be educated and trained based on research findings to improve infection prevention and control techniques, as well as to respond promptly and effectively to cases of AMR, germ circulation between hospital wards and nosocomial infections.

5. Continuous monitoring and evaluation

Hospitals can use this data to continuously monitor and evaluate the effectiveness of infection control measures and to adapt work protocols to the circulation of germs and their resistance.

6. Improving public health policies

Analysis of microbiological alerts can provide information about infection trends and outbreaks, enabling the hospital and competent authorities to take prompt action to limit the spread of infections.

FUTURE RESEARCH DIRECTIONS

On the basis of the initial study on germ circulation in a hospital, the following research directions could be explored in the future:

1. Actual implementation of the WHONET program in the microbiology department within 1 year maximum.
2. Monitoring, according to the objectives of the 3 studies, of germ circulation in the ICU and surgical wards, 9 months.
3. Extension of the study over a period of at least 6 months in the ICU and surgical wards on the evolution trends of germ circulation in these wards related to AMR, nosocomial or community origin and patient care.
4. Organization by the microbiologist of an interdisciplinary team consisting of department heads, infectologist, epidemiologist, pharmacist, to analyze together the familiarization with the WHONET program, the analysis of the evolutionary trends of germ circulation in the wards proposed for research and the antibiotic resistance profile of the identified germs and their nosocomial potential.

DISSEMINATION OF RESULTS

Dissemination and capitalization of the results materialized through:

- Publication of 2 papers as first author and participation in 2 papers as co-author.

SELECTIVE BIBLIOGRAPHY

1. Essentials of Glycobiology. 2nd edition.Varki A, Cummings RD, Esko JD, et al., editors.Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
7. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016;14(8):e1002533. Published 2016 Aug 19. doi:10.1371/journal.pbio.1002533
49. BARBER M. Hospital infection yesterday and today. J Clin Pathol. 1961;14(1):2-10. doi:10.1136/jcp.14
52. Haque M, Sartelli M, McKimm J, Abu Bakar M. Healthcare-associated infections - an overview. Infect Drug Resist. 2018;11:2321–2333. doi: 10.2147/IDR.S177247
56. Ricchizzi E, Latour K, Kärki T, et al. Antimicrobial use in European long-term care facilities: results from the third point prevalence survey of healthcare-associated infections and antimicrobial use, 2016 to 2017. Euro Surveill. 2018;23(46):1800394. doi: 10.2807/1560-7917.ES.2018.23.46.1800394
122. Todar, K. (2007) Todar's Online Textbook of Bacteriology. http://www.textbookofbacteriology.net/normalflora.html
125. Siegel SJ, Weiser JN. Mechanisms of Bacterial Colonization of the Respiratory Tract. Annu Rev Microbiol. 2015;69:425-444. doi:10.1146/annurev-micro-091014-104209
126. Petersen AM. Gastrointestinal dysbiosis and Escherichia coli pathobionts in inflammatory bowel diseases. APMIS. 2022;130 Suppl 144(Suppl 144):1-38. doi:10.1111/apm.13256
131. Halim AS, Khoo TL, Saad AZ. Wound bed preparation from a clinical perspective. Indian J Plast Surg. 2012;45(2):193-202. doi:10.4103/0970-0358.101277
133. Sikora A, Zahra F. Nosocomial Infections. In: StatPearls. Treasure Island (FL): StatPearls Publishing; April 27, 2023.

135. National Institute of Public Health. Antibiotic Consumption, Microbial Resistance and Health Care Associated Infections in Romania - 2021. National Institute of Public Health; 2021. Availa ble at: https://example.com/CARMIAAM-ROM-2021.
136. Raoofi S, Pashazadeh Kan F, Rafiei S, et al. Global prevalence of nosocomial infection: A systematic review and meta-analysis. PLoS One. 2023;18(1):e0274248. Published 2023 Jan 27. doi:10.1371/journal.pone.0274248
155.McGowan JE Jr. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. Rev Infect Dis. 1983;5(6):1033-1048. doi:10.1093/clinids/5.6.1033
163. WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 – 2020 data. Copenhagen: WHO Regional Office for Europe; 2022.
178. Almagor J, Temkin E, Benenson I, Fallach N, Carmeli Y; DRIVE-AB consortium. The impact of antibiotic use on transmission of resistant bacteria in hospitals: Insights from an agent-based model. PLoS One. 2018;13(5):e0197111. Published 2018 May 14. doi:10.1371/journal.pone.0197111
180. Antibiotic Resistance Threats in the United States, 2019 https://www.cdc.gov/ncezid/ index.html
186. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front Microbiol. 2019;10:539. Published 2019 Apr 1. doi:10.3389/fmicb.2019.00539
192. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x
288. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev. 2011;24(1):141-173. doi:10.1128/CMR.00027-10
334. Chandler CIR. Current accounts of antimicrobial resistance: stabilisation, individualisation and antibiotics as infrastructure. Palgrave Commun. 2019;5(1):53. doi:10.1057/s41599-019-0263-4
336. Joshi S. Hospital antibiogram: a necessity. Indian J Med Microbiol. 2010;28(4):277-280. doi:10.4103/0255-0857.71802
342. O'Brien TF, Stelling J. Integrated Multilevel Surveillance of the World's Infecting Microbes and Their Resistance to Antimicrobial Agents. Clin Microbiol Rev. 2011;24(2):281-295. doi:10.1128/CMR.00021-10

LIST OF PUBLICATIONS

1 Ioana Fluturu, Adriana Magdalena Dulbabă, Elena Parapiru, Codruța Nemet , Klebsiella pathogen eskape - bacteriological profile of emergency hospital wards, Acta Medica Transilvanica; 29(1):7-10 DOI 10.62764/amtsb-2024-0003 Online ISSN 2285-7079

2. Ioana Fluturu, Adriana Magdalena Dulbabă, Tiberiu Marius Marin, Codruța Nemet Epidemiological considerations regarding the circulation of multidrug-resistant germs in a high nosocomial risk hospital, Acta Medica Transilvanica; Sept 2023, Vol. 28 Nr 3, p11-14, ISSN 14531968

3. Dulbabă Adriana Magdalena, Fluturu Ioana, Nemet Codruța (2024). Causes of Failure in Peripheral Venous Venous Catheter Insertion: a Prospective Epidemiologic Study. Acta Medica Transilvanica, Transilvania University of Brașov.

4. Adriana Magdalena Dulbabă, Ioana Fluturu, Tiberiu Marius Marin,Codruța Nemet, Peripheral venous catheter - omissions in assembly, care, mounting, disposal, Acta Medica Transilvanica; Sept 2023, Vol. 28 Number 3, p29-32, ISSN 14531968