République Algérienne Démocratique et Populaire Ministère de l'enseignement supérieur et de la recherche scientifique

Université ABOU BAKR BELKAID de Tlemcen

Faculté des sciences de la nature et de la vie et des sciences de la terre et de l'univers

Département de Biologie

Laboratoire de **M**icrobiologie **A**ppliquée à l'**A**gro-alimentaire, au **B**iomédical et à l'**E**nvironnement

THESIS

For obtaining

MASTER'S DEGREE IN BIOLOGICAL SCIENCES

OPTION: Microbiology and Quality Control

THEME

Evaluation of biofilm formation by strains isolated from medical devices from Tlemcen University Hospital Center

Presented by

- **MUSAAZI KEITH**
- **TARINGA TAKUDZWA LATOYAH**

Before the jury composed of:

President:	BELLIFA Samia	M.C.A Tlemcen University
Supervisor:	SAKER Meriem	Professor Tlemcen University
Co supervisor:	MEDJADI Sidi Mohamed	Professor Tlemcen University Hospital
Examiner:	CHARIF ANTER Asma	M.C.A Tlemcen University (ISTA)

Academic year 2024-2025

Acknowledgments

First and most importantly, we are really grateful and thankful to the Almighty God, for the greatest gift of life and for enabling us reach this milestone despite all conditions. All glory and honor go back to You, Lord.

To all of the esteemed jury members:

We would like to express our sincere appreciation to Professor SAKER Meriem, for not only accepting to be our research supervisor when we reached out to her but also being the best supervisor, we could ask for. A very big thank you for your attentive supervision, precious advice, constant help and your professionalism which greatly inspires us. Your kindness and willingness to step in allowed us to stay focused and continue moving forward. We are incredibly grateful for your generosity and trust you have shown in us.

We would also like to share out heartfelt gratitude to Dr. BELLIFA Samia for being the finest co- supervisor and mentor throughout our years spent at the faculty. Even when we were faced with difficulties, you were always there ready to listen to us and find a solution the best way you could. We appreciate you for welcoming us to the laboratory, the regular guidance and hard work in ensuring our research work is top notch.

We extend our deepest appreciation to Professor CHARIF ANTER Asma, and the members of the Jury, for the honor that they have given us as they accepted to evaluate our work, for your time, vigor and your constructive remarks.

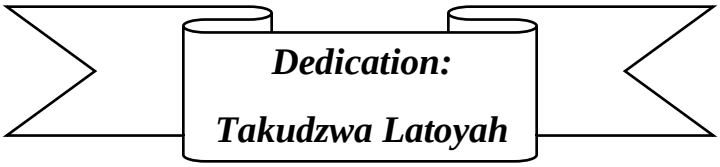
This thesis is the result of months of work, observation, and investigation at the Laboratoire de **Microbiologie Appliquée à l'Agro-alimentaire, au Biomédical et à l'Environnement (LAMAABE)** - University of Tlemcen where Dr Bellifa introduced us to team (Equipe 2). We would like to extend our earnest thanks to

Mohammed Soufiane Boushaba, Amel Chiali, and Zahra Douli, for being more than just colleagues, but friends in a place far from home. Your unwavering support, patient guidance, and shared laughter turned challenges into lessons and moments into memories. We will always be grateful for the kindness and camaraderie that made this lab feel like family.

Additionally, we want to express our gratitude to the Tlemcen University Hospital Center's (CHU) Anesthesia-resuscitation department for assisting us in obtaining the samples we required for our study. Special thanks goes to Dr MEDJADI Sidi Mohammed, a kind and wholesome gentleman, always ready to help us the best way he could and also thank you for accepting us to do this research in your department. To his team of professional medical personnel, Dr Hicham, Dr Meriem, your assistance was unmeasurable, thank you so much, we are grateful!

We would also like to extend our earnest gratitude to all our lecturers for your teachings and great contributions our academic journey.

Finally, to our families and friends, your immeasurable love and support has always kept us going. May we all live to see best time has to offer. Thank you all for being part of our journeys.😊



Firstly, I want to thank God Almighty, whose grace, guidance, and unending blessings have sustained me throughout this journey.

I dedicate this work to my beloved parents, thank you for your unconditional love, prayers, and unwavering support. Your sacrifices and encouragement have always been my greatest source of strength.

To my Dad, William Taringa, who made me his little angel, his princess, never said no and did everything to shape me into a woman I am today. I carry your strength and kindness with me in all that I do.

To my mom, the strongest woman I know, your love and prayers keeps me moving.

To my younger siblings, you are my constant source of joy and light. Your laughter and innocence bring happiness even on the toughest days.

To my older brother, Tafara Romeo, your love, encouragement, and moral support have meant the world to me. You have never always believed in me. Your cheer never failed to lift my spirit.

To my best friend, Progress Hambirepi, thank you for the countless laughs and shared memories. Our mutual encouragement and shared drive to grow have pushed me to aim higher and do better.

Finally, to all my family members, I am endlessly
grateful to each one of you.

To my dear friends I had the joy of meeting in Algeria, thank you for making this journey unforgettable. Your friendship, kindness, and the moments we shared turned challenges into memories and everyday life into something truly special.

To my binôme, Musaazi Keith, thank you for being the most patient, understanding, and reliable research partner I could ever ask for. I genuinely value every moment, every discussion, the joyful moments we shared, the time we spent working tirelessly and every challenge we overcame together during this process.

This achievement is ours!

With the blessings and help of the Lord, God Almighty, and all the people who supported me, I was able to accomplish this fine work that I dedicate to:

To most loving and mighty God, who gave me the greatest gift of life and for always blessing and protecting me. Thank you, God!

To the woman that will always love me no matter what, my mom Mrs Jesca Kadapao. I love you mom. And to the memory my late dad Mr. Musaazi Robert, your love and comfort made memories that will last forever.

To my forever young grandmother Mrs. Lubwama Christine, your guidance and words of courage kept me going on. And to the memory of my late Grandfather Dr Lubwama Henry, you are my inspiration to achieve the highest honors that life and education has got to offer.

To my beloved family parents who filled the gap that was big in my life, daddy Ntege Ronald and mummy Ntege Rose. You have loved me like your own child, given your whole, always supported and guided me to be the man that I am today.

To my lovely aunties; Mrs Crystal, Mrs Josephine, Mrs Lillian, Mrs Vickie, Mrs Grace, Mrs Immy, Mrs Florence, Mrs Rose, Mrs Brenda, Mrs Agnes and dearest uncles; Mr Richard, Mr Julius, Mr Emma. Your love and support have pushed me to a better version of myself every day.

To my adorable brothers and sisters: Natasha, Daniella, Druscilla, Elisha, Jorum, Jordan. We share a bond that's unlike any other, thank you for always bringing joy to my life. And to the memory of my little baby angel sister, Darlene Faith Nalubwama , the warmth of your hugs, cute smiles and love will forever be part of the lasting memories in my heart. You taught me to be the strongest and never let anyone put me down in whatever situation.

To my lovely cousin brothers and sisters, I love you all.

To my dear friends; Original day1s, Algerie friends, cube Chipolopolo and all with whom I have shared moments of my life. You were all important to me, thank you for sharing this journey called life with me.

To my dear binome, Taringa Takudzwa Latoyah. Thank you for not only being the best research colleague one could ask for but also a true friend. The shared hustles, challenges, work hours, discussions, good happenings and laughter have built life long memories to cherished.

To all those that I know, either close or far.

Table of contents

Chapter 3: Materials and methods

Chapter 4: Results and discussions

Abstract

Catheter-related infections (CRIs) pose a significant threat in healthcare settings, particularly in intensive care units, where indwelling medical devices are frequently colonized by biofilm-forming pathogens. This study investigated microbial colonization, antibiotic resistance patterns, and biofilm formation on urinary catheters, central venous catheters, and tracheal tubes from patients at University Hospital of Tlemcen (CHU), Algeria.

Using culture based methods and VITEK 2 system; we identified predominant pathogens including *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Enterobacter cloacae complex, Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Candida* species. Antimicrobial susceptibility testing revealed that multidrug resistance in *Acinentobacter baumannii* and *Enterobacter cloacae complex*, while *P. aeruginosa* remained broadly susceptible. *Staphylococcus* showed resistance to tetracycline but sensitive to vancomycin.

Biofilm formation was assessed via Congo Red Agar (CRA) and Tissue Culture Plate (TCP) methods. TCP analysis classified most isolates as weak biofilm producers with *A. baumannii* exhibiting moderate biofilm production. CRA showed lower sensitivity, underscoring TCP's reliability for biofilm detection. A correlation between biofilm formation and antibiotic resistance was observed, highlighting the protective role of biofilms in bacterial persistence.

These findings emphasize the clinical challenge of CRIs caused by resilient, biofilm-forming pathogens. The study advocates for enhanced infection control measures, antimicrobial stewardship, and research into anti-biofilm strategies to mitigate device-associated infections.

Keywords: Catheter-related infections, biofilm, antimicrobial resistance, extracellular polymeric substances (EPS), intensive care unit, catheters.

Résumé

Les infections liées aux cathéters (IRC) constituent une menace importante dans les établissements de santé, en particulier dans les unités de soins intensifs, où les dispositifs médicaux à demeure sont fréquemment colonisés par des agents pathogènes formant un biofilm. Cette étude a examiné la colonisation microbienne, les profils de résistance aux antibiotiques et la formation de biofilms sur les cathéters urinaires, les cathéters veineux centraux et les tubes trachéaux de patients de l'hôpital universitaire de Tlemcen (CHU), en Algérie.

À l'aide de méthodes basées sur la culture et du système VITEK 2, nous avons identifié des agents pathogènes prédominants, notamment *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* et *les espèces Candida*. Les tests de sensibilité aux antimicrobiens ont révélé une multirésistance aux médicaments chez *A. baumannii* et *E. cloacae*, tandis que *P. aeruginosa* est demeuré largement sensible. Les staphylocoques ont montré une résistance à la tétracycline mais une sensibilité à la vancomycine.

La formation du biofilm a été évaluée par les méthodes de la gélose rouge du Congo (CRA) et de la plaque de culture tissulaire (TCP). L'analyse du TCP a classé la plupart des isolats comme de faibles producteurs de biofilm, A. *baumannii* présentant une production modérée de biofilm. L'CRA a montré une sensibilité plus faible, ce qui souligne la fiabilité du TCP pour la détection des biofilms. Une corrélation entre la formation de biofilms et la résistance aux antibiotiques a été observée, mettant en évidence le rôle protecteur des biofilms dans la persistance bactérienne.

Ces résultats soulignent le défi clinique des IRC causés par des agents pathogènes résilients et formant un biofilm. L'étude préconise des mesures améliorées de contrôle des infections, une gestion des antimicrobiens et la recherche de stratégies anti-biofilm pour atténuer les infections associées aux dispositifs.

Mots-clés : Infections liées aux cathéters, biofilm, résistance aux antimicrobiens, substances polymères extracellulaires (EPS), unité de soins intensifs, cathéters.

ملخص

تشكل الالتهابات المرتبطة بالقسطرة (CKDs) تهديدا كبيرا في أماكن الرعاية الصحية ، خاصة في وحدات العناية المركزة ، حيث يتم استعمار الأجهزة الطبية الساكنة بشكل متكرر بواسطة مسببات الأمراض المكونة للأغشية الحيوية. فحصت هذه الدراسة الاستعمار الميكروبي وأنماط مقاومة المضادات الحيوية وتكوين الأغشية الحيوية على القسطرة البولية والقسطرة الوريدية المركزية وأنابيب القصبة الهوائية للمرضى في مستشفى تلمسان الجامعي (CHU) ، الجزائر.

باستخدام الأساليب القائمة على الثقافة ونظام VITEK 2 ، حددنا مسببات الأمراض السائدة ، بما في ذلك *Pseudomonas aeruginosa* و *Acinetobacter baumannii* و *Enterobacter cloacae* و *Staphylococcus epidermidis* و *Staphylococcus haemolyticus* و *Candida الأنواع*. كشف اختبار الحساسية لمضادات الميكروبات عن مقاومة للأدوية المتعددة في *A. baumannii* و *E. cloacae* ، بينما *ظلت المتصورة الزنجارية* عرضة إلى حد كبير. أظهرت المكورات العنقودية مقاومة للتتراسيكلين ولكن حساسية للفانكومايسين.

تم تقييم تكوين الأغشية الحيوية بواسطة طرق الكونغو الأحمر أجار (CRA) ولوحة زراعة الأنسجة (TCP). صنف تحليل TCP معظم العزلات على أنها منتجة منخفضة للأغشية الحيوية ، مع *A. baumannii* أظهر إنتاجا معتدلا للأغشية الحيوية. أظهر ARC حساسية أقل ، مما يؤكد موثوقية TCP للكشف عن الأغشية الحيوية. لوحظ وجود علاقة بين تكوين الأغشية الحيوية ومقاومة المضادات الحيوية ، مما يسلط الضوء على الدور الوقائي للأغشية الحيوية في ثبات البكتيريا.

تسلط هذه النتائج الضوء على التحدي السريري لأمراض الكلى المزمنة الناجمة عن مسببات الأمراض المرنة المكونة للأغشية الحيوية. تدعو الدراسة إلى تحسين تدابير مكافحة العدوى ، والإشراف على مضادات الميكروبات ، والبحث في استراتيجيات مكافحة الأغشية الحيوية للتخفيف من العدوى المرتبطة بالجهاز.

الكلمات المفتاحية: الالتهابات المرتبطة بالقسطرة ، الأغشية الحيوية ، مقاومة مضادات الميكروبات ، مواد البوليمر خارج الخلية (EPS) ، وحدة العناية المركزة ، القسطرة.

List of Abbreviations

AI:	Autoinducer
AIP:	Autoinducing peptide
AMR:	Antimicrobial resistance
C:	*Candida*
CAUTIs:	Catheter-associated urinary tract infections
CPR:	Cardiopulmonary resuscitation
CRBIs:	Catheter-related bloodstream infections
CRIs:	Catheter related infections
CVC:	Central Venous catheters
DGCs:	Diguanylate cyclases
E.COLI:	*Escherichia coli.*
eDNA:	Extracellular DNA
EPS:	Extracellular polymeric substances
ESBLs:	β-lactamases
ESBLs:	Extended-Spectrum β-lactamases
ETs:	Endotracheal tubes
FV:	Femoral vein
HAIs:	Healthcare-associated infections
ICU:	Intensive Care Unit
KPC:	*Klebsiella pneumoniae carbapenemase*
LPS:	Lipopolysaccharides
MBLs:	Metallo-β-lactamases

MDR:	Multidrug-resistant
MRSA:	*Methicillin-resistant S. aureus*
PDE:	Phosphodiesterase
PICC:	Peripherally inserted central catheters.
PM:	*Proteus mirabilis*
QS:	Quorum sensing
TIVAP:	Totally implantable venous access ports
UC:	Urinary catheter
UPEC:	uropathogenic *Escherichia coli*
UTIs:	Urinary tract infections
VRE	*Vancomycin-resistant enterococci*

List of figures

List of tables

Introduction

Introduction

In modern healthcare settings, the use of medical devices such as central venous catheters, endotracheal tubes, and urinary catheters is vital. However, these devices are susceptible to colonization by microorganisms that form biofilms, leading to persistent and hard-to-treat infections **(Bouhrour *et al.,* 2024).** Defined as complex communities of microorganisms embedded within a self-produced extracellular polymeric substance (EPS) matrix, biofilms confer increased resistance to antimicrobial agents and host immune responses which causes significant challenges in clinical management **(Assefa and Amare, 2022).**

The intensive care unit (ICU), particularly the Anesthesia and Resuscitation Unit, represents one of the most vulnerable environments within the hospital due to high use of invasive devices and immunocompromised patients. Critically ill patients often require prolonged intubation, mechanical ventilation, central venous access, and urinary catheterization, all of which serve as potential entry points and surfaces for microbial colonization and biofilm formation **(Lanaghan and Stenhouse, 2024)**. For instance, central venous catheters are frequently colonized by biofilm-forming pathogens, leading to catheter-related bloodstream infections (CRBSIs). These hospital-acquired infections remain a major global health concern, leading to patient morbidity, prolonged hospital stays and increased healthcare costs (**Silvetti *et al.,* 2018)**.

Common pathogens associated with biofilm formation on medical devices include both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus* , *Staphylococcus epidermidis*, *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumoniae* These organisms possess various virulence factors that facilitate adherence to device surfaces and biofilm maturation. Moreover, the presence of biofilms significantly increases the resistance of these pathogens to antibiotics, often necessitating the removal of the infected device **(Codru *et al.,* 2024; Zhao *et al.,* 2023).**In addition to bacterial pathogens, fungal organisms such as *Candida* species have also been implicated in biofilm-associated infections on medical devices. **(Wang *et al.,* 2024).**

Antibiotic resistance has become a pressing concern with its continued growth associated to numerous reasons, including self-medication without prescription and the misuse of antibiotics in the environment, animals, agricultural sector and. the community. Furthermore, drug breakdown processes, the slow growth rate of bacteria, and the difficulty of antibiotics entering the biofilm may all contribute to the high rates of antibiotic resistance seen among biofilm producers **(Hoceini *et al.,* 2023)**. The process of biofilm formation on medical devices is complex and dynamic. Once

bacteria adhere to a surface, they undergo phenotypic changes that trigger the production of extracellular polymers, leading to the establishment of mature biofilms. This mode of growth not only enhances bacterial survival but also promotes genetic exchange and the development of multi-drug resistance. Recent studies have highlighted the correlation between biofilm formation and antibiotic resistance. Conventional microbiological methods often fail to detect these sessile communities, leading to underestimation of their impact **(Thinina *et al*., 2020).**

Emerging strategies to combat biofilm-associated infections include the use of bacteriophages, quorum-sensing inhibitors, and novel antimicrobial coatings on medical devices. These approaches aim to disrupt biofilm formation and enhance the efficacy of existing antimicrobial therapies. Furthermore, understanding the genetic and molecular mechanisms underlying biofilm development can inform the design of targeted interventions to prevent and treat these persistent infections multi-drug strains, underscoring the clinical significance of biofilm assessment **(Assefa and Amare, 2022).**

Recognizing the clinical importance of biofilm-producing organisms, this study aims to evaluate the biofilm-forming ability of bacterial strains isolated from medical devices, with a specific focus on samples obtained from Tlemcen University Hospital. It also explores the relationship between biofilm-forming capacity and antibiotic resistance, with the goal of contributing to mitigate the spread of antibiotic resistance by understanding their antibiotic susceptibility.

Literature review

CHAPTER 1: CATHETERIZATION

1. Types of catheters

The most common used medical devices in the world are catheters and can either be indwelling or intermittent catheters**(Cortese *et al.*, 2018).** The insertion of medical devices in the hospital setting for various patient treatment purposes has increased the incidence of nosocomial infections, including the urinary catheters, various types of central venous catheters (CVCs), and tracheal catheters (**Figure 1) (Cangui-Panchi *et al.*, 2022)**.

A urinary catheter (UC) is a flexible tube, typically made of silicon or latex, inserted into the urethra to manage urine flow based on patient needs. While useful, these catheters are prone to infections **(Cortese *et al.*, 2018)**, especially catheter-associated urinary tract infections (CAUTIs). UTIs account for 32% of all healthcare-associated infections (HAI) and affect around 150 million people globally each year (**Elpern *et al.*, 2009; Werneburg, 2022)**. About 80% of hospital-acquired UTIs are linked to indwelling urethral catheters. These infections can range from uncomplicated to severe, depending on patient factors and catheter use **(Ha and Cho, 2006).**

A central venous catheter (CVC) is a medical device inserted into a large vein to deliver treatments like chemotherapy, parenteral nutrition, or for blood sampling and pressure monitoring. Common types include non-tunneled and tunneled catheters, implantable ports, and PICC lines (peripherally inserted central catheters). While essential, CVCs carry infection risks, especially when placed in the femoral vein. Infections are often linked to biofilm formation, making them difficult to treat and a major cause of hospital-acquired infections **(Gominet *et al.*, 2017; Silvetti *et al.*, 2018)**. Cancer patients frequently require CVCs for treatment and nutrition but face a heightened risk of infections due to immunosuppression. Signs of infection can include local symptoms like swelling or redness, and systemic ones such as fever and low blood pressure **(Böll *et al.*, 2021)**.

Tracheal tubes are essential for establishing artificial airways and supporting ventilation but also create ideal conditions for bacterial adhesion and biofilm formation. First observed in 1967, biofilms on endotracheal tubes (ET) were later visualized microscopically by 1986. These tubes are used in procedures like anesthesia, cardiopulmonary resuscitation (CPR), and respiratory therapy (**Chen *et al.*, 2022).** When patients cannot maintain an open airway, intubation with endotracheal or tracheostomy tubes becomes necessary. Due to oral insertion, endotracheal tubes

are exposed to the oral microbiome, increasing the risk of microbial colonization **(Deshmukh-Reeves *et al.*, 2025)**.

Intubation weakens airway defenses by impairing cough, mucociliary clearance, and damaging the tracheal lining, allowing biofilm-forming pathogens to colonize endotracheal tubes. Contaminated secretions above the cuff can seep into the lower airway, increasing the risk of ventilator-associated pneumonia **(Walsh *et al.*, 2024)**. Tracheostomy tubes, often colonized by biofilms within days, are difficult to clean and can lead to serious complications like airway obstruction, pneumonia, or sepsis **(Ścibik *et al.*, 2022; Raveendra *et al.*, 2022).**

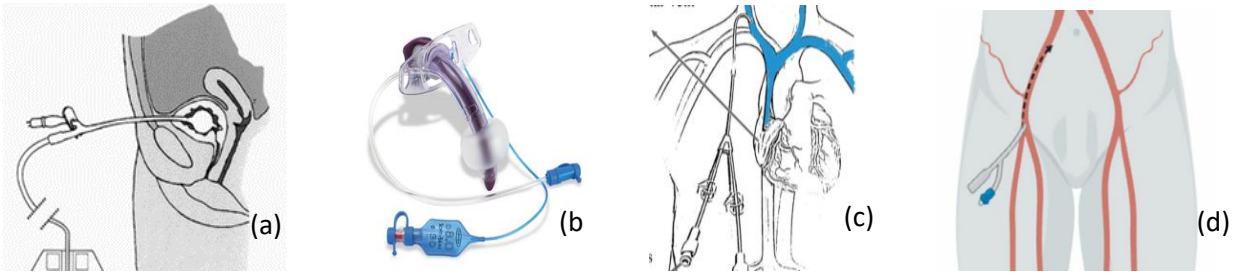


Figure 1 : different types of catheters (a) urinary catheter (b) tracheal catheter (c) central venous catheter (d) femoral vein catheter **(Feneley *et al.*, 2015; Balikci *et al.*, 2021; Ghattas *et al.*, 2021).**

1.1. Catheter related infections (CRIs)

CRI is the most important complication since it is the main reason for morbidity, high risk of sepsis, and mortality in intensive care units (ICUs). These hospital acquired infections (HAI) also cause long hospitalization time and high cost of treatment with 30% of hospitalized patients in acquiring at least one infection **(Garvey ,2023).**

CRI has three common types: CRBSI, tunnel infection, and exit site infection. When the biofilm reaches maturity, the planktonic bacteria, which spread from the biofilm to other parts of the body through the blood circulation cause CRBSI. CRBSI is characterized by fever higher than 37.8ºC, hypotension, pain, shortness of breath, weakness, and pain in the general body. In exit-site infection, there is pain, localized erythema, redness, bloating at the insert site of the catheter, and the culture test at the exit site of the catheter is positive, while it is negative for the patient's blood **(Balikci *et al.*, 2021).**

Tunnel infection is characterized by the presence of an inflamed discharge from the tunnel, redness, tenderness, and positive catheter culture. The bacteria that cause the infection may be from the patient's bacterial flora, which is called endogenous infection. In some cases, the source of infection may come from other patients, health care personnel, contaminated dialysis fluids, equipment, and inadequate hospital and surgery conditions. This type of infection is called exogenous infection. Most of the microorganisms that cause infection are caused by the patient's microflora. The skin integrity of the patient is impaired by inserting the catheter. Consequently, pathogens that are leaking from this area cause infection **(Balikci *et al.*, 2021).**

Both, Gram-positive and Gram-negative bacteria **(Schulze *et al.*, 2021)** commonly cause HAIs**.** A major concern is the group of ESKAPE pathogens, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp*, which are associated with high morbidity and mortality (**Deshmukh-Reeves *et al.*, 2025**). Fungal organisms like *Candida albicans*, an oral commensal, are also significant contributors, particularly in catheter-related bloodstream infections (**Walsh *et al.*, 2024**). Research has shown that, beyond commonly identified intensive care units (ICU) pathogens, various other microbes may inhabit device-associated biofilms as shown in **Table Nº 1** (**Codru *et al.*, 2024**).

Table N^{O} 1 : Various medical devices and common pathogens from biofilm **(Mishra *et* al., 2024).**

Medical Device	Common Pathogen
Respiratory equipment, Indwelling catheters	*Acinetobacter baumanii*
Bronchoscopy	*Klebsiella pneumonia*
UTIs devices,	*Enterobacter spp.*
Intravascular medical device	*Enterobacter spp.*
Respiratory device	*Aspergillus fumigatusi*
Cardiac medical device	*Cryptococcus neoformans*
Urinary catheters	*Enterococcus faecalis*
Catheters, prosthetic devices	*Aspergillus fumigatus*
Central venous catheter	*Staphylococcus epidermidis*
Cerebrospinal shunts	*Staphylococcus aureus, Propionibacteriuìtl*
Dental implants	*Prevotella intermedia, Actinobacillus*
Orthopedic devices	*Staphylococci, Gram-negative bacilli*

Endotracheal cubes	*Pseudomonas aeruginosa, Staphylococcus aureus*
Contact lenses	*Pseudomonas aeruginosa, Staphylococci*
Intravascular catheters	*Staphylococci, Enterococci, Gram-negative bacilli*
Valves, pacemaker	*Staphylococci, Streptococci*

2. Common pathogens involved in CRIs

2.1Gram-positive bacteria

2.1.1 *Enterococci spp.*

Enterococci are Gram-positive, facultative anaerobes commonly found in the gastrointestinal tract. Although they are typically harmless in a healthy person, they pose a serious threat for nosocomial infections, particularly in hospitalized patients with indwelling devices or those receiving broad-spectrum antibiotics. Of the many species, *Enterococcus faecalis* and *Enterococcus faecium*, are mainly responsible for most enterococci infections, especially the UTIs which originate feacally. In some cases, these bacteria may persist as asymptomatic colonizers without causing noticeable symptoms **(Heintz *et al.*, 2010)**.

A significant concern with enterococcal infections is their ability to form biofilms, which contribute to their persistence and antibiotic resistance. They can easily attach to medical devices like urinary catheters. Key virulence factors in biofilm formation include Esp (extracellular surface protein), Asa1 (aggregation substance), and Ebp (endocarditis and biofilm-associated pili), which enhance bacterial adherence and colonization. These traits contribute to the development of antimicrobial resistance, complicating treatment and increasing the risk of recurrent UTIs and other infections **(Fallah *et al.*, 2017).**

2.1.2 *Staphylococcus spp.*

Staphylococcus aureus (S. aureus) is a gram-positive bacterium, **(Gunaratnam *et al.*, 2020)** identified by the WHO as a critical antibiotic-resistant pathogen due to its role in both community and healthcare-associated infections (HAIs). A major factor in its persistence is its ability to form biofilms, which shield it from antibiotics and immune responses**.** *S. aureus* commonly colonizes

medical devices like catheters, prostheses, and pacemakers. It adheres easily to both coated and uncoated surfaces, forming biofilms **(Suresh *et al.*, 2019)**.

Methicillin-resistant strains (MRSA) are especially concerning due to their link with severe infections, including catheter-related bloodstream infections (CRBSIs) **(Mandolfo *et al.*, 2020;Kitano *et al.*, 2021)**. MRSA strains are also emerging as uropathogens, particularly in elderly and catheterized patients, contributing to urinary tract infections (UTIs), bacteremia, and urosepsis. These infections are difficult to manage and often lead to complications **(Paudel *et al.*, 2023)**.

Staphylococcus epidermidis (S. epidermidis) and *staphylococcus haemolyticus* are coagulase-negative, gram-positive bacteria and part of the normal skin microbiota. However, in hospital settings, particularly in immunocompromised patients, it is a major cause of device-related infections. Its ability to form biofilms on central venous catheters makes it a key contributor to CRBSIs **(Van kerckhoven *et al.*, 2016)**. One of *S.epidermidis* key biofilm-promoting proteins is the accumulation-associated protein (Aap), which helps stabilize biofilm structures and supports persistent infections **(Yarawsky *et al.*, 2020**). Promising approaches, such as bacteriophage-coated catheters, are being explored to combat these infections caused by these bacteria (**Curtin *et al.*, 2006**).

The main cause of staphylococcal colonization of medical equipment is the polysaccharide intercellular adhesin (PIA), which is produced by the ica locus. Environmental factors influence the synthesis of PIA, which is essential for bacterial cell adhesion, biofilm matrix structure, bacterial attachment to biomaterial surfaces, and immune response evasion (**Hoceini et al., 2023**).

2.2Gram-negative bacteria

2.2.1 *Escherichia coli*

Escherichia coli (E. coli) is a facultative anaerobic bacterium from the Enterobacteriaceae family and is frequently associated with diseases such as sepsis, meningitis, diarrhea, and urinary tract infections (UTIs) **(Jacobsen *et al.*, 2008)**. Among the different strains, uropathogenic *E. coli* (UPEC) is the main cause of UTIs, especially in both community-acquired and hospital-associated cases, including catheter-associated UTIs (CAUTIs) **(Guiton *et al.*, 2012)**. Urinary catheterization increases the risk of *E. coli* colonization, and its ability to form biofilms increases its resistance to treatment (**Zou *et al.*, 2023)**.

UPEC strains possess several virulence factors that enhance their ability to colonize and persist in the urinary tract **(Soto *et al.,* 2006)**. These include adhesins for epithelial attachment, iron uptake systems for survival in iron-poor environments, cytotoxins that harm host cells, and specific serotypes that help evade immune responses **(Kanamaru *et al.,* 2006)**. Such features allow UPEC to cause both asymptomatic bacteriuria (ABU) and symptomatic infections with varying degrees of severity.

ABU is defined as the presence of *E. coli* in urine without the typical symptoms of a UTI and is common in both healthy and elderly individuals. While ABU strains often do not cause harm, more virulent UPEC strains can lead to painful and severe infections. The variability in pathogenicity underscores the complexity of managing *E. coli*-related urinary tract conditions and the importance of identifying strain-specific characteristics **(Hancock *et al*., 2007)**.

2.2.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (PA) is a gram-negative bacterium that causes a wide range of acute and chronic illnesses in both plants and animals. It's an opportunistic pathogen, meaning it often infects individuals who are already sick or have weakened immune systems (**Rasamiravaka *et al*., 2015**). It produces three main polysaccharides-alginate that retains nutrients and reinforces the biofilm, aligate, polysaccharides synthesis local(Psl) and pellicle (Pel), which form its foundational framework that support and stabilize the biofilm structure (**Rasamiravaka *et al.,* 2015**).

P. aeruginosa strains that are not mucoid form biofilms without the aid of alginate production. These biofilms can colonize solid surfaces and form mushroom structures in flow cells, rings in culture tubes and microtiter plates, or pellicles at the air-liquid interface. Although researchers have discovered several factors in *Pseudomonas* that promote biofilm growth in lab settings, it is still not understood how each of these components contributes to biofilm formation in cases of CAUTI. Urea, a significant component of urine, and may be able to stimulate in vitro the formation of biofilm in vitro by exopolysaccharide-deficient *Pseudomonas*. A fraction of *P. aeruginosa* cells rounded and lysed when exposed to urea. Both the mutant strains and PA14 may develop biofilms as a result of the leaked eDNA **(Cole *et al.,* 2014)**.

2.2.3 *Proteus mirabilis*

Proteus mirabilis (PM) is a motile Gram-negative, rod-shaped bacterium from the Enterobacteriaceae family commonly found in the environment. It's best known for two key traits:

its ability to swarm across surfaces and its strong urease activity. It has a unique ability to transform its shape and behavior depending on its environment. On solid surfaces, short rod-shaped cells can rapidly differentiate, developing thousands of flagella and elongating into swarm cells that move collectively **(Yuan *et al.*, 2021**).

With its peripheral flagella, PM is a motile organism that can differentiate from a single short rod-like "swimming cell" into multicellular, elongated "colony cells." These cells get stacked in a sequential fashion to form rafts of cells that can move quickly and cooperatively across slid surfaces. Consequently, group movement may encourage PM migration from the vicinity of the urethra along the catheter's surface into the urethra and bladder, which could result in CAUTI. It is an opportunistic pathogen that accounts for <0.005% of the human intestinal flora in healthy subjects **(Yuan *et al.*, 2021).**

Due to PM's special capacity to form crystalline biofilms, these infections eventually result in crusts and obstruction on the catheter's surface. If the infection worsens and causes cystitis and pyelonephritis, this can cause sepsis and septic shock, as well as urinary retention and reflux. Furthermore, removing the crystalline catheter could harm the mucosa of the bladder and urethra (**Jacobsen and Shirtliff, 2011**).

Another study showed that 62% of patients with recurring *P. mirabilis* catheter encrustation developed bladder stones. These stones then become a source of infection, leading to the rapid colonization of replacement catheters by the same bacteria (**Sabbuba *et al.*, 2004).**

2.3 Fungi

2.3.1*Candida spp.*

Candida (C) species are known to produce opportunistic infections with significant fatality rates, particularly in immunocompromised persons, and are found as normal flora in healthy individuals. Of the *Candida* species that can cause both superficial and systemic infections, *Candida albicans* is the most common. *C. tropicalis, C. glabrata, C. parapsilosis, and C. krusei* are other harmful species of Candida that cause 25%, 8%, 7%, and 4% of candidiasis, respectively (**Marak, 2018**).

Among all identified species, *Candida spp*. emerged as the most prevalent pathogen group. These findings align with previous multicenter studies on CAUTIs, which also identified *Candida spp*. as the most commonly isolated uropathogen.

Candida albicans forms complex, highly organized biofilms containing three distinct cellular forms: yeast, pseudohyphae, and hyphae. The ability to develop hyphae, express hypha-specific genes, and construct biofilms represents key virulence factors. These characteristics enable the fungus to invade host tissues, evade immune responses, and ultimately cause significant tissue damage and systemic infection.

C. albicans biofilm development begins when yeast cells attach to a surface and form microcolonies. These colonies then expand through the growth of pseudohyphae and hyphae. The transition to hyphal growth is stimulated by environmental cues like serum exposure, neutral pH, and elevated CO_2 levels. This critical morphological shift is regulated by key transcription factors, including enhanced filamentous growth protein 1 (Efg1), which acts as a master controller of this process **(La Bella *et al*., 2023).**

Due to their increased resistance to antifungal medication and ability to elude host defenses, Candida biofilm formation on medical devices is a major concern.

As shown in **Table Nº 2**, the percentage of bacteria isolated in North Algeria was documented in a study by **(Saadi *et al*., 2022)** providing insight into the distribution and prevalence of bacterial species in different medical devices.

Table Nº 2: Total bacteria isolated from hospital devices in Algeria.

Type	Bacterial species	Medical Equipment	Total % of species
Gram	*Staphylococcus*	2	7,41
Positive	*Staphylococcus aureus*	3	15,74
	Staphylococcus xylosus	1	2,78
	Staphylococcus saprophyticus	1	6,48
	Bacillus spp.	2	5,56
Gram	*Escherichia coli*	3	11,11
Negative	*Clebsiella pneumonia*	4	6,48
	Providencia rettgeri	2	6,48
	Providencia stuartii	3	4,63
	Serratio marscense	2	1,85

	Proteus mirabilis	2	3,70
	Proteus vulgaris		1,85
	Shigella spp.	1	3,70
	Pontoea spp.		2,78
	Acinetobacter	4	11,11
	Pseudomonas stutzeri	1	0,93
	Pseudomonas .aeruginosa	3	7,41
Total		34	100

CHAPTER 2: BIOFILMS

1. Biofilm structure

Biofilm was first discovered in the 17th century by Anthony van Leeuwenhoek using his microscope **(Bouhrour *et al.*, 2024).** It is defined as surface-associated microbial communities of microcolonies with heterogene functions **(Ren *et al.*, 2018; Pelling *et al.*, 2019)** embedded in a self-produced extracellular polymeric matrix(EPS). They adhere to the surface of an organic or inorganic structure **(Niveditha, 2012; Sabir *et al.*, 2017).** Bacterial and fungal species are known to have the intrinsic ability to form biofilms **(Cangui-Panchi *et al.*, 2022).** For bacteria it depends with species within the biofilm, it can be classified into two types: Gram-positive and Gram-negative biofilms. Gram-positive bacteria have teichoic acid (TA) in their cell walls, while Gram-negative bacteria possess lipopolysaccharides (LPS) in their outer membranes(**Vani *et al.*, 2023)**.

The major component of biofilms is EPS and it consists of many types of polymers, including polysaccharides, extracellular nucleic acids (eDNA), proteins, amyloids, and amphiphilic surfactants **(Caldara *et al.*, 2022)**. It is said that biofilms consist of about 80% EPS which plays an important role in biofilm formation. EPS is comprised of water channels that assist in distribution of nutrients, oxygen and protection from external environmental stresses. EPS is a basic platform for surface attachment and stimulates the growth and adherence of bacterial species by facilitating the functioning of intercellular signaling molecules (**Veerachamy *et al.*, 2014;Khatoon *et al.*, 2018).**

2. Biofilm formation

The stages of biofilm formation are defined by the organized accumulation of cells, each exhibiting distinct structure and physiological characteristics **(Goudarzi *et al.*, 2021).** The initial stage of surface colonization is carried out by free-floating planktonic bacteria. These bacteria adhere to the surface, multiply, transition into a sessile state, develop new traits depending on environmental conditions **(Sharma *et al.*, 2023)**. There are 5 different main stages in the development of biofilms **(Figure 2)**; reversible and irreversible fixation, microcolony formation, maturation, and dispersion/detachment (**Mishra *et al.*, 2024)**.

The first stage of biofilm formation is initial adhesion **(Li *et al.*, 2023)** of planktonic bacteria to the substrate surface**.** This phase is called reversible adsorption because bacteria attach to the biomaterial surface only for a short period and then detach from it **(Tsehaye *et al.*, 2016)**, with adhesion being mainly influenced by the surface properties of the substrate **(Mancuso *et al.*, 2024**). In the initial attraction, planktonic bacteria are influenced by non-specific physical interactions such as Van der Waals attractive forces, electrostatic forces (attractive or repulsive), hydrophobic interactions, Brownian motion, and gravitational forces **(Bouhrour *et al.*, 2024;Wang *et al.*, 2024)**.

The second stage of adherence is referred to as the anchoring or latching phase, and it involves a binding that is molecularly coordinated among particular adhesins and the outermost layer **(Sharma *et al.*, 2023)**. Reversible adhesion becomes irreversible. During this process there is secretion of EPS that form the biofilm matrix which consist of polysaccharides, proteins, nucleic acids, and lipids. These substances allow bacteria to stick to surfaces and to each other **(Abdallah *et al.*, 2014).** This phase is accomplished through bacterial cell surface hydrophobicity, hydrogen bonding, covalent bonding, ionic bonding, and dipole-dipole interactions **(Zhao *et al.*, 2023)**. At this stage, bacteria have special surface parts like pili, fimbriae, and flagella that help them stick to surfaces.

The attachment between cells and to surfaces relies on EPS. When the cell population is dense, the biofilm utilizes cell signaling methods known as quorum sensing, which involve various signal molecules and bis-(3′, 5)-cyclic dimeric guanosine monophosphate (Cyclic-di-GMP). Quorum sensing plays a key role in the maturation of the biofilm, as it enables bacteria to sense their cell density and coordinate group behaviors accordingly **(Alotaibi, 2021).** Once bacteria have irreversibly attached to a surface, the biofilm begins to grow and mature **(Mancuso *et al.*, 2024**).

Following irreversible attachment, cells multiply and start producing biofilm matrix components, forming small aggregates of bacteria called microcolonies. Overtime, these develop into large cellular aggregates encased by a matrix **(Armbruster and Parsek, 2018).** The biofilm matures in parallel with the accumulation of extracellular polymeric substances(**Preda and Săndulescu, 2019)**. During this phase of biofilm maturation, specific genes are activated to produce substances essential for the biofilm's 3D structure, spaces or voids begin to form within the matrix. These water-filled channels function like a circulatory system, helping to deliver nutrients and eliminate waste from the clusters of microbial colonies within the biofilm **(Jamal *et al.,* 2018).**

Bacteria are able to recognize the dimensions and proximity of adjoining groups, which aids them in forming clusters that can bond with nearby cells more effectively **(Sharma *et al.,* 2023)**. In summary, the maturation stage involves EPS production, aggregation of cells, chemical interactions, quorum sensing and formation of micro and macrocolonies **(Khatoon *et al.,* 2018)**. The mature biofilm is generally visible as a slimy coating on the surface **(Krishnan, 2015)**.

Dispersal is the final stage of the biofilm process where attached cells detach and disperse to colonize a new niche due to nutrient limitation, fluid dynamics and shear effects of the bulk fluid, secretory proteins and catabolite repression (**Joo and Otto, 2012)**, or mechanical stress, formation of flagella, degradation of EPS, or the formation of toxins **(Ali *et al.,* 2023).** This stage involves sloughing, erosion, and abrasion. Erosion is the gradual detachment of cells or small biofilm fragments, while sloughing releases larger chunks due to nutrient or oxygen depletion, or sudden nutrient surges. Abrasion occurs when suspended particles physically remove parts of the biofilm **(Alotaibi, 2021)**.

This dispersal process may occur actively or passively. Active dispersal is driven by changes in gene expression in response to various environmental signals, including temperature and pH fluctuations, nitric oxide, nutrient scarcity, oxygen limitation, and other stress conditions **(Schulze *et al.,* 2021)**. Passive behavior refers to shedding and erosion dispersion mediated by external forces. Shedding dispersion refers to the abrupt shedding of a large proportion of the bacterial biofilm, and erosion dispersion refers to releasing a portion of bacterial cells in a bacterial biofilm **(Zhao *et al.,* 2023)**. During the detachment process, microbial communities within the biofilm produce different saccharolytic enzymes that help to release the surface of the microbes into a new area for colonization **(Jamal *et al.,* 2018**). Different bacteria use various mechanisms to disperse

biofilms, which is a crucial step in their transmission both between and within hosts, facilitating the spread of biofilm infections **(Mancuso *et al.*, 2024).**

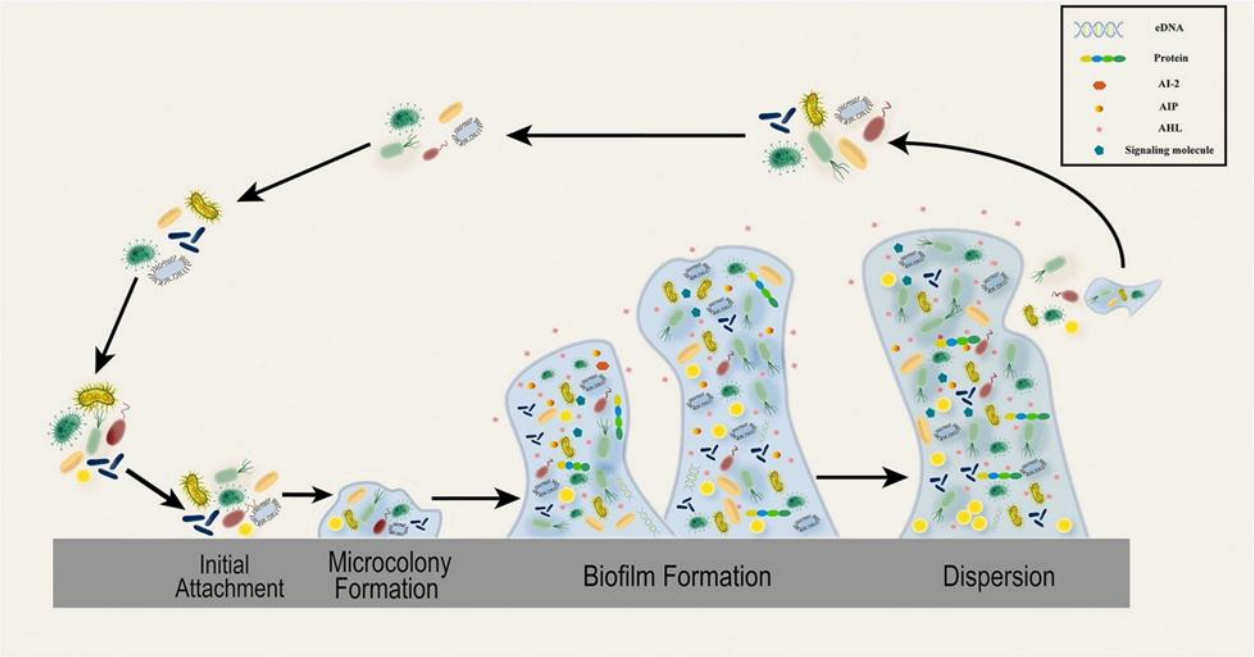


Figure 2: Different stages of biofilm formation **(Wang *et al.*, 2024**).

3. Quorum sensing

From within the matrix bacterial cells secrete quorum sensing molecules, which are cytokines that direct the gene expression of the bacteria within the community **(Wolcott, 2021).**

The processes of QS and biofilm formation are interdependent. When the QS gene is activated, it causes the biofilm to develop and then coordinates its maturation and breakdown. The concentration of autoinducer signaling molecules released by bacteria within a microcolony reflects the bacterial population density in that specific volume **(Sharma *et al.*, 2023)**. This auto-regulation enables the bacteria to synchronize within its sessile microbial community in order to optimize adaption and resilience (e.g. luminescence, virulence, and biofilm formation) **(Schulze *et al.*, 2021).**

QS systems regulate bacterial behaviour in the population through signaling mediated by autoinducer molecules. The AI-2 and AI-3 (autoinducers 2 and 3) quorum sensing is used in both

Gram-positive and Gram-negative species **(Ali *et al.,* 2023).** Many Gram-negative bacteria use N-acyl-L-homoserine lactones (AHLs) to communicate with each other. These bacterial signaling molecules are typically composed of a mixture of several AHLs, with one main component that is unique to each species, since the structure of its N-acyl chain differs between species **(Dickschat, 2010)**. Gram-positive bacteria use autoinducing peptide (AIP) QS system while autoinducer-2(AI-2) system is for both gram-negative and gram-positive bacteria (**Preda and Săndulescu, 2019)**.

As part of their cooperation and communication, microorganisms have the ability to sense and translate the signals from distinct strains in AI-2 or autoinducer-2 interspecific signals, catalyzed by LuxS synthase. In addition, LuxS plays a key role in initiating the methylation cycle and has been shown to regulate the expression of hundreds of genes involved in microbial activities such as surface adhesion, detachment, and toxin production (**Preda and Săndulescu, 2019)**. QS signaling enables bacteria to collectively modify their behavior, including the production of virulence factors and biofilms, in response to changes in cell density and community composition **(Mishra *et al.,* 2024)**.

4. Bis-(3′, 5)-cyclic dimeric guanosine monophosphate (Cyclic-di-GMP)

The bacterial second messenger c-di-GMP is pivotal in biofilm development (**Nair *et al.,* 2017**). This molecule has been identified as a key regulator in the transition between a free-swimming, planktonic mode of life and a stationary, biofilm-dwelling state. Its function in mediating the shift from motility to a sessile lifestyle has been documented across multiple bacterial species **(Alotaibi and Bukhari, 2021)**.

Numerous bacteria are capable of producing c-di-GMP, which has been demonstrated to govern a variety of processes, such as bacterial adhesion and biofilm formation, EPS synthesis, bacterial motility, and virulence regulation (**Sisti *et al*., 2013).**

Low levels of c-di-GMP can boost bacterial motility, encourage biofilm disintegration, and trigger the activation of virulence pathways, while high levels are linked to bacterial attachment to surfaces, EPS generation, biofilm development, and a sessile lifestyle. Cyclic di-GMP (c-di-GMP) plays a regulatory role in biofilm dispersion as well. For instance, in *Pseudomonas aeruginosa* biofilms, nitric oxide (NO)-releasing compounds trigger dispersal by enhancing

phosphodiesterase (PDE) activity, which subsequently reduces intracellular c-di-GMP levels **(Alotaibi and Bukhari, 2021)**.

The cyclic di-GMP (c-di-GMP) signaling system comprises three key components: (1) enzymes responsible for its synthesis (diguanylate cyclases, DGCs) and degradation (phosphodiesterases, PDEs), (2) effector proteins that bind c-di-GMP, and (3) downstream targets that mediate cellular responses. In this regulatory circuit, DGCs and PDEs act as signal transducers, converting environmental or intracellular cues into dynamic c-di-GMP concentrations. These fluctuating c-di-GMP levels subsequently modulate the activity of c-di-GMP-binding effectors, ultimately driving physiological and behavioral adaptations in the bacterial cell (**Mills *et al*., 2011**).

5. Factors influencing biofilm formation

Biofilm formation is a well-organized process that depends on the effects of surface conditioning, environmental characteristics, and microbial cell properties (**Martinez, 2007)** as shown and summarized in **Table N^{O}3.**

Table N^{O}3: Showing the different factors influencing biofilm formation **(Alotaibi and Bukhari, 2021)**.

PROPERTIES OF THE SUBSTRATUM	PROPERTIES OF THE BULK FLUID	PROPERTIES OF THE CELL
Hydrophobicity	Temperature	Cell surface hydrophobicity
Conditioning film	pH	Extracellular appendages, such as fimbriae and flagella
Texture or roughness	Flow velocity and nutrient availability	Extracellular Polymeric substances

5.1. Surface characteristics

The roughness, chemical properties of a surface, and the prior presence of protein films on it influence the attachment of bacteria to this surface and the formation of a biofilm. Surface roughness, in particular, promotes biofilm formation by enhancing microbial attachment. A textured substratum can facilitate uniform biofilm coverage, leading to the establishment of a thin yet metabolically active biofilm layer. Additionally, surface irregularities can protect adhered cells from shear stress and provide microenvironments conducive to biofilm stability **(Pal and Lavanya, 2022)**.

Furthermore, because the material's irregularities are less susceptible to external influences like surface currents, they make better places for bacteria to implant (**Baillif *et al.*, 2010**). Smooth surfaces could, in fact evade colonization (**Donlan *et* Costerton, 2002a).**

5.1.2. Surface Hydrophobicity or Wettability

Wettability (hydrophobicity or hydrophilicity) refers to the quantity of wastewater or biomass that comes into contact with the surface of a biofilm carrier. Research indicates that bacterial adhesion is strongly influenced by surface hydrophobicity, with microorganisms exhibiting preferential attachment to hydrophobic, non-polar substrates (e.g., Teflon and similar polymers) compared to hydrophilic surfaces. This phenomenon can be attributed to hydrophobic interactions, which minimize repulsive forces between bacterial cells and the substrate **(Pal and Lavanya, 2022)**.

Additionally, substances including blood, tears, urine, saliva, interstitial fluid, and respiratory secretions that have previously been present on a protein film biomaterial affect how bacteria adhere to its surface and encourage the creation of biofilms **(Rima *et al*., 2024).**

5.2. Environmental characteristics

Environmental conditions can influence both bacterial properties (mediated by changes in gene regulation and/or cell surface physicochemical properties) and surface properties (mainly through physicochemical changes)

It is commonly known that the environment in which bacteria live and develop can have a significant impact on the behavior of planktonic cells, including resistance, proliferation, and the generation of toxins. Temperature, nutrition, metals, osmolytes, water activity, pH, redox potential,

microbial populations, host interaction, stressors, antimicrobials, and so forth are some examples of these variables **(Pal and Lavanya, 2022)**.

Temperature can influence cell physiological state, physical properties of the compounds within and surrounding the cells. Temperature has an impact on EPS properties like the viscosity of the polysaccharides; raising the temperature of the polysaccharides can result in the creation of a gel-like substance that progressively gains strength until it reaches a critical point where the gel turns into a solution **(Villain-Simonnet *et al*., 2000).**

Researchers manipulate temperature to observe bacterial adhesion patterns. Above 35°C, most cells express a single flagellum, while cooler temperatures induce multiflagellation - enhancing surface contact area and initial attachment probability. Interestingly, while lower temperatures stabilize polysaccharide matrices (promoting biofilm maturation), they simultaneously decrease cell surface hydrophobicity (inhibiting biofilm initiation) **(Pal and Lavanya, 2022)**.

The pH of the surrounding environment can significantly affect the development of biofilms mostly in the initial stage of biofilm formation (**Pompilio *et al*., 2008**). It has been demonstrated that the pH of the medium influences the development of bacterial biofilm slime, which can impact enzyme function because each enzyme has an optimal pH **(Alotaibi and Bukhari, 2021)**.

Oxygen availability directly influences bacterial energy metabolism, thereby modulating biofilm development. For instance, a lack of oxygen reduction can reduce the metabolic activity of bacterial biofilms. Lower oxygen availability usually triggers active dispersal, which is critical for the biofilm life cycle **(Alotaibi and Bukhari, 2021)**.

An increase in nutrient concentration raises the microbial attachment rate. It has been demonstrated that variations in the availability of vital nutrients affect the physiology of bacteria in developing biofilms **(Alotaibi and Bukhari, 2021)**.

5.3. Cell properties

Physical and chemical interactions govern whether microorganisms adhere to a surface, which, depending on the complex interactions between the chemistries of the bacterial and substratum surfaces as well as the aqueous phase, can be either attracting or repulsive **(Pal and Lavanya, 2022)**.

Cell surface hydrophobicity, presence of fimbriae and flagella, and production of EPS **(Donlan, 2002)**, such as proteins or surface-associated polysaccharides, may give one organism in a mixed microbial community a competitive edge **(Alotaibi and Bukhari, 2021)** thus all influence the rate and extent of attachment of microbial cells.

Cell surface hydrophobicity influences hydrophobic interactions between the abiotic surface and cell, which mediate bacterial attachment (**Fan *et al.*, 2020)**. Like the majority of natural surfaces, bacterial cell surfaces are usually negatively charged and vary in their hydrophobicity **(Pal and Lavanya, 2022)**.

In general, bacteria with hydrophobic properties prefer hydrophobic material surfaces; those with hydrophilic characteristics prefer hydrophilic surfaces **(Katsikogianni *et* Missirlis, 2004).** However, it seems that hydrophobic bacteria are better at adhering than hydrophilic ones **(Biallif *et al.*, 2010).**

Flagellum-mediated motility is crucial for the generation of biofilms, and flagella are involved as surface adhesins in strain surface attachment. Mutants with reduced motility, like the flaA (flagellin A) and motB (motor protein B deficient) mutants, have issues forming biofilms **(Fan *et al.*, 2020).**

6. Clinical significance of biofilms in medical devices, mainly catheters

Bacterial biofilms are thought to be responsible for over 80% of chronic infections and 65% of nosocomial infections (NIs), demonstrating the well-known impact that biofilms have on medicine through the development of HAIs (hospital acquired infections) **(Jamal *et al.*, 2018).**

Biofilms can develop on both external surfaces and internal lumens of these devices. Bacteria typically gain access through two primary routes: (a) migrating along the outer catheter surface (extraluminal route) or (b) traveling through the inner channel (intraluminal route), ultimately leading to device-associated infections **(Sharma *et al.*, 2023**).

At the moment of catheter insertion, host proteins, extracellular matrix proteins and some bacterial proteins from blood and interstitial liquid cover its surface. Platelets on the surface of the catheter have integrins, which serves as a recognizer for proteins like fibrinogen, and collagen. The bacteria may colonise the catheter or remain free depending on various factors. In addition to these factors,

the type of material selected for the catheter also affects the risk of biofilm formation. For example, silicone-based catheters are more prone to biofilm formation and susceptible to infection when compared to catheters with poly(tetrafluoroethylene) (PTFE) poly(urethane) (PU), poly(vinyl chloride) (PVC) based catheters **(Balikci *et al*., 2021).**

Biofilms can contribute to infectious diseases in several ways. Detached cells or cell clusters from biofilms can enter the bloodstream or urinary tract, causing infections or emboli. Within biofilms, bacteria can exchange resistance plasmids and become less susceptible to antimicrobial agents. Additionally, biofilm-associated bacteria may release endotoxins and provoke immune resistance in the host **(Sharma *et al*., 2023)**.

The adverse effects that biofilms have on in-dwelling medical devices have been visualized by scientists as shown by **Figure 3.**

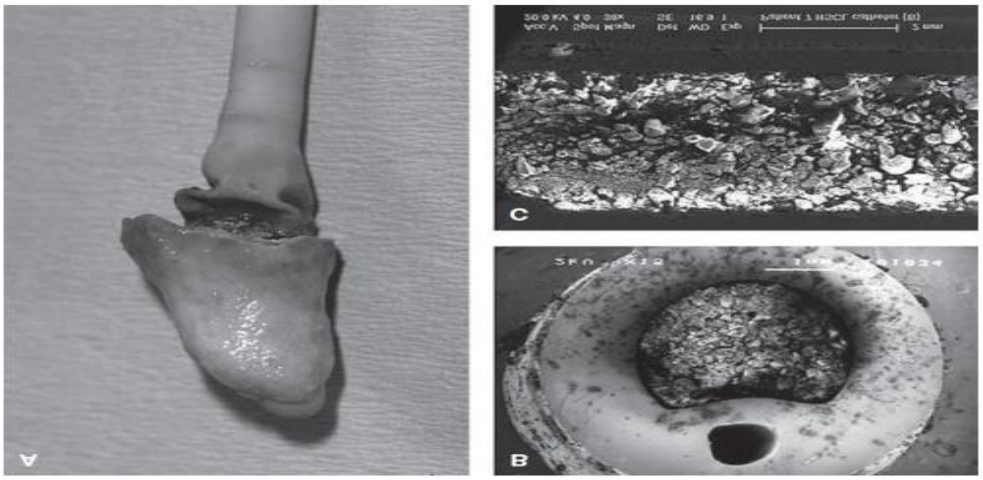


Figure 3: Showing examples of crystalline biofilm on blocked catheters taken from patients **(Stickler, 2008). (A)** A catheter that had been indwelling suprapubically for 6 months. It was removed surgically. Crystalline material completely covered the eyehole and balloon of the hydrogel-coated latex catheter. Image kindly supplied by Professor Roger Feneley. **(B)** A cross-section of a silicone catheter that had been indwelling for 8 weeks. The image shows that the central lumen is occluded by crystalline biofilm. **(C)** A longitudinal section of a silver-hydrogel-coated latex catheter that was blocked after 11 days in situ.

7. Correlation between biofilm formation and antimicrobial resistance

The microorganisms that make up the biofilm and its structural features are what cause antibiotic or medication resistance. When compared to their planktonic stage, bacteria that live in biofilms exhibit a 10–1000-fold increase in drug resistance, particularly antibiotic resistance. Antimicrobial resistance is caused by a variety of factors, including varying growth rates of the biofilm microbiological organisms, delayed or insufficient diffusion of the antimicrobial drugs through the biofilms, and other functional changes. A variety of unique elements combines to reduce or eliminate a drug's effectiveness within a biofilm, which increases resistance. Drug resistance in bacterial biofilm colonies is associated with strategies such reduced drug penetration, a subgroup of microorganisms in a biofilm that display a form of differentiation resembling spore production, and a changing chemical microenvironment of the biofilm **(Sharma *et al.*, 2023**).

Recalcitrance is another name for this phenomenon, which happens when bacteria in the biofilm are able to withstand large drug dosages by combining a number of distinct mechanisms. The development of antibiotic resistance in biofilms is also facilitated by the maintenance of a high number of bacterial cells that survive antibiotic treatment because of tolerance of the slow-growing population and persisters, a high mutation rate, the presence of antimicrobial-selective pressure, and localized competition between mutants in the compartmentalized structure of the biofilms **(Sharma *et al.*, 2023**).

Antibiotics that spread through biofilms can have their activity inhibited by EPS through the phenomena of diffusion-reaction inhibition. Treatment resistance is further induced by the EPS component of the biofilm, which either slows the penetration process or reacts with the antimicrobial agent and changes its efficacy. Yet another method of antibiotic resistance of biofilm microorganisms is the horizontal gene transfer, which is a process by which the bacteria acquires genes for resistance **(Sharma *et al.*, 2023**).

The slower growth rate of bacteria associated with biofilms is another possible cause for antibiotic resistance. Slower growth causes antimicrobial medicines to be absorbed more slowly, which results in intracellular medication concentrations that are not as effective at killing bacteria. The faster-growing cells in younger biofilms are more susceptible to the antimicrobials than those in older biofilms **(Macia *et al.*, 2014).**

Furthermore, the interaction between different bacterial species can further complicate the biofilm formation. Co-colonization between *S. aureus* and *P. aeruginosa*, for example, increases the creation of biofilms and causes the differential expression of many genes related to defense, development, signaling, inflammation, and immune response. Therefore, biofilm cells are shielded from phagocytosis by thick layers of exopolymeric matrix (**Nachimuthu *et al.*, 2016**).

Since antibiotic resistance can spread to other classes of antibiotics and is not exclusive to any one antibiotic, it has become a serious worry. One of the main causes of antibiotic resistance, which eventually reduces the efficacy of current treatments, is the overuse and improper prescription of antibiotics (**Hoceini *et al.*, 2023).**

Compared to antibiotic-treatable infections, antibiotic resistant infections require significantly more healthcare resources - including higher costs, longer hospital stays, and greater medical interventions - while also causing increased patient suffering. As antimicrobial resistance (AMR) continues to grow, the range of effective empirical treatments keeps shrinking. This makes it crucial to understand current AMR patterns in ICU-associated CAUTIs; as such, data would provide valuable guidance for clinical decision-making (**Peng *et al.*, 2018**).

Materials and Methods

1. Presentation of the site: Centre Hospitalo-Universitaire (CHU) de Tlemcen

The Dr. Tidjani Damerdji University Hospital is a suburban structure situated in the city of Tlemcen, which is in the northwest of Algeria, close to the Moroccan border (**Figure 4**). The facility takes up thirteen hectares of land. It serves a population of 1.5 million people and has 646 beds with 44 specialized departments and laboratories (***Centre Hospitalo-Universitaire Dr Tidjani Damerdji De Tlemcen, n.d*.).**

Sampling was done at the Anesthesia-resuscitation department (intensive care unit) which is under Dr MEDJADI Sidi Mohammed **(Figure 5)** .This ward contains 9 beds. This department was chosen because of its high exposure to nosocomial infections and frequent use of invasive devices like catheters, ventilators and central lines, which offers a rich and varied source of clinical microbiological samples.

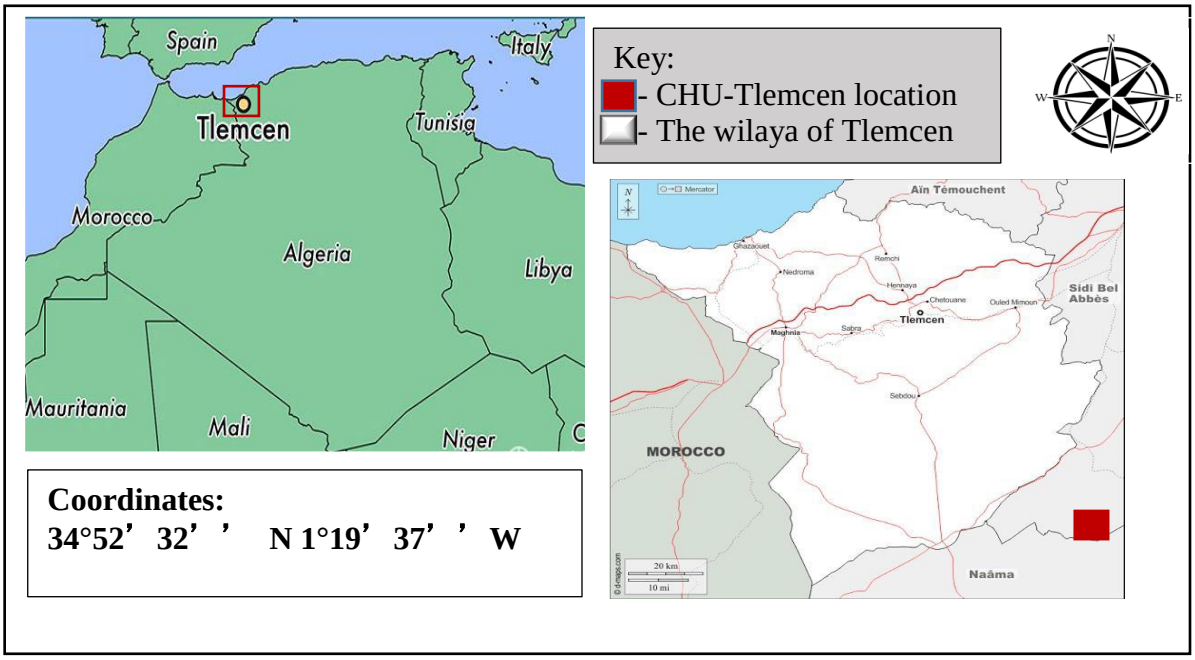


Figure 4: Geographic location of the Dr. Tidjani Damerdji University Center Hospital-Tlemcen.

Figure 5: Anesthesia-resuscitation department

2. Samples collection and processing

In a 3-month period from February 2025 to April 2025, 15 different sets of medical devices (central venous catheters, urinary catheters and intubation catheters) were collected from patients hospitalized for more than 48 hours in the (intensive care unit) ward of Anesthesia and resuscitation of the Tlemcen University Hospital, Algeria. The patients' ages ranged from 19 to 52 years, suffering from different types of healthy issues including accidents.

All catheters had been removed as part of the routine procedure to remove foreign material when the patient experienced clinical and laboratory signs of infection. At the bedside, the last 3cm of the tip of the catheter was separated from the bulk of the catheter by cutting with sterile scissors and transported in sterile tubes to the microbiology laboratory (Laboratoire de Microbiologie Appliquée à l'Agro-alimentaire, au Biomédical et à l'Environnement) within a maximum of two hours to guarantee the samples' reliability and validity **(Figure 6).**

After the medical equipment were removed, the "Brun-Buisson" technique was used to conduct a microbiological analysis (**Brun-Buisson, 1994**). The catheter tip was then placed in Brain Heart Infusion broth (BHIB) and vortexed at 150 rpm to remove the bacteria from the catheter and then incubated up to 24h at 35^0C.

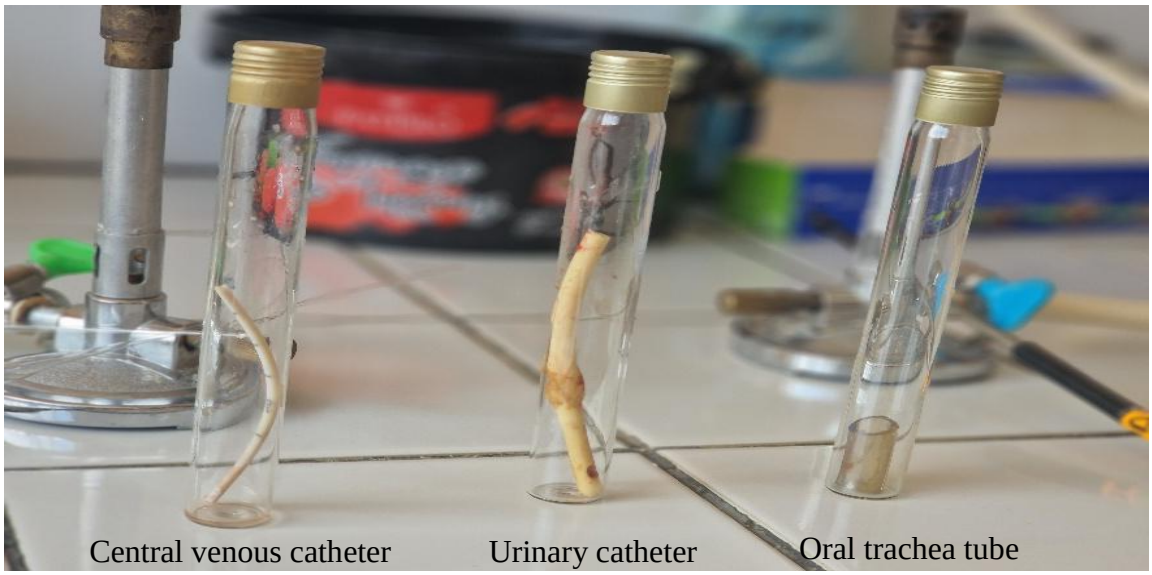


Figure 6: The sterile tubes with samples from the hospital.

3. Isolation and identification of bacteria

3.1 Plate streaking

A platinum wire loop that has been sterilized by flaming in a red-hot Bunsen burner, was dipped into the test tube containing the sample catheter and BHIB (**Figure Nº 6**), and used to streak on Nutrient agar (GN) for confirmation of the infection. Subsequently on three selective media which are Chapman agar for the isolation of Gram-positive Staphylococci, Mac Conkey agar for isolation of Gram-negative bacteria and Cetrimide agar for isolation of *Pseudomonas aeruginosa.* The Mac Conkey agar and Cetrimide agar plates are then incubated at 35^0C for 24 hours while the Chapman agar plates were incubated for 48 hours.

3.2. Purification

After isolation on the three selective media, the bacterial colonies obtained were purified, based on their macroscopic appearance, by sub culturing on the same selective media to obtain pure and identical strains in order to begin bacterial identification.

3.3. Identification of bacteria

Bacterial identification is conducted with usual microbiological techniques. Identification begins with a macroscopic examination of cultural characteristics (shape, color, size, appearance and texture) and microscopic observation using an electronic microscope.

3.3.1. Catalase test

The catalase test is a method for differentiating most aerobic bacteria and facultative anaerobes, particularly for differentiating staphylococci from streptococci.

From a pure culture, a small amount was taken and placed in a tube containing hydrogen peroxide. The release of oxygen bubbles is considered indicative of catalase activity, the reaction of which proceeds according to the following reaction **(Ekawati *et al.*, 2020).**

$$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2$$ Catalase reaction **(Heck *et al.*, 2010)**

3.3.2. Identification of strains by VITEK 2 system

Due to identification difficulties and efficiency, it was suggested that the following steps of identification be concluded by VITEK in order to obtain accurate results. Young purified bacterial colonies on the different selective media were prepared within a 24h period and delivered to the private laboratory to be identified by the VITEK system (**Figure Nº 7**).

VITEK refers to a series of automated microbiology analyzers for microbial identification (ID) and antibiotic sensitivity testing (AST) as it offers quicker AST results and an effective process. Its completely integrated ID and AST strategy guarantees exceptional performance to confidently and quickly guide therapy. The VITEK 2 system uses fluorescence-based techniques to identify metabolic changes, making it easier to identify gram-negative bacteria in less than three hours. This device uses a special algorithm to determine MICs while tracking the kinetics of bacterial growth **(Joyanes et al., 2001**).

Isolated colonies were dissolved in a special saline solution to a standardized turbidity (0,5 McFarland standard). The inoculum was mixed well and the density was checked with densicheck.

The inoculum was transferred into a VITEK 2 card which contains 64 microwells with biochemical substrates for identification (ID) and antibiotics at different concentrations for antimicrobial susceptibility testing (AST). For filling and Loading the card into VITEK 2 System, all the test tubes containing cards and suspension were set on a cassette and from computer work station cassette worksheet was printed and job ID and bar code for each card was recorded **(Khullar *et al*., 2016).**

3.4 The interpretation

The Global CLSI guidelines were used for the interpretation of the bacterial isolates being resistant, susceptible, or intermediate **(M100 ED34 | Performance Standards for Antimicrobial Susceptibility Testing, 2021)** and natural resistance as therapeutic interpretation policy. A bacterial isolate was regarded as multidrug-resistant when it showed resistance to 2 or more classes of antibiotics.

Figure 7: VITEK 2–compact 15 from the medical analysis laboratory of Dr Chahida BENHAMIDAT.

3.5 Conservation of strains

The pure strains are sub-cultured onto slanted nutrient agar tubes and stored at 4°C to keep the strains viable for the required amount of time.

5. Biofilm Detection Methods

Two methods were employed to detect the presence of biofilms in the collected medical devices. One being a qualitative method and the other being a quantitative method.

5.I. Congo Red Agar method (CRA)

To investigate slime production, CRA medium was used in accordance with Freeman et *al.* (1989) To produce the medium, 10 g/L of agar, 50 g/L of sucrose, 37 g/L of BHIB, and 0.8 g/L of Congo Red indicator were added. After being suspended and inoculated in the medium, the strains were incubated for twenty-four hours at 37°C.

Black colonies emerged from strains that produced slime, while red colonies showed no slime production. The strains were classified as significantly biofilm-forming if they displayed different morphologies, such as colonies with red centers and black outlines or colonies with black centers and red outlines (**Hoceini *et al.*, 2023).**

5.2. Tissue culture plate method (TCP)

The Christensen et *al*. technique is regarded as the standard for quantitative biofilm detection. (**Christensen et *al*., 1982**) This technique uses a colorimetric test following the washing, staining, and destaining of sessile cells in tubes or a microtiter with crystal violet or safranin.

Initially, the strains were cultivated and cultured in Brain Heart Infusion (BHI) media for an entire night. A final optical density of 0.08-0.1 at 590 nm was achieved by adjusting 200 µL of the strain suspension in the wells of a sterile 96-well polystyrene microplate. To guarantee sterility and non-specific media binding, sterile broths were utilized as blanks. After that, the microplates were sterilely coated and incubated at 37 °C for 24 hours. The plates were lightly tapped to get rid of any remaining bacteria after incubation, and then washed with deionized water up to three times.

The biofilm-forming bacterial cells were stained using a 0.1% (w/v) crystal violet solution for 10 minutes. Following staining, the solution was aspirated and the wells underwent three deionized water washes before air-drying. Subsequently, 200 µl of 95% ethanol was added to each well to solubilize the bound dye. The optical density (OD) of the stained biofilm was quantified using a micro-ELISA auto-reader (BioTek, ELx808) (**Figure 8)** at 630 nm.

Stepanovic et al. interpreted biofilm production into four categories: no biofilm producer (OD < ODc), weak biofilm producer (ODc < OD < 2xODc), moderate biofilm producer (2xODc < OD < 4xODc), and strong biofilm producer (4xODc < OD). The cut-off absorbance (ODc) was calculated as the average OD of the negative control plus three times the negative control's standard deviation. (**Stepanovic et *al.*, 2000**)

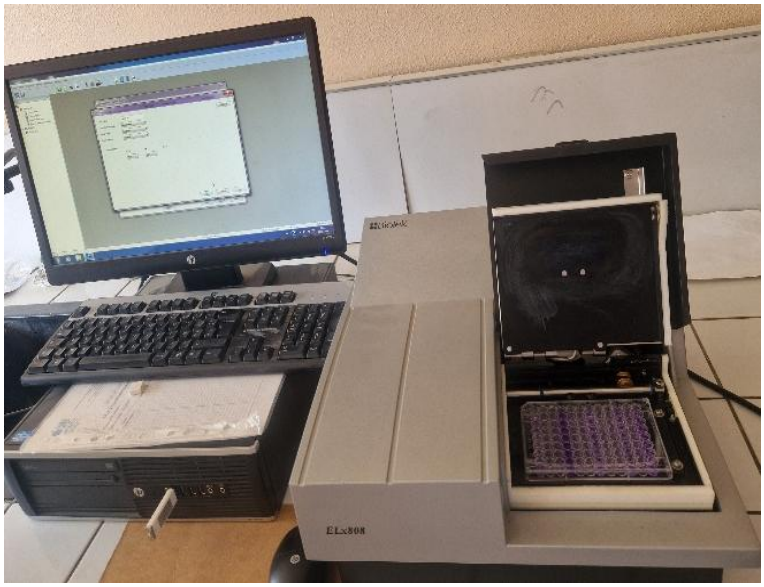


Figure 8: Micro-ELISA autoreader (BioTek ELx808)

Results and Discussion

1. Isolated bacteria in catheters

The bacteria from catheter tips, which were placed in Brain Heart Infusion broth (BHIB), showed a robust growth after incubation up to 24h at 35^0C, indicating successful detachment from catheters.

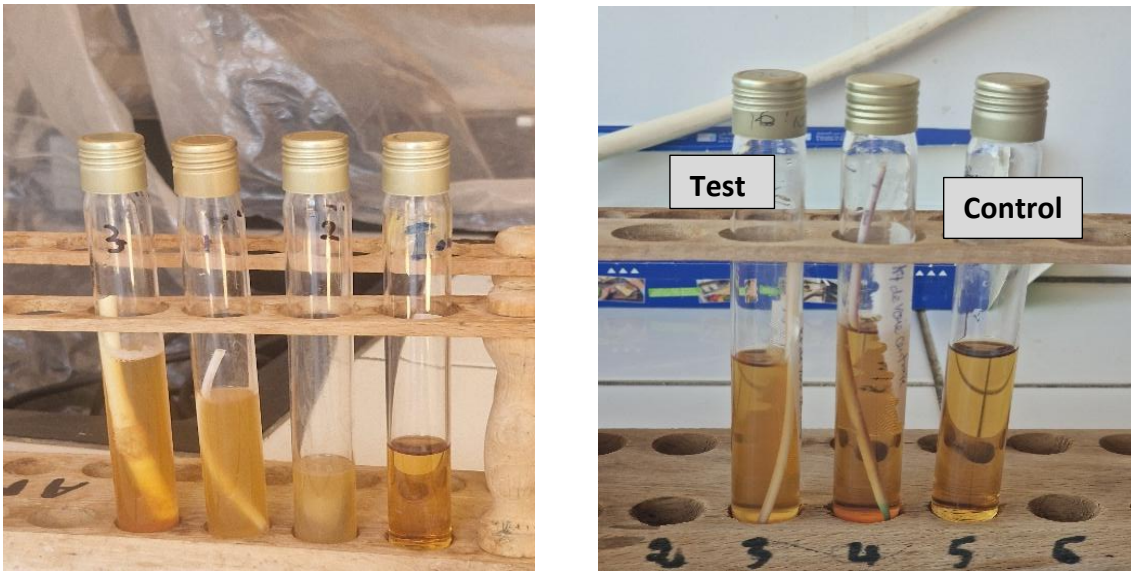


Figure 9: Growth of bacteria in nutrient broth

1.2. Macroscopic characterization of isolated bacteria

Following sub-culturing to obtain pure cultures, the bacterial colonies were visually examined for morphological characteristics to allow for preliminary identification of the isolates.

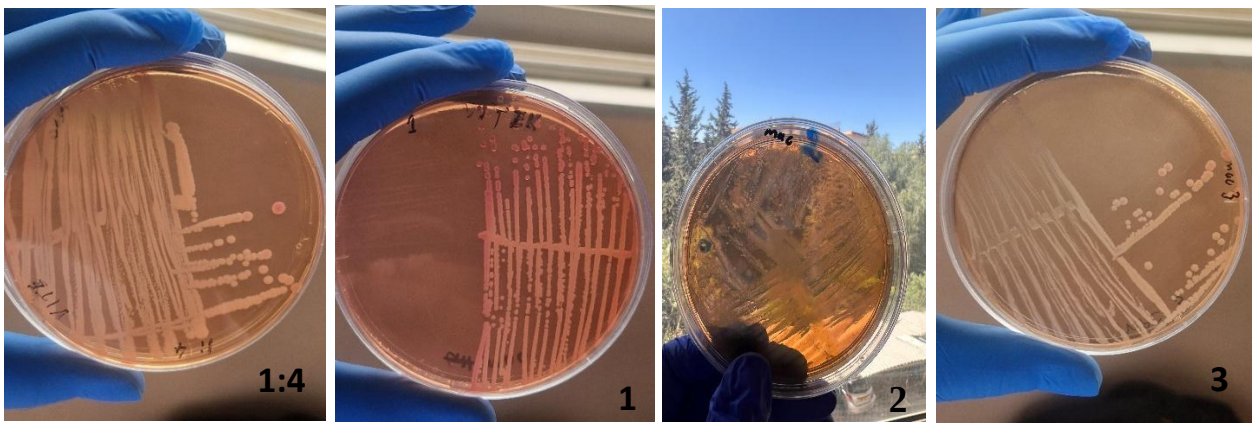
The isolates exhibited diverse morphological features. Identification was mainly based on visual assessment of colony traits such as size, color, texture and other distinctive attributes.

1.2.1. Isolates - MacConkey Agar plates

After approximately 24 hours of incubation at 37°C on MacConkey Agar plates, the isolates displayed various macroscopic characteristics. These differences are summarized in **Table Nº 4** and illustrated in **Figure 10.**

Table Nº 4: Description of the macroscopic aspects of the isolates selected on Mac Conkey agar from the Anesthesia -resuscitation unit

The isolate	**Macroscopic aspect on Mac Conkey Agar**
Mac-1:4 (*Enterobacter cloacae complex***)**	Large, round and slimy colonies that are pink in color. Other colonies were light pink. Agar turned pale red.
Mac-1 (*Acinetobacter baumannii***)**	The colonies are pink, small, and round. The agar remained red.
Mac-2 (*Enterobacteriaceae***)**	Notable mucoid texture, shiny glistening, transparent, grey colonies with a distinct odor.
Mac-3 (*Enterobacter cloacae complex***)**	Medium in size, pinkish color, and shiny colonies. The agar changed slightly changed color



Enterobacter cloacae complex *Acinetobacter baumannii* *Enterobacteriaceae* *Enterobacter cloacae complex*

Figure 10: Photos of the macroscopic aspect of the Gram- negative isolates after 24 hours of incubation

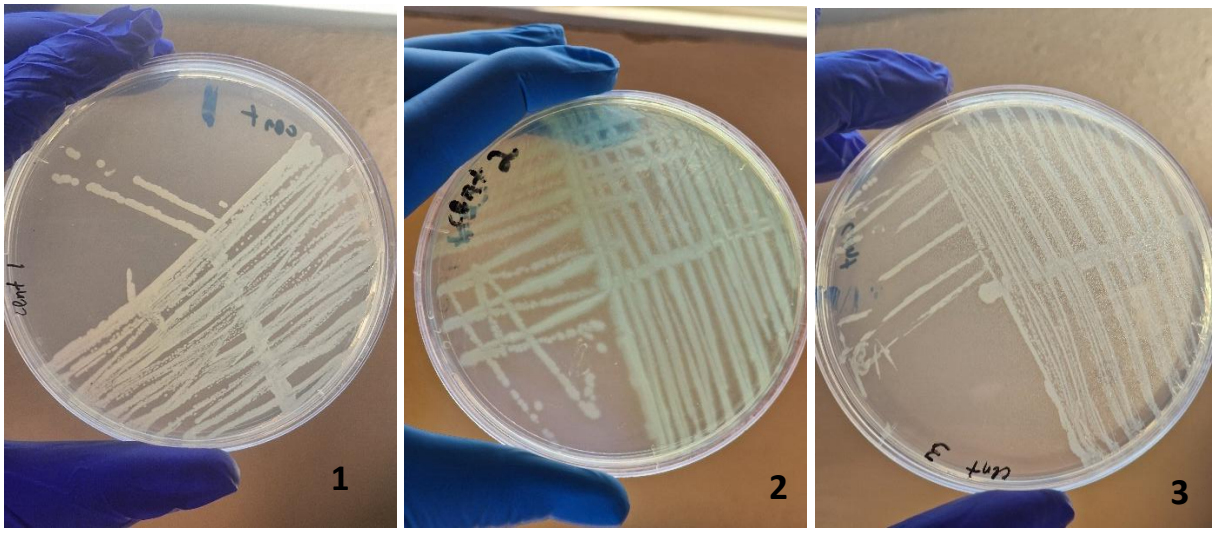
1.2.2. Isolates- Cetrimide Agar plates

On Cetrimide Agar plates (CA), after 24 hours of incubation at 37°C, some appearances were observed.

The macroscopic aspects of the Isolates are summarized in **Table Nº 5** presented in **Figure 11.**

Table Nº 5: Description of the macroscopic aspects of the isolates selected on Cetrimide Agar from the hospital.

The isolate	**The macroscopic aspects on Cetrimide agar plates**
Cent-1 (*Pseudomonas spp.***)**	The colonies were grey, small and appearing mucoidal and shiny No color change of the agar.
Cent-2 (*Pseudomonas aeruginosa***)**	Green slimy, mucoid, small colonies
Cent-3 (*Pseudomonas spp.***)**	Very small ,white colonies, slightly moist No significant coloration on the plate.



Pseudomonas spp. *Pseudomonas aeruginosa* *Pseudomonas spp.*

Figure 11: Photos of the macroscopic aspect of the *Pseudomonas spp*. isolates after 24 hours of incubation.

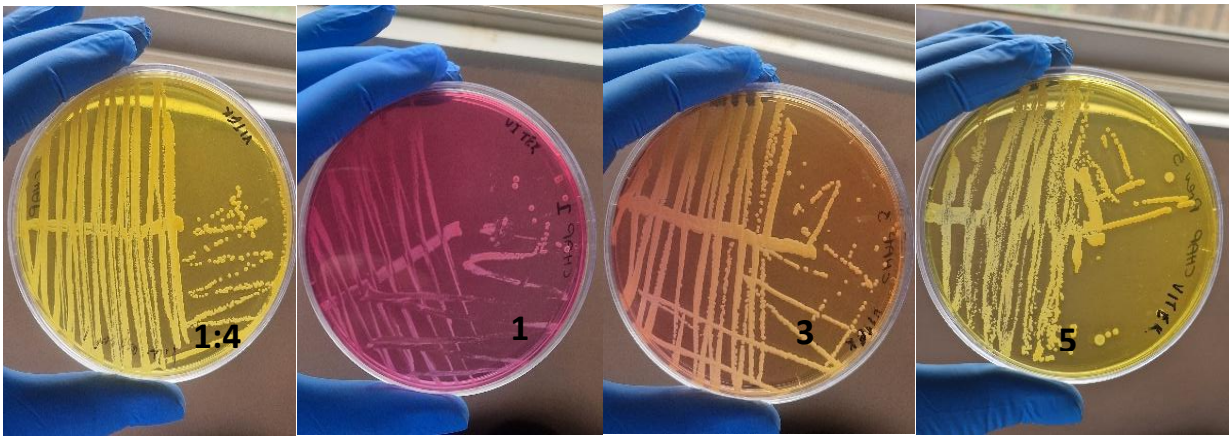
Cent-1: *Pseudomonas spp.* isolates from the central venous catheter

Cent-2*: Pseudomonas aeruginosa* isolates from the oral trachea tube

Cent-3: *Pseudomonas spp.* isolates from the urinary catheter

1.2.3. Isolates – Chapman Agar plates

The isolates showed some appearances after about 24 to 48 hours of incubation at 37°C on Mannitol Salt Agar plates. The different macroscopic aspects are summarized in **Table Nº 6** and presented in **Figure 12.**



Leuconostoc mesenteroides ssp. | *Staphylococcus epidermidis* | *Staphylococcus spp.* | *Staphylococcus haemolyticus*

Figure 12: Photos of the macroscopic aspect of the isolates after 48 hours of incubation.

Table Nº 6: Description of the macroscopic aspects of the isolates selected on Mannitol Salt Agar from the hospital.

THE ISOLATE	**THE MACROSCOPIC ASPECT ON MANNITOL SALT AGAR PLATES**.
CHAP-1:4 (*Leuconostoc mesenteroides ssp.***)**	Some colonies were very small and so close to each other. The colonies were small and yellowish in colour The agar completely turned yellow (indicating acid production due to fermentation by *Leuconostoc mesenteroides*.)
CHAP-1 (*Staphylococcus epidermidis* **)**	Small, round colonies, no distinct smell, pink in color with pale edges colonies. The agar remained pink indicating that mannitol fermentation did not occur.
CHAP-3 (*Staphylococcus spp.***)**	The colonies were small, smooth glistening and very distinct from each other. They were pale in color. Agar slightly changed the color.
CHAP-5 (*Staphylococcus haemolyticus***)**	Some colonies were big and round ,pale in color Other colonies were small, round and yellow in color. Mannitol salt Agar changed color to yellow from pink showing acid production from mannitol fermentation by *Staphylococcus haemolyticus.*

The conformation of the automated biochemical identification was carried out by the catalase test in which all bacteria of the genus *Staphylococcus spp.* are catalase positive, which is reflected by the release of the air bubbles **(Figure 13(A)).**

Microscopic observation of candida was also done, which shows opaque cells, dark oval and polymorphic features **(Figure 13(B)).**

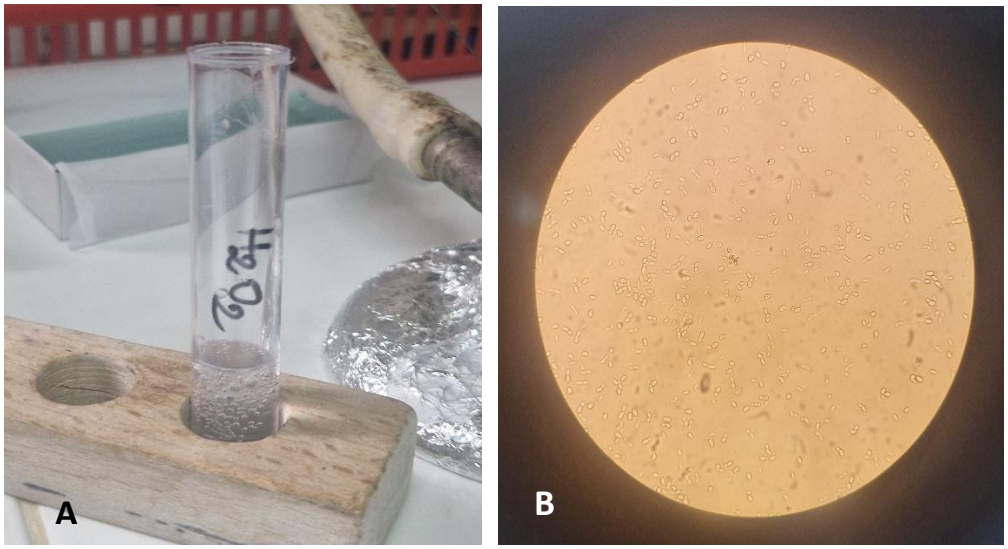


Figure Nº 13: A) Production of catalase by isolates (reaction positive, presence of *staphylococcus* bacteria)

B) Observation microscopic of *Candida* (Enlargement × 100)

1.2.4. Growth of Bacteria on Nutritive Agar plates

Nutritive Agar was utilized as a general-purpose medium that supports the growth of a broad spectrum of bacteria from hospital samples. This non-selective medium served as a control enabling the evaluation of the overall bacterial load and diversity present in the samples.

After incubating the Nutritive Agar plates for 24 hours at 37°C, a diverse array of bacterial colonies was observed the plates.

The colonies varied significantly in their appearance, which was indicative of the heterogeneity of the bacterial population. Notable variations included the colonies' size, shape, texture, and color differences in all the plates (**Figure 14)**. Other colonies were large, round, smooth-edged with a mucoid texture, and others were small, round with different colors, some whitish, and pale, others translucent and some were yellow. Some were very distinct and some were compact.

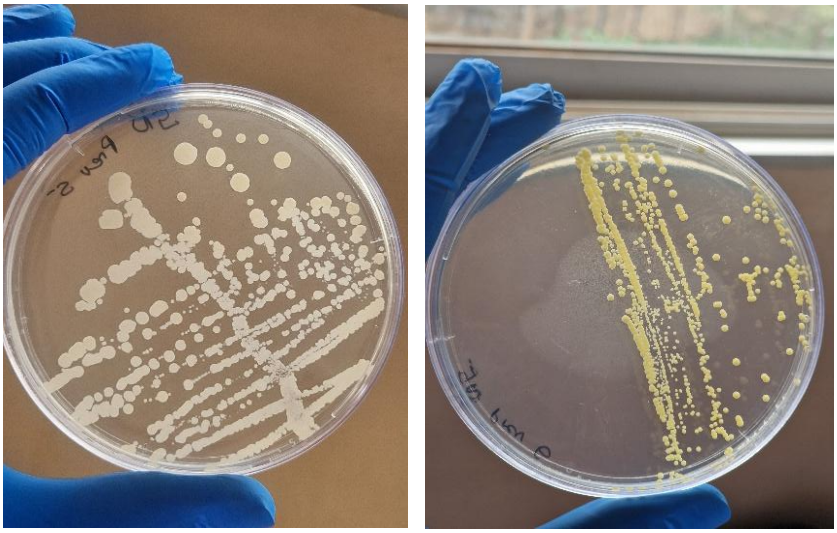


Figure 14: Photos of the macroscopic aspect of different bacteria on Nutritive Agar after 24 hours of incubation.

1.3. Identification by VITEK 2 system

Due to the insufficiency of the panels required for identification, some other strains were not identified and only 8 isolates (3 gram-positive bacteria, 4 gram-negative bacteria and 1 fungi) were identified. The identified isolates from different medical devices are shown on the table below and the biochemical reactions of each bacteria and fungi are shown in **annex 1**

Table Nº 7: The names of the identified isolates, along with their identification probability, reliability, and the type of medical device from which they were screened by VITEK 2

Germs selected	**Probability**	**Identification reliability**	**Type of medical device**
Pseudomonas aerugisa	91%	Good	Oral trachea tube
Acinetobacter baumannii complex	N/A	Excellent	Central venous catheter(CVC)
***Enterobacter cloacae complex* ODC(94)**	N/A	Very good	Urinary catheter
Enterobacter cloacae complex	N/A	Excellent	Central venous catheter
Staphylococcus haemolyticus	95%	Very good	Femoral venous catheter
Staphylococcus epidermidis	98%	Excellent	Central venous catheter
Leuconostoc mesenteroides ssp.	Very low	R/D	Central venous catheter
Candida + Staphylococcus spp.	N/A	Acceptable	Urinary catheter

N/A: unclear **R/D**: reliable discrimination

2. Antimicrobial susceptibility test of isolated bacteria by VITEK 2 System

2.1. Study of the sensitivity of isolates to antibiotics

The sensitivity of the isolates was tested using the installed VITEK 2 Systems Version: 9.02, following Global CLSI-based MIC Interpretation Standard. The analyzation of results was done by Advanced Expert System Parameter set named Global CLSI-based + Natural Resistance, following Therapeutic Interpretation Policy: NATURAL RESISTANCE with R standing for resistance, I and S as intermediate sensitivity and sensitive, respectively.

The antibiotics tested were, oxacillin, gentamycin, kanamycin, levofloxacin, résistance inducible à la clindamycin, erythromycin, clindamycin, quinupristin/dalfopristin, linezolid, test de depistage de la cefoxitin, daptomycin, teicoplanin, vancomycin,tetracyclin, fosfomycin, nitrofurantoin, fusidic acid, rifampicin, trimethoprim/sulfamethoxazole (T/S), ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, cefepime, ertapeneme, imipeneme, meropeneme, amikacin, tobramycin, ticarcillin, aztreonam, ciprofloxacin, pefloxacin, minocyclin, colistin.

2.1.1. The sensitivity of *Enterobacter cloacae complex* isolates to antibiotics

Table Nº 8: The results of the antibiogramme tests of the *Enterobacter cloacae complex* isolates.

Antibiotic	CM1	Interpretation	Antibiotic	CMI	Interpretation
Ampicillin			Imipeneme	≥16	R
Amoxicillin/clavunic acid	≥ 32	R	Meropeneme	≥16	R
Piperacillin/tazobactam	≥ 128	R	Amikacin	4	S
Cefazolin	≥64	R	Gentamicin	≥16	R
Cefotaxime	≥ 64	R	Tobramycin	≥16	R
Ceftazidime	≥64	R	Ciprofloxacin	≥4	R
Cefepime	≥32	R	Nitrofurantoin	64	I
Ertapeneme	≥ 8	R	Trimethoprim/ sulfamethoxazole	40	S

2.1.2. Table N⁰9: The sensitivity of *Acinetobacter baumanii complex* isolates to antibiotics

Antibiotic	CMI	Interpretation	Antibiotic	CMI	Interpretation
Ampicillin			Imipeneme	≥ 16	R
Amoxicillin/clavunic acid			Meropeneme	≥16	R
Piperacillin/tazobactam	≥128	R	Amikacin	≥64	R
Cefazolin	≥64	R	Gentamicin	≥16	R
Cefotaxin	≥64	R	Tobramycin	2	S
Ceftazidime	≥64	R	Ciprofloxacin	≥ 4	R
Cefepime	16	I	T/S	≥ 120	R

2.1.3. Table Nº 10: The sensitivity of *Pseudomonas aeruginosa* isolates to antibiotics

Antibiotic	CMI	Interpretation	Antibiotic	CMI	Interpretation
Ticarcillin	32	S	Amikacin	≤2	S
Ticarcillin/clavunic acid	16	S	Gentamicin	2	S
Piperacillin	≤ 4	S	Tobramycin	≤1	S
Piperacillin/tazobactam	8	S	Ciprofloxacin	≤0,25	S
Ceftazidime	2		Pefloxacin		
Cefepime	≤ 1	S	Minocycline		
Aztreonam			Colistin	2	S
İmipeneme	1	S	Rifampicin		
Meropename	≤0,25	S	Trimethoprim/ sulfamethoxazole		

2.1.4. The sensitivity of *staphylococcus haemolyticus* isolates to antibiotics

Table Nº 11: Shows the results of the antimicrobial susceptibility tests of *staphylococcus haemolyticus* Isolates

Antibiotic	CMI	Interpretation	Antibiotic	CMI	Interpretation
Test cefoxitin screen	NEG		Daptomycin		S
Oxacillin	≤0,25	S	Teicoplanin	≤8	S
Gentamicin	≤0,5	S	Vancomycin	≤0.5	S
Kanamycin		S	Tetracyclin	≥16	R
Levofloxacin	≤O, 12	S	Fosfomycin		
Inducible resistance to clyindamycin	NEG		Nitrofurantoin	≤16	S
Erythromycin			Acide fusidique		S
Clindamycin	≤0,25	S	Rifampicin	≤0,03	S
Quinupristin/dalfopristin	≤0,25	S	Trimethoprim/ sulfamethoxazole	≤10	S
Linezolid	1	S			

2.1.5. The sensitivity of *staphylococcus epidermidis* isolates to antibiotics

Table Nº 12: Shows the results of the antimicrobial disc susceptibility tests of *staphylococcus epidermidis* Isolates

Antibiotic	CMI	Interpretation	Antibiotic	CMI	Interpretation
Test cefoxitine screen	NEG	*+	Daptomycin	0,5	S
Oxacillin	0,5	R	Teicoplanin	≥32	R
Gentamicin	≤0,5	S	Vancomycin	1	S
Kanamycin	≤4	S	Tetracyclin	2	S
Levofloxacin	≤0,12	S	Fosfomycin		
Inducible resistance to clindamycin	NEG	-	Nitrofurantoin	≤16	S
Erythromycin			Fusidic acid	≤0,5	S
Clindamycin	≤0,12	S	Rifampicine	≤0,03	S
Quinupristine/dalfopristin	<= 0,25	S	Trimethoprim/ sulfamethoxazole	>= 320	R
Linezolid	1	S			

3. Evaluation of biofilm formation

The ability of strains to form a biofilm was evaluated using two different methods, the Congo Red technique and the TCP method. In this case, only identified strains were detected.

3.1. The Congo Red Agar (RCA) method

This is a qualitative method based on the phenotypic character of the strains shown on Congo Red Agar. Investigation of production of slime on congo red medium revealed that among the 8 identified strains, 4(*S. epidermidis, Staphylococcus spp + Candida*, *Enterobacter cloacae complex*, *Enterobacter cloacae complex* ODC(94)) were slime producing strains while 4 were non-slime producing strains. The slime producing strains had a variable or positive phenotype as shown in the table below**.**

Table Nº 13: Results of slime production on Congo Red medium

	Number of strains	Percentages	Observed phenotype
Slime producer	3	37,5%	
Variable	1	12,5%	
Non-slime producer	4	50%	

3.2 Tissue culture plate (TCP) technique

The 96-well microplate technique allows not only a quantitative but also a qualitative assessment of biofilm formation. After quantifying the OD with the ELISA reader, the OD values were calculated and biofilm production was graded into strong, moderate and weak (**Table Nº14**). This technique revealed that of the 8 strains identified, 1 was a moderate biofilm-forming, and 7 of the strains were weakly adherent. This screening is consistent with that obtained by observing the coloration of the inoculated wells **(Figure 15)**. The OD obtained for Gram-negatives was between 0.180 to 0.40 and between for Gram-positives 0.150 to 0.20 **(Figure 16).**

Table Nº14: Grading of biofilm formation by TCP method

Optical densities values	Biofilm formation
< 0.1425	Non
0.1425 – 0.285	Weak
0.285 – 0.57	Moderate
>0.57	Strong

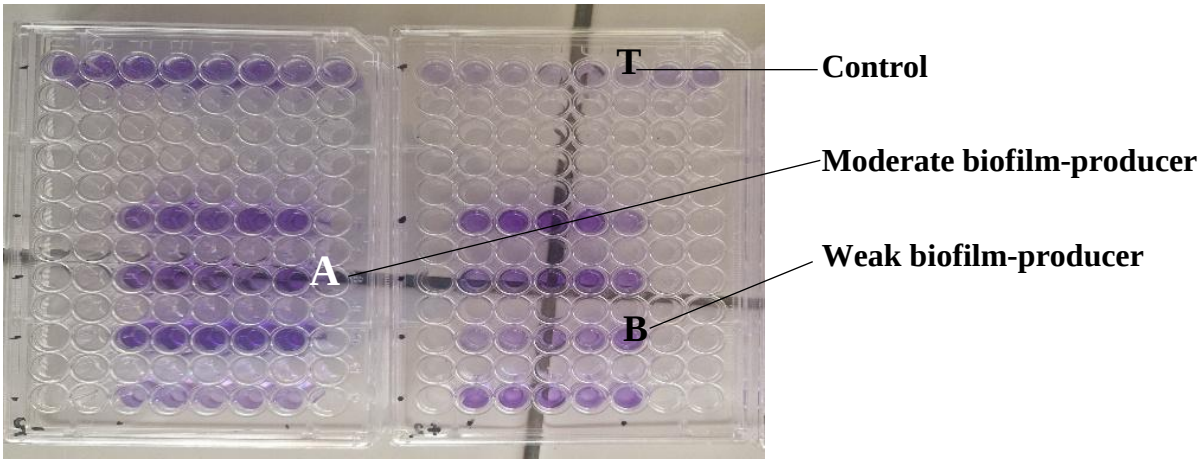
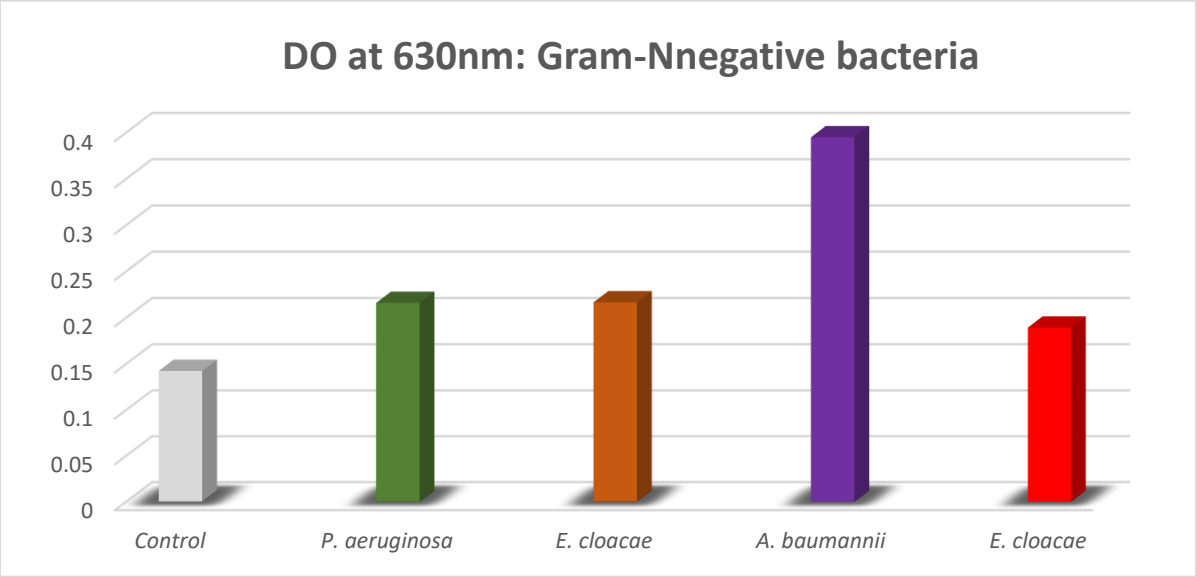


Figure 15: Evaluation of biofilm production by TCP method



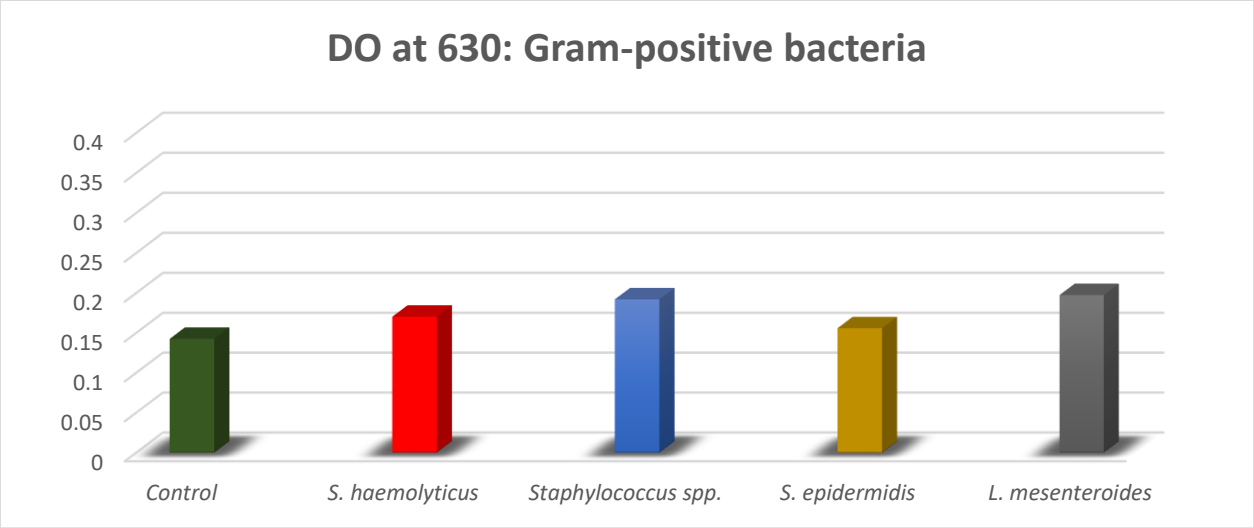


Figure16: Quantification of biofilm formation by Gram-negative and Gram-positive bacteria.

4. Discussion

This study was carried out with one of the aims of searching for the presence of bacteria species in different medical devices from the Tlemcen Hospital. The bacterial organisms present in the medical devices (catheters) sampled from the Anesthesia-resuscitation unit comprised of Gram-positive bacteria, *Staphylococcus haemolyticus, Staphylococcus epidermidis, Leuconostoc mesenteroides ssp.*; Gram-negative enteric bacteria, *Enterobacter cloacae complex, Pseudomonas aeruginosa, Acinetobacter baumannii and Acinetobacter nosocomialis* and other fungi species isolated on different agars including *Candida*.

Our study showed that most catheters had mostly one or two types of microorganism present based on the bacterial strains identified by VITEK 2 **(Table Nº 7).** Our results are in line with prior research by **Gunardi *et al*. (2021)** that found that the majority of catheters had a single bacterium, such as *Candida spp., S. aureus, E. coli, E. faecalis, P. aeruginosa, S. epidermidis,* and *S. haemolyticus.* Furthermore, according to a study by **Gunardi *et al.* (2021**) only 26.1% and 11.1% of the catheter cultures were polymicrobial, including two and three germs, respectively, whereas 62.8% of the cultures had a single bacterium.

4.1. Identification of Gram- negative bacteria

Pseudomonas aeruginosa is one of the six ESKAPE bacterial pathogens. It is also known to be a significant contributor to chronic infections because of its capacity to create biofilms. Patients with cystic fibrosis and chronic obstructive lung disease develop persistent lung infections due to P. aeruginosa **(Ciofu and Tolker-Nielsen, 2019)**.

The *P. aeruginosa* strains identified were isolated from an oral tracheal tube, which according to **Pericolini *et al*. (2018)** is among several devices, that is particularly vulnerable to the production of biofilms. Due to the simultaneous breakdown of the skin/mucosa barrier and endo-tracheal tube exposure to respiratory mucus and blood, which may further facilitate bacterial adherence to such an abiotic surface.

We were able to isolate *Acinetobacter baumannii* from a central venous catheter. CVC have been found to be a common source of bloodstream infections, and the presence of *A. baumannii* in CVCs

is associated with higher healthcare expenses, longer hospital stays, and increased morbidity according to **Jang *et al*. (2009).**

Strains of *A. baumannii* that have been tested can adhere to glass and plastic surfaces and create biofilms. This pathogen's obligatory aerobic nature encouraged extensive cell aggregation at the air–liquid interface. Antibiotic resistance is known to be linked to the presence of plasmids in *A. baumannii*. This also enhances the ability of these isolates to transfer resistance markers to the other clinical strains in mixed infections by transformation or conjugation **(Pour *et al*., 2011)**.

Among the other isolates, that we identified from a central venous catheter and urinary catheter was *Enterobacter cloacae* complex. This finding is comparable to **Štefánek *et al*. (2022)** who found rod-shaped *bacilli E*. cloacae complex isolates belonging to ST599 and were identified as *Enterobacter bugandensis* from CVC.

4.2. Identification of Gram-positive bacteria and fungi

We found that the two most prevalent Gram-positive species in venous catheters are *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* and *Leuconostoc mesentoroides* at low quantity. Candida was also found dominant in urinary catheter**.**

Vuong and Otto, (2002) mentioned that *Staphylococcus epidermidis* is the most frequently isolated member of the group of coagulase-negative staphylococci and has gained substantial interest in recent years due to its cause of nosocomial infections and its ability to form biofilm on indwelling devices is considered to be the main virulence factor.

The finding of *S. epidermidis* CVC aligns well with **Siciliano *et al.* (2023)** who found that most coagulase-negative staphylococcal (CoNS) bloodstream infections are the result of infections of intravascular catheters. *S. epidermidis* infections associated with the presence of an indwelling device, assessment regarding whether the device should be removed is an important component of management.

Among CNS, Staphylococcus haemolyticus is the second most frequently isolated from human blood cultures and plays an important role in hospital-acquired opportunistic infections related to implanted medical devices in the words of **Fredheim *et al*. (2009).** *S. haemolyticus* causes

bacteremia following the central catheter-related bloodborne infection and the presence of venous catheters or medical devices increases the risk of infections according to **Eltwisy *et al.* (2022)**.

A 2023 study by Osório *et al*. found that *Staphylococcus epidermidis* exhibits increased expression of surface proteins that promote its attachment to catheters, which likely contributes to its predominance in catheter-related infections. In contrast, *Staphylococcus haemolyticus*, though also clinically significant, tends to persist less on catheter surfaces due to weaker colonization mechanisms. However, comparative research by **Pereira-Ribeiro *et al*. (2022)** indicates that both species are capable of adhering to catheter surfaces, with *S. haemolyticus* showing greater resilience under antibiotic pressure. This suggests that, under certain conditions, *S. haemolyticus* may have a stronger capacity for colonization despite its lower surface adherence efficiency.

The results also showed low discrimination of *Leuconostoc mesenteroides ssp.* a Gram-positive, non-motile, vancomycin-resistant bacteria from the family of Streptococcaceae. They naturally exist in food and are important in the sauerkraut, milk and wine industries due to their role in fermentation. *Leuconostoc mesenteroides* can be considered as a causative agent in community-acquired infections. Risk factors for *Leuconostoc* infections are immunocompromised status and indwelling medical devices. Central venous catheter insertion is also a risk factor for introducing this bacteria into the body **(Usta-Atmaca *et al.*, 2015)**.

The results by VITEK 2 System has shown that urinary catheter was colonized by *Candida.* **Lee *et al.* (2017)** found that in cases where urinary catheters are inserted for a short period or over a long term, most infections start with single microorganism and *Candida* can also be involved. Localized and systemic fungal infections typically involve opportunistic *Candida.*

Similarly to **Gnardi *et al.* (2021)**, *Candida sp* is the second most isolated microorganism from urinary catheters at 17,8% while other studies have also reported monomicrobial isolates of *Candida spp.* at 95,1% from urine samples.

4.3. Antimicrobial susceptibility test of isolated bacteria by VITEK 2 System

Enterobacter cloacae complex isolated from CVC and urinary catheter was resistant to almost all antibiotics as shown above and sensible to only two antibiotics, amikacin and trimethoprim/sulfamethoxazole while having an intermediate sensitivity to nitrofurantoin.

In this study, *Enterobacter cloacae complex* demonstrated a multiplex pattern of antibiotic susceptibility, as it has shown high resistance to most antibiotics used during antibiogram as being shown above, while retaining sensitivity to only Amikacin and Trimethoprim/ sulfamethoxazole hence confirmation of the AMR phenotypes observed for this bacterium. According to some research, *E. cloacae* isolates have comparatively low rates of TMP/SMX resistance, which suggests that in some situations, it may be a useful therapeutic alternative. For instance, *E. cloacae* had a low percentage of TMP/SMX resistance (4.96%) in urinary tract infections in a Spanish hospital, suggesting that this antibiotic is still a useful empirical treatment in that setting **(Rodríguez *et a*l., 2023).**

A. baumannii was resistant to more than 2 classes of antibiotics used hence proving it's also a multi-drug resistant bacteria. Our result align with a research by **(Gounden *et al.* (2009)** ,where in a cohort of ICU patients, over half of the *A. baumannii* isolates were only susceptible to tobramycin and colistin, highlighting the unique efficacy of tobramycin against otherwise resistant strains. Unlike in our research where the antibiotic colistin wasn't used , **Pour *et al*. (2011)** revealed that all strains were sensitive to colistin and had high resistance rates to different classes of antibiotics that were tested.

All of the antibiotics tested against the isolate from oral trachea catheter, *P. aeruginosa* were all effective. Our results correlate to an extent with research done in Algeria by **Hoceini *et al*. (2023)** where both biofilm-producing and non-producing *P. aeruginosa* strains showed susceptibility to nearly all of the antibiotic groups used, except for ticarcillin, piperacillin, imipenem and rifampicin. However, the among the 4 antibiotics mentioned previously, 3 were used in our antibiogram and showed sensibility to the antibiotics (ticarcillin, piperacillin, imipenem) hence contradicting the research done in the Algerian hospitals by **Hoceini *et al.* (2023).**

Nevertheless, prior studies have demonstrated a high vulnerability to colistin and resistance to a number of antibiotics. Because colistin causes nephrotoxicity due to oxidative stress and the activation of inflammatory and apoptotic pathways, especially in renal proximal tubules, its restricted usage as a last resort treatment for major MDR infections is linked to its high susceptibility (**Hoceini *et al.*, 2023).**

The Antibiogram of *S. haemolyticus* revealed that the bacteria was sensible to most of the antibiotics and resistant to only one antibiotic named Tetracycline **(Table Nº 11).** According to **Eltwisy *et al.* (2022),** teicoplanin is effective in the treatment of *S. haemolyticus* infections, with rare homogenous resistance reports. Their study showed that *S. haemolyticus* isolated from both reports were also sensitive to vancomycin. Linezolid is also recommended in cases of severe bacterial infections caused by staphylococci as it inhibits bacterial protein synthesis by interfering with the peptidyl transferase of 23S rRNA in the 50S ribosomal subunit.

Another important and inexpensive parameter to evaluate in the diagnosis of *S. epidermidis* infections is the resistance phenotype and indeed, the oxacillin resistance seems to correlate with the *S. epidermidis* true BSI based on **Siciliano *et al.*** (**2023**). Thirty-six *S. epidermidis* specimens isolated from implanted port catheters (12, 33%) from the University Hospital of Montpellier were included in the study by **Pouget *et al.*** (**2023**). The resistance profile showed that all isolates were 83% were resistant to oxacillin and 64% to erythromycin. According to **Noshak *et al.*** (**2023**) , *S. epidermidis* had a higher rate of resistance to trimethoprim-sulfamethoxazole (83.9%) which is in accord with our findings.

In another study, the antibiotic-resistance profiles of CoNS isolated from a hospital environment in South Italy were evaluated. *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* were the most prevalent CoNS isolated from clinical samples. The strains did not exhibit any resistance to linezolid. No clinical isolate was resistant to the glycopeptides vancomycin. The high susceptibility rate against these antibiotics could be due to the preferential use of linezolid by hospital clinicians. Rifampicin was said to be one of the antibiotics of choice for the treatment of joint and bone infections, due to its ability to penetrate staphylococcal biofilm and trimethoprim-sulfamethoxazole as it exerts a bacteriostatic effect against staphylococci and susceptibility to this

antibiotic combination is highly variable. Regarding tetracycline, in this study of **Nicolosi *et al.* (2020)**, 24% of the strains exhibited resistance.

4.4. Evaluation of biofilm formation

The quantitative determination of biofilm formation in all our strains was carried out under standard growth conditions, using BHIB growth medium without supplementation. Indeed, we noticed that biofilm production exist in medical devices, strains were all biofilm-forming, (**Figure 15**). Our findings are somewhat similar to an investigation done by **Folliero *et al*., (2021)** which reported that 56.4% of bacterial isolates from medical devices were biofilm producers, and these strains were more likely to exhibit multidrug resistance compared to non-biofilm producers

Comparing the results obtained from the two TCP and RCA techniques, we found that the strains considered non-slime-forming *S. aureus, P. aeruginosa, A. baumannii* and *L. mesenteroides* were found as positive biofilm producers. These were false-negative results reported by RCA, while the other strains were true-positive biofilm producers by both techniques. A difference in the results was also observed by **(Sultan & Nabiel, 2018).** These authors considered TCP as the best for detection of biofilm production since interpretation of results by ELISA reader eliminates the subjective errors seen with other phenotypic tests. In their study, TCP detected 9% isolates as strong biofilm producers and 20.7% as moderate biofilm producers while CRA detected 11.0% and 32.4% isolates as strong and moderate biofilm producers respectively.

In this current study, among 8 bacterial strains from medical devices, the standard method TCP detected 7 (87,5%) isolates as weak and 1 (12,5%) isolate as moderate biofilm producers .Biofilm producing Gram-negative organisms were 75% weak and 25% moderate producers. Gram-positive strains were all (100%) weak biofilm producers. 3 (37.5%), 1(12, 5%), and 4(50%) strains were identified as strong, variable and weak/non-producers respectively by CRA method **(Figure17).** Both Gram-negative and Gram-positive strains had a percentage of 50 as biofilm producers in CRA technique. Figure and table shows the percentages of biofilm and non-biofilm production identified by CRA and TCP. There was a variable significant correlation between TCP and CRA method although the CRA assay can well detect biofilm-producing strains, weak producers were difficult to discriminate from biofilm negative isolates **(Mohsenzadeh *et al*., 2021).**

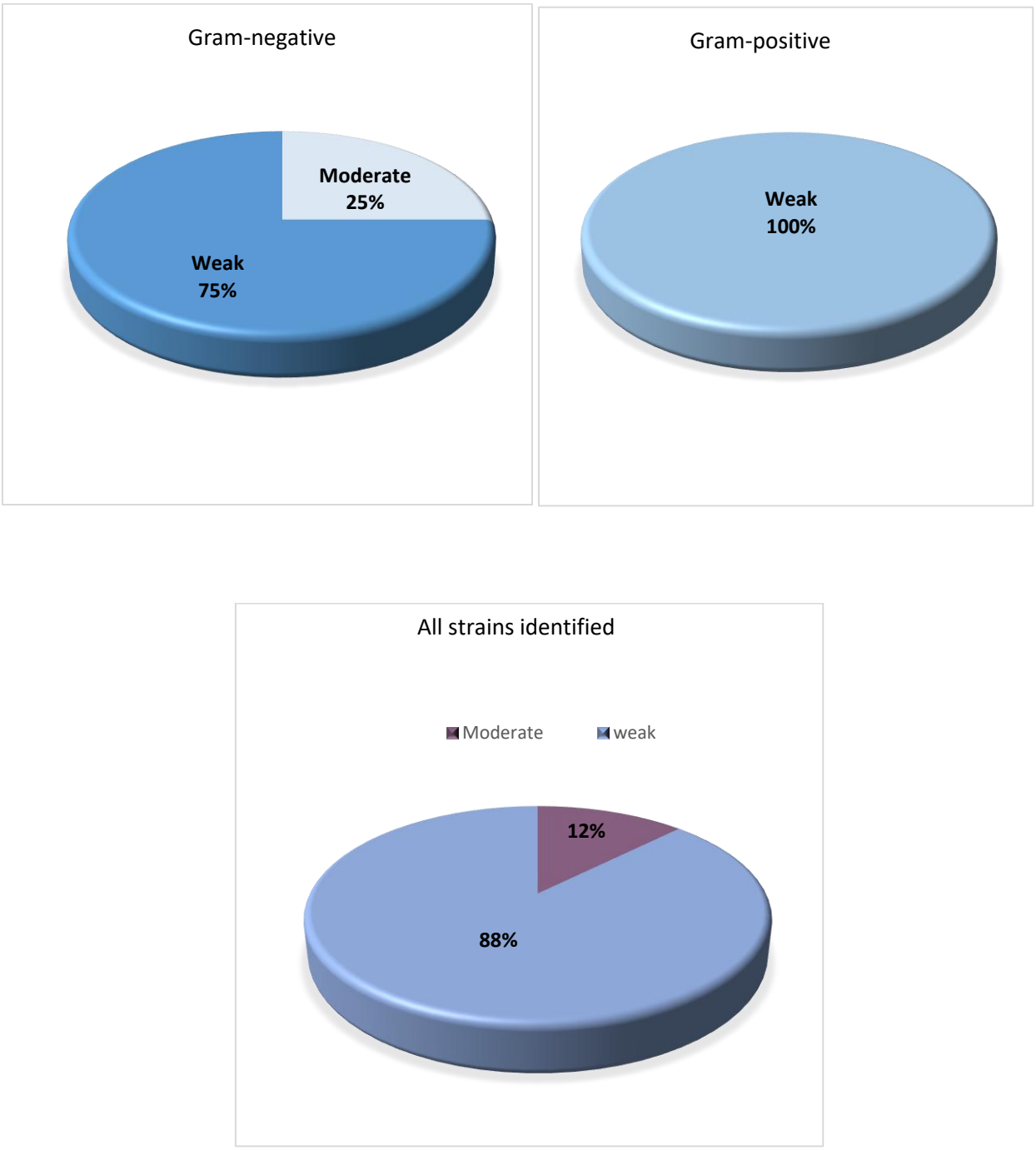


Figure 17: Biofilm production rate by the TCP method.

According **to Juliana *et al.*, (2022)**, a true biofilm producing organism is considered an organism that shows biofilm formation in both methods. The same author mentioned that in most articles where a strong correlation was found between biofilm formation, the biofilm formed was either strong or moderate. These studies did not correlate with our findings. In this current study *S. epidermidis, Staphylococcus spp. + Candida* and *E. cloacae* were the common biofilm-producing organisms in both TCP and RCA methods and were all considered weak biofilm producers.

We found that *staphylococcus spp.* and *candida* combined was detected by TCP and CRA as a biofilm producer. This has been proven by **Vila *et al.* (2021)** in their study that demonstrated that *S. aureus* exhibits high affinity to the *C. albicans* hyphal form as these species co-adhere and interact synergistically, forming a dense and architecturally complex biofilm. *C. albicans* induced the activation of the *S. aureus* biofilm formation network via down-regulation of the lrg operon, repressor of autolysis, and up-regulation of the ica operon and production of polysaccharide intercellular adhesin (PIA), indicating an increase in eDNA production, and extracellular polysaccharide matrix, respectively.

High resistance pattern was observed between the moderate biofilm producers in comparison with weak-biofilm producers. In this study, we compared antibiotic sensitivity pattern of *A. baumannii* complex a moderate biofilm producer and 3 weak producers (*P. aeruginosa, S. epidermidis* and *S. haemolyticus*). We have observed a higher antibiotic resistance in moderate biofilm-producing bacteria than in weak-biofilm producers. **(Table Nº9)** illustrates the resistant pattern of the Gram-negative bacterial (*A. baumannii*).Our findings were proven by **M'hamed *et al*. (2021)** who found a high resistance rate (>80%) to amoxicillin, ampicillin, cefazolin, cefoxitin and cefotaxime, with 4 isolates resistant to imipenem. These strains were also resistant to aminosides with rates ranging from 36.36 % for amikacin to 50 % for gentamycin. Resistance rates of isolates to trimethoprim and cloramphénicol were 36.36 % and 63.63 % respectively. This same author found that *staphylococcus* strains isolated at Mohamed Boudiaf Hospital revealed of Ouargla (Algeria) were resistance to vancomycin, which did not align with our results. In our study, *staphylococcus* strains were sensible to vancomycin and resistant to tetracycline. **(Table Nº11 and Table Nº12)** illustrates the resistance pattern of the Gram-positive isolates. This reveals that the susceptibility pattern of an organism is dependent on the strength of the biofilm formed by that organism.

A significant correlation was detected between the antibiotic resistance exposure and the biofilm-production capacity, in medical devices. Mostly all of the strong biofilm producers and most weak biofilm producers showed multi drug resistance, confirming that the production of biofilms made the pathogen sensitive to a limited spectrum of antibiotics **(Revi *et al.*, 2022)**. The higher antibiotic resistance pattern showed by biofilm-producing bacteria could be due to restricted and limited antibiotics penetration into biofilms, the high expression of efflux pump and expression and exchange of resistance genes among bacteria within a biofilm **(M'hamed *et al.*, 2021).**

Biofilm-associated mixed infections, such as those on indwelling medical devices, are particularly challenging to treat due to their inherent heterogeneity, which can be further amplified by synergistic inter-species interactions. Combined, these findings provide mechanistic insights into the therapeutic implications of interspecies interactions, underscoring the need for novel strategies to overcome limitations of current therapies.

General Conclusion

General conclusion

This study evaluated the biofilm-forming capacity of bacterial strains isolated from medical devices, with a specific focus on samples collected exclusively from the ICU unit of Centre Hospitalo-Universitaire (CHU) of Tlemcen, Algeria. The findings confirm the widespread presence of biofilm-producing organisms in clinical environments, especially on indwelling medical devices, underscoring their role in persistent and hard-to-treat infections.

Using phenotypic methods for assessing biofilm formation such as the Tissue Culture Plate (TCP) and Congo Red Agar (CRA) assays, this study confirmed that a majority of the isolates possess biofilm-forming abilities, with varying intensities. TCP provided a more sensitive and objective measurement in detecting weak producers, while CRA was less reliable for borderline cases, highlighting the importance of method selection in accurate biofilm assessment. Both the Gram-negative and Gram-positive bacteria isolated from devices demonstrated biofilm production, with certain strains like *A. baumannii* showing moderate to strong biofilm-forming potential. Notably, a correlation was observed between biofilm production capacity and multidrug resistance, suggesting that biofilm formation enhances bacterial survival in hostile environments, including those with high antibiotic pressure.

The findings underscore the clinical relevance of routine biofilm screening for isolates from medical devices to guide effective antimicrobial therapy and infection control. They also highlight the urgent need for alternative therapeutic approaches, including stricter catheter management protocols, anti-biofilm strategies and improved biomaterials that resist microbial colonization.

One of the major limitations of this study was the narrow scope of sample collection confined to a single hospital ward (Anesthesia and Resuscitation unit). While this focus allowed for in-depth analysis of a high-risk environment, it restricted the diversity of microbial strains examined and may not fully represent the biofilm formation trends across other departments. Broader sampling across multiple wards or hospitals would be needed to draw conclusions that are more generalizable. Future research must overcome these obstacles in order to produce more reliable and representative results.

Furthermore, several operational and environmental challenges affected the consistency and progress of the work. During the usage the Tissue Culture Plate (TCP) method as the standard for

quantifying biofilm formation, we encountered some technical challenges. The lower readings may have been influenced by inconsistent washing, residual staining, or variations in the plate reader calibration (630nm) instead of (590nm), making interpretation difficult in indeterminate cases, especially among weak biofilm producers. These issues highlight the need for stringent standardization and operator consistency when using TCP to ensure reproducible and accurate results.

In spite of these limitations, our study reinforces the critical importance of early and accurate detection of biofilm production and resistance profiles of clinical isolates. It also highlights the ongoing need for standardized biofilm quantification methods and broader surveillance to inform infection control strategies. Addressing these challenges in future researches will be vital for improving biofilm detection, understanding its clinical impact, and developing more effective treatment protocols for device-associated infections.

References

References

Abdallah, M., Benoliel, C., Drider, D., Dhulster, P., & Chihib, N.-E. (2014). Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. *Archives of Microbiology*, *196*(7), 453–472. https://doi.org/10.1007/s00203-014-0983-1

Ali, A., Zahra, A., Kamthan, M., Husain, F. M., Albalawi, T., Zubair, M., Alatawy, R., Abid, M., & Noorani, M. S. (2023). Microbial Biofilms: Applications, Clinical Consequences, and Alternative Therapies. *Microorganisms*, *11*(8), 1934. https://doi.org/10.3390/microorganisms11081934

Alotaibi, G. F. (2021). Factors Influencing Bacterial Biofilm Formation and Development. *American Journal of Biomedical Science & Research*, *12*(6), 617–626. https://doi.org/10.34297/AJBSR.2021.12.001820

Alotaibi, G. F., & Bukhari, M. A. (2021). Factors Influencing Bacterial Biofilm Formation and Development. *American Journal of Biomedical Science & Research*, *12*(6), 617.

Armbruster, C. R., & Parsek, M. R. (2018). New insight into the early stages of biofilm formation. *Proceedings of the National Academy of Sciences*, *115*(17), 4317–4319. https://doi.org/10.1073/pnas.1804084115

Assefa, M., & Amare, A. (2022). Biofilm-Associated Multi-Drug Resistance in Hospital-Acquired Infections: A Review. *Infection and Drug Resistance*, *Volume 15*, 5061–5068. https://doi.org/10.2147/IDR.S379502

Balikci, E., Yilmaz, B., Tahmasebifar, A., Baran, E. T., & Kara, E. (2021). Surface modification strategies for hemodialysis catheters to prevent **c** atheter- **r** elated infections: A review. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *109*(3), 314–327. https://doi.org/10.1002/jbm.b.34701

Baillif-S., Hartmann-D., Freney-J., Kodjikian-L. (2010). Implant intraoculaire et adhésion bactérienne : influence des conditions environnementales, des propriétés bactériennes et des caractéristiques du matériau. *Journal français d'ophtalmologie*. 33: 210-221

Böll, B., Schalk, E., Buchheidt, D., Hasenkamp, J., Kiehl, M., Kiderlen, T. R., Kochanek, M., Koldehoff, M., Kostrewa, P., Claßen, A. Y., Mellinghoff, S. C., Metzner, B., Penack, O., Ruhnke, M., Vehreschild, M. J. G. T., Weissinger, F., Wolf, H.-H., Karthaus, M., & Hentrich, M. (2021). Central venous catheter–related infections in hematology and oncology: 2020 updated guidelines on diagnosis, management, and prevention by the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO). *Annals of Hematology*, *100*(1), 239–259. https://doi.org/10.1007/s00277-020-04286-x

Bouhrour, N., Nibbering, P. H., & Bendali, F. (2024). Medical Device-Associated Biofilm Infections and Multidrug-Resistant Pathogens. *Pathogens*, *13*(5), 393. https://doi.org/10.3390/pathogens13050393

Brun-buisson, C. (1994). Analyse critique des méthodes diagnostiques d'infection liée au cathéter sur matériel enlevé. Réanimation Urgence, 3, 343-346

Caldara, M., Belgiovine, C., Secchi, E., & Rusconi, R. (2022). Environmental, Microbiological, and Immunological Features of Bacterial Biofilms Associated with Implanted Medical Devices. *Clinical Microbiology Reviews*, *35*(2), e00221-20. https://doi.org/10.1128/cmr.00221-20

Cangui-Panchi, S. P., Ñacato-Toapanta, A. L., Enríquez-Martínez, L. J., Reyes, J., Garzon-Chavez, D., & Machado, A. (2022). Biofilm-forming microorganisms causing hospital-acquired infections from

References

intravenous catheter: A systematic review. *Current Research in Microbial Sciences*, *3*, 100175. https://doi.org/10.1016/j.crmicr.2022.100175

***Centre Hospitalo-Universitaire Dr Tidjani Damerdji de Tlemcen*. (n.d.).** https://chutlemcen

Ciofu, O., & Tolker-Nielsen, T. (2019). Tolerance and Resistance of Pseudomonas aeruginosa Biofilms to Antimicrobial Agents—How P. aeruginosa Can Escape Antibiotics. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.00913

Cho, S.-H., Naber, K., Hacker, J., Ziebuhr, W., 2002. Detection of the icaADBC gene cluster and biofilm formation in Staphylococcus epidermidis isolates from catheter-related urinary tract infections. Int. J. Antimicrob. Agents 19, 570–575. https://doi.org/10.1016/S0924-8579(02)00101-2

Chen, X., Ling, X., Liu, G., & Xiao, J. (2022). Antimicrobial Coating: Tracheal Tube Application. *International Journal of Nanomedicine*, *Volume 17*, 1483–1494. https://doi.org/10.2147/ijn.s353071

Christensen, G., Bisno, A., Simpsom, W., Beachey, E. (1982). Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infection and Immunology, 37, 318-326.

Codru, I. R., Vintilă, B. I., Sava, M., Bereanu, A. S., Neamțu, S. I., Bădilă, R. M., & Bîrluțiu, V. (2024). Optimizing Diagnosis and Management of Ventilator-Associated Pneumonia: A Systematic Evaluation of Biofilm Detection Methods and Bacterial Colonization on Endotracheal Tubes. *Microorganisms*, *12*(10), 1966. https://doi.org/10.3390/microorganisms12101966

Cole, S., Records, A., Orr, M., Linden, S., & Lee, V. (2014). Catheter-Associated Urinary Tract Infection by Pseudomonas aeruginosa Is Mediated by Exopolysaccharide-Independent Biofilms. *Infection and Immunity*, *82*. https://doi.org/10.1128/IAI.01652-14

Cortese, Y. J., Wagner, V. E., Tierney, M., Devine, D., & Fogarty, A. (2018). Review of Catheter-Associated Urinary Tract Infections and *In Vitro* Urinary Tract Models. *Journal of Healthcare Engineering*, *2018*, 1–16. https://doi.org/10.1155/2018/2986742

Curtin, J., & Donlan, R. (2006). Using Bacteriophages To Reduce Formation of Catheter-Associated Biofilms by Staphylococcus epidermidis. *Antimicrobial Agents and Chemotherapy*, 50, 1268 - 1275. https://doi.org/10.1128/AAC.50.4.1268-1275.2006.

Deshmukh-Reeves, E., Gourlay, C., Shaw, M., & Bilsby, C. (2025). *Biofilm formation on endotracheal and tracheostomy tubing: A systematic review and meta-analysis of culture data and sampling method.* Authorea, Inc. **https://doi.org/10.22541/au.174048689.91496251/v1**

Dickschat, J. S. (2010). Quorum sensing and bacterial biofilms. *Natural Product Reports*, *27*(3), 343. https://doi.org/10.1039/b804469b

Donlan Rodney M., J-William Costerton. (2002a). Relevant Microorganisms Biofilms: Survival Mechanisms of Clinically. *Clinical Microbiology Reviews.* 15(2):167

Donlan, R. M. (2002). Biofilms: Microbial Life on Surfaces. *Emerging Infectious Diseases*, *8*(9), 881–890. https://doi.org/10.3201/eid0809.020063

Elpern, E. H., Killeen, K., Ketchem, A., Wiley, A., Patel, G., & Lateef, O. (2009). Reducing Use of Indwelling Urinary Catheters and Associated Urinary Tract Infections. *American Journal of Critical Care*, *18*(6), 535–541. https://doi.org/10.4037/ajcc2009938

References

Eltwisy, H. O., Twisy, H. O., Hafez, M. H., Sayed, I. M., & El-Mokhtar, M. A. (2022). Clinical Infections, Antibiotic Resistance, and Pathogenesis of Staphylococcus haemolyticus. *Microorganisms*, *10*(6), 1130. https://doi.org/10.3390/microorganisms10061130

Ekawati, E. R., Darmanto, W., & Wahyuningsih, S. P. A. (2020). Detection of Staphylococcus aureus in wound infection on the skin surface. *IOP Conference Series: Earth and Environmental Science*, *456*(1), 012038. https://doi.org/10.1088/1755-1315/456/1/012038

Fallah, F., Yousefi, M., Pourmand, M. R., Hashemi, A., Nazari Alam, A., & Afshar, D. (2017). Phenotypic and genotypic study of biofilm formation in Enterococci isolated from urinary tract infections. *Microbial Pathogenesis*, *108*, 85–90. https://doi.org/10.1016/j.micpath.2017.05.014

Fan, Y., Qiao, J., Lu, Z., Fen, Z., Tao, Y., Lv, F., Zhao, H., Zhang, C., & Bie, X. (2020). Influence of different factors on biofilm formation of Listeria monocytogenes and the regulation of cheY gene. *Food Research International*, *137*, 109405. https://doi.org/10.1016/j.foodres.2020.109405

Feneley, R. C. L., Hopley, I. B., & Wells, P. N. T. (2015). Urinary catheters: History, current status, adverse events and research agenda. *Journal of Medical Engineering & Technology*, *39*(8), 459–470. https://doi.org/10.3109/03091902.2015.1085600

FerriÃ¨res, L., Hancock, V., & Klemm, P. (2007). Specific selection for virulent urinary tract infectious *Escherichia coli* strains during catheter-associated biofilm formation. *FEMS Immunology & Medical Microbiology*, *51*(1), 212–219. https://doi.org/10.1111/j.1574-695X.2007.00296.x

Fisher, L.E., Hook, A.L., Ashraf, W., Yousef, A., Barrett, D.A., Scurr, D.J., Chen, X., Smith, E.F., Fay, M., Parmenter, C.D.J., Parkinson, R., Bayston, R., 2015. Biomaterial modification of urinary catheters with antimicrobials to give long-term broadspectrum antibiofilm activity. J. Controlled Release 202, 57–64. https://doi.org/10.1016/j.jconrel.2015.01.037

Folliero, V., Franci, G., Dell'Annunziata, F., Giugliano, R., Foglia, F., Sperlongano, R., De Filippis, A., Finamore, E., & Galdiero, M., 2021. Evaluation of Antibiotic Resistance and Biofilm Production among Clinical Strain Isolated from Medical Devices. *International Journal of Microbiology*, 2021. https://doi.org/10.1155/2021/9033278

Fredheim, E. G. A., Klingenberg, C., Rohde, H., Frankenberger, S., Gaustad, P., Flægstad, T., & Sollid, J. E. (2009). Biofilm Formation by *Staphylococcus haemolyticus*. *Journal of Clinical Microbiology*, *47*(4), 1172–1180. https://doi.org/10.1128/JCM.01891-08

Garvey, M. (2023). Medical Device-Associated Healthcare Infections: Sterilization and the Potential of Novel Biological Approaches to Ensure Patient Safety. *International Journal of Molecular Sciences*, *25*(1), 201. https://doi.org/10.3390/ijms25010201

Ghattas, C., Alsunaid, S., Pickering, E. M., & Holden, V. K. (2021). State of the art: Percutaneous tracheostomy in the intensive care unit. *Journal of Thoracic Disease*, *13*(8), 5261–5276. https://doi.org/10.21037/jtd-19-4121

Gominet, M., Compain, F., Beloin, C., & Lebeaux, D. (2017). Central venous catheters and biofilms: Where do we stand in 2017? *APMIS*, *125*(4), 365–375. https://doi.org/10.1111/apm.12665

Goudarzi, M., Navidinia, M., Khadembashi, N., & Rasouli, R. (2021). Biofilm Matrix Formation in Human: Clinical Significance, Diagnostic Techniques, and Therapeutic Drugs. *Archives of Clinical Infectious Diseases*, *16*(3). https://doi.org/10.5812/archcid.107919

References

Gounden, R., Bamford, C., Van Zyl-Smit, R., Cohen, K., & Maartens, G. (2009). Safety and effectiveness of colistin compared with tobramycin for multi-drug resistant Acinetobacter baumannii infections. *BMC Infectious Diseases*, *9*(1), 26. https://doi.org/10.1186/1471-2334-9-26

Guiton, P. S., Cusumano, C. K., Kline, K. A., Dodson, K. W., Han, Z., Janetka, J. W., Henderson, J. P., Caparon, M. G., & Hultgren, S. J. (2012). Combinatorial Small-Molecule Therapy Prevents Uropathogenic Escherichia coli Catheter-Associated Urinary Tract Infections in Mice. *Antimicrobial Agents and Chemotherapy*, *56*(9), 4738–4745. https://doi.org/10.1128/AAC.00447-12

Gunaratnam, G., Spengler, C., Trautmann, S., Jung, P., Mischo, J., Wieland, B., Metz, C., Becker, S. L., Hannig, M., Jacobs, K., & Bischoff, M. (2020). Human blood plasma factors affect the adhesion kinetics of Staphylococcus aureus to central venous catheters. *Scientific Reports*, *10*(1), 20992. https://doi.org/10.1038/s41598-020-77168-x

Gunardi, W. D., Karuniawati, A., Umbas, R., Bardosono, S., Lydia, A., Soebandrio, A., & Safari, D. (2021). Biofilm-Producing Bacteria and Risk Factors (Gender and Duration of Catheterization) Characterized as Catheter-Associated Biofilm Formation. *International Journal of Microbiology*, *2021*, 1–10. https://doi.org/10.1155/2021/8869275

Ha, U.-S., & Cho, Y.-H. (2006). Catheter-associated urinary tract infections: New aspects of novel urinary catheters. *International Journal of Antimicrobial Agents*, *28*(6), 485–490. https://doi.org/10.1016/j.ijantimicag.2006.08.020

Hancock, V., FerriÃ¨res, L., & Klemm, P. (2007). Biofilm formation by asymptomatic and virulent urinary tract infectious *Escherichia coli* strains. *FEMS Microbiology Letters*, *267*(1), 30–37. https://doi.org/10.1111/j.1574-6968.2006.00507.x

Heck, D. E., Shakarjian, M., Kim, H. D., Laskin, J. D., & Vetrano, A. M. (2010). Mechanisms of oxidant generation by catalase. *Annals of the New York Academy of Sciences*, *1203*(1), 120–125. https://doi.org/10.1111/j.1749-6632.2010.05603.x

Heintz, B. H., Halilovic, J., & Christensen, C. L. (2010). Vancomycin-Resistant Enterococcal Urinary Tract Infections. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *30*(11), 1136–1149. https://doi.org/10.1592/phco.30.11.1136

Hoceini, A., Benbaha, K., Adoul, H., Bensaber, A., Tahraoui, H., Chelghoum, H., Amrane, A., Zhang, J., 2023. Evaluation of Biofilm Forming Potential and Antimicrobial Resistance Profile of *S. aureus* and *P. aeruginosa* Isolated from Peripheral Venous Catheters and Urinary Catheters In Algeria, in vitro Study. Adv. Res. Life Sci. 7, 83–92. https://doi.org/10.2478/arls-2023-0010

Holá, V., Ruzicka, F., Horka, M., 2010. Microbial diversity in biofilm infections of the urinary tract with the use of sonication techniques: Table 1. FEMS Immunol. Med. Microbiol. 59, 525–528. https://doi.org/10.1111/j.1574-695X.2010.00703.x

Ibtissem, K.T., Hafida, H., Salwa, O., Samia, B., Imen, M., Meriem, L., Mohammed, T., 2013. Detection of icaA and icaD genes and biofilmformation in Staphylococcus spp. isolated from urinary catheters at the University Hospital of Tlemcen (Algeria). Afr. J. Microbiol. Res. 7, 5350–5357. https://doi.org/10.5897/AJMR2013.5873

Jacobsen, S. M., & and Shirtliff, M. E. (2011). Proteus mirabilis biofilms and catheter-associated urinary tract infections. *Virulence*, *2*(5), 460–465. https://doi.org/10.4161/viru.2.5.17783

References

Jacobsen, S. M., , D. J., Mobley, H. L. T., & Shirtliff, M. E. (2008). Complicated Catheter-Associated Urinary Tract Infections Due to *Escherichia coli* and *Proteus mirabilis*. *Clinical Microbiology Reviews*, *21*(1), 26–59. https://doi.org/10.1128/CMR.00019-07

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M., & Kamil, M. A. (2018). Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association*, *81*(1), 7–11. https://doi.org/10.1016/j.jcma.2017.07.012

Jang, T., Lee, S., Huang, C., Lee, C., & Chen, W. (2009). Risk factors and impact of nosocomial Acinetobacter baumannii bloodstream infections in the adult intensive care unit: a case-control study.. *The Journal of hospital infection*, 73 2, 143-50 . https://doi.org/10.1016/j.jhin.2009.06.007

Joo, H.-S., & Otto, M. (2012). Molecular Basis of In Vivo Biofilm Formation by Bacterial Pathogens. *Chemistry & Biology*, *19*(12), 1503–1513. https://doi.org/10.1016/j.chembiol.2012.10.022

Joyanes, P., Del Carmen Conejo, M., Martínez-Martínez, L., Perea, E.J., 2001. Evaluation of the VITEK 2 System for the Identification and Susceptibility Testing of Three Species of nonfermenting Gram-Negative Rods Frequently Isolated from Clinical Samples. J. Clin. Microbiol. 39, 3247–3253. https://doi.org/10.1128/JCM.39.9.3247-3253.2001

Juliana, A., Leela, K. V., Gopinathan, A., & T. Jayaprakash, T. J. (2022). Biofilm Formation and its Association with Gram Negative Sepsis Pathogenicity. *Biomedical and Pharmacology Journal*, *15*(4), 2099–2106. https://doi.org/10.13005/bpj/2546

Kanamaru, S., Kurazono, H., Terai, A., Monden, K., Kumon, H., Mizunoe, Y., Ogawa, O., & Yamamoto, S. (2006). Increased biofilm formation in Escherichia coli isolated from acute prostatitis. *International Journal of Antimicrobial Agents*, *28*, 21–25. https://doi.org/10.1016/j.ijantimicag.2006.05.006

Katsikogianni-M., YF-Missirlis. (2004). Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteriamaterial interactions. *European Cells and Materials Journal*. 8: 37-57. http://doi.org/10.22203/eCM.v008a05

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T.-F., & Alarcon, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*, *4*(12), e01067. https://doi.org/10.1016/j.heliyon.2018.e01067

Khullar, S., Rathore, L., Khatri, P. K., Parihar, R. S., Meena, S., Bora, A., Maurya, V., & Sharma, N. (2016). Identification and Antibiogram of Various Gram Positive Bacterial Isolates from Pyogenic Samples by VITEK® 2 Compact System. *International Journal of Current Microbiology and Applied Sciences*, *5*(2), 66–77. https://doi.org/10.20546/ijcmas.2016.502.007

Kitano, H., Shigemoto, N., Koba, Y., Hara, T., Seiya, K., Omori, K., Shigemura, K., Teishima, J., Fujisawa, M., Matsubara, A., & Ohge, H. (2021). Indwelling catheterization, renal stones, and hydronephrosis are risk factors for symptomatic Staphylococcus aureus-related urinary tract infection. *World Journal of Urology*, *39*(2), 511–516. https://doi.org/10.1007/s00345-020-03223-x

Krishnan, S. (2015). Biofilm Formation on Medical Devices and Infection: Preventive Approaches. In H. Kanematsu & D. M. Barry (Eds.), *Biofilm and Materials Science* (pp. 93–108). Springer International Publishing. https://doi.org/10.1007/978-3-319-14565-5_12

References

La Bella, A. A., Andersen, M. J., Gervais, N. C., Molina, J. J., Molesan, A., Stuckey, P. V., Wensing, L., Nobile, C. J., Shapiro, R. S., Santiago-Tirado, F. H., & Flores-Mireles, A. L. (2023). The catheterized bladder environment promotes Efg1- and Als1-dependent *Candida albicans* infection. *Science Advances*, *9*(9), eade7689. https://doi.org/10.1126/sciadv.ade7689

Lanaghan, F., & Stenhouse, P. (2024). Nosocomial infections in the intensive care unit. *Anaesthesia & Intensive Care Medicine*, *25*(10), 652–656. https://doi.org/10.1016/j.mpaic.2024.07.003

Lee, K.-H., Park, S. J., Choi, S., Uh, Y., Park, J. Y., & Han, K.-H. (2017). The Influence of Urinary Catheter Materials on Forming Biofilms of Microorganisms. *Journal of Bacteriology and Virology*, *47*(1), 32. https://doi.org/10.4167/jbv.2017.47.1.32

Li, P., Yin, R., Cheng, J., & Lin, J. (2023). Bacterial Biofilm Formation on Biomaterials and Approaches to Its Treatment and Prevention. *International Journal of Molecular Sciences*, *24*(14), 11680. https://doi.org/10.3390/ijms241411680

Macia, M. D., Rojo-Molinero, E., & Oliver, A. (2014). Antimicrobial susceptibility testing in biofilm-growing bacteria. *Clinical Microbiology and Infection*, *20*(10), 981–990. https://doi.org/10.1111/1469-0691.12651

Maharjan, G., Khadka, P., Siddhi Shilpakar, G., Chapagain, G., Dhungana, G.R., 2018. Catheter-Associated Urinary Tract Infection and Obstinate Biofilm Producers. Can. J. Infect. Dis. Med. Microbiol. 2018, 1–7. https://doi.org/10.1155/2018/7624857

Mancuso, G., Trinchera, M., Midiri, A., Zummo, S., Vitale, G., & Biondo, C. (2024). Novel Antimicrobial Approaches to Combat Bacterial Biofilms Associated with Urinary Tract Infections. *Antibiotics*, *13*(2), 154. https://doi.org/10.3390/antibiotics13020154

Mandolfo, S., Anesi, A., Maggio, M., Rognoni, V., Galli, F., & Forneris, G. (2020). High success rate in salvage of catheter-related bloodstream infections due to *Staphylococcus aureus* , on behalf of project group of Italian society of nephrology. *The Journal of Vascular Access*, *21*(3), 336–341. https://doi.org/10.1177/1129729819875323

Marak, M., & B., D. (2018). Antifungal Susceptibility and Biofilm Production of Candida spp. Isolated from Clinical Samples. *International Journal of Microbiology*, *2018*, 1–5. https://doi.org/10.1155/2018/7495218

Martinez Luis-R., Arturo Casadevall. (2007). Cryptococcus neoformans Biofilm Formation Depends on Surface Support and Carbon Source and Reduces Fungal Cell Susceptibility to Heat, Cold, and UV Light. *Applied and Environmental Microbiology Journal*. 73(14): 4592– 4601.

Mills E, Pultz I, Kulasekara HD, Miller S (2011) The bacterial second messenger c-di-GMP: mechanisms of signalling. Cell Microbiol 13(8): 1122-1129

Mishra, A., Aggarwal, A., & Khan, F. (2024). Medical Device-Associated Infections Caused by Biofilm-Forming Microbial Pathogens and Controlling Strategies. *Antibiotics*, *13*(7), 623. https://doi.org/10.3390/antibiotics13070623

Mohsenzadeh, A., Fazel, A., Bavari, S., Borji, S., Pourasghar, S., Azimi, T., & Sabati, H. (2021). Detecting of biofilm formation in the clinical isolates of Pseudomonas aeruginosa and Escherichia coli: An evaluation of different screening methods. *Journal of Current Biomedical Reports*, *2*(2), 56–61. https://doi.org/10.52547/JCBioR.2.2.56

References

Nachimuthu, R., Subramani, R. Maray, S. Gothandam, K.M. Sivamangala, K. Manohar, P. and Bozdogan B. (2016). Characterizationofcarbapenemresistant Gram-negative bacteria from Tamil Nadu. J. Chemother, 28, 371–374

Nair H, Periasamy S, Yang L, Kjelleberg S, Rice SA, et al. (2017) Real time, spatial, and temporal mapping of the distribution of c-di-GMP during biofilm development. The Journal of Biological Chemistry 292(2): 477487

Nicolle, L.E., 2014. Catheter associated urinary tract infections. Antimicrob. Resist. Infect. Control 3, 23. https://doi.org/10.1186/2047-2994-3-23

Nicolosi, D., Cinà, D., Di Naso, C., D'Angeli, F., Salmeri, M., & Genovese, C. (2020). Antimicrobial Resistance Profiling of Coagulase-Negative Staphylococci in a Referral Center in South Italy: A Surveillance Study. *The Open Microbiology Journal*, *14*(1), 91–97. https://doi.org/10.2174/1874285802014010091

Niveditha, S. N. (2012). The Isolation and the Biofilm Formation of Uropathogens in the Patients with Catheter Associated Urinary Tract Infections (UTIs). *JOURNAL of CLINICAL AND DIAGNOSTIC RESEARCH*. https://doi.org/10.7860/JCDR/2012/4367.2537

Noshak, M. A., Ahangarzadeh Rezaee, M., Hasani, A., Mirzaii, M., Memar, M. Y., Azimi, T., Ebrahimi, M., & Dolatyar Dehkharghani, A. (2023). Molecular Detection and Characterization of the *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* Isolated from Hospitalized Patients and Healthcare Workers in Iran. *BioMed Research International*, *2023*(1), 3775142. https://doi.org/10.1155/2023/3775142

Osório, N., Oliveira, V., Costa, M. I., Santos-Costa, P., Serambeque, B., Gama, F., Adriano, D., Graveto, J., Parreira, P., & Salgueiro-Oliveira, A. (2023). Short Peripheral Venous Catheters Contamination and the Dangers of Bloodstream Infection in Portugal: An Analytic Study. *Microorganisms*, *11*(3), 709. https://doi.org/10.3390/microorganisms11030709

Pal, M. K., & Lavanya, M. (2022). Microbial Influenced Corrosion: Understanding Bioadhesion and Biofilm Formation. *Journal of Bio- and Tribo-Corrosion*, *8*(3), 1–13. https://doi.org/10.1007/s40735-022-00677-x

Paudel, S., Guedry, S., Obernuefemann, C. L. P., Hultgren, S. J., Walker, J. N., & Kulkarni, R. (2023). Defining the Roles of Pyruvate Oxidation, TCA Cycle, and Mannitol Metabolism in Methicillin-Resistant Staphylococcus aureus Catheter-Associated Urinary Tract Infection. *Microbiology Spectrum*, *11*(4), e05365-22. https://doi.org/10.1128/spectrum.05365-22

Pelling, H., Nzakizwanayo, J., Milo, S., Denham, E. L., MacFarlane, W. M., Bock, L. J., Sutton, J. M., & Jones, B. V. (2019). Bacterial biofilm formation on indwelling urethral catheters. *Letters in Applied Microbiology*, *68*(4), 277–293. https://doi.org/10.1111/lam.13144

Peng, D., Li, X., Liu, P., Luo, M., Chen, S., Su, K., Zhang, Z., He, Q., Qiu, J., & Li, Y. (2018). Epidemiology of pathogens and antimicrobial resistanceof catheter-associated urinary tract infections in intensivecare units: A systematic review and meta-analysis. *American Journal of Infection Control*, *46*(12), e81–e90. https://doi.org/10.1016/j.ajic.2018.07.012

Pereira-Ribeiro, P. M. A., Cabral-Oliveira, G. G., Olivella, J. G. B., Motta, I. C. D. M., Ribeiro, F. C., Nogueira, B. A., Santos, L. S. D., Sued-Karam, B. R., & Mattos-Guaraldi, A. L. (2022). Biofilm

formation, multidrug-resistance and clinical infections of Staphylococcus haemolyticus: A brief review. *Research, Society and Development*, *11*(11), e228111133605. https://doi.org/10.33448/rsd-v11i11.33605

Pompilio A, Piccolomini R, Picciani C, D'antonio D, Savini V, et al. (2008) Factors associated with adherence to and biofilm formation on polystyrene by Stenotrophomonas maltophilia: the role of cell surface hydrophobicity and motility. FEMS Microbiol Lett 287(1): 41-47

Pouget, C., Chatre, C., Lavigne, J.-P., Pantel, A., Reynes, J., & Dunyach-Remy, C. (2023). Effect of Antibiotic Exposure on Staphylococcus epidermidis Responsible for Catheter-Related Bacteremia. *International Journal of Molecular Sciences*, *24*(2), 1547. https://doi.org/10.3390/ijms24021547

Pour, N. K., Dusane, D. H., Dhakephalkar, P. K., Zamin, F. R., Zinjarde, S. S., & Chopade, B. A. (2011). Biofilm formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters. *FEMS Immunology & Medical Microbiology*, *62*(3), 328–338. https://doi.org/10.1111/j.1574-695X.2011.00818.x

Preda, V. G., & Săndulescu, O. (2019). Communication is the key: Biofilms, quorum sensing, formation and prevention. *Discoveries (Craiova, Romania)*, *7*(3), e100. https://doi.org/10.15190/d.2019.13

Rasamiravaka, T., Labtani, Q., Duez, P., and El Jaziri, M. (2015). The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. Biomed Research International, 20, 15-20

Raveendra, N., Rathnakara, S. H., Haswani, N., & Subramaniam, V. (2022). Bacterial Biofilms on Tracheostomy Tubes. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *74*(S3), 4995–4999. https://doi.org/10.1007/s12070-021-02598-6

Ren, Y., Wang, C., Chen, Z., Allan, E., Van Der Mei, H. C., & Busscher, H. J. (2018). Emergent heterogeneous microenvironments in biofilms: Substratum surface heterogeneity and bacterial adhesion force-sensing. *FEMS Microbiology Reviews*, *42*(3), 259–272. https://doi.org/10.1093/femsre/fuy001

Rima, M., Villeneuve-Faure, C., Soumbo, M., El Garah, F., Pilloux, L., Roques, C., & Makasheva, K. (2024). Towards a better understanding of the effect of protein conditioning layers on microbial adhesion: A focused investigation of fibronectin and bovine serum albumin layers on SiO_2 surfaces. *Biomaterials Science*, *12*(12), 3086–3099. https://doi.org/10.1039/D4BM00099D

Rodríguez-Guerrero, E., Cabello, H., Expósito-Ruíz, M., Navarro-Marí, J., & Gutiérrez-Fernández, J. (2023). Antibiotic Resistances of Enterobacteriaceae with Chromosomal Ampc in Urine Cultures: Review and Experience of a Spanish Hospital. *Antibiotics*, 12. https://doi.org/10.3390/antibiotics12040730

Saadi, S., Allem, R., Sebaihia, M., Merouane, A., & Bakkali, M. (2022). Bacterial contamination of neglected hospital surfaces and equipment in an Algerian hospital: An important source of potential infection. *International Journal of Environmental Health Research*, *32*(6), 1373–1381. https://doi.org/10.1080/09603123.2021.1885631

Sabbuba NA, Stickler DJ, Mahenthiralingam E, Painter DJ, Parkin J, Feneley RC. Genotyping demonstrates that the strains of Proteus mirabilis from bladder stones and catheter encrustations of patients undergoing long-term bladder catheterization are identical. J Urol 2004; 171:1925-8; PMID:15076313; http://dx.doi. org/10.1097/01.ju.0000123062.26461.f9.

References

Sabir, N., Ikram, A., Zaman, G., Satti, L., Gardezi, A., Ahmed, A., & Ahmed, P. (2017). Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. *American Journal of Infection Control*, *45*(10), 1101–1105. https://doi.org/10.1016/j.ajic.2017.05.009

Salim, N., Mishra, B., & Revi, D. (2022). Evaluation of Biofilm Production and Antibiotic Resistance Pattern Between Biofilm Producing and Non-biofilm Producing Isolates of Staphylococcus aureus. *Acta Scientific Medical Sciences*, *6*(3), 147–152. https://doi.org/10.31080/ASMS.2022.06.1176

S**chulze, A., Mitterer, F., Pombo, J. P., & Schild, S. (2021).** Biofilms by bacterial human pathogens: Clinical relevance - development, composition and regulation - therapeutical strategies. *Microbial Cell*, *8*(2), 28–56. https://doi.org/10.15698/mic2021.02.741

Ścibik, Ł., Ochońska, D., Golda-Cepa, M., Brzychczy-Włoch, M., & Kotarba, A. (2022). Microbiological analysis of tracheostomy tube biofilms and antibiotic resistance profiles of potentially pathogenic microorganisms. *Otolaryngologia Polska*, *76*(5), 8–21. https://doi.org/10.5604/01.3001.0015.8827

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023a). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, *11*(6), 1614. https://doi.org/10.3390/microorganisms11061614

Sharma, S., Mohler, J., Mahajan, S. D., Schwartz, S. A., Bruggemann, L., & Aalinkeel, R. (2023b). Microbial Biofilm: A Review on Formation, Infection, Antibiotic Resistance, Control Measures, and Innovative Treatment. *Microorganisms*, *11*(6), Article 6. https://doi.org/10.3390/microorganisms11061614

Siciliano, V., Passerotto, R. A., Chiuchiarelli, M., Leanza, G. M., & Ojetti, V. (2023). Difficult-to-Treat Pathogens: A Review on the Management of Multidrug-Resistant Staphylococcus epidermidis. *Life*, *13*(5), 1126. https://doi.org/10.3390/life13051126

Silvetti, S., Aloisio, T., Cazzaniga, A., & Ranucci, M. (2018). Jugular vs femoral vein for central venous catheterization in pediatric cardiac surgery (PRECiSE): Study protocol for a randomized controlled trial. *Trials*, *19*(1). https://doi.org/10.1186/s13063-018-2717-1

Soto, S. M., Smithson, A., Horcajada, J. P., Martinez, J. A., Mensa, J. P., & Vila, J. (2006). Implication of biofilm formation in the persistence of urinary tract infection caused by uropathogenic Escherichia coli. *Clinical Microbiology and Infection*, *12*(10), 1034–1036. https://doi.org/10.1111/j.1469-0691.2006.01543.x

Štefánek, M., Wenner, S., Borges, V., Pinto, M., Gomes, J. P., Rodrigues, J., Faria, I., Pessanha, M. A., Martins, F., Sabino, R., Veríssimo, C., Nogueira, I. D., Carvalho, P. A., Bujdáková, H., & Jordao, L. (2022). Antimicrobial Resistance and Biofilms Underlying Catheter-Related Bloodstream Coinfection by Enterobacter cloacae Complex and Candida parapsilosis. *Antibiotics*, *11*(9), Article 9. https://doi.org/10.3390/antibiotics11091245

Sultan, A. M., & Nabiel, Y. (2018). Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: Which one could be better? *African Journal of Clinical and Experimental Microbiology*, *20*(1), 60. https://doi.org/10.4314/ajcem.v20i1.9

Soumia, D., Khedoudja, K., M'hamed, B., & Bouziane, A. (2021). Detection of Biofilm Production and Antibiotic Resistance Pattern In Clinical Isolates from Indwelling Medical Devices. *Egyptian Academic*

References

Journal of Biological Sciences. C, Physiology and Molecular Biology, *13*(2), 229–239. https://doi.org/10.21608/eajbsc.2021.209999

Suresh, M. K., Biswas, R., & Biswas, L. (2019). An update on recent developments in the prevention and treatment of Staphylococcus aureus biofilms. *International Journal of Medical Microbiology*, *309*(1), 1–12. https://doi.org/10.1016/j.ijmm.2018.11.002

Texto_completo.pdf, n.d. The catheterized bladder environment promotes Efg1- and Als1-dependent Candida albicans infection [WWW Document], n.d. URL https://www.science.org/doi/epdf/10.1126/sciadv.ade7689 (accessed 3.22.25)

Tsehaye,HaduAyichew Teshalesh,Sibehat Haddis, A. T. (n.d.). Review on Biofilm and Microbial Adhesion. *2016*. https://doi.org/10.5829/idosi.ijmr.2016.63.73

Usta-Atmaca, H., Akbas, F., Karagoz, Y., & Piskinpasa, M. E. (2015). A rarely seen cause for empyema: Leuconostoc mesenteroıdes. *The Journal of Infection in Developing Countries*, *9*(04), 425–427. https://doi.org/10.3855/jidc.5237

Van kerckhoven, M., Hotterbeekx, A., Lanckacker, E., Moons, P., Lammens, C., Kerstens, M., Ieven, M., Delputte, P., Jorens, P. G., Malhotra-Kumar, S., Goossens, H., Maes, L., & Cos, P. (2016). Characterizing the *in vitro* biofilm phenotype of *Staphylococcus epidermidis* isolates from central venous catheters. *Journal of Microbiological Methods*, *127*, 95–101. https://doi.org/10.1016/j.mimet.2016.05.009

Vani, S., Vadakkan, K., & Mani, B. (2023). A narrative review on bacterial biofilm: Its formation, clinical aspects and inhibition strategies. *Future Journal of Pharmaceutical Sciences*, *9*(1), 50. https://doi.org/10.1186/s43094-023-00499-9

Veerachamy, S., Yarlagadda, T., Manivasagam, G., & Yarlagadda, P. K. (2014). Bacterial adherence and biofilm formation on medical implants: A review. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, *228*(10), 1083–1099. https://doi.org/10.1177/0954411914556137

Vila, T., Kong, E. F., Montelongo-Jauregui, D., Van Dijck, P., Shetty, A. C., McCracken, C., Bruno, V. M., & Jabra-Rizk, M. A. (2021). Therapeutic implications of *C. albicans-S. aureus* mixed biofilm in a murine subcutaneous catheter model of polymicrobial infection. *Virulence*, *12*(1), 835–851. https://doi.org/10.1080/21505594.2021.1894834

Villain-Simonnet, A., Milas, M., and Rinaudo, M. (2000). A new bacterial exopolysaccharide (YAS34). II. Influence of thermal treatments on the conformation and structure. Relation with gelation ability. Int J Biol Macromol 27, 77-87. http://dx.doi.org/10.1016/S01418130(99)00119-1

Vuong, C., & Otto, M. (2002). Staphylococcus epidermidis infections. *Microbes and Infection*, *4*(4), 481–489. https://doi.org/10.1016/S1286-4579(02)01563-0

Walsh, D., Parmenter, C., Bakker, S. E., Lithgow, T., Traven, A., & Harrison, F. (2024). A new model of endotracheal tube biofilm identifies combinations of matrix-degrading enzymes and antimicrobials able to eradicate biofilms of pathogens that cause ventilator-associated pneumonia. *Microbiology*, *170*(8). https://doi.org/10.1099/mic.0.001480

Wang, X., Chen, C., Hu, J., Liu, C., Ning, Y., & Lu, F. (2024). Current strategies for monitoring and controlling bacterial biofilm formation on medical surfaces. *Ecotoxicology and Environmental Safety*, *282*, 116709. https://doi.org/10.1016/j.ecoenv.2024.116709

References

Wolcott, R. (2021). Biofilm and catheter-related bloodstream infections. *British Journal of Nursing*, *30*(8), S4–S9. https://doi.org/10.12968/bjon.2021.30.8.S4

Yarawsky, A., Johns, S., Schuck, P., & Herr, A. (2020). The biofilm adhesion protein Aap from Staphylococcus epidermidis forms zinc-dependent amyloid fibers. *The Journal of Biological Chemistry*, 295, 4411 - 4427. https://doi.org/10.1074/jbc.RA119.010874

Yuan, F., Huang, Z., Yang, T., Wang, G., Li, P., Yang, B., & Li, J. (2021). Pathogenesis of ***Proteus mirabilis*** in Catheter-Associated Urinary Tract Infections. *Urologia Internationalis*, *105*(5–6), 354–361. https://doi.org/10.1159/000514097

Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*, *13*, 1137947. https://doi.org/10.3389/fcimb.2023.1137947

Zou, Z., Potter, R. F., McCoy, W. H., Wildenthal, J. A., Katumba, G. L., Mucha, P. J., Dantas, G., & Henderson, J. P. (2023). E. coli catheter-associated urinary tract infections are associated with distinctive virulence and biofilm gene determinants. *JCI Insight*, *8*(2), e161461. https://doi.org/10.1172/jci.insight.161461

Annexure

Annexure

Annex 1: **Reports of biochemical reactions for identification of the different bacteria as identified by VITEK 2**

Origine du germe	VITEK 2
Germe sélectionné	**Slashline** Profil biochimique : 0241010103500352 Fiabilité : Excellente identification
Germes identifiés et tests discriminants : Acinetobacter baumannii complex	

Détails biochimiques

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	+	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	+			

Germe sélectionné	91% de probabilité **Pseudomonas aeruginosa** Profil biochimique : 0043553103500272 Fiabilité : Bonne identification
Germes identifiés et tests discriminants :	
Commentaire sur l''ident. :	
Tests à l''encontre Pseudomonas aeruginosa GGAA(1),BGLU(1),	

Détails biochimiques

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	+	18	dMAL	-	19	dMAN	(+)	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	+	62	ELLM	-	64	lLATa	+			

Informations sur l''identification	**Carte :** GN	**N° de lot :** 2412668103	**Péremption :** 17 mars 2025 12:00 WAT		
	État : Final	**Heure de l''analyse :** 4,98 heures	**Terminée le :** 8 mai 2023 09:02 WAT		
Origine du germe	VITEK 2				
Germe sélectionné	**Slashline** Profil biochimique : 0627734553571010 Fiabilité : Excellente identification				
Germes identifiés et tests discriminants : Enterobacter cloacae complex					

Détails biochimiques

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	(+)
46	GlyA	+	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Annexure

Informations sur l''identification	Carte : GN	N° de lot : 2412668103	Péremption : 17 mars 2025 12:00 WAT
	État : Final	Heure de l''analyse : 4,77 heures	Terminée le : 8 mai 2023 08:50 WAT
Origine du germe	VITEK 2		
Germe sélectionné	Slashline Profil biochimique : 0627734553531010		Fiabilité : Très bonne identification

Tests à l''encontre
Enterobacter cloacae complex ODC(94),

Détails biochimiques

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Origine du germe	VITEK 2
Germe sélectionné	95% de probabilité **Staphylococcus haemolyticus** Profil biochimique : 050046003760231 Fiabilité : Très bonne identification
Germes identifiés et tests discriminants :	
Commentaire sur l''ident. :	
Tests à l''encontre Staphylococcus haemolyticus BGURr(9),	

Détails biochimiques

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	+	25	AGAL	-	26	PyrA	+	27	BGUR	+
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	lLATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	-	50	NC6.5	+	52	dMAN	+	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Origine du germe	VITEK 2
Germe sélectionné	98% de probabilité **Staphylococcus epidermidis** Profil biochimique : 010000012620251 Fiabilité : Excellente identification
Germes identifiés et tests discriminants :	
Commentaire sur l''ident. :	
Tests à l''encontre	

Détails biochimiques

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	lLATk	+	42	LAC	-	44	NAG	-	45	dMAL	+	46	BACI	+
47	NOVO	(-)	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	-	63	ADH2s	+
64	OPTO	+															

Annexure

Informations sur l''identification	**Carte :** GP	**N° de lot :** 2422942503	**Péremption :** 16 déc. 2025 12:00 WAT
	État : Final	**Heure de l''analyse :** 7,97 heures	**Terminée le :** 9 mai 2023 12:54 WAT
Origine du germe	VITEK 2		
Germe sélectionné	**Low Discrimination** **Leuconostoc mesenteroides ssp**		
	Profil biochimique : 000000000040021	Fiabilité : Faible discrimination	

Détails biochimiques

2	AMY	(-)	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	+	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	-	59	SAL	-	60	SAC	-	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Tests à l''encontre Candida

~~Streptococcus sobrinus~~ NAG(18),ADH1(1),ILATk(1),dXYL(1),

Détails biochimiques

2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	-	11	AGLU	+
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Annex 2: Culture media.

The theoretical formula of this culture medium in g/L of purified water is:

Nutrient agar. pH = 7.4

Peptic digest of animal tissue…….5g

Beef extract……..……..1.5g

Yeast extract……..……..1.5g

Sodium chloride…….……...…….5g

Agar……..……15g

MacConkey Agar. pH =7.1

Pancreatic digest of gelatin …….……17g

Peptone from meat …….……1.5g

Peptone from Casein …….…….1.5g

Lactose ……...…….10g

Sodium chloride ……5g

Bile salts …….1.5g

Agar …….……15g

Neutral red ……...……0.03g

Crystal violet …….…….0.001g

Mannitol salt agar (chapman agar). pH = 7.4

Protease peptone …….10g

Beef extract …….……..1g

Sodium chloride …….…….75g

D-Mannitol ……..…….10g

Phenol red ……..……0.025g

Agar ……...……..15g

Cetrimide agar. pH =7.2

Peptone from gelatin ……..…….20g

Magnesium chloride ……..……..1.4g

N-Cetyl-N,N,N-trimethyl-ammonium bromide (cetrimide)……..…… 0.3g

Agar-agar ……..……13.6g

Potassium sulfate ……...…….10g

Annexure 3

Software Version	2.04.11
Experiment File Path:	
Protocol File Path:	
Plate Number	Plate 1
Date	1/1/2004
Time	0:17:19
Reader Type:	ELx808
Reader Serial Number:	Unknown
Reading Type	Reader

Procedure Details

Plate Type	96 WELL PLATE
Read	Absorbance Endpoint
	Full Plate
	Wavelengths: 490, 630
	Read Speed: Normal

Results

GRAM +

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.104	0.041	0.041	0.042	0.041	0.043	0.042	0.041	0.043	0.041	0.043	0.043	490
	0.148	0.109	0.11	0.111	0.089	0.115	0.114	0.094	0.113	0.11	0.102	0.116	630
B	0.094	0.042	0.047	0.041	0.042	0.041	0.041	0.044	0.041	0.04	0.042	0.041	490
	0.145	0.111	0.116	0.111	0.113	0.114	0.113	0.113	0.119	0.108	0.111	0.108	630
C	0.08	0.041	0.042	0.043	0.04	0.102	0.042	0.18	0.042	0.153	0.041	0.188	490
	0.142	0.091	0.115	0.116	0.088	0.151	0.117	0.174	0.116	0.172	0.107	0.19	630
D	0.082	0.043	0.044	0.042	0.043	0.155	0.041	0.198	0.043	0.099	0.042	0.145	490
	0.14	0.118	0.118	0.118	0.116	0.165	0.114	0.197	0.118	0.147	0.112	0.175	630
E	0.073	0.041	0.042	0.041	0.043	0.191	0.042	0.198	0.041	0.1	0.042	0.141	490
	0.139	0.114	0.114	0.113	0.122	0.168	0.115	0.205	0.118	0.15	0.111	0.165	630
F	0.073	0.043	0.042	0.043	0.046	0.208	0.041	0.148	0.043	0.096	0.042	0.189	490
	0.14	0.097	0.117	0.115	0.105	0.181	0.116	0.162	0.122	0.143	0.105	0.194	630
G	0.081	0.044	0.042	0.043	0.043	0.145	0.043	0.109	0.044	0.091	0.043	0.224	490
	0.149	0.124	0.121	0.121	0.125	0.167	0.125	0.156	0.125	0.154	0.124	0.208	630
H	0.089	0.042	0.042	0.041	0.041	0.048	0.044	0.041	0.041	0.042	0.043	0.042	490
	0.152	0.111	0.121	0.115	0.105	0.127	0.12	0.106	0.114	0.118	0.116	0.114	630

Gram -

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.136	0.042	0.043	0.043	0.043	0.044	0.045	0.043	0.045	0.048	0.042	0.042	490
	0.168	0.101	0.114	0.111	0.089	0.115	0.119	0.09	0.113	0.12	0.088	0.114	630
B	0.132	0.044	0.041	0.041	0.042	0.194	0.04	0.185	0.04	0.538	0.042	0.127	490
	0.165	0.115	0.11	0.112	0.111	0.198	0.113	0.189	0.111	0.389	0.119	0.174	630
C	0.126	0.044	0.048	0.044	0.044	0.169	0.044	0.126	0.04	0.582	0.038	0.146	490
	0.162	0.103	0.116	0.119	0.097	0.183	0.117	0.149	0.113	0.422	0.088	0.18	630
D	0.139	0.041	0.042	0.041	0.041	0.145	0.042	0.18	0.041	0.496	0.041	0.14	490
	0.178	0.113	0.115	0.117	0.11	0.179	0.117	0.206	0.113	0.374	0.116	0.183	630
E	0.152	0.042	0.041	0.041	0.046	0.165	0.041	0.247	0.044	0.419	0.041	0.147	490
	0.187	0.116	0.11	0.113	0.129	0.199	0.114	0.231	0.117	0.329	0.116	0.189	630
F	0.13	0.042	0.042	0.043	0.041	0.263	0.043	0.236	0.06	0.47	0.044	0.148	490
	0.166	0.106	0.122	0.117	0.106	0.251	0.115	0.213	0.145	0.36	0.114	0.196	630
G	0.132	0.041	0.042	0.045	0.044	0.043	0.043	0.044	0.044	0.045	0.044	0.044	490
	0.17	0.117	0.116	0.121	0.122	0.122	0.119	0.119	0.12	0.123	0.123	0.118	630
H	0.123	0.04	0.047	0.042	0.041	0.041	0.043	0.04	0.041	0.041	0.041	0.041	490
	0.166	0.104	0.124	0.118	0.105	0.114	0.121	0.1	0.112	0.111	0.111	0.114	630

ملخص

تشكل الالتهابات المرتبطة بالقسطرة (CKDs) تهديدا كبيرا في أماكن الرعاية الصحية ، خاصة في وحدات العناية المركزة ، حيث يتم استعمار الأجهزة الطبية الساكنة بشكل متكرر بواسطة مسببات الأمراض المكونة للأغشية الحيوية. فحصت هذه الدراسة الاستعمار الميكروبي وأنماط مقاومة المضادات الحيوية وتكوين الأغشية الحيوية على القسطرة البولية والقسطرة الوريدية المركزية وأنابيب القصبة الهوائية للمرضى في مستشفى تلمسان الجامعي (CHU) ، الجزائر.

باستخدام الأساليب القائمة على الثقافة ونظام VITEK 2 ، حددنا مسببات الأمراض السائدة ، بما في ذلك *Pseudomonas aeruginosa* و *Acinetobacter baumannii* و *Enterobacter cloacae* و *Staphylococcus epidermidis* و *Staphylococcus haemolyticus* و *Candida* *الأنواع*. كشف اختبار الحساسية لمضادات الميكروبات عن مقاومة للأدوية المتعددة في *A. baumannii* و *E. cloacae* ، بينما *ظلت المتصورة الزنجارية* عرضة إلى حد كبير. أظهرت المكورات العنقودية مقاومة للتتراسيكلين ولكن حساسية للفانكومايسين.

تم تقييم تكوين الأغشية الحيوية بواسطة طرق الكونغو الأحمر أجار (CRA) ولوحة زراعة الأنسجة (TCP). صنف تحليل TCP معظم العزلات على أنها منتجة منخفضة للأغشية الحيوية ، مع *A. baumannii* أظهر إنتاجا معتدلا للأغشية الحيوية. أظهر ARC حساسية أقل ، مما يؤكد موثوقية TCP للكشف عن الأغشية الحيوية. لوحظ وجود علاقة بين تكوين الأغشية الحيوية ومقاومة المضادات الحيوية ، مما يسلط الضوء على الدور الوقائي للأغشية الحيوية في ثبات البكتيريا.

تسلط هذه النتائج الضوء على التحدي السريري لأمراض الكلى المزمنة الناجمة عن مسببات الأمراض المرنة المكونة للأغشية الحيوية. تدعو الدراسة إلى تحسين تدابير مكافحة العدوى ، والإشراف على مضادات الميكروبات ، والبحث في استراتيجيات مكافحة الأغشية الحيوية للتخفيف من العدوى المرتبطة بالجهاز.

الكلمات المفتاحية: الالتهابات المرتبطة بالقسطرة ، الأغشية الحيوية ، مقاومة مضادات الميكروبات ، مواد البوليمر خارج الخلية (EPS) ، وحدة العناية المركزة ، القسطرة.

Abstract

Catheter-related infections (CRIs) pose a significant threat in healthcare settings, particularly in intensive care units, where indwelling medical devices are frequently colonized by biofilm-forming pathogens. This study investigated microbial colonization, antibiotic resistance patterns, and biofilm formation on urinary catheters, central venous catheters, and tracheal tubes from patients at University Hospital of Tlemcen (CHU), Algeria.

Using culture based methods and VITEK 2 system; we identified predominant pathogens including *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Enterobacter cloacae complex, Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Candida* species. Antimicrobial susceptibility testing revealed that multidrug resistance in *Acinentobacter baumannii* and *Enterobacter cloacae complex*, while *P. aeruginosa* remained broadly susceptible. *Staphylococcus* showed resistance to tetracycline but sensitive to vancomycine.

Biofilm formation was assessed via Congo Red Agar (CRA) and Tissue Culture Plate (TCP) methods. TCP analysis classified most isolates as weak biofilm producers with *A. baumannii* exhibiting moderate biofilm production. CRA showed lower sensitivity, underscoring TCP's reliability for biofilm detection. A correlation between biofilm formation and antibiotic resistance was observed, highlighting the protective role of biofilms in bacterial persistence.

These findings emphasize the clinical challenge of CRIs caused by resilient, biofilm-forming pathogens. The study advocates for enhanced infection control measures, antimicrobial stewardship, and research into anti-biofilm strategies to mitigate device-associated infections.

Keywords: Catheter-related infections, biofilm, antimicrobial resistance, extracellular polymeric substances (EPS), intensive care unit, catheters.

Résumé

Les infections liées aux cathéters (IRC) constituent une menace importante dans les établissements de santé, en particulier dans les unités de soins intensifs, où les dispositifs médicaux à demeure sont fréquemment colonisés par des agents pathogènes formant un biofilm. Cette étude a examiné la colonisation microbienne, les profils de résistance aux antibiotiques et la formation de biofilms sur les cathéters urinaires, les cathéters veineux centraux et les tubes trachéaux de patients de l'hôpital universitaire de Tlemcen (CHU), en Algérie.

À l'aide de méthodes basées sur la culture et du système VITEK 2, nous avons identifié des agents pathogènes prédominants, notamment *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* et *les espèces Candida*. Les tests de sensibilité aux antimicrobiens ont révélé une multirésistance aux médicaments chez *A. baumannii* et *E. cloacae*, tandis que *P. aeruginosa* est demeuré largement sensible. Les staphylocoques ont montré une résistance à la tétracycline mais une sensibilité à la vancomycine.

La formation du biofilm a été évaluée par les méthodes de la gélose rouge du Congo (CRA) et de la plaque de culture tissulaire (TCP). L'analyse du TCP a classé la plupart des isolats comme de faibles producteurs de biofilm, A. *baumannii* présentant une production modérée de biofilm. L'CRA a montré une sensibilité plus faible, ce qui souligne la fiabilité du TCP pour la détection des biofilms. Une corrélation entre la formation de biofilms et la résistance aux antibiotiques a été observée, mettant en évidence le rôle protecteur des biofilms dans la persistance bactérienne.

Ces résultats soulignent le défi clinique des IRC causés par des agents pathogènes résilients et formant un biofilm. L'étude préconise des mesures améliorées de contrôle des infections, une gestion des antimicrobiens et la recherche de stratégies anti-biofilm pour atténuer les infections associées aux dispositifs.

Mots-clés : Infections liées aux cathéters, biofilm, résistance aux antimicrobiens, substances polymères extracellulaires (EPS), unité de soins intensifs, cathéters.