Popular Democratic Republic of Algeria
Ministry of High Education and Scientific Research
Abbes Laghrour University- Khenchela-
Natural and life sciences Faculty
Molecular and Cellular Biology Department

Serial N°: .....

ACADEMIC MASTER'S THESIS

Field: Natural and life science
Sector : Biological science
Specialty : Applied Biochemistry

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THEME

BIOCHEMISTRY STUDY OF NOSOCOMIAL INFECTIONS IN MATERNITY SALHI BELKACEM.

Thesis defended on **17/06/ 2025** before the jury composed of:

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University year: 2024/2025

Acknowledgements

First and foremost, we extend my sincere thanks to Dr. YAHIA Massinissa, our supervisor, for his invaluable guidance, patience, and insightful feedback throughout this research.

We sincerely thank the jury members for their time, expertise and fair judgment.

Our appreciation goes to the faculty of biology for their mentorship and encouragement. Since we had difficulties to realize this study.

We would also like to thank the members of the hospital EL BALSEM EL CHAFY, Dr. LAHMARI, Dr. BOUZIDI and Dr. SLIMANI for their collaboration, assistance, time and for giving us a chance to practice in the laboratory and guided us to do it in the right method

This work would not have been possible without the support of director of the maternity and hygiene team especially the president of the group

Finally, we would like to thank all those who, directly or indirectly, contributed to the completion of this work

Dedication

To my beloved father, Ahmed whose unwavering love, wisdom, and sacrifices have been the foundation of everything I have achieved. You taught me the true meaning of hard work, honesty, and perseverance, values that have guided me not only in my studies, but in every step of my life. Your constant encouragement and quiet strength gave me the courage to keep going, even when the path was difficult. This work is as much yours as it is mine.

To my dear mother, Nadjet, whose prayers, blessings, and unconditional love have been my light during the most challenging times. You have done more than any mother could do to guide your children toward the right path in life and education. May God grant you both good health, long life, and happiness.

To my brothers, Mahdi, Aymen, Ramy, Moudh, mounsif and Akram, you have been my role models, my protectors, and my greatest source of strength. Through every challenge and every victory, your support, advice, and love reminded me that I was never alone. Thank you for always standing by ma side and believing in me.

To Haneen, who walked this path with me. Without you, this journey would have been far more difficult. Thank you.

To my besties, Dhoha, Radja, Aya, Wassila, Chaima, Rayan, and Manar, this journey would have been far more difficult without your love, support, and encouragement. You stood by me through every challenge, celebrated every small victory, and reminded me that I was never alone. Thank you for being there whether with a listening, a word of encouragement, or just your presence. To all of you: I am forever grateful.

And to everyone who believed in me, supported me, or simply prayed for me during this important chapter of my life, thank you. Your love and encouragement carried me through every challenge and made this achievement possible.

Yassmine

Dedication

First and foremost, I thank God for granting me the strength, patience, and determination to carry out this work.

I dedicate this modest work to the memory of my mother, whose wisdom, values, and unconditional love continue to guide my steps. Her memory remains an enduring source of strength and inspiration.

To my beloved father, Dr GHELANI Laala, for his unwavering support and invaluable presence.

To my sisters and brother, for their affection and constant encouragement.

To my project partner, with whom I shared this journey in a spirit of solidarity and determination.

And to all those who, in one way or another, contributed to the completion of this work.

RACHIDA

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Table of Contents

PRACTICAL PART

Chapter ONE: Materials and methods

CHAPTER TWO

List of figures

List of tables

Introduction

Introduction

Nosocomial infections, also known as healthcare-associated infections (HAIs), were first described in the nineteenth century and represent a critical challenge to patient safety and a significant public health concern within healthcare institutions **(1)**. Even with the efforts made in recent years, including various strategies to control these infections, data and statistics show that approximately 5% of hospitalized patients still acquire an infection during their medical care **(2).**

Among the factors complicating the management of HAIs is the growing issue of antimicrobial resistance. While resistance is not uniformly distributed across all bacteria, it is particularly concentrated in a limited number of bacterial species. These species, collectively referred to as the "ESKAPE" pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species), are recognized for their drug-resistant properties **(3).**

According to clinical and biological criteria established by the Centers for Disease Control and Prevention (CDC) and the National Healthcare Safety Network (NHSN), healthcare-associated infection sites are classified into 13 primary categories **(4)**. The most common types of nosocomial infections (NI) that could occur in a hospital set up are: Urinary Tract Infections, Surgical Site Infections, Pneumonia, and Bacteremia.

Previous studies indicate that nosocomial infections affect more than 1.4 million patients worldwide **(5)**. In Algeria, nosocomial infection rates show variability due to limited data availability. Nationally, 14% of hospitalized patients were reported to contract such infections in 2005. For example, a 2013 survey conducted at CHU Bab El Oued-Algiers, a major university hospital in Algiers, revealed a prevalence rate of 11.3%. Surgical site infections were the most common (38%), followed by pulmonary infections (23.8%), urinary tract infections (19%), and catheter-related infections (9.5%). Notably, two decades earlier, the same hospital had reported a higher prevalence rate of 16.2%, suggesting potential improvements in infection control practices over time **(6).**

In many cases, patients do not die from their illness but rather from infections spread inside the hospital environment. This is an alarming rate that necessitates the implementation of measures to combat and prevent such infections **(5).**

The incidence of nosocomial infections in pregnant women is a growing concern, particularly in healthcare settings with limited resources, where factors such as improper hygiene, invasive procedures, and antibiotic misuse contribute to higher infection rates.

This study aims to determine the incidence of nosocomial infections and the associated risk factors in pregnant women at the maternity wards in Khenchela, identify the specific microorganisms responsible for these infections, and analyze their antibiotic resistance patterns. It is based on four tests performed: urine cytobacteriological examinations (UCBE), C-reactive protein (CRP) assays, vaginal smears, prolactin levels.

Our manuscript is structured into three chapters:

- The first chapter provides an overview of nosocomial infections.
- The second chapter describes the materials used and the methodology adopted to carry out this work.
- Finally, the results obtained are presented and discussed in a concluding section, followed by a general conclusion that wraps up this thesis.

Literature Review
Chapter ONE

Nosocomial infections

I. Definition of Nosocomial Infections:

Nosocomial infections, also called health-care-associated or hospital-acquired infections, are a subset of infectious diseases acquired in a health-care setting. **(7)**

The term "Nosocomial" derived from the Greek words “nosos” meaning disease and “komein” meaning hospital. **(8)**

The term "Infection" refers to the invasion and spread of pathogenic microorganisms, such as bacteria, viruses, fungi, or parasites, within a living organism (the body). An infection is more likely to develop when an individual's immune defenses are weakened. This represents a disruption of balance, corresponding to the weakening of the host's (individual's) defense mechanisms by a microorganism. **(5)**

Nosocomial infections can be defined as infections that develop in patients during their stay in a hospital or other healthcare facility, which were not present or incubating at the time of admission **(9).** These infections generally occur within 48 hours of hospital admission, 3 days after discharge, or 30 days following surgery. They impact approximately 1 in 10 patients admitted to the hospital **(10).**

According to the World Health Organization (WHO), nosocomial infections (NIs) can be defined as "infections occurring in a patient within a hospital or another healthcare facility, where the infection was not present or incubating at the time of admission**."(11)**

II. Main Types of Nosocomial Infection

1. Types based on Source nosocomial infections

Can be classified into two main types based on their origin :

1.1. Endogenous Infections

These infections occur when the source of the infection is the patient’s own microbiota. In this case, the infection develops internally, without any external influence Factors such as the patient’s medical condition (e.g., age and underlying diseases), ongoing treatments, the quality of care received, and the presence of pathogenic germs in vulnerable patients contribute to the development of these infections **(6).** Figure 1**(13)**

1.2. Exogenous Infections

These infections originate from an external source outside the patient’s body. They can be transmitted through various means, such as contact with another infected patient (cross-infection), germs carried by healthcare workers, or airborne pathogens **(6).**

Both endogenous and exogenous nosocomial infections are primarily caused by either inadequate hygiene and asepsis practices or the overuse of advanced medical and

surgical techniques. For instance, performing aggressive medical or surgical procedures on immunocompromised patients can increase the risk of infection **(6).** Figure2 **(13)**

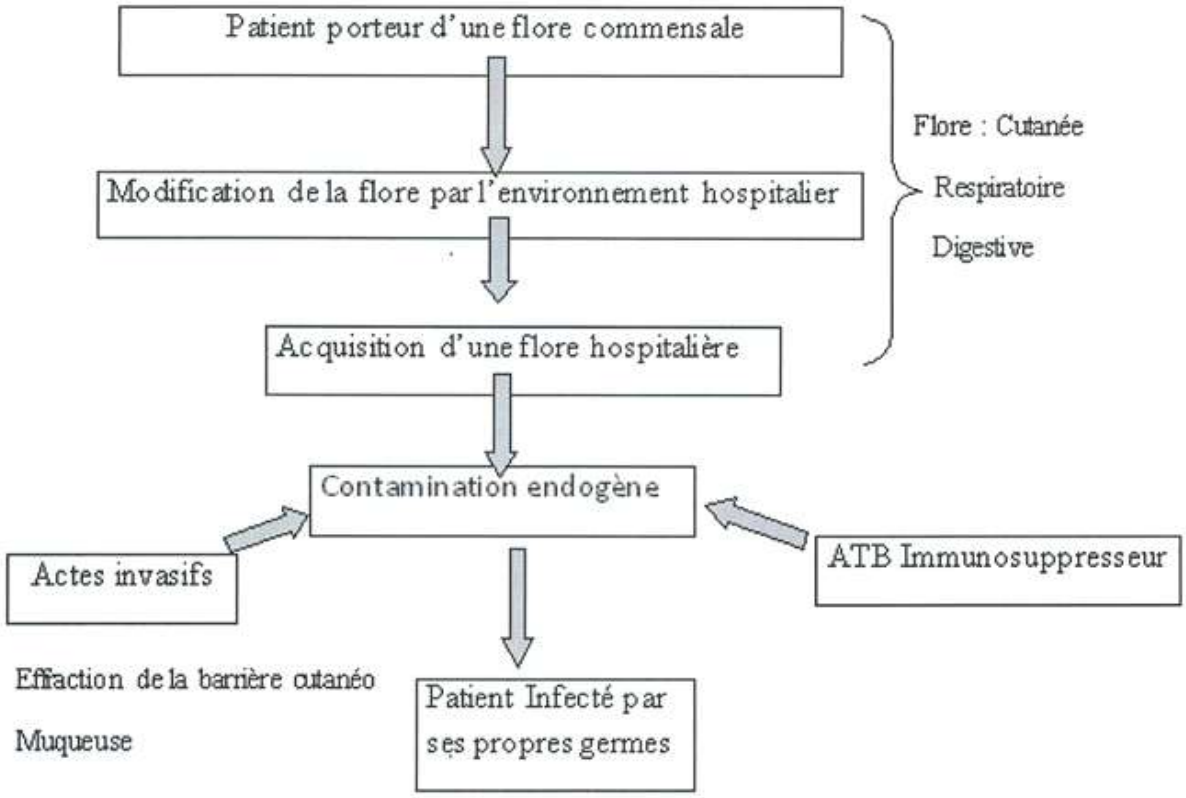

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Figure1: Endogenous Infections.

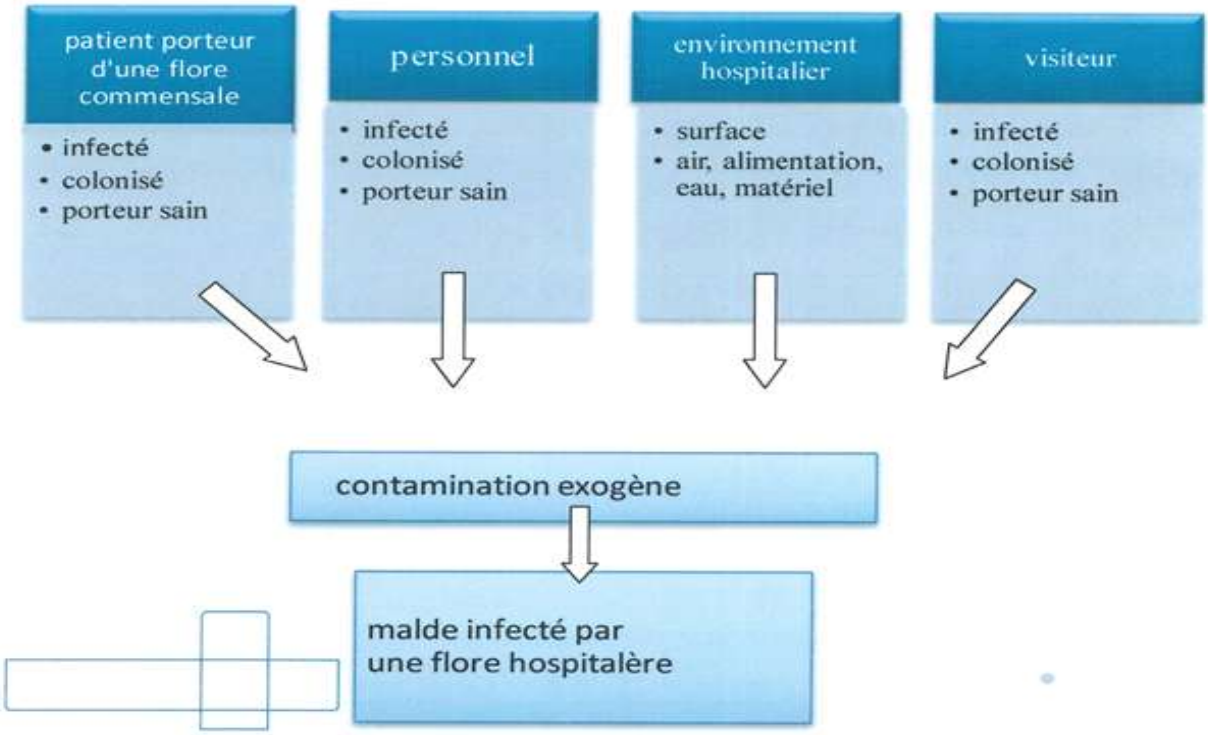

Figure 2: Exogenous Infections.

2. Types Based on Anatomical Site

2.1. Urinary Tract Infections

Urinary tract infection (UTI) is a general term that refers to any infection affecting any part of the urinary tract, including the kidneys, ureters, bladder, and urethra (12). UTIs are among the most common bacterial infections, affecting 150 million people worldwide each year **(14).**

2.1.1. Pathogenic bacteria

Urinary tract infections (UTIs) are caused by various pathogens, including Gram-negative and Gram-positive bacteria, as well as fungi. **Uncomplicated UTIs** typically occur in healthy women, children, and elderly patients, while **complicated UTIs** are associated with catheters, urinary tract abnormalities, immunosuppression, or antibiotic exposure. The primary causative agent for both types is **Uropathogenic Escherichia Coli (UPEC) (14)**.

Other pathogens for **uncomplicated UTIs** (in order of prevalence) include:

- Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, Group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida spp **(14).**

For **complicated UTIs**, the other causative agents (in order of prevalence) are:

- Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis, P. aeruginosa, and GBS **(14).**

a. Bladder Infection: Uropathogenic Escherichia coli (UPEC) employs a range of virulence mechanisms to establish bladder infections. Initially, Type 1 Pili (FimH) facilitate bacterial adhesion by binding to mannosylated uroplakins and integrins on bladder umbrella cells, triggering actin rearrangement via RHO-family GTPases to enable invasion. Once inside, UPEC escapes into the cytoplasm, evading host defenses and antibiotics, and forms intracellular bacterial communities (IBCs). These IBCs mature and disperse, infecting neighboring cells. Additionally, UPEC establishes quiescent intracellular reservoirs (QIRs), where non-replicating bacteria encased in F-actin can persist for months within transitional cells. To support growth, UPEC secretes toxins such as α-haemolysin (HlyA), which lyses host cells to release iron and nutrients, while siderophores scavenge iron during urinary tract infections (UTIs). HlyA also induces epithelial exfoliation, shedding surface layers to expose deeper tissues for QIR establishment or further UPEC spread. Furthermore, cytotoxic necrotizing factor 1 (CNF1) activates RHO GTPases (RAC1, RHOA, CDC42), causing cytoskeletal rearrangements and preventing apoptosis of infected cells. Under certain conditions, UPEC adopts a filamentous morphology to resist neutrophil killing and survive extracellularly.

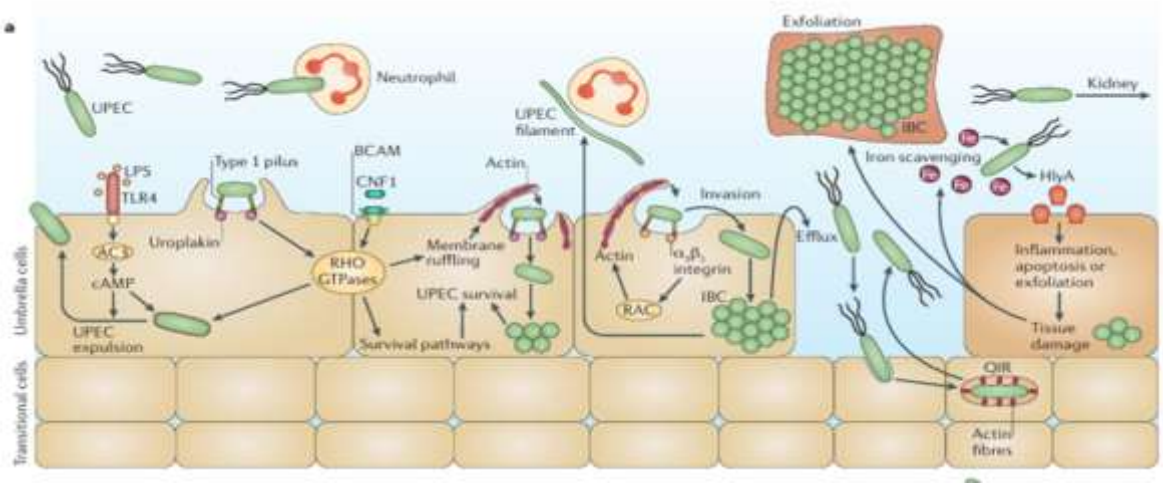

Figure 3: Virulence Factors of Uropathogenic.

b. **Bladder Infection (14).**

c. **Kidney Infection:** Colonization of the kidneys by UPEC depends on the expression of pyelonephritis-associated (P) pili, which bind to globoside-containing glycolipids present in the renal tissue lining. The P pilus adhesin, PapG, also interacts with TLR4, leading to a decrease in the expression of the polymeric immunoglobulin receptor (PIGR). This reduction impairs the transport of immunoglobulin A (IgA) across the epithelial layer, thereby modulating the local secretory antibody immune response and preventing opsonization and clearance of UPEC **(14).**

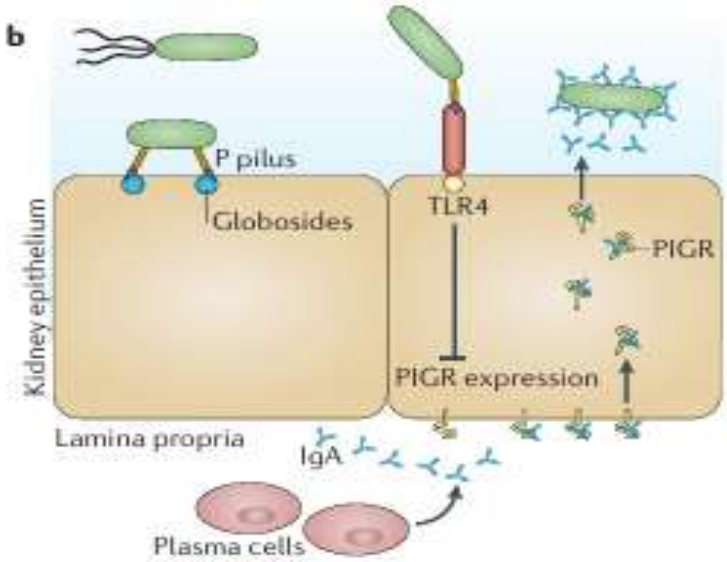

Figure 3: Virulence Factors of Uropathogenic
b. Kidney Infection (14)

2.1.2. Pathogenesis of Urinary Tract Infections

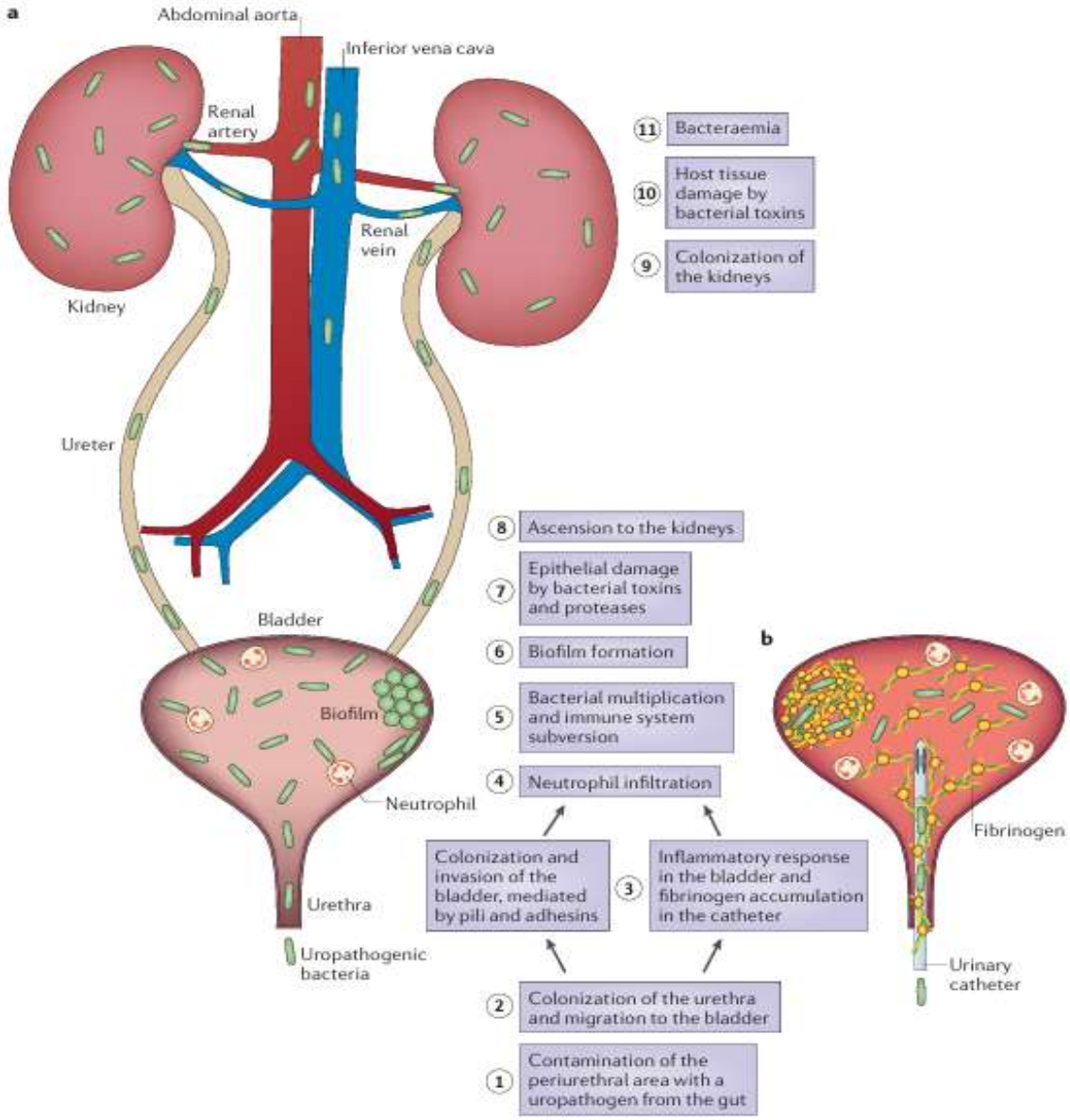

Figure 4: Pathogenesis of urinary tract infections (14).

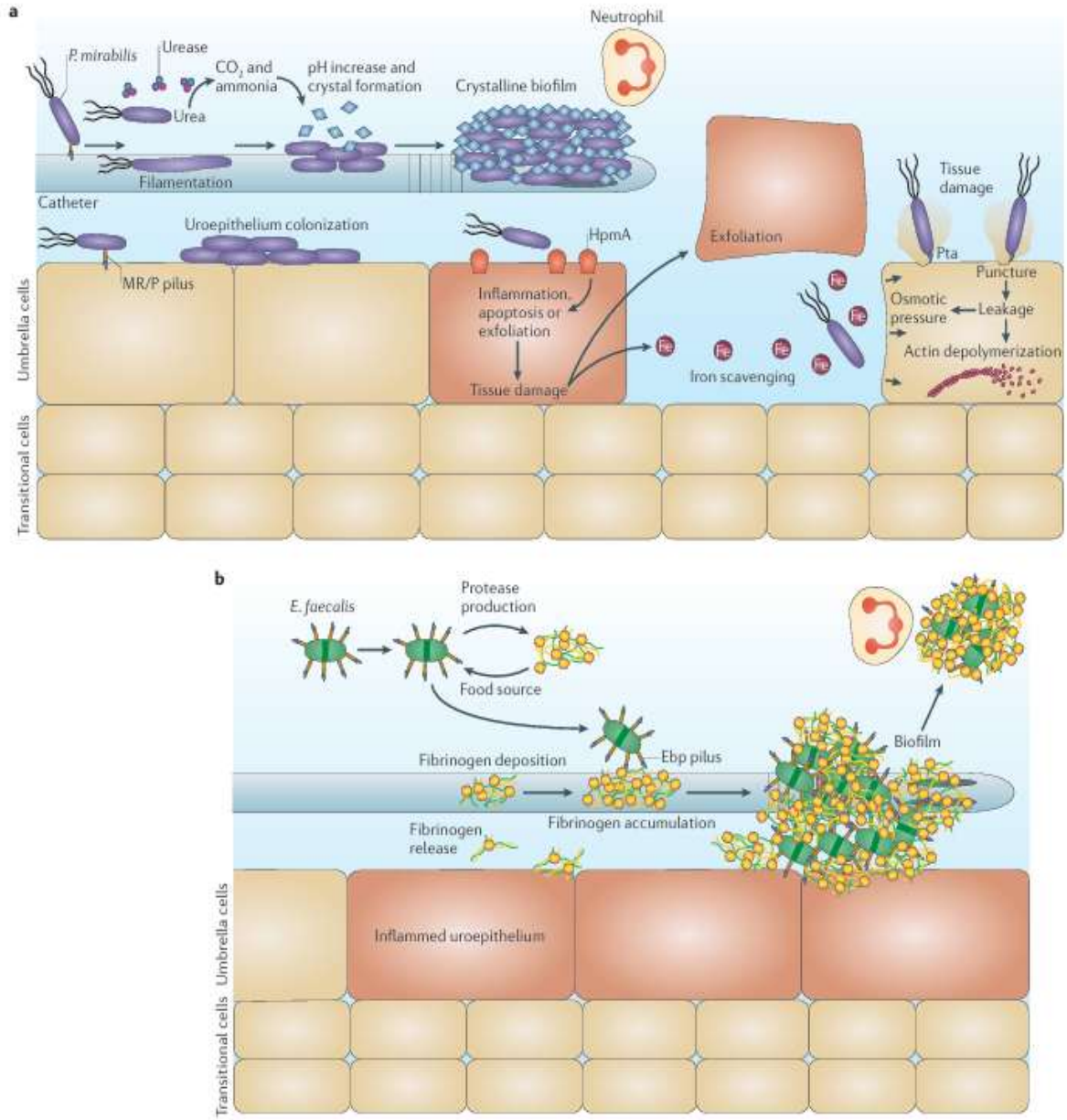

Figure 5: Mechanisms of Pathogenesis in Catheter-Associated Urinary Tract (14).

a) Uncomplicated Urinary Tract Infections: Uncomplicated urinary tract infections begin when uropathogens residing in the gut contaminate the periurethral area (**Step 1**).

b) These pathogens then colonize the urethra and migrate to the bladder (**Step 2**). Through the expression of structures such as pili and adhesins, they can colonize and invade the superficial umbrella cells of the bladder (**Step 3**). In response, the host's inflammatory mechanisms, including the infiltration of neutrophils (**Step 4**), eliminate extracellular bacteria. However, some bacteria evade the immune system either by invading host cells or by altering their morphology to resist neutrophils. These surviving bacteria multiply (**Step 5**) and form protective biofilms (**Step 6**). They produce toxins and proteases that damage host cells (**Step 7**), releasing essential nutrients that promote

bacterial survival and ascent toward the kidneys (**Step 8**). Colonization of the kidneys (**Step 9**) leads to increased production of bacterial toxins and tissue damage in the host (**Step 10**). If left untreated, the infection can progress to bacteremia if the pathogen crosses the tubular epithelial barrier in the kidneys (**Step 11**).

c) **Complicated Urinary Tract Infections:** Complicated urinary tract infections follow the same initial steps described for uncomplicated infections, including colonization of the periurethral area (**Step 1**), migration through the urethra to the bladder (**Step 2**). However, for a complicated infection to occur, the bladder must be compromised. The most common cause of this compromise is the insertion of a urinary catheter. Catheterization triggers a strong immune response (**Step 3**), leading to the accumulation of fibrinogen on the surface of the catheter. This deposition provides an ideal environment for the attachment of uropathogens expressing fibrinogen-binding proteins. The infection also triggers neutrophil infiltration (**Step 4**). After their initial attachment to the fibrinogen-coated catheter, the bacteria multiply (**Step 5**), form biofilms (**Step 6**), and cause epithelial damage (**Step 7**). These biofilms can spread the infection to the kidneys (**Steps 8 and 9**), where toxin production results in tissue damage (**Step 10**). If untreated, complicated urinary tract infections can also progress to bacteremia when the pathogen crosses the tubular epithelial barrier in the kidneys (**Step 11**) **(14).**

Infections (CAUTIs). a. Proteus mirabilis : is a key pathogen in catheter-associated urinary tract infections (CAUTIs) due to its ability to form biofilms and induce complications. It utilizes Mannose-Resistant Proteus-like pili (MR/P) for attachment to catheter surfaces and bladder epithelial cells, facilitating biofilm formation. A hallmark of P. mirabilis infection is the production of urease, which hydrolyzes urea into ammonia, leading to the formation of crystalline biofilms. These biofilms not only protect the bacteria from host defenses and antibiotics but also contribute to urinary reflux and severe complications such as pyelonephritis or sepsis. Additionally, P. mirabilis secretes toxins, including Haemolysin toxin (HpmA) and Proteus toxic agglutinin (Pta). HpmA causes pore formation, tissue damage, exfoliation, and nutrient release, while Pta punctures host cell membranes, inducing cytosol leakage, osmotic stress, and actin depolymerization. These mechanisms aid bacterial spread and enhance iron scavenging, further promoting infection.

b. Enterococcus faecalis: exploits the inflammatory environment caused by catheter use to thrive in the urinary tract. It utilizes fibrinogen, released during catheter-induced inflammation, as a nutrient source through proteolytic activity. The bacteria bind to

fibrinogen using Endocarditis- and Biofilm-associated pili (Ebp), enabling robust biofilm formation on catheter surfaces. These biofilms provide a protective niche against immune defenses and environmental stresses, allowing E. faecalis to persist and establish chronic infections **(14).**

2.1.3. Diagnostic Approaches for Urinary Tract Infections (UTIs): "Tools and Considerations"

- Urinalysis:
  - **a.** Diagnosis should not rely solely on urine appearance; cloudy urine may be aseptic, and clear urine can still be infected.
  - **b.** Dipstick testing is used to assess pH, nitrites, leukocyte esterase, and blood:
    - **i.** Nitrites: Indicate bacteria that convert nitrates to nitrites but are not produced by all bacteria.
    - **ii.** Leukocyte esterase: Suggests white blood cells (WBCs) in the urine, though it may also indicate non-infectious inflammation.
    - **iii.** Blood: Helps differentiate UTIs from conditions like vaginitis or urethritis.
  - **c.** Microscopic examination checks for bacteria and WBCs; more than 10 WBCs per high-power field (HPF) suggests infection.
- Urine Cultures:
  - **d.** Not always required for uncomplicated UTIs but recommended due to rising antibiotic resistance.
  - **e.** A threshold of ≥1000 CFU/mL in symptomatic patients is diagnostic.
  - **f.** Cultures are essential for men, pregnant women, diabetics, immunocompromised individuals, and recurrent UTI cases.
  - **g.** Useful for guiding treatment if initial antibiotics fail.
- Other Tests:
  - **h.** Imaging and cystoscopy: Not routinely needed for uncomplicated UTIs but may help rule out stones or structural abnormalities in recurrent or complicated cases **(15).**

2.1.4. Risk factors:

One of the major risk factors for urinary tract infections (UTIs) is the use of urinary catheters. UTIs are also frequently observed following kidney transplants, where

immunosuppressive medications and vesicoureteral reflux play significant roles in increasing susceptibility. Other contributing factors include antibiotic use, which can promote the emergence of drug-resistant bacterial strains, and diabetes mellitus, which further elevates the risk of infection **(15).**

Table 1: Key Aspects of Urinary Tract Infections (UTIs): Risk Factors, Differential **Diagnoses, and Complications (15).**

Other risk factors	The differential diagnoses of an UTI	Complications of uncomplicated UTIs
• Abnormal urination (eg, incomplete emptying and neurogenic bladder) • Abnormal urinary tract anatomy or function • Antibiotic use and increasing bacterial resistance • Cystocele • Dehydration • Diabetes • Diarrhea • First UTI before age 15 • Frequent pelvic examinations • Incomplete bladder emptying • Immune system suppression or inadequacy	• Bladder stones • Complicated UTI • Food or dietary issues • Herpes simplex infection • Medication adverse effects • Overactive bladder • Pelvic inflammatory disease • Prostatitis • Pyelonephritis • Recurrent UTI • Relapsing UTI • Renal infarction • Renal stones • Sexually transmitted infections • Urethritis • Vaginitis	• Chronic prostatitis • Emphysematous pyelonephritis and cystitis • Focal renal nephronia • Hypertension • Incontinence • Persistent lower urinary tract symptoms • Progression to complicated UTIs • Prostatic abscess • Pyelonephritis • Renal abscess • Renal failure • Staghorn urinary calculi

2.2. Surgical Site Infections

Surgical site infections (SSIs) are nosocomial infections that occur in 2%–5% of patients undergoing surgery. These represent the second most common type of NI, primarily caused by Staphylococcus aureus, and are associated with prolonged hospital stays and an increased risk of mortality. The pathogens responsible for SSIs typically originate from the

patient's endogenous microflora. The incidence of SSIs can reach up to 20%, depending on the surgical procedure and the surveillance criteria employed **(16).**

There are three types of surgical site infections (SSIs):

2.2.1. Superficial Incisional Infection

This type of infection occurs within 30 days following surgery and affects the skin, mucous membranes, and tissues located above the fascia. It is characterized by at least one of the following signs:

- Purulent drainage from the incision or drain.
- Isolation of microorganisms through culture of fluid from a closed wound or tissue sample.
- Deliberate opening of the wound by the surgeon due to localized pain, swelling, redness, or warmth (unless cultures taken during reopening are negative).
- Diagnosis confirmed by the surgeon or physician **(17).**

2.2.2. Deep Incisional Infection

This type of infection occurs within 30 days following surgery (or up to 12 months if prosthetic material is involved) and affects the deep soft tissues at or below the fascial level. It is characterized by at least one of the following signs:

- Purulent drainage originating from the subfascial area (e.g., via a drain).
- Spontaneous dehiscence of the wound or deliberate opening of the wound by the surgeon due to fever (temperature > 38°C) or localized pain (unless cultures are negative).
- Presence of an abscess (detected intraoperatively or radiologically).
- Diagnosis confirmed by the surgeon or physician **(17).**

2.3. Organ/Space Infection:

This type of infection occurs within 30 days following surgery (or up to 12 months if prosthetic material is involved) and affects the organ, site, or space (e.g., serosal cavity) that was opened or manipulated during the procedure. It is characterized by at least one of the following signs:

- Purulent drainage originating from the organ (e.g., via a drain).
- Isolation of microorganisms through culture of a sample taken from the organ.
- Clear clinical or radiological evidence of infection affecting the organ (e.g., detected intraoperatively or radiologically).
- Diagnosis confirmed by the surgeon or physician **(17).**

2.4. Pneumonia

Pneumonia was first described by Hippocrates (460–370 BC). However, the clinical and pathological characteristics of the disease were not described until 22 centuries later, in 1819, by Laennec. According to Harrison's textbook of internal medicine, pneumonia is defined as an infection of the pulmonary parenchyma caused by a variety of organisms **(18).**

Pneumonia represents the outcome of a complex process in which the lower respiratory tract is invaded by an infectious microorganism. This condition can be acquired either in the community or within the hospital environment and may result from the aspiration or inhalation of a pathogenic organism. To ensure effective clinical and therapeutic management of patients, it is crucial to identify the specific role of the causative microorganism in the etiology of pneumonia. Globally, Streptococcus pneumoniae (pneumococcus) is recognized as the most prevalent pathogen responsible for community-acquired pneumonia. In the 2014 global report on antibiotic resistance published by the World Health Organization (WHO), pneumococcus was classified as one of nine bacteria of international concern [3]. Conversely, a wide array of pathogens—whether originating from the patient or the hospital environment—can lead to nosocomial pneumonia. However, in these cases, Gram-negative bacteria are more frequently implicated than Gram-positive bacteria. This intricate interplay underscores the importance of a precise understanding of the causes and transmission mechanisms of pneumonia to guide diagnostic and treatment strategies effectively **(19).**

2.5. Bacteremia

Bacteremia, in its strictest definition, refers to the presence of viable bacteria in the blood. Asymptomatic bacteremia can occur during normal daily activities, such as oral hygiene, or following minor medical procedures. In healthy individuals, these clinically harmless infections are temporary and do not lead to further complications. However, when immune response mechanisms fail or become overwhelmed, bacteremia can progress into a bloodstream infection, manifesting in a wide range of clinical symptoms and is then classified as septicemia **(20).** Healthcare-associated bacteremia (HAB) represents only a small proportion of nosocomial infections (approximately 5%). Nevertheless, it is a serious condition with significant consequences, as case fatality rates exceed 50% for certain microorganisms **(21).** This underscores the critical need for early detection and the implementation of strict preventive measures **(2).**

The Two Main Categories of HAB:

1. **Primary Bacteremia:** These are by far the most common and occur in the absence of other infections at different anatomical sites. This category includes infections related to the insertion of intravenous or intra-arterial catheters.
2. **Secondary Bacteremia:** These are infections found at another anatomical site (e.g., urinary tract infections, pneumonia, skin infections, etc.). The pathogen is present at that anatomical site and in the blood. Although less common than primary bacteremia, these infections are very serious and associated with a high mortality rate **(2).**

2.5.1. Pathogenic Agents

the four main microorganisms (Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa) accounted for 48.3% of isolated pathogens from infections. **(21)**

2.5.2. Diagnostic

The diagnosis of healthcare-associated bacteremia (HAB) is confirmed through laboratory testing, specifically by obtaining at least one positive blood culture (or two in the case of low-pathogenicity commensal microorganisms) at least 48 hours after hospital admission. **(2).**

Differential diagnosis

- Acute prostatitis / abscess
- Community-acquired pneumonia (with asplenia)
- Deep neck space infection abscess
- Empyema
- Instrumentation in patients with bacteriuria
- Intra- or perinephric abscess
- Lung abscess
- Peritonitis abscess
- Pyelonephritis
- Renal calculi
- Renal insufficiency
- Urinary tract obstruction (20)

2.5.3 Risk factors: The main risk factors include the length of catheterization, level of asepsis during insertion and continuing catheter care. **(21)**

2.5.4 Complication: Meningitis, Endocarditis, Osteomyelitis, Sepsis, Cellulitis, Peritonitis **(20).**

2.5.5 Prevention

Reducing nosocomial bacteremia primarily depends on preventing infections associated with intravascular devices. Indications for device placement and maintenance should be limited. Placement and handling must strictly adhere to aseptic protocols, including hand disinfection, the use of alcohol-based antiseptics, and disinfection of ports or taps before any manipulation. For prevention, systematic samples (e.g., nasal swabs, urine) are collected at hospital admission to ensure patients are not carriers of these pathogens**. (21)**

2.6. Other Nosocomial Infections

These are the four most frequent and important nosocomial infections, but there are many other potential sites of infection. Below are examples of less common but significant nosocomial infections:

2.6.1 Skin and Soft Tissue Infections: Open sores, such as ulcers, burns, and bedsores, can lead to bacterial colonization and may progress to systemic infections.

2.6.2 Gastroenteritis In children, rotavirus is a leading cause of nosocomial gastroenteritis. In adults, particularly in developed countries, Clostridium difficile is the primary pathogen responsible for nosocomial gastroenteritis.

2.6.3 Sinusitis and Other Respiratory Tract Infections: Sinusitis and other infections of the respiratory tract can occur, especially in patients with prolonged hospital stays or those on ventilators.

2.6.4 Eye and Conjunctival Infections: Infections of the eye and conjunctiva may arise due to improper hygiene or contaminated medical equipment.

2.6.5 Reproductive Organ Infections: Endometritis and other infections of the reproductive organs can occur following childbirth or gynecological procedures **(21).**

III. Microorganisms responsible for Nosocomial Infections (Causative Organisms)

The pathogens responsible for nosocomial infections encompass a range of microorganisms, including bacteria, viruses, and fungi. Each type of microorganism possesses distinct characteristics that make it more likely to cause specific infections in susceptible hosts. The frequency of infections caused by specific microorganisms varies

based on factors such as the location of the healthcare facility, the nature of the healthcare setting, and the characteristics of the patient population. Generally, bacteria are the predominant pathogens associated with nosocomial infections, followed by fungi and viruses.

1. Bacteria

Bacterial pathogens responsible for infections can originate from either exogenous (external) or endogenous (internal) sources, with the latter often being part of the body's natural flora. Opportunistic bacterial infections typically arise when there is a compromise in the host's immune system function, allowing normally harmless commensal organisms to become pathogenic.

Among the most frequently encountered Gram-positive organisms are coagulase-negative Staphylococci, Staphylococcus aureus, various species of Streptococcus, and Enterococcus species (e.g., Enterococcus faecalis and Enterococcus faecium). On the other hand, Gram-negative bacteria represent a significant group of pathogens, particularly within healthcare settings. Common examples include members of the Enterobacteriaceae family, such as Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Proteus mirabilis, and Enterobacter species. Additionally, non-fermenting Gram-negative bacilli like Pseudomonas aeruginosa, Acinetobacter baumannii, and Burkholderia cepacia are notable pathogens **(22).**

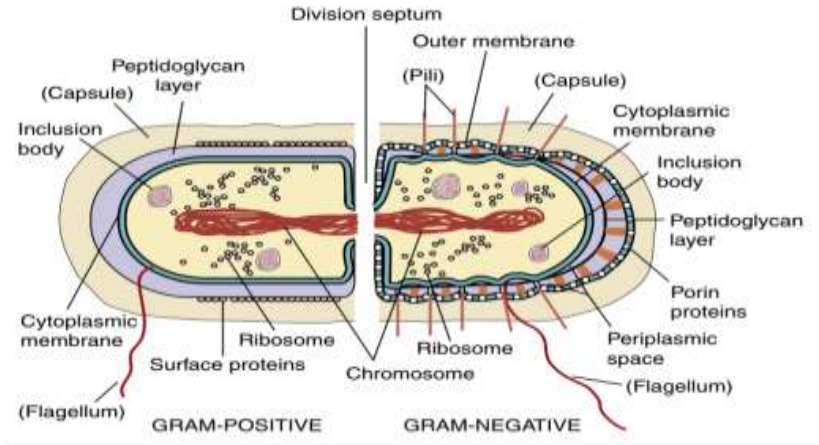

Figure 6: Gram-positive and gram-negative bacteria (23).

A Gram-positive bacterium has a thick layer of peptidoglycan (filling the purple space) (left). A gram-negative bacterium has a thin peptidoglycan layer (single black line)

and an outer membrane (right). Structures in parentheses are not found in all bacteria. On cell division, the membrane and peptido-glycan grow toward each other to form a division septum to separate the daughter cells **(23).**

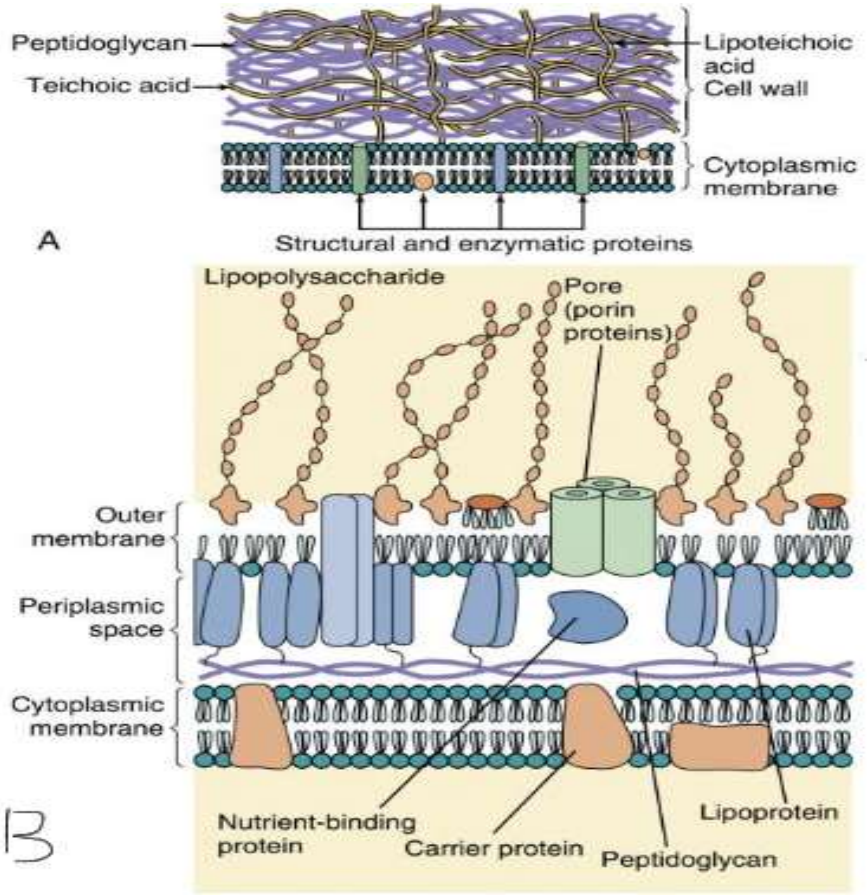

Figure 7: Comparison of gram-positive, gram-negative (4).

A. A Gram-positive bacterium has a thick peptidoglycan layer that contains teichoic and lipoteichoic acids.

B. A Gram-negative bacterium has a thin peptidoglycan layer and an outer membrane that contains lipo-polysaccharide, phospholipids, and proteins. The periplasmic space between the cytoplasmic and outer membranes contains transport, degradative, and cell wall synthetic proteins. The outer membrane is joined to the cytoplasmic membrane at adhesion points and is attached to the peptidoglycan by lipoprotein links **(23).**

Based on the available data, Escherichia coli and Staphylococcus aureus are identified as the most frequently isolated nosocomial pathogens. However, the distribution of these bacteria varies significantly depending on the type of infection. For instance, while E. coli is responsible for approximately a quarter of urinary tract infection (UTI) cases, it is

less commonly isolated from other infection sites. Conversely, S. aureus is rarely associated with UTIs but is frequently implicated in infections at other body sites. In the context of bloodstream infections (BSI), coagulase-negative staphylococci are isolated nearly twice as often as S. aureus. Meanwhile, Enterococcus spp are commonly encountered in surgical site infections (SSI) and bloodstream infections but are seldom found in respiratory tract infections. Additionally, Pseudomonas aeruginosa accounts for roughly 10% of all infections and appears to affect major infection sites evenly, with the notable exception of the bloodstream, where it is isolated less frequently **(4).**

2. Fungi

Fungal pathogens are usually linked to opportunistic infections, particularly in immunocompromised patients or those with indwelling medical devices, such as central venous catheters or urinary catheters. Among the most frequently encountered fungal organisms associated with healthcare-associated infections (HAIs) are species of **Candida**, including C. albicans, C. parapsilosis, and C. glabrata. Notably, Candida auris has emerged as a significant global health concern due to its multidrug-resistant properties, which contribute to high morbidity and mortality rates. This is exacerbated by diagnostic challenges and frequent treatment failures. Collectively, **Candida species** rank as the fourth most common pathogen across all types of HAIs **(22).** In addition to Candida, **Aspergillus fumigatus** can be acquired through airborne environmental contamination, particularly in healthcare settings undergoing construction or renovation. However, infected hospitalized patients may also serve as a primary source of transmission **(22).**

3. Viruses

Viral infections represent the least reported type of healthcare-associated infections (HAIs), accounting for only 1% to 5% of all HAI pathogens. Healthcare-acquired hepatitis B and C, as well as human immunodeficiency virus (HIV), have been linked to unsafe needle practices or improper use of medical instruments. Globally, an estimated 5.4% of all HIV infections are associated with healthcare settings, with a higher prevalence in developing countries **(22).** Other viral pathogens that have been reported include rhinovirus (a common cause of the common cold), cytomegalovirus, herpes simplex virus, rotavirus, and influenza viruses **(22).**

4. Parasites

Parasites are recognized as causative agents of diarrhea, with protozoa being the most common offenders. Examples include Cryptosporidium sp., Entamoeba histolytica, Giardia lamblia, and Blastocystis, which cause infections often transmitted through food and water contaminated. Additionally, Toxoplasma gondii has been identified as a potential agent of waterborne nosocomial parasitic diseases. Another possible source of nosocomial infections is transmission via blood transfusions, with Plasmodium species being the most notable blood parasite transmitted in this manner. Nosocomial parasitic infections can also arise from contaminated hospital equipment. Reports have shown that Plasmodium, a genus of protozoa, can be spread through contaminated injectors, gloves, and even bedside glucometers. Furthermore, Strongyloides stercoralis has been reported to be transmitted via contaminated endoscopes. Therefore, hospital staff must exercise thorough vigilance to prevent transmission through medical equipment **(24).**

IV. Factors influencing the development of nosocomial infection

Risk factors for nosocomial infections are multifaceted, including the healthcare environment, patient susceptibility and clinical conditions, and inadequate implementation of infection control protocols by medical staff.

1. Environmental Factors

Poor hygienic conditions and inadequate waste management in healthcare settings represent significant environmental risk factors. These issues contribute to the proliferation of pathogens and increase the likelihood of nosocomial infections **(16).**

Airborne spores from environmental fungi, such as Aspergillus species, pose a significant risk to immunocompromised patients. Hospital water distribution systems are also susceptible to colonization by pathogens like Legionella pneumophila and Pseudomonas aeruginosa. Contaminated water used for drinking or washing can lead to infections caused by these and other similar environmental organisms in vulnerable patients. Individuals with impaired swallowing reflexes, lung diseases, or weakened immune systems are particularly at risk of developing Legionnaires' pneumonia. Additionally, contaminated food and water can result in gastroenteritis caused by foodborne bacteria (e.g., Salmonella, Campylobacter, E. coli O157) and viruses (e.g., norovirus) **(25).**

2. Patient susceptibility

The occurrence of a nosocomial infection is influenced by the patient's medical condition, which depends on several key factors:

- Age and Underlying Pathology:

  Certain populations are particularly vulnerable to infections due to their physiological or pathological state. These include:

  a. Elderly individuals, whose immune systems may be weakened by age.
  b. Immunocompromised patients, such as those undergoing chemotherapy or living with HIV/AIDS.
  c. Newborns, especially premature infants, whose immune systems are not fully developed.
  d. Polytrauma patients, who experience multiple severe injuries.
  e. Severe burn victims, whose skin barrier is compromised.
  f. Diabetic patients, who often have impaired wound healing and immune responses.
  g. Individuals with morbid obesity, which can increase susceptibility to infections.

- Specific Treatments:

  Certain therapeutic interventions can predispose patients to infections:

  a. Antibiotics, which can disrupt the patient’s natural microbiota, leading to the overgrowth of resistant bacteria.
  b. Immunosuppressive treatments, which reduce the body’s ability to fight off infections.

- Invasive Medical Procedures:

  Necessary for patient care, these procedures can inadvertently increase the risk of infection:

  a. Urinary catheterization, which can introduce pathogens into the urinary tract.
  b. Central venous catheter placement, associated with bloodstream infections.
  c. Mechanical ventilation, linked to ventilator-associated pneumonia (VAP).
  d. Surgical interventions, which create open wounds that are susceptible to contamination.

The development of nosocomial infections is closely tied to the interplay between the patient’s baseline health, treatments received, and the nature of invasive procedures performed **(2).**

3. Unawareness

A lack of awareness and improper practices further exacerbate the risk of infections. This includes the incorrect use of injection techniques, insufficient knowledge of basic infection control measures, inappropriate utilization of invasive devices (e.g., catheters), and the absence of robust control policies. In low-income countries, these risks are compounded by systemic challenges such as poverty, limited financial resources, understaffed healthcare facilities, and a shortage of essential medical equipment **(16).**

V. Mode of transmission of nosocomial infections

The transmission of nosocomial infections occurs through various pathways, each with distinct mechanisms and implications for infection control. Below is a detailed classification of the primary modes of transmission:

1. Contact Transmission

Contact transmission is the most common and significant mode of nosocomial infection spread. It is subdivided into two categories: direct-contact transmission and indirect-contact transmission.

1.1.Direct-Contact Transmission:

This involves direct physical contact between an infected or colonized individual and a susceptible host. Examples include healthcare workers turning patients, bathing them, or performing other care activities that require close personal interaction. Direct transmission can also occur between two patients, where one serves as the source of infection and the other as the susceptible recipient.

1.2.Indirect-Contact Transmission

This occurs when a susceptible host contacts a contaminated intermediate object, typically inanimate. Common examples include contaminated medical instruments, needles, dressings, or gloves not changed between patients. Additionally, improper use of saline flush syringes, vials, and intravenous bags has been linked to disease transmission, even in settings where disposable equipment and protective gloves are available **(26).**

1.3.Droplet Transmission

Droplet transmission occurs when large droplets containing infectious microorganisms are expelled from an infected person and travel short distances (usually less than 1 meter) through the air before landing on a susceptible host's mucosal surfaces (e.g., eyes, nose, or mouth). These droplets are typically generated during coughing, sneezing, talking, or

certain medical procedures like bronchoscopy. Unlike airborne transmission, droplet transmission does not involve prolonged suspension of particles in the air **(26).**

1.4. Airborne Transmission

Airborne transmission involves the dissemination of microorganisms through small-particle droplet nuclei (≤5 μm in size) or dust particles that remain suspended in the air for extended periods. These particles can be carried over long distances by air currents and inhaled by susceptible hosts within the same room or even farther away, depending on environmental conditions. To prevent airborne transmission, specialized air-handling systems and ventilation are required. Examples of pathogens transmitted via airborne routes include Legionella pneumophila, Mycobacterium tuberculosis, and viruses causing measles (rubeola) and chickenpox (varicella) **(26).**

1.5.Common Vehicle Transmission

This mode of transmission occurs when microorganisms are spread to hosts via contaminated items such as food, water, medications, devices, or equipment. For instance, improperly sterilized surgical instruments or contaminated intravenous fluids can serve as vehicles for infection. The scale of transmission depends on the extent of contamination and the number of exposed individuals **(26).**

1.6.Vector-Borne Transmission

Vector-borne transmission occurs when infectious microorganisms are carried and transmitted by vectors such as mosquitoes, flies, rats, and other vermin. While less common in modern healthcare settings, this mode remains relevant in certain environments where vector control measures are inadequate. For example, rodents or insects may introduce pathogens into healthcare facilities, posing risks to both patients and staff **(26).**

VI. Biochemical tests for pathogens identification

The primary objective of detection and identification in a clinical setting is to answer the question: "Is this a bacterial infection or not?" If it is, the goal is to provide a confident identification of the bacteria responsible.

When identifying bacteria in a clinical sample after culture, the genus (e.g., Staphylococcus or Streptococcus) is determined using a combination of morphological features (e.g., colony size and color), microscopy (e.g., Gram stain), and rapid biochemical tests (e.g., for catalase and/or oxidase activity) with simple reagents.Subsequently, identification at the species level (e.g., S. aureus) is performed using targeted biochemical or serological tests (e.g., latex agglutination tests).

This step is critical because different species within the same genus can exhibit significantly different pathogenicity and resistance profiles, leading to infections of varying severity and requiring distinct antibiotic treatment regimens **(27).**

VII. Antibiotic susceptibility test (antibiograms)

Most infectious diseases are of bacterial origin. With the development of laboratory techniques for cultivating these microorganisms using appropriate growth media commonly referred to as "culture" it has become essential to determine the susceptibility and resistance patterns of specific pathogens to a wide range of antimicrobial agents. This allows clinicians to promptly initiate the most appropriate treatment regimen for each patient.An timicrobial susceptibility testing (AST) is a critical laboratory procedure performed by medical laboratory technologists (clinical laboratory scientists) to identify the most effective antimicrobial agent against a particular bacterial isolate **(28).**

The antibiogram is the result of antimicrobial susceptibility testing, in which a microorganism is tested in vitro to determine its sensitivity to various antibiotics at different concentrations. The main methods used are the Kirby-Bauer disk diffusion method, broth microdilution (BMD), and the gradient method (E-test) **(29).**

The Kirby-Bauer method involves measuring the zones of inhibition around antibiotic-impregnated disks. However, the broth microdilution method, which can also be automated, is more commonly used and is considered the reference standard. To accelerate the reporting of results and manage the high volume of tests received in centralized laboratories, automated systems such as Vitek2 are increasingly being utilized **(29).**

1. Main Classes of Antibiotics

1.1. Beta-Lactams

B-lactams antibiotics represent one of the most important and widely used classes of antimicrobial agents. Their significance stems not only from the large number and variety of available molecules, but also from their extensive use in both treatment and prevention of bacterial infections. The popularity of this antibiotic family is due to several key advantages: multiple routes of administration, a broad spectrum of antibacterial activity, bactericidal action, good tissue penetration, favorable safety profiles, and relatively few drug interactions **(30).**

1.2. Aminoglycosides

Aminoglycosides are antibiotics originally derived from soil bacteria such as Streptomyces and Micromonospora. Some, like amikacin, are semi-synthetic. These antibiotics are often used in combination with β-lactams to enhance their rapid bactericidal

effect. They are commonly prescribed for serious infections such as septicemia, pneumonia, meningitis, and urinary or abdominal infections in both humans and animals **(30).**

1.3. Macrolides

Macrolides are a class of antimicrobial agents widely used in the treatment and management of various bacterial infections. Commonly prescribed macrolide antibiotics include azithromycin, clarithromycin, and erythromycin, which are frequently used to treat conditions such as pneumonia, sinusitis, pharyngitis, and tonsillitis **(31).**

These compounds are naturally derived and consist of a macrocyclic lactone ring linked to deoxy sugar moieties. Some macrolides exhibit antibacterial activity, while others possess antifungal properties, making them valuable in pharmaceutical antimicrobial therapy. Erythromycin, introduced in 1952, was the first macrolide antibiotic to be used clinically **(31).**

1.4. Fluoroquinolones

Fluoroquinolones constitute a class of broad-spectrum systemic antibacterial agents that are widely used in the treatment of respiratory and urinary tract infections. These antibiotics demonstrate activity against a diverse range of aerobic gram-positive and gram-negative bacterial pathogens **(32).**

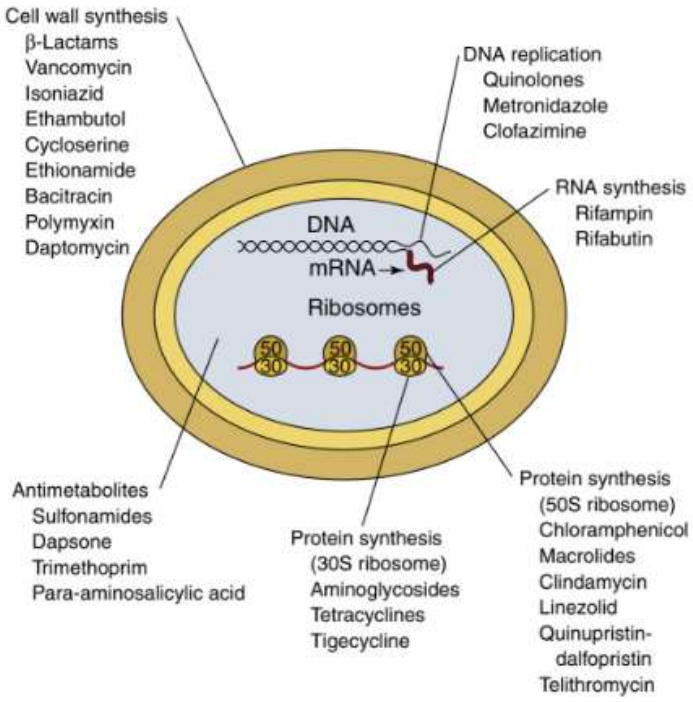

Figure 8: basic sites of antibiotic activity (23).

2. Antibiotic resistance

Antibiotic resistance represents a major challenge to the effectiveness of antimicrobial therapy. Resistance mechanisms are highly diverse and may include alterations in the drug's target site, reduced bacterial membrane permeability, active efflux of the antibiotic through pump systems, or direct inactivation of the drug **(29).**

A single microorganism can possess multiple resistance mechanisms simultaneously. These mechanisms may arise from one or more-point mutations in target genes, or through the acquisition of new genetic material via horizontal gene transfer, such as plasmids or transposons **(29)**

Resistance mechanisms can be either constitutive meaning they are continuously expressed or inducible, occurring only in the presence of a specific antibiotic. Inducible mechanisms can be particularly difficult to detect using standard in vitro susceptibility testing **(29).** When resistance develops through high-frequency mutations, there is a significant risk that it will emerge during monotherapy in an individual patient. This risk is influenced by the infection site, especially increasing in locations where achieving effective drug concentrations is challenging **(29).**

Multidrug-resistant bacteria are frequently encountered in healthcare-associated infections (HAIs) and are associated with significantly increased morbidity and mortality. Studies indicate that nearly 20% of all reported pathogens exhibit resistance to multiple antibiotics. Among the most concerning organisms are methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-intermediate S. aureus (VISA), and vancomycin-resistant S. aureus (VRSA). Also notable are members of the Enterobacteriaceae family that produce extended-spectrum beta-lactamases (ESBLs), leading to resistance to broad-spectrum cephalosporins, as well as vancomycin-resistant Enterococcus (VRE), carbapenem-resistant Enterobacteriaceae, and multidrug-resistant Acinetobacter species and Pseudomonas aeruginosa. These pathogens pose major challenges in clinical settings due to limited treatment options and their ability to spread rapidly **(22).**

VIII. Prevention of Hospital-Acquired Infections

Hospital-acquired infections (HAIs) represent a significant healthcare challenge that requires comprehensive and integrated preventive strategies. Below are the key measures that can be implemented to reduce the spread of these infections:

- Adherence to Hand Hygiene Protocols:
  Hand hygiene is one of the most effective methods for preventing the transmission of infections within healthcare settings and should be an integral part of all healthcare practices.
- Use of Aseptic Techniques for Inserting Medical Devices:
  Aseptic techniques must be strictly followed when inserting intravenous or urinary catheters to prevent secondary infections.
- Compliance with Antimicrobial Use Guidelines:
  Strict policies should be implemented to ensure the appropriate use of antibiotics, minimizing the emergence of drug-resistant strains.
- Provision of Optimal Patient Care:
  High-quality care, including personal hygiene and wound management, should be provided to patients to reduce the risk of infection.
- Minimizing Hospital Stays:
  Reducing the duration of hospital stays lowers the likelihood of patients acquiring hospital-related infections.
- Early Removal of Invasive Devices:
  Devices such as intravenous catheters or ventilator tubes should be removed as soon as they are no longer necessary, as their prolonged use increases the risk of infection.
- Isolation of Infectious Patients:
  Patients diagnosed with or suspected of having contagious infections should be isolated to prevent transmission to others.
- Vaccination of Healthcare Staff:
  Vaccinating healthcare workers against common infectious diseases, such as influenza and other hospital-acquired pathogens, helps reduce the spread of infections.
- Adequate Sterilization and Disinfection of Surgical Instruments:
  All medical and surgical instruments must undergo thorough sterilization and disinfection processes to ensure patient safety.
- Active Surveillance for Hospital-Acquired Infections:
  Implementing active surveillance programs to detect early cases of HAIs allows for prompt intervention and prevents further spread **(33).**

PRACTICAL PART
Methods and Materials

I. Context of study

1. Study Design

This study conducted to investigate the prevalence, identify the causative microorganisms, and determine antimicrobial resistance patterns of bacterial isolates obtained from pregnant women suffering from infections at Salhi Belkasem Specialized Hospital in Khenchela.

2. Study Location and Period

The study was carried out over a two-month period, from March to April 2025. The first phase involved sample collection at Salhi Belkasem Specialized Hospital, where clinical specimens were obtained from pregnant women admitted to the Gynecology and High-Risk Pregnancy (GHR) wards. Out of 100 pregnant women hospitalized during March, five were diagnosed with infections, including urinary tract infections, surgical site infections, and vaginal infections. In the second month, the collected samples were transferred to the microbiology laboratory at Al-Balsam Al-Chafy Clinical Center, Khenchela, for microbiological and biochemical analysis.

3. Study Population

The study population consisted of hospitalized patients who presented with signs and symptoms of infection during their hospital stay. A total of 140 patients were included in the study, from which 5 positive cases were identified based on clinical and microbiological findings.

4. Ethical Considerations

This study was conducted after submitting a formal request from the university and obtaining approval from the Salhi Belkasem Hospital Institution. Written informed consent was obtained from all participating women after explaining the objectives and procedures of the study. Patient confidentiality was maintained throughout the study period, and all data were used exclusively for research purposes.

5. Inclusion and Exclusion Criteria

- ✓ **Inclusion Criteria:**

The study included pregnant women of any age or gestational stage who presented with clinical signs suggestive of infection, such as fever, abnormal discharge, pain, or abnormal laboratory findings. Only those participants who provided written informed consent were included in the study.

✓ **Exclusion Criteria:**

Women who had received antibiotic treatment within 48 hours prior to sample collection were excluded from the study. Additionally, cases with incomplete medical records or those who did not provide written informed consent were also excluded.

6. Data collection

Data were collected using a structured questionnaire that included demographic information (age, gestational stage, and place of residence), clinical profile (type of infection, symptoms, and duration of hospital stay). Samples were collected under sterile conditions and properly preserved. Patient confidentiality was maintained by using identification codes instead of names.

II. Materials

1. Biological material

The biological materials used in this study comprised urine samples, pus samples, and vaginal swabs, which were collected for microbiological analysis, and blood samples

2. Chemical reagent and equipment

Table 2: Chemical reagents and instrumentation used in our study.

Chemical reagents and solvents	Materials	Equipment used
Gentian violet (Crystal violet)	Culter media HEKTOEN CHAPMAN	Integra 400 Plus Biochemistry Analyzer
Lugol's solution (Iodine solution)	Mueller-Hinton agar	Cobas e411 Immunoassay Analyzer
Ethanol / Alcohol (usually 95%)	API 20 E And reagents TDA/IND/ VP (1 and 2)	Optical microscope / Light microscope
Fuchsin (basic fuchsin or carbol fuchsin)		
Sterile distilled water	Culture tube	
Oxygenated water	Pasteur pipette	
	Microscope slide	
	Coverslip	
	Oxidase reagent/test	

III. Sample Collection Technic

1) Urine Sampling

Patient samples were collected during routine clinical care as part of asymptomatic bacteriuria (ASB) screening, performed prior to any diagnostic examination **(34).**

Urine samples were collected from patients at the maternity ward in Khenchela. All specimens were obtained 24 hours after admission and were collected into 100-mL sterile containers without any chemical preservatives **(35).** Patients were instructed to collect the first morning urine **(36)**, ensuring that the container was filled to approximately 30 mL **(37)**. Following collection, samples must be transported to the laboratory as soon as possible. If immediate transport is not possible, samples may be stored in a refrigerator at +4°C maximum for 3 hours **(38).**

Fig 9: Urine sample for CBEU(Original2025).

2) Pus samples

To collect pus samples, begin by removing any bioburden and surface slough to expose viable tissue that is more likely to harbor active pathogens and less likely to be contaminated with normal skin flora. Rinse the collection site thoroughly with sterile normal saline, then carefully excise the specimen using aseptic technique.

In cases of sinus tracts, use a curette to obtain the sample as close to the base of the tract as possible for optimal diagnostic yield.

Place collected specimens on sterile gauze moistened with saline to prevent drying, and ensure the container is tightly sealed. If a swab must be used, it should be taken from the advancing edge of the lesion rather than from pus or exudate alone.

Firmly swab the lesion margin and abscess walls, making sure to avoid contact with intact surrounding skin to reduce contamination risk **(39).**

3) vaginal swabs

During the routine pelvic examination, and before conducting a sterile digital assessment **(34).**

Before beginning the procedure, wash your hands thoroughly with warm water and soap. Place a clean paper towel on the counter or in the sink and set the collection tube and the swab package on it. Open the swab package carefully, pulling the swab out by the handle without letting the tip touch anything. Hold the swab in the middle using your thumb and index finger. Note that the swab has a score line in the middle, which will make it easy to snap after collecting the sample do not break it before collection **(40).**

Hold only the plastic end of the swab and position yourself comfortably either standing with one foot resting on a toilet or bench or sitting on a firm surface with your legs spread apart. With your other hand, gently separate the labia (the folds of skin around the vaginal opening). Insert the soft tip of the swab approximately 2 inches (5 centimeters) into the vagina. Rotate the swab for 10 to 30 seconds, ensuring that it is touching the vaginal walls **(40).** After collecting the sample, remove the cap from the collection tube, which contains a liquid preservative. Insert the swab into the tube with the soft tip facing downward and snap the swab at the score line using the edge of the tube. Discard the top portion of the swab, then securely replace the cap on the tube to prevent leakage. Finally, wash your hands again with soap and water to complete the procedure **(40).**

IV. Experimental Methods

1. Cytobacteriological examination of urine (CBEU)

The Cytobacteriological Examination of Urine (ECBU) is a commonly requested urine test used in the diagnosis and monitoring of infections in the urinary tract, which is typically a sterile system. This analysis helps confirm the presence of a urinary tract infection and identify the responsible pathogen **(41).**

- **Macroscopic examination:**

The Cytobacteriological Urine Examination or ECBU begins with a macroscopic examination of the urine sample which allows for the observation of its general characteristics including whether it is clear cloudy or contains red blood cells as well as its color which can range from pale yellow to dark yellow and may reflect the concentration of the urine although certain medications can also alter its appearance **(41).**

- **Cytological examination:**

cytological examination which involves the identification and counting of different types of cells present in the urine: Normal values for urine cytobacteriological examination:

- Leukocytes (white blood cells): < 10^3/mL.
- Erythrocytes (red blood cells): < 10^3/mL.
- Normal urinary pH: 5.4 – 7.2.

The presence of leukocytes is considered a key indicator of urinary tract infection and underlying inflammation known as leukocyturia. A small number of red blood cells in the urine is generally normal but when found in large quantities this may suggest an infection although it does not constitute a definitive diagnostic element and is referred to as hematuria. Hyaline casts are not typically associated with pathological conditions whereas leukocyte casts indicate inflammation within the renal parenchyma and waxy casts may be observed in cases of chronic kidney disease. The presence of crystals is usually not of clinical significance except in specific situations such as uric acid crystals in cases of acute kidney injury due to hyperuricemia or cystine crystals which are indicative of cystinuria. Additionally, the presence of vaginal epithelial cells suggests possible contamination of the sample which may compromise the reliability of the results **(41)**.

- **Bacteriological examination**

The bacteriological examination includes microscopic analysis using Gram staining as well as the identification and quantification of microorganisms expressed in colony-forming units per milliliter or CFU/mL. Escherichia coli remains the most isolated pathogen in urinary tract infections accounting for between 70 and 80 percent of all cases **(41).**

- **Culture**

The remaining fragments are crushed in a sterile mortar using a pestle, working between two Bunsen burners or under a laminar flow hood.

- **Antibiogram**

Also known as an Antibiotic susceptibility testing, is performed to determine the sensitivity of the isolated bacteria to various antibiotics thereby guiding appropriate antimicrobial therapy **(41).** Urinary tract infections can be broadly classified into two main categories. The first category includes “simple” infections such as acute cystitis and acute pyelonephritis while the second category consists of “complicated” infections which occur in the presence of risk factors such as anatomical or functional abnormalities certain

medical conditions like diabetes or immunosuppression or physiological states such as male sex advanced age with comorbidities or pregnancy **(41).**

In pregnant women : asymptomatic bacteriuria is relatively common and for this reason systematic screening should be carried out at around four months of gestation initially using a urine dipstick test and followed by an ECBU if the result is positive. Women who are at high risk for gestational urinary tract infections due to underlying uropathy a history of recurrent acute cystitis voiding difficulties or diabetes should undergo monthly monitoring throughout pregnancy **(41).**

In cases of acute cystitis during pregnancy diagnosis should be based directly on an ECBU without relying on preliminary dipstick testing. In cases of acute pyelonephritis during pregnancy urgent performance of an ECBU is necessary to confirm the diagnosis and further investigations including ultrasound imaging and fetal assessment should also be carried out to evaluate maternal and fetal outcomes **(41).**

- **Biochemical examination: this include API 20E biochemical gallery**
- **Oxidase and catalase test**

2. C-Reactive protein (CRP)

C-reactive protein (CRP), first discovered by Tillett and Francis in 1930, is named for its ability to react with the C carbohydrate antigen found in the capsule of Streptococcus pneumoniae during episodes of acute inflammation **(42).**

Immunoturbidimetric Determination of C-Reactive Protein (CRP)

CRP levels were measured using an immunoturbidimetric method on the Olympus AU2700 biochemical analyzer. Serum Protein Multi-calibrator and System Reagents for CRP (OSR 6147) and CRP latex (hs-CRP, OSR 6185) were supplied by Olympus (Rungis, France). The reagents and calibrators were used according to the manufacturer's instructions, with analytical measurement ranges of 5–200 mg/L for standard CRP testing and 0.5–20 mg/L for the hs-CRP test. Calibrations for CRP and hs-CRP were performed once a week and once every two weeks, respectively **(43).**

3. Prolactin levels

The term "prolactin" refers to a hormone secreted by the anterior lobe of the pituitary gland, also known as the "pituitary gland." This endocrine gland is in a small bony cavity at the base of the brain. Prolactin is secreted in both women and men **(44).**

❖ **Electrochemiluminescence Immunoassay (ECLIA)**

Prolactin is measured using an electrochemiluminescence immunoassay (ECLIA) with the Roche Cobas Prolactin II assay. This assay has been shown to exhibit no high-dose hook effect at prolactin concentrations up to approximately 12,690 ng/mL **(45).**

Samples are tested both undiluted and after a 100-fold dilution using the appropriate diluent. A high-dose hook effect is considered unlikely if the result from the diluted sample (adjusted for dilution factor) is not significantly higher than the result from the undiluted sample. A 100-fold dilution effectively minimizes any potential hook effect and can aid in distinguishing between a large prolactinoma and a nonfunctioning pituitary adenoma **(45).**

The blood concentration of prolactin is normally less than 20 ng/mL (or 400 mIU/L). When it is too high, it is referred to as hyperprolactinemia **(44)**

4. Cytobacteriological study of pus (CBP)

The cytobacteriological analysis of pus is an essential step in identifying the microbial agents responsible for infection.

➢ **Macroscopic Appearance**

During the macroscopic examination of pus, one observes its consistency, color, general appearance, and viscosity. Pus can vary in texture it may be thick, viscous, elastic, mixed with blood or not, fluid, or even serous. The color can range from chocolate brown to white, and some types of pus may appear greenish or bluish **(46).**

When enough sample is available, the macroscopic examination particularly the odor and color of the pus can provide valuable diagnostic clues. For example, pus containing anaerobic bacteria often has a foul, unpleasant smell; streptococcal pus tends to have a granular and poorly cohesive appearance, while staphylococcal or pneumococcal pus usually presents as creamy. These features are important indicators that should be considered during analysis **(46).**

➢ **Microscopic Analysis**

Microscopic examination plays a crucial role in the analysis of pus and is considered essential. It helps guide the diagnosis and informs which further tests should be carried out. Although it only provides presumptive results, it is highly valuable in determining the appropriate culture media to use **(46).**

➢ **Direct Examination**

This includes staining techniques such as:

- Gram staining, which helps identify the type of bacterial flora present,

The examination can be performed on one of two swabs collected, or directly on pus obtained from a tube or syringe, sterile implant material, or drainage fluid. If the pus cannot be collected directly (e.g., from a prosthetic device), 1 mL of Brain Heart Infusion Broth (BHIB) should be added, followed by careful mixing, using a vortex if necessary. A direct examination of the broth is then carried out, and 2–3 drops are inoculated onto various culture media **(46).**

Culture:

The remaining fragments are crushed in a sterile mortar using a pestle, working between two Bunsen burners or under a laminar flow hood. The same culture media used for pus samples are then inoculated **(46).**

- **Direct Examination in Fresh State:**

A drop of the sample is placed on a glass slide, covered with a coverslip, and examined under an optical microscope at ×40 magnification. This examination makes it possible to distinguish accompanying cells such as neutrophils or lymphocytes, both qualitatively and quantitatively. It also allows for the assessment of cell condition whether intact or altered and the observation of bacterial morphology and motility, if bacteria are present **(46).** Cytological examination involves evaluating the degree of alteration and number of neutrophilic polymorphonuclear cells, as well as the possible presence of other types of cells **(46).**

- **Direct Examination After Staining:**

The stained preparation is observed under an optical microscope at ×100 magnification **(46).**

- **Gram Staining:**

During Gram staining, the following characteristics are noted: the presence or absence of bacteria (either one or multiple species), their morphology (such as cocci or bacilli), whether they are located inside or outside cells, and in cases of polymicrobial infection, the predominant species and the overall bacterial load are described **(46).**

- **Culture Media**

Due to the diversity of bacteria that may be present in pus samples, enriched culture media as well as selective media will be necessary especially for samples contaminated with commensal flora **(47).** Culture media should be kept for at least **48 hours** under aerobic conditions and up to **5 days** for deep-seated infections or when slow-growing organisms are suspected. Media incubated under anaerobic conditions should be

maintained for at least **5 to 10 days** to allow sufficient time for the growth of anaerobic bacteria to become detectable **(47).**

5. Bacteriological Examination of Vaginal Samples

- **culture of Clinical Samples:**

All clinical samples were inoculated onto **HEKOEN**

Isolates were identified based on macroscopic and microscopic examination, as well as conventional biochemical tests.

The swabs were stained using Gram's method and cultured. The cultures were incubated aerobically and examined after 18 to 24 hours **(48).**

- **Gram Stain Examination**

The Gram-stained smears were analyzed for the presence of pus cells, vaginal epithelial cells, various bacterial morphotypes, clue cells, and yeasts. Each microbial morphotype was semi-quantitatively assessed under an oil immersion objective (1000× magnification)

V. Identification of Pathogens and Study of Their Antibiotic Sensitivity

1. Isolation and Identification of Bacterial Isolates

The swabs were directly streaked onto appropriately Medium (HEKTOEN / CHAPMAN) and incubated at 37°C for 24 hours. Initial identification of bacterial isolates was based on colony morphology and hemolytic patterns observed on culture media available. Further identification and characterization of the isolates were carried out using standard microbiological methods, including Gram staining, assessment of colony characteristics, and biochemical tests such as API 20 E biochemical gallery, catalase and oxidase reactions.

2. Microbiological methods

2.1. Gram staining

Gram staining is one of the most essential differential staining techniques in microbiology. Developed by Hans Christian Gram in 1884, it differentiates bacteria based on the structural differences in their cell walls. This method plays a crucial role in bacterial classification and is widely used in clinical laboratories to guide diagnosis and inform antimicrobial treatment decisions.

To prepare a smear for Gram staining, a small amount of bacterial sample is transferred using a sterile inoculation loop onto a clean microscope slide. If the sample comes from solid media, a drop of sterile water should first be placed on the slide to facilitate even spreading. The mixture is then spread into a thin, even film approximately

15 mm in diameter. Up to four smears can be prepared on a single slide for comparative analysis. After air-drying, the smear is heat-fixed by passing the slide through a Bunsen burner flame 3–4 times using circular motions to avoid overheating. Proper heat fixation ensures that the cells adhere firmly to the slide without causing distortion. It is important to allow the slide to cool completely before proceeding to staining **(49).**

The Gram staining procedure involves four key steps. First, the smear is flooded with crystal violet for 30–60 seconds, acting as the primary stain, and then rinsed gently with water. Next, Gram's iodine is applied as a mordant for one minute, which complexes with crystal violet to form an insoluble compound within the cell wall. Excess iodine is removed with a gentle water rinse. The critical decolorization step follows, during which ethanol-acetone is added dropwise for 5–10 seconds until the runoff appears clear. This step removes the stain from Gram-negative bacteria due to their thinner peptidoglycan layer, while Gram-positive organisms retain the dye. Finally, a counterstain, such as safranin or basic fuchsin, is applied for 40–60 seconds to impart color to the now-colorless Gram-negative cells. The slide is then rinsed with water and allowed to air dry **(49).**

During microscopic examination, begin with the 40× objective to evaluate smear quality and locate suitable areas for detailed observation. Switch to the 100× oil immersion objective for high-resolution visualization. Focus on regions where the smear is thin and cells are arranged in a single layer for accurate interpretation. While examining the stained smear, it is important to note that human cells may also appear in the sample white blood cells typically stain Gram-negative (pink), while epithelial cells may stain Gram-positive (purple) **(49).**

Quality control is vital to ensure accurate results. Known Gram-positive and Gram-negative control organisms should be included alongside test samples. Decolorization time must be carefully monitored, as this is the most critical step in the process. Additionally, proper lighting and regular calibration of the microscope contribute to reliable outcomes **(49).**

Expected results are distinct: Gram-positive bacteria appear purple or violet due to the retention of the crystal violet-iodine complex, whereas Gram-negative bacteria appear pink or red after counterstaining. However, several factors can lead to inaccurate results. Over-decolorization may cause Gram-positive organisms to appear falsely negative, while under-decolorization may result in Gram-negative organisms appearing falsely positive. Thick smears or old cultures may yield inconsistent staining patterns, reducing diagnostic accuracy **(49).**

When performed correctly by trained personnel using standardized reagents and technique, Gram staining provides rapid and valuable information about the morphology and staining characteristics of microorganisms. Mastery of this fundamental skill requires consistent practice, attention to procedural detail, and an understanding of potential sources of error **(49).**

Figure 10: Gram staining (original 2025).

3. Biochemical tests

3.1.Catalase reactions

Place a drop of 3% hydrogen peroxide (H□O□) onto a clean glass slide using a sterile Pasteur pipette. Using a sterile inoculation loop that has been flamed and allowed to cool, carefully pick an isolated bacterial colony from a solid culture medium. If possible, avoid using colonies from blood agar, as red blood cells can produce false-positive results due to their intrinsic catalase activity **(50).**

Gently emulsify the colony in the hydrogen peroxide drop and observe immediately for the rapid release of oxygen bubbles (gas formation), which indicates a positive catalase reaction **(50).**

Avoid using metal instruments directly in the hydrogen peroxide solution, as they may induce false-positive reactions. Instead, use wooden sticks or plastic loops when available for safer and more accurate results **(50).**

3.2.Oxidase reactions

Using sterile, flamed forceps, place a dry, non-impregnated filter paper disk on a clean glass slide. Add one drop of oxidase reagent (e.g., tetramethyl-p-phenylenediamine) to the center of the disk. Using a sterile Pasteur pipette, moisten the disk with the reagent **(50).**

With a sterile Pasteur pipette or a plastic loop, carefully pick an isolated colony from a solid culture medium preferably a Gram-negative organism grown on non-blood-

containing media and smear it onto the moistened area of the disk. A positive result is indicated by a rapid development of a dark blue or purple color within 10–30 seconds, signifying the presence of cytochrome c oxidase **(50).** Avoid using metal inoculating loops, as they may cause false-positive reactions. Interpret results promptly, as the reagent can undergo spontaneous oxidation over time, which may interfere with accurate interpretation **(50).**

3.3.API20 Biochemical gallery

the API (Analytical Profile Index) gallery is a miniaturized and standardized set of biochemical tests, designed to be used with comprehensive identification databases. One of the most well-known systems is API 20E, which assesses 20 characteristics specifically for Enterobacteriaceae **(51).**

The API 20E consists of a plastic strip containing 20 mini-wells, each filled with dehydrated media having chemically defined compositions. These media are designed to detect various enzymatic activities, primarily related to carbohydrate fermentation or the catabolism of proteins and amino acids by the inoculated organisms **(51).**

Preparation of the inoculum:

- Collect 1 isolated colony
- Suspend in 5 ml physiological water (turbidity ≈ 0.5 McFarland)

- **Inoculation**

Fill the cups with the suspension.

Add mineral oil to the ADH, LDC, ODC, URE (anaerobic) wells.

- **Incubation**

18-24h at 37°C.

Add reagents if necessary:

- TDA (1 drop of FeCl□).
- IND (1 drop of Kovacs).
- VP (VP1 + VP2, wait 10 min).

- **Interpretation:**

- Reading these reactions depends on colour variations
- Convert the results into a numerical code (3 to 7 digits).
- Compare with API database for identification.

Fig 11: API20 E biochemical gallery's technic (original 2025).

Fig 12: API 20 E' reagent (original 2025).

4. Antibiogram

The antibiotic susceptibility testing process begins with the preparation of Mueller-Hinton agar plates, which must be poured to a uniform thickness of 4 mm and allowed to dry thoroughly before use. High-quality antibiotic disks, each clearly labeled with their respective identification codes, are selected for the test **(52).**

To prepare the inoculum, several well-isolated colonies from an 18–24-hour pure culture are suspended in sterile 0.9% saline, and the turbidity is adjusted to match that of a 0.5 McFarland standard, corresponding to approximately 1 × 10□ CFU/mL **(52).**

The surface of the agar plate is then evenly inoculated by flooding it with the standardized bacterial suspension, after which excess liquid is carefully removed. Using sterile forceps, the antibiotic disks are placed aseptically onto the inoculated agar surface and gently pressed to ensure proper contact. The plates are left undisturbed for pre-

diffusion, typically for 30 minutes to 2 hours at room temperature, before being incubated under controlled conditions (specific in terms of temperature, atmosphere, and duration) appropriate for the organism being tested **(52).** After incubation, zones of inhibition around each disk are measured to the nearest millimeter. These measurements are interpreted using the latest CLSI or EUCAST guidelines, allowing classification of the organism as Susceptible (S), Intermediate (I), or Resistant (R) to each tested antimicrobial agent. Strict adherence to this standardized procedure ensures accurate, consistent, and reproducible antimicrobial susceptibility results **(52).**

Fig 13: Technic of antibiogram (Original 2025).

Results and Discussion

1. **Overall distribution of positive cases :**

140 cases analysed, 10 were positive, representing a rate of 7%, while 130 samples were negative, representing a rate of 93%. This indicates a relatively low prevalence of nosocomial infections in the sampled population, but several factors must be considered when interpreting these results.

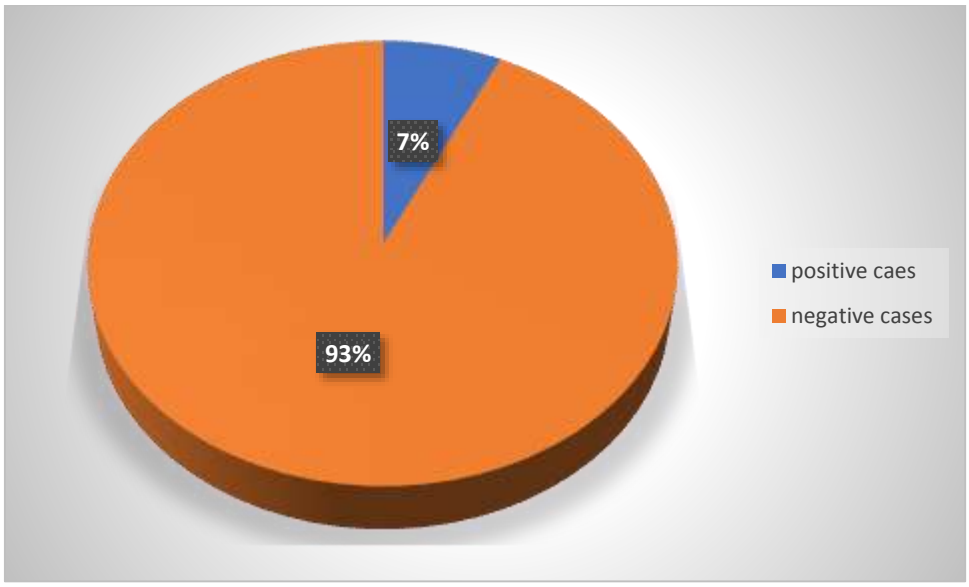

Figure 14 : Distribution of positive and negative sample cases (original 2025).

The 7% infection rate is lower than the global average reported by the World Health Organization (WHO), which estimates that 7-10% of hospitalized patients in developed countries and over 10% in developing countries acquire nosocomial infections **(53)**. A study in Algeria reported a nosocomial infection rate of 9.2% in intensive care units (ICUs) **(54)**, suggesting that Khenchela's rate may be lower than other Algerian regions.

However, differences in hospital hygiene protocols, surveillance methods, and reporting accuracy could influence these variations. Larger, multi-center studies across different hospital departments would provide a more comprehensive picture.

2. **Analysis by type of sample**

During the study period, 10 samples were positive , the highest rate being represented by pus samples (7cases) then urine samples (2 cases), followed by vaginal samples (1 case). This distribution suggests specific patterns of infection that may reflect common healthcare-associated infections (HAIs) in the region.

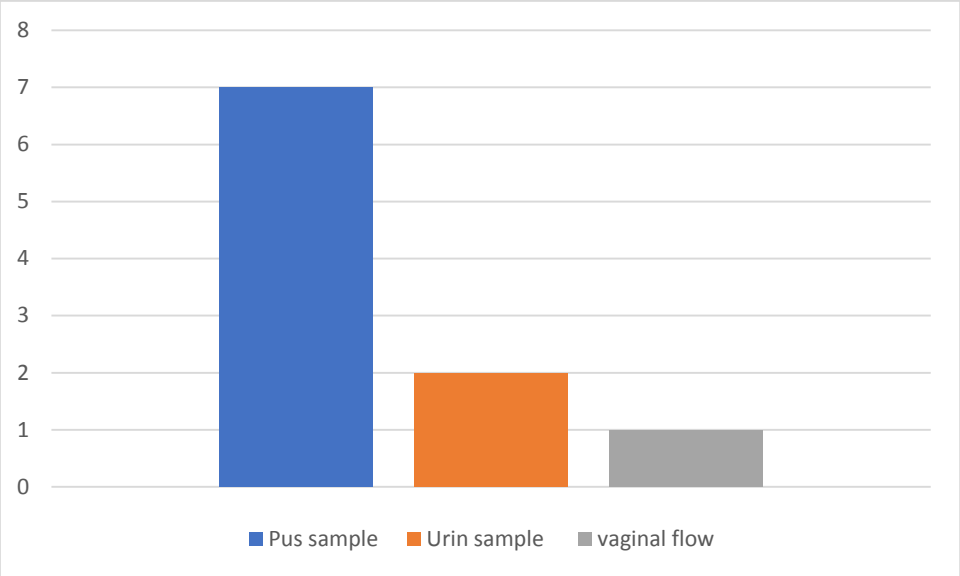

Figure 15 : Distribution of infections by type of sample (original 2025).

The high proportion of pus-positive cases likely indicates surgical site infections (SSIs) or skin/soft tissue infections, which are among the most common HAIs globally. It's proabaly refering to post-surgical infections: Poor sterile techniques, inadequate wound care, or antibiotic misuse could contribute **(53)**. Other study in Algeria, studies report high resistance in wound isolates **(55).**

Riguarding Urinary Tract Infections (UTIs) it can be linked to Catheter-associated UTIs (CAUTIs), a leading cause of HAIs **(53)**, or poor catheter hygiene or prolonged use **(56).**For regional context a study in Algiers found E. coli was the most common UTI pathogen, with high resistance to cephalosporins **(57).**

3. Distribution of infections by department

During the hospitalisation 7 cases developped an infection in the surgical departement ,2 cases for GHR department and 1 case for GYN department .

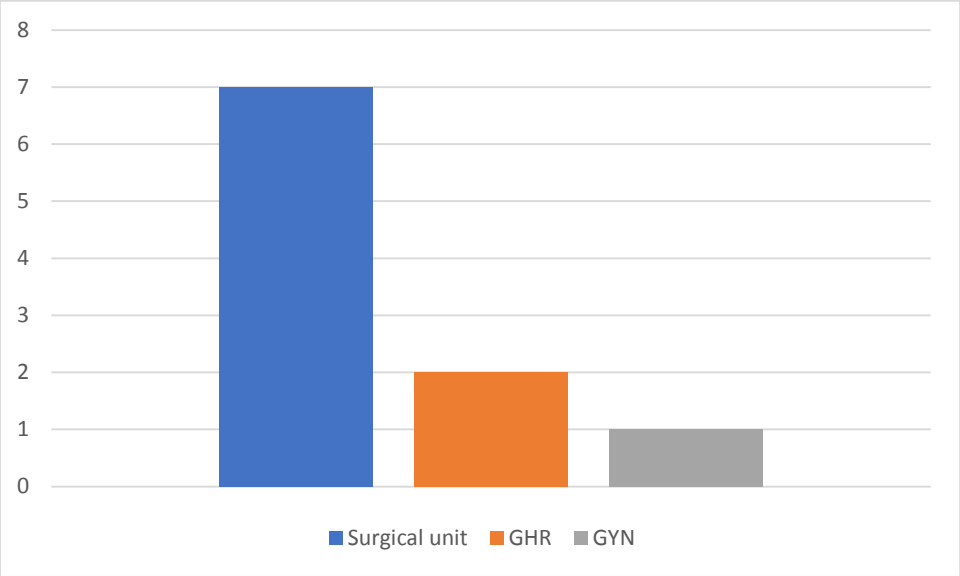

Figure 16: Distribution of infections by department (original 2025).

4. positive cases who developed an infected post-operative wound

Among the 120 cases operated on in the surgical unit , 7 developed an infected wound postoperatively after a medical check-up.

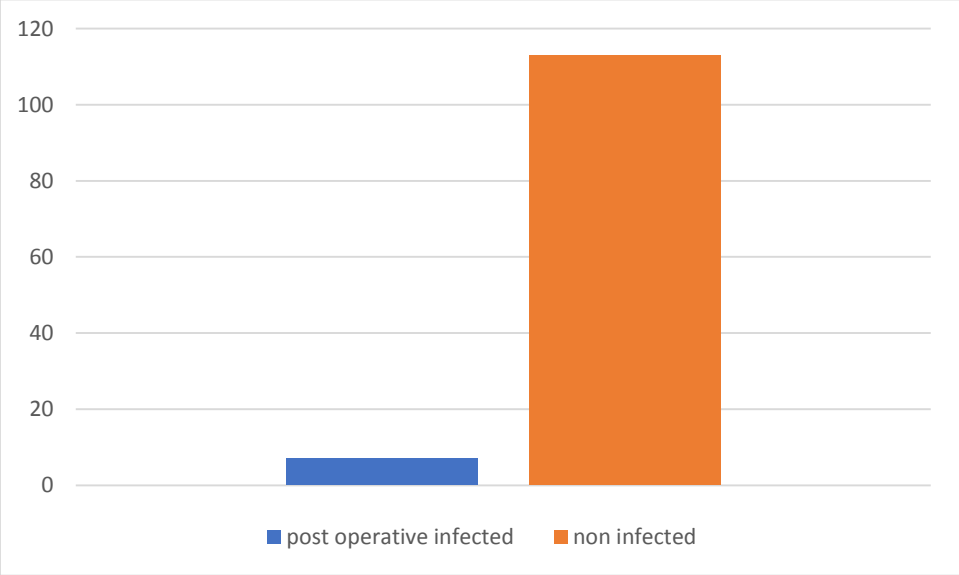

Figure 17 : ditribution of infected patient post oprative (original 2025).

5. positive cases who developed a urinary tract infection on the department

During hospitalisation, patients in the high-risk pregnancy department were examined to diagnose a urinary tract infection after elimination of 30 cases that were positive cases on the admission; so 2 patients developped a urinary tract infection on the department and 8 cases were negtive

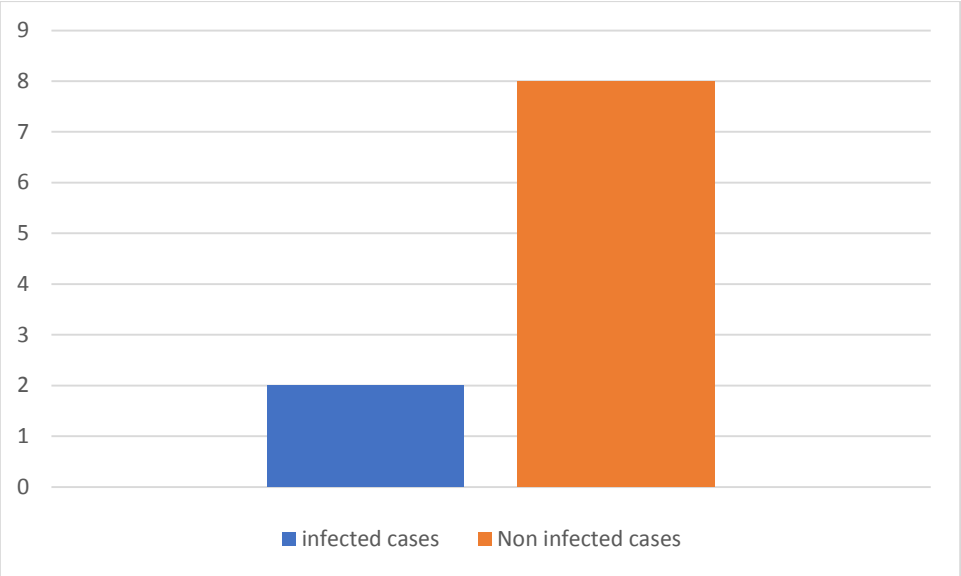

Figure 18 : Distribution of patients that developed a urinary tract infection on the department (original 2025).

6. positive cases who developed a post- curttage infection

After the post ABRT curttage procedure, one case was examined which presented a vaginal discharge among 10 patients who benefited from the same procedure in the same period.

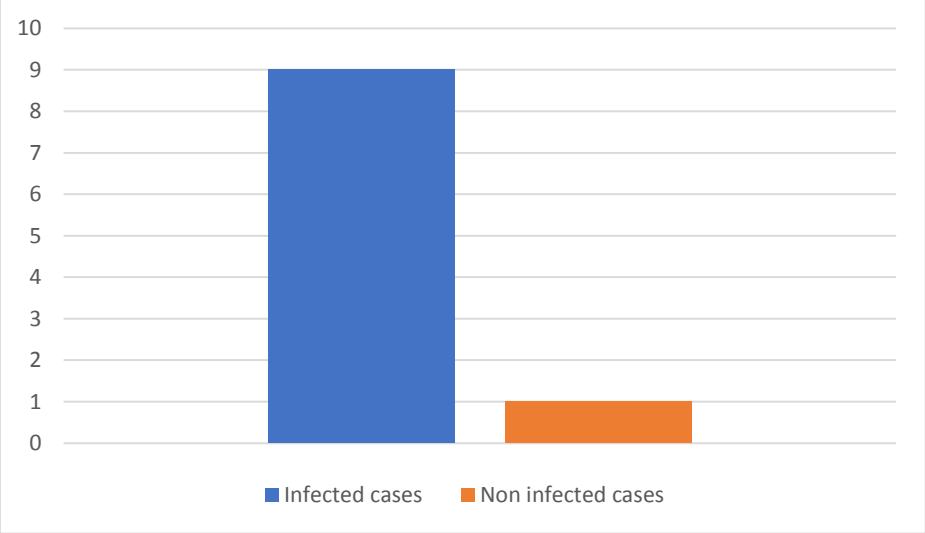

Figure 19 : Distribution of infected cases post-cuttage procedure(Original 2025) .

7. Distribution according to germs responsible

breakdown by germ shows that Escherichia coli is the main germ responsible for infections, with a rate of 4 Cases . Pseudomonas aeruginosa and Staphylococcus aureus are small and equal, representing 3 cases respectively.

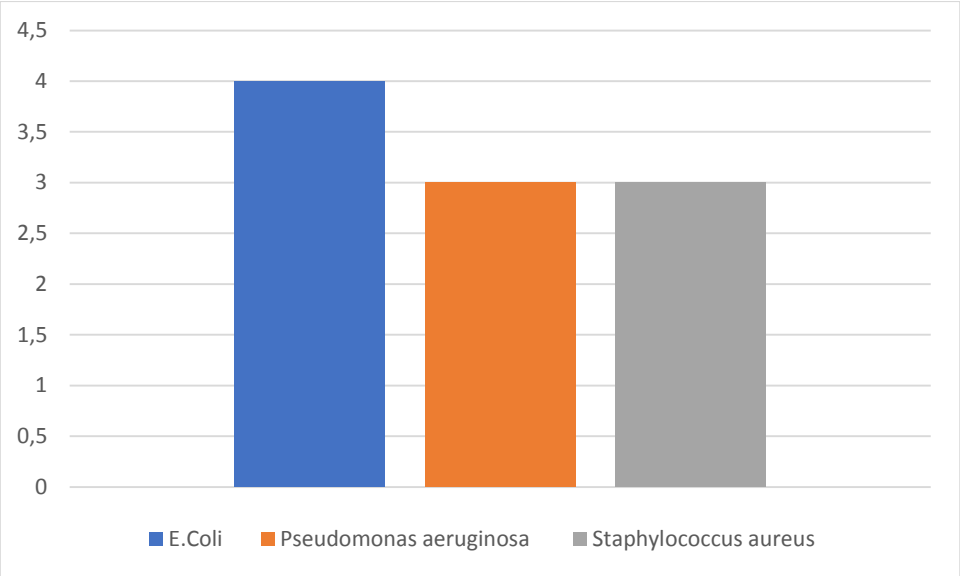

Figure 20 : distributiion of germs responsible of infections (original 2025).

8. Distribution of microorganisms by type of sample

8.1.E.Coli

The distribution of E.Coli is equal in both of urine and pus samples

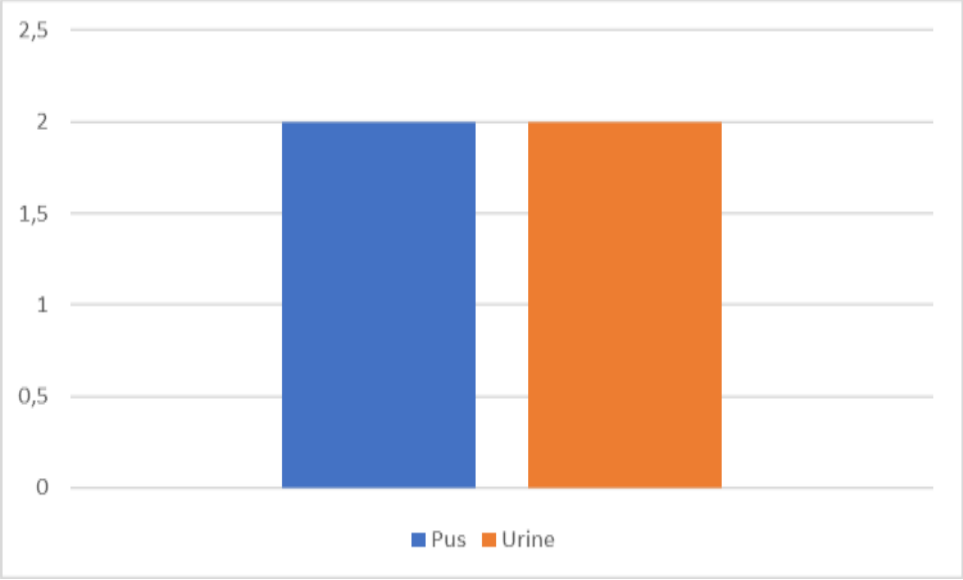

Figure 21 : distribution of E.Coli in the samples(original 2025).

The finding that Escherichia coli (E. coli) was equally distributed in urine samples (UTIs) and pus samples (wound/surgical infections) is clinically significant and warrants further analysis. While E. coli is classically associated with urinary tract infections (UTIs), its presence in pus samples suggests possible shifts in bacterial behavior, antibiotic resistance patterns, or healthcare-associated transmission routes. E. coli is the most common cause of UTIs (70-90% of cases), particularly catheter-associated UTIs (CAUTIs) **(58).** A study in Algiers found high resistance rates in E. coli, particularly to ciprofloxacin (62%) and third-gen cephalosporins (45%) **(57).** E. coli strains (e.g., ESBL producers) are increasing in Algerian hospitals **(55).**

E. coli in both urine and pus suggests possible hospital-wide dissemination, either via healthcare workers' hands (poor hand hygiene) or contaminated surfaces (catheters, surgical instruments).Genomic studies (e.g., whole-genome sequencing) could confirm if the same strain is circulating **(59).**

Figure 22 : macroscopic aspect of E.Coli isolated n HEKTEON medium (original 2025) .

❖ **Catalase test**

The observation that isolated E. coli strains tested positive for catalase is unusual because E. coli is typically classified as a catalase-positive member of the Enterobacteriaceae family, but its catalase activity is generally weaker compared to bacteria like Staphylococcus aureus. Below, we discuss the implications of this finding, possible explanations, and its clinical significance.

Figure 23: Positive catlase test of E.Coli isolated (original 2025).

E. coli produces catalase to break down hydrogen peroxide (H□O□), a byproduct of aerobic metabolism **(60).**

However, its catalase activity is less vigorous than in Staphylococcus or Bacillus species.

❖ **Oxidase test**

The observation that the isolated E. coli strains tested negative for oxidase is an expected and confirmatory result that aligns with standard microbiological identification criteria for this bacterium. Below is a detailed discussion of this finding and its significance:

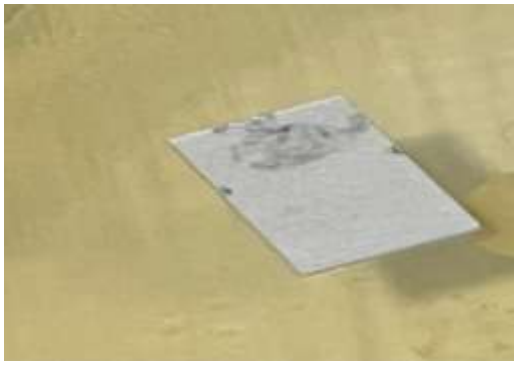

Figure 24 : Negative Oxidase test of E.Coli isolated (original 2025).

E. coli is a facultative anaerobe that primarily uses fermentation for energy production, even in aerobic conditions. Unlike Pseudomonas or Neisseria (which rely heavily on aerobic respiration), E. coli lacks cytochrome c oxidase, making it oxidase-negative **(60)**.This is a key distinguishing feature of Enterobacteriaceae (e.g., E. coli, Klebsiella, Proteus), all of which are oxidase-negative.

Since E. coli is a leading cause of UTIs, surgical site infections, and bloodstream infections, its oxidase-negative status helps streamline lab workflows for rapid identification and antibiotic susceptibility testing **(61).**

- **Antibiotic susceptibility test results**

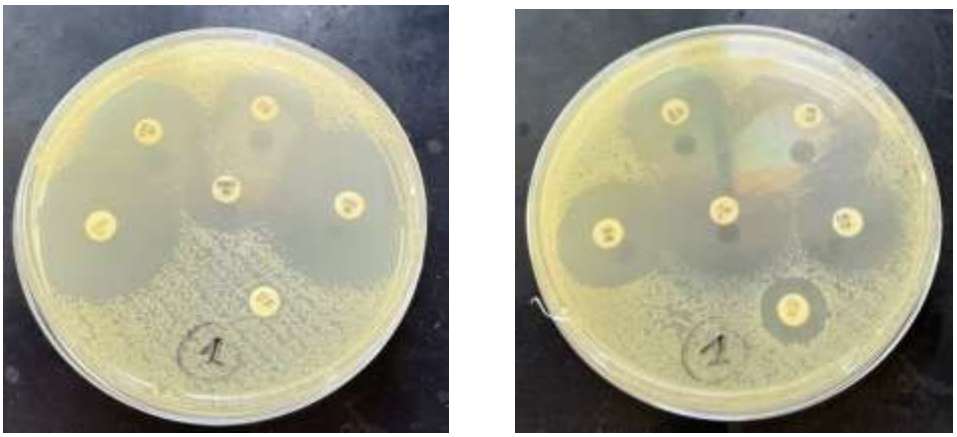

Figure 25: Antibiotic susceptibility test results of E.Coli in MH midium (original 2025).

The antibiotic susceptibility results reveal a resistant E. coli strain with resistance to ampicillin and ticarcillin but susceptibility to all other tested antibiotics, including β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanate), cephalosporins, carbapenems, aminoglycosides, and colistin.

Table 3: Antibiotic susceptibility test results of E.Coli

Antibiotics tested	Ampicilin	Ticarcilin	Amoxicilin-Clavulanic asid	cefazolin	cephoxitin	cephotaxim	Imipinem
Interpretation	R	R	S	S	S	S	S
Antibiotics tested	Gentamycin	Naldisic asid	Ciproflaxin	Trimethoprim-sulfamethoxazole	Fosfomycin	colsitin	
Interpretation	S	S	S	S	S	S	

For the esistance to Penicillins (Ampicillin, Ticarcillin) E. coli commonly acquires plasmid-encoded β-lactamases (e.g., TEM-1), hydrolyzing ampicillin/ticarcillin **(62).**Ticarcillin resistance suggests possible Pseudomonas-coverage β-lactamases (e.g., PSE-1), though rare in E. coli. Ampicillin is obsolete for empirical UTI treatment in many regions due to high resistance **(53)**. In Aalgeria ESBL rates in E. coli: ~30–40% in hospitals **(57)**.

- **API 20 E bichemical gallery results**

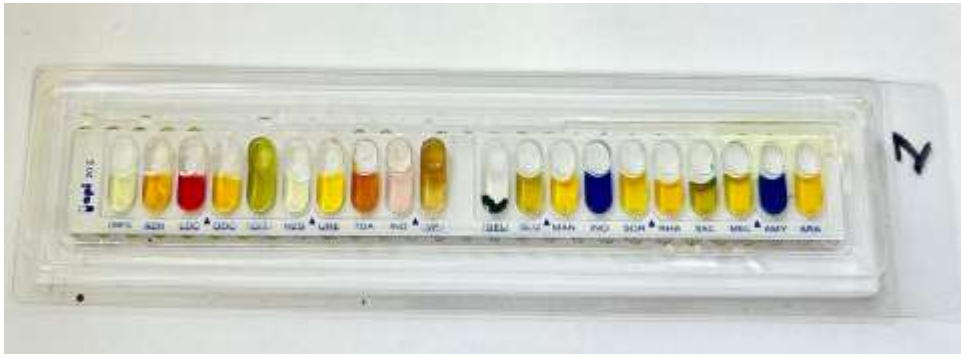

Figure 26: API 20 E biochemical gallery results of E.Coli isolated (orginal 2025).

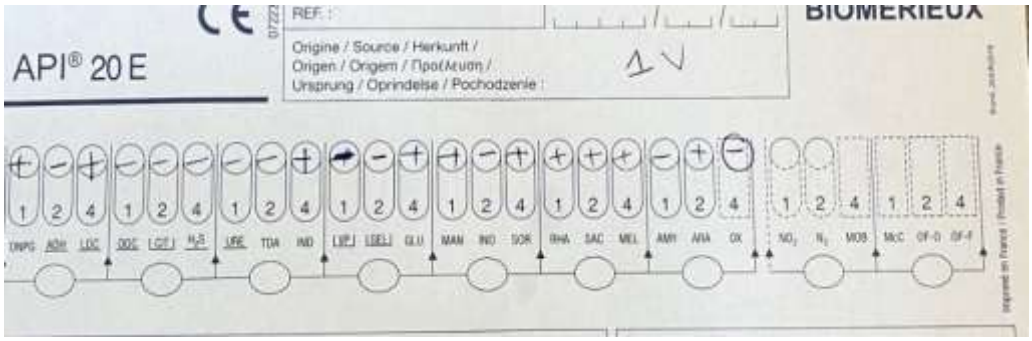

Figure 27 : results page of API 20 E bichemical gallery (original 2025).

The API 20E system is a standardized biochemical panel used to identify Enterobacteriaceae, including E. coli. Below is a breakdown of expected results for E. coli, their significance, and how they confirm the isolate's identity.

Rapid Identification: API 20E provides ~95% accuracy for E. coli **(63).**

several results are complementary to our results , Citrate-positive E. coli: Reported in 5–12% of Algerian isolates, often linked to ESBL or MDR strains **(54)**. Sorbitol-negative E. coli: Found in 35% of ESBL producers in Annaba, suggesting possible enterohemorrhagic (EHEC) contamination **(64).**

Atypical profiles: Some Algerian isolates show TDA+ (tryptophan deaminase), mimicking Proteus **(57).**

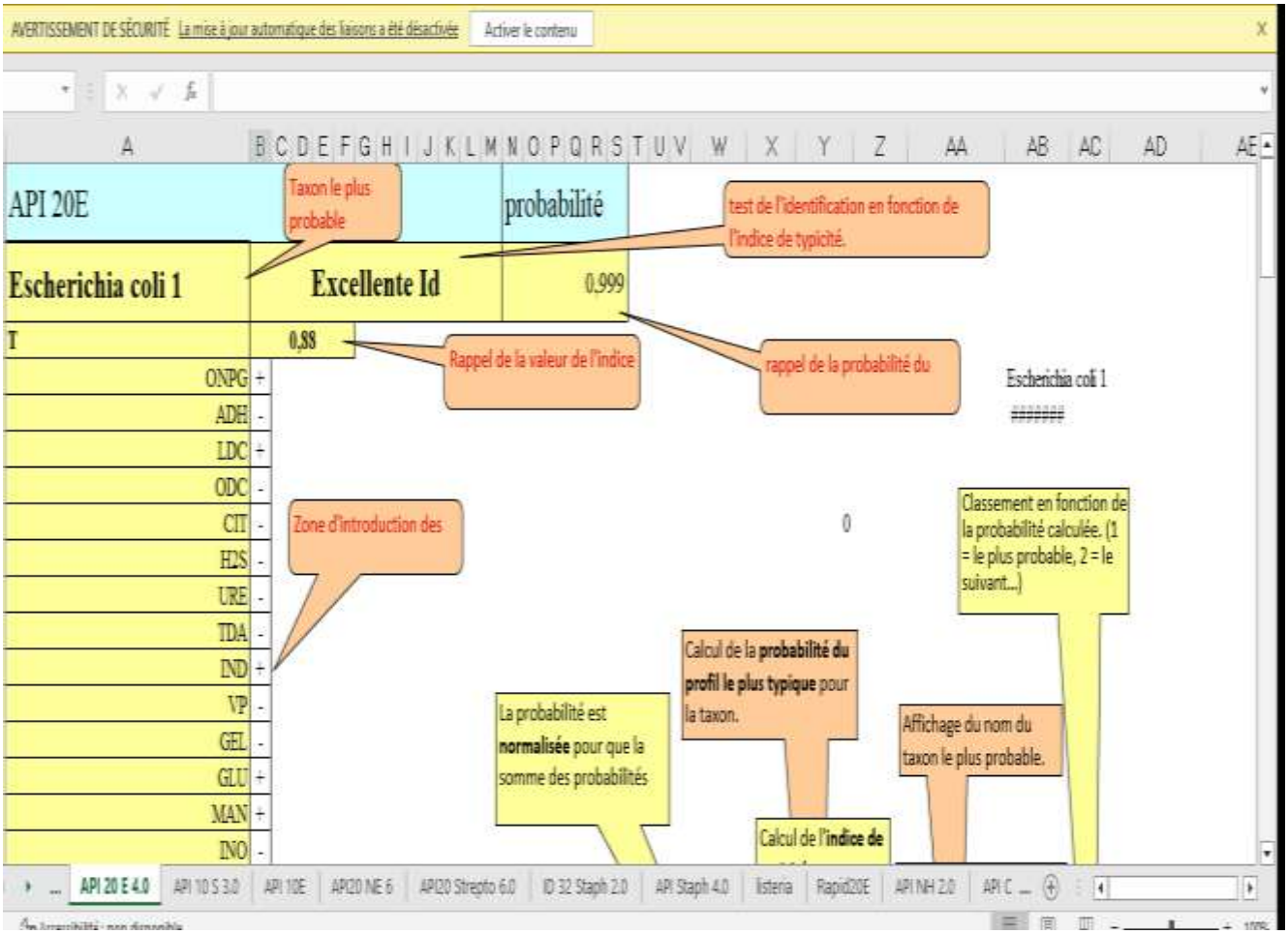

Figure 28: microbial identification worksheet of E.Coli (original 2025).

8.2. Pseudomonas aeruginosa

The distribution of the germ in the samples is distributed in the pus in 2 cases and rsponsible for one infection case for the post currtage

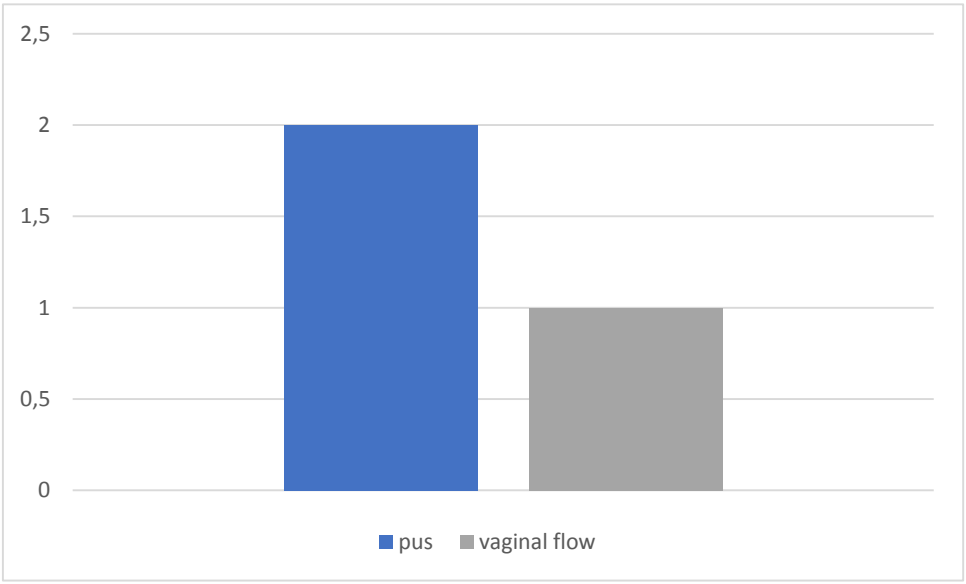

Figure 29 : distribution of Pseudomonas aeruginosa in the samples (original 2025).

Figure 30 : macroscopic aspect of Pseudomonas aeruginosa in HEKTOEN midium (original 2025).

❖ **Catalase test**

Figure 31: positive catalase test of Pseudomonas aeruginosa (original 2025).

❖ Oxydase test

Figure 32: positive oxidase test of Pseudomonas aeruginosa (original 2025).

❖ **Antibiotic susceptibility test results**

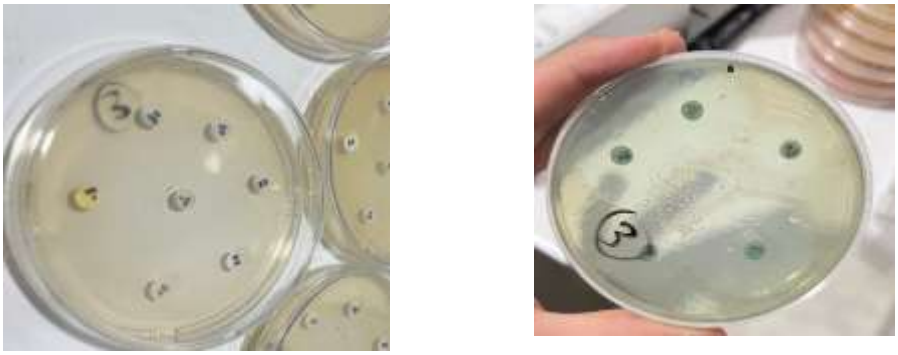

Figure 33: Antibiotic susceptibility test results of Pseudomonas aeruginosa isolated in MH midium (original 2025)**.**

Table 4 : Antibiotic susceptibility test results of Pseudomonas aeruginosa (original 2025)

Antibiotics tested	Interpretation	Antibitis tested	Interpretation
Ticarcilin	I	Aztreonam	S
Piperacillin	I	Ciproflaxin	S
Ticaecilin -clavulanic acid	I	Gentamicyn	S
Ceftazidine	I	Tobramycin	S
Imipinem	S	Colsitin	S

❖ **API 20 E bichemical gallery results**

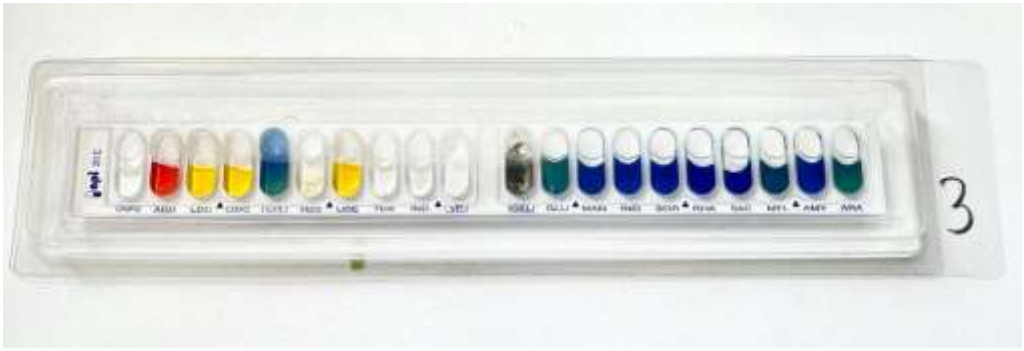

Figure 34: API 20 E bichemical gallery results of Pseudomonas aeruginosa (original 2025).

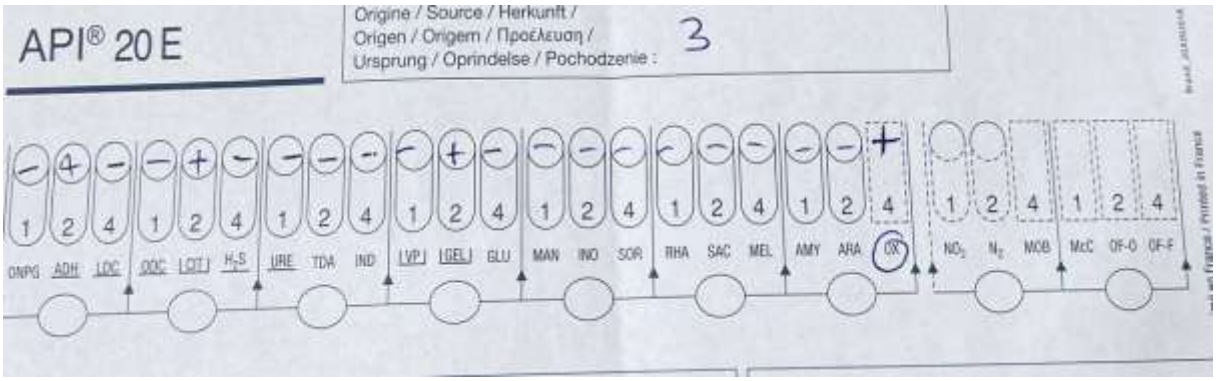

Figure 35: results page of API 20 E bichemical gallery (original 2025).

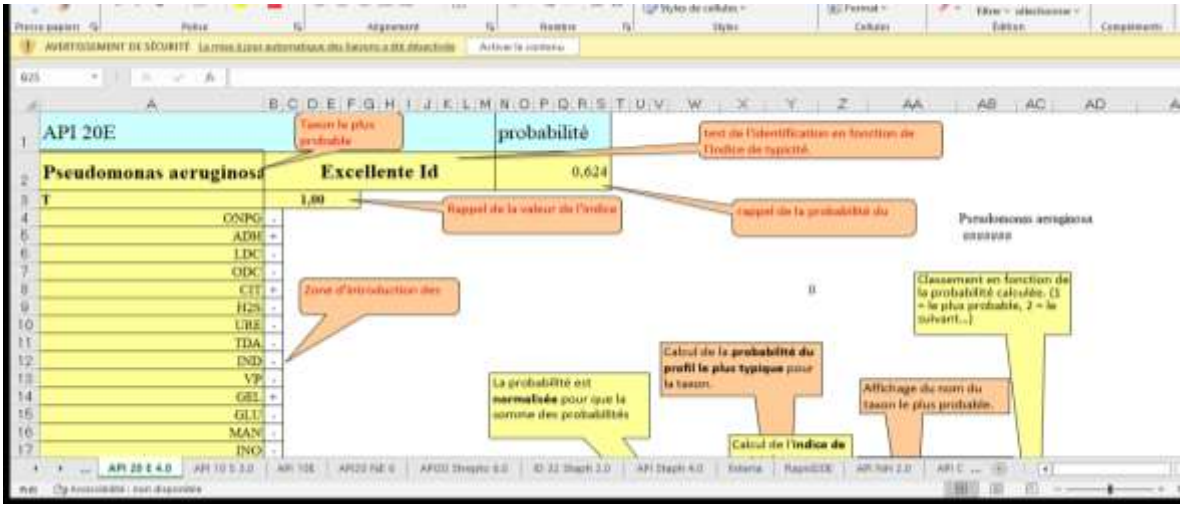

Figure 36: microbial identification worksheet of Pseudomonas aeruginosa (original 2025).

This study identified three cases of Pseudomonas aeruginosa infection in a hospital setting, with two isolates recovered from pus samples and one from a post-curettage infection. The distribution pattern aligns with the organism's known predilection for causing opportunistic infections in healthcare environments **(53)**. The isolation from pus samples is clinically significant as P. aeruginosa is a well-documented cause of wound infections, particularly in burn patients and those with post-surgical complications **(59)**.

The post-curettage case represents a potentially serious nosocomial infection that warrants investigation of sterilization protocols and possible environmental contamination in gynecological procedures **(65).**

Biochemical characterization revealed typical phenotypic markers of P. aeruginosa, with positive results for both catalase and oxidase tests. The catalase positivity reflects the bacterium's defense mechanism against host oxidative stress **(60)**, while the oxidase positivity confirms the presence of cytochrome c oxidase, a key differentiating feature from Enterobacteriaceae **(66).** These biochemical characteristics are consistent with standard identification criteria for P. aeruginosa and help distinguish it from other Gram-negative pathogens commonly encountered in clinical settings **(63)**.

Antimicrobial susceptibility testing yielded notably favorable results, with all isolates showing no resistance to the tested antibiotics. This finding is somewhat unexpected given P. aeruginosa's notorious reputation for developing multidrug resistance, particularly in hospital environments (**53)**. The susceptibility pattern may indicate one of several scenarios: (1) these represent community-acquired strains with less antibiotic exposure

(59),(2) effective antimicrobial stewardship programs in the hospital are limiting resistance development **(65)**, or (3) the antibiotic panel may not have included certain drugs for which resistance is commonly encountered **(61)**. The isolates' sensitivity to commonly used anti-pseudomonal agents such as piperacillin, ceftazidime, and ciprofloxacin suggests these remain viable treatment options in this setting **(67)**.

he clinical implications of these findings are significant. The post-curettage infection case highlights potential gaps in infection control measures that require immediate attention, including review of instrument sterilization procedures and evaluation of water sources in the gynecological unit **(59).** The absence of antimicrobial resistance in these isolates presents a therapeutic advantage, though continued surveillance is essential to monitor for emerging resistance patterns **(53).**

From a public health perspective, these results suggest that while P. aeruginosa remains a concerning pathogen in this hospital, current infection control measures may be effectively limiting the spread of resistant strains **(65)**. However, the detection of even susceptible isolates in clinical specimens underscores the need for ongoing microbiological surveillance, particularly in high-risk procedures **(61).** Future investigations should include environmental sampling to identify potential reservoirs **(59),** expanded antimicrobial testing to include last-resort agents like colistin (**67)**, and molecular characterization to detect virulence factors associated with severe infections **(60)**.

8.3. Staphylococcus aureus

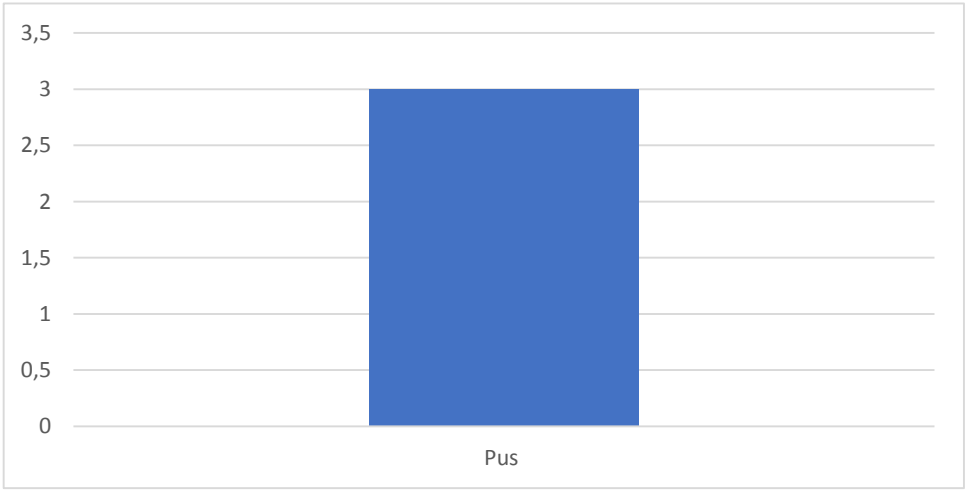

Figure 37 : distribution of Staphylococcus aureus in the samples (original 2025).

Figure 38: macroscopic aspect of Staphylococcus aureus isolated in CHAPMAN medium (original 2025).

On chapman medium Staphylococcus aureus produces yellow colonies this is because it ferments mannitol,producing acid as a byproduct , which lowers the pH and causes the phenol red indicator it turn yellow

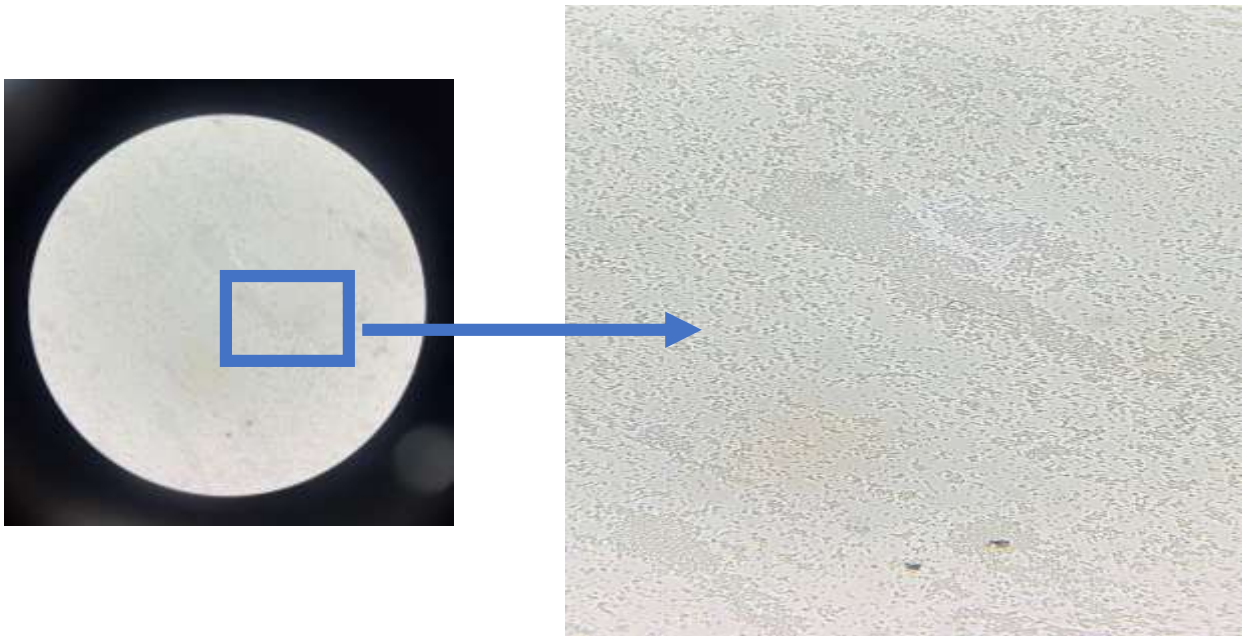

Figure 39: microscopic aspect of S.aureus (original 2025).

❖ **Gram stain**

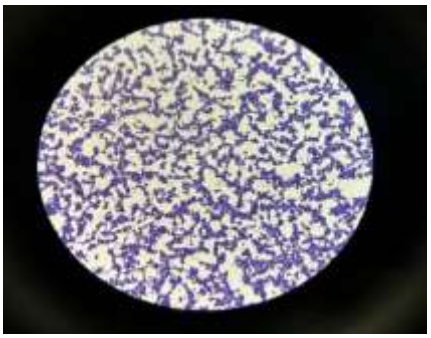

Figure 40 : Gram positive coccus (original 2025).

❖ **Coagulase test**

S.aureus produces coagulase;a, enzyme that clots plasma

Figure 41 : positive coagulase test (original 2025).

❖ **Catalse test**

Figure 42 : postive catalse test of .aureus (original 2025).

❖ **Oxydase test**

Figure 43: positive oxydase test of S.aureus(original 2025).

❖ **Antibiotic susceptibility test results**

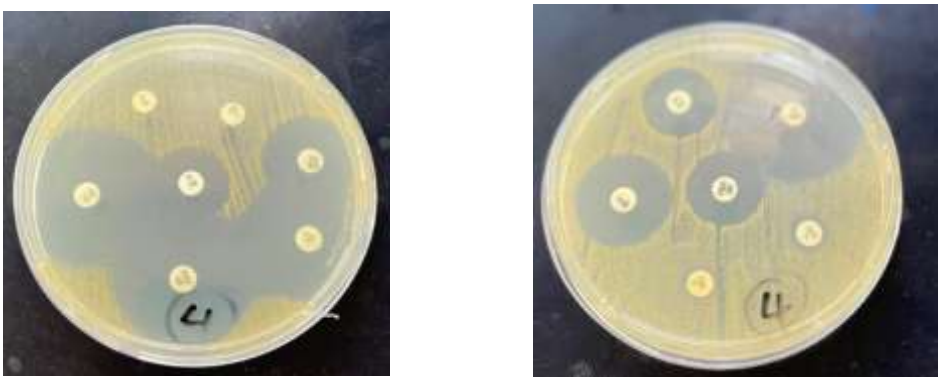

Figure 44: Antibiotic susceptibility test results of S.aureus in MH medium (original 2025).

Table 5: Antibiotic susceptibility test results of S.aureus (original 2025)

Antibiotics tested	Interpretation	Antibitics tested	Interpretation
penicilin	R	Gentamycin	S
Oxacilin	S	Fusidic acid	S
Cephotaxime	S	Vancomycine	S
Erythromycin	R	Rifamycin	S
clindamycin	R	Ciproflaxin	R
Teicoplanin	S	Ofloxacin	R
Kanamycin	S		

The present study identified Staphylococcus aureus isolates through comprehensive laboratory characterization using multiple diagnostic methods. Growth on Chapman medium, a selective and differential medium for staphylococci, demonstrated the

organism's ability to ferment mannitol, as evidenced by yellow colony formation **(60).** This observation is particularly significant as it represents a key diagnostic feature that helps differentiate S. aureus from other staphylococcal species **(66).**

Microscopic examination revealed Gram-positive cocci arranged in characteristic clusters, a morphological pattern typical of staphylococci **(68).** This finding was further supported by positive results in three critical biochemical tests: coagulase, catalase, and oxidase. The positive coagulase test, which detects the production of free coagulase or clumping factor, serves as a primary marker for S. aureus identification **(69).** This virulence factor contributes to the bacterium's pathogenicity by promoting fibrin clot formation, thereby protecting the organism from host immune defenses **(70).**

The catalase test yielded positive results, indicating the presence of the catalase enzyme that breaks down hydrogen peroxide into water and oxygen **(60).** This biochemical property distinguishes staphylococci from catalase-negative streptococci **(66).** Interestingly, the oxidase test also showed positive reactivity, which is somewhat unusual for S. aureus as most strains are typically oxidase-negative **(68).** This atypical result warrants further investigation as it may suggest the presence of rare oxidase-positive variants or potential technical issues during testing **(69).**

Of particular clinical concern was the detection of penicillin resistance in all isolates. This finding aligns with global surveillance data reporting high rates of penicillin resistance among S. aureus clinical isolates, with recent studies indicating resistance rates exceeding 90% in many healthcare settings **(71).** The resistance mechanism typically involves production of β-lactamase (penicillinase), encoded by the blaZ gene, which hydrolyzes the β-lactam ring of penicillin **(72).** This resistance pattern has significant therapeutic implications, as it renders penicillin ineffective for treatment of infections caused by these isolates **(69).**

The combined findings of typical morphological characteristics, biochemical profile (with the exception of the oxidase result), and predictable antibiotic resistance pattern strongly support the identification of these isolates as Staphylococcus aureus **(68).** The penicillin resistance observed underscores the importance of routine antimicrobial susceptibility testing to guide appropriate therapy **(71).** Furthermore, the unusual oxidase positivity suggests the need for additional confirmatory testing, such as MALDI-TOF mass spectrometry or molecular methods, to rule out potential misidentification or the presence of rare variants **(60).**

From a clinical perspective, these results highlight several important considerations. First, the confirmation of S. aureus as the etiological agent in clinical specimens necessitates careful evaluation of infection control measures, particularly in healthcare settings **(70).** Second, the penicillin resistance pattern suggests that alternative antimicrobial agents, such as β-lactamase-stable penicillins (e.g., oxacillin) or other classes of antibiotics, should be considered for empirical therapy **(72).** Finally, the atypical oxidase result emphasizes the value of using multiple diagnostic methods to ensure accurate microbial identification in clinical microbiology laboratories **(66).**

9. CRP and PROLACTINE results

Table 6: CRP and PRL results (original 2025)

CRP (mg/L)	32	32	48	24	12	48	12	32	24	12
PRL ng/Ml	**120,6**	**50,7**	**126,5**	**125**	**135**	**41,35**	**37,8**	**41,2**	**48**	**39,19**

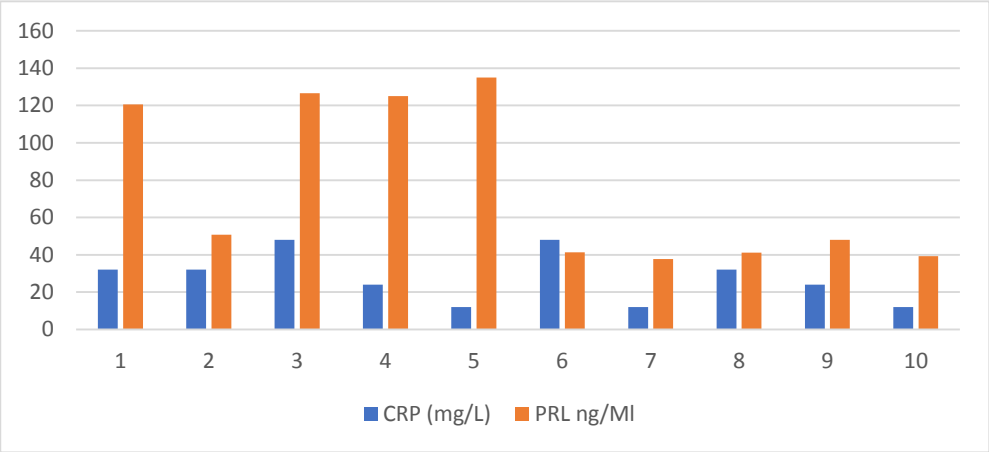

Figure 45: CRP and PRL results (original 2025).

The CRP measurements showed considerable variability among participants, with values ranging from 12 mg/L to 48 mg/L (mean: 27.6 mg/L, median: 24 mg/L). These elevated levels (normal range: <10 mg/L) indicate a significant systemic inflammatory response in all cases **(73) ;(74).** The bimodal distribution of values, with clusters around 12-24 mg/L and 32-48 mg/L, suggests two distinct groups of inflammatory response intensity **(75).**

The persistently elevated CRP levels across all measurements are clinically significant, as values >10 mg/L are strongly associated with active infection or tissue damage **(76)**. The highest recorded value of 48 mg/L represents a marked acute phase response, typically seen in severe bacterial infections or significant tissue trauma **(74)**. The repeated measurements showing sustained elevation (e.g., multiple 32 mg/L and 48 mg/L readings) indicate ongoing inflammatory processes rather than transient responses **(75).**

The prolactin levels demonstrated extreme variability, ranging from 37.8 ng/mL to 135 ng/mL (mean: 72.6 ng/mL, median: 48 ng/mL). These values are substantially elevated compared to normal ranges (typically <20 ng/mL in men and non-pregnant women) **(77); (78).** The distribution shows two distinct patterns: a cluster of moderate elevations (37.8-50.7 ng/mL) and a group of marked hyperprolactinemia (120.6-135 ng/mL) **(79).**

The highest recorded value of 135 ng/mL is particularly notable, as levels >100 ng/mL are strongly suggestive of prolactin-secreting pituitary adenomas (prolactinomas) **(79)**. The lower but still elevated values (37.8-50.7 ng/mL) may indicate medication effects, stress responses, or mild hypothalamic-pituitary dysfunction **(78).**

General Conclusion

General Conclusion

This comprehensive investigation of nosocomial pathogens in khenchela hospital setting maternity has yielded significant findings with important clinical and public health implications. The study characterized multiple bacterial isolates, including Staphylococcus aureus and Pseudomonas aeruginosa, while also analyzing systemic inflammatory and endocrine markers through CRP and prolactin measurements.

The microbiological analysis revealed crucial patterns in pathogen distribution and resistance profiles. Staphylococcus aureus isolates demonstrated typical morphological characteristics on Chapman medium and through Gram staining, with biochemical testing confirming coagulase and catalase positivity, although the unexpected oxidase positivity warrants further investigation. The universal penicillin resistance among these isolates reflects the well-documented global trend of β-lactam resistance in S. aureus and underscores the necessity for routine antimicrobial susceptibility testing to guide appropriate therapy.

The Pseudomonas aeruginosa isolates showed a concerning clinical distribution, particularly in post-curettage infections, suggesting potential gaps in sterilization protocols. While these isolates maintained typical biochemical profiles (catalase and oxidase positive), their surprising antibiotic susceptibility presents both therapeutic opportunities and questions about resistance patterns in this healthcare setting. This finding may indicate effective antimicrobial stewardship or possibly the circulation of less resistant community-acquired strains in the hospital environment.

The analysis of inflammatory markers revealed consistently elevated CRP levels (12-48 mg/L) across all measurements, indicating significant systemic inflammation in the study population. The prolactin measurements demonstrated marked hyperprolactinemia (37.8-135 ng/mL), with the highest values suggesting possible prolactinomas or significant stress-induced pituitary dysfunction. The correlation between elevated CRP and prolactin levels may indicate an important interaction between systemic inflammation and neuroendocrine regulation that merits further investigation.

Several key recommendations emerge from these findings:

- Implementation of enhanced infection control measures, particularly in surgical and gynecological procedures
- Ongoing antimicrobial resistance surveillance with expanded testing panels

- Consideration of pituitary imaging for patients with prolactin levels >100 ng/mL
- Regular review of antibiotic stewardship programs
- Further research into the oxidase-positive S. aureus isolates to determine if this represents a novel variant or technical artifact

The study's limitations include its single-center design, relatively small sample size for some pathogens, and lack of molecular characterization of resistance mechanisms. Future research should incorporate genomic analysis of bacterial isolates, longitudinal monitoring of inflammatory markers, and correlation with clinical outcomes.

These findings collectively contribute to our understanding of nosocomial infections in this healthcare setting while raising important questions about pathogen behavior, antibiotic resistance patterns, and the complex interplay between infection and endocrine response. The results emphasize the need for continued vigilance in infection control practices, judicious antibiotic use, and comprehensive diagnostic approaches to ensure optimal patient care and public health protection.

This research provides a foundation for future studies examining the epidemiological trends of nosocomial pathogens, the evolution of antibiotic resistance, and the biological connections between infection, inflammation, and endocrine function in hospitalized patients.

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Formulaire d'échantillonnage

Cette étude destinée aux patientes hospitalisées dans l'**établissement hospitalier spécialisé SALHI BELKACEM** au niveau des services : GHR/GYNECO/SUITE DE COUCHE

N ° :

Nom :

Prénom :

Age :

Consentement de patient, pour autoriser les étudiantes à utiliser les échantillons dans l'étude .

Date d'admission	Le :
Motif d'hospitalisation	
Unité d'hospitalisation	
Type d'intervention	
Nombre de parité	
ATCD d'infection	
ATCD médicaux	
ATCD chirurgicaux	
Antibiothérapie	
Échantillons	Sang Urines Pus
Bilans réalisés	CRP PROLATINE
Résultats	CRP PROACTINE
Date de sortie	Le :

Abstract

Nosocomial infections, or healthcare-associated infections (HAIs), were first identified in the nineteenth century and remain a serious threat to patient safety and a major public health challenge in healthcare settings. Despite ongoing efforts and various control strategies implemented in recent years, data indicate that about 10% of hospitalized patients still contract an infection during their stay. One of the key factors complicating the management of HAIs is the rising problem of antimicrobial resistance, which, although not present in all bacterial species, is notably concentrated among a few types.This study was carried out over a one-month period,on March 2025. This phase involved sample collection at Salhi Belkasem Specialized Hospital, where clinical specimens were obtained from pregnant women admitted to the Gynecology and High-Risk Pregnancy (GHR) wards. Higher than 100 pregnant women hospitalized during March, 10 were diagnosed with infections, including urinary tract infections, surgical site infections, and vaginal infections. Them the collected samples were transferred to the microbiology laboratory at Al-Balsam Al-Chafy Clinical Center, Khenchela, for microbiological and biochemical analysis. The study reveals that the identified germs, characterised by multiple approaches, cause major biochemical alterations in the body, highlighting their critical pathogenic efect.

Keywords

Nosocomial infections pregnant woman antimicrobial resistance biochemical analysis

Résumé

Les infections nosocomiales, ou infections associées aux soins (IAS), ont été identifiées pour la première fois au XIXe siècle et restent aujourd'hui une menace sérieuse pour la sécurité des patients ainsi qu'un défi majeur de santé publique dans les établissements de soins. Malgré les efforts constants et les diverses stratégies de contrôle mises en œuvre ces dernières années, les données indiquent que près de 10 % des patients hospitalisés contractent encore une infection durant leur séjour. L'un des facteurs clés qui complique la gestion des IAS est le problème croissant de résistance aux antimicrobiens, qui, bien qu'il ne concerne pas toutes les espèces bactériennes, est particulièrement concentré chez quelques types seulement. L'étude a été menée sur une période d'un mois, en mars 2025. Cette phase a consisté à collecter des échantillons au niveau de l'hôpital spécialisé Salhi Belkasem, où des prélèvements cliniques ont été réalisés auprès des femmes enceintes admises dans les services de gynécologie et grossesse à haut risque (GHR). Superieur à 100 femmes enceintes hospitalisées en mars, 10 ont été diagnostiquées comme infectées, notamment avec des infections urinaires, des infections du site opératoire et des infections vaginales. Les échantillons recueillis ont ensuite été transférés au laboratoire de microbiologie du centre clinique Al-Balsam Al-Chafy, à Khenchela, pour des analyses microbiologiques et biochimiques. L'étude révèle que les germes identifiés, caractérisés par plusieurs approches, provoquent des altérations biochimiques majeures dans l'organisme, soulignant ainsi leur l'effet pathogène critique.

Mots-clés :

Infections nosocomiales, femme enceinte, résistance aux antimicrobiens, analyse biochimique

ملخـــــــص

العدوى المكتسبة من المستشفى، أو ما تُعرف بالعدوى المرتبطة بالرعاية الصحية (HAIs)، تم التعرف عليها لأول مرة في القرن التاسع عشر ولا تزال تشكل تهديداً جدياً لسلامة المرضى وتشكل تحدياً كبيراً للصحة العامة في مؤسسات الرعاية الصحية .وعلى الرغم من الجهود المتواصلة والاستراتيجيات المختلفة لمكافحة العدوى التي تم تطبيقها في السنوات الأخيرة، فإن المعطيات تشير إلى أن حوالي 10%من المرضى المنومين يصابون بعدوى خلال فترة إقامتهم في المستشفى .إن أحد العوامل الرئيسية التي تُعقّد من إدارة هذه العدوى هو مشكلة ارتفاع معدلات مقاومة مضادات الميكروبات، والتي وإن لم تكن موجودة في جميع الأنواع البكتيرية، فهي مركزة بشكل لافت لدى بعض الأنواع فقط .أُجريت الدراسة على مدى شهر واحد، في مارس 2025.شملت هذه المرحلة جمع العينات في مستشفى "البوعلي سلحي بلقاسم "المتخصص، حيث تم أخذ عينات سريرية من النساء الحوامل اللواتي دخلن إلى قسم طب النساء والحملات ذوات الخطورة العالية .أكثر من 100 امرأة حامل تم نقلهن إلى المستشفى خلال شهر مارس، تم تشخيص إصابة 10منهن بعدوى، ومن ضمنها التهابات المسالك البولية، والتهابات مواضع العمليات الجراحية، والالتهابات المهبلية .ثم نُقلت العينات المجمعة إلى المختبر الميكروبيولوجي التابع لمركز "الباسم الشافي "السريري بخنشلة، لإجراء الفحوصات الميكروبيولوجية والكيميائية الحيوية .وقد كشفت الدراسة أن الجراثيم المُحددة، والمُميزة عبر عدة مناهج، تُسبب تغييرات كيميائية حيوية كبيرة في الجسم، مما .يبرز دورها المرضي الجوهري .

الكلمات المفتاحية

العدوى المستشفيّة، المرأة الحامل، مقاومة مضادات الميكروبات، التحليل الكيميائي الحيوي