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Antibiotics Sensitivity of Bacteria Types Isolated from burns and Wounds in Babylon Governorate

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Annotation: The current study involved collecting 100 clinical samples, taken directly from burn and wound swabs, from patients of various ages and sexes, between the beginning of November 2025 and the end of December 2025, from both inpatients and outpatients at Al-Hilla Teaching Hospital. The samples were cultured on blood agar and MacConkey agar. Bacterial isolates were identified using selective and differential media. Biochemical tests were then performed to confirm the diagnosis of each isolate. Based on the results of biochemical diagnosis, the bacterial species were Gram-positive: 22 isolates of Staphylococcus aureus, and Gram-negative, comprising 4 bacterial species: 26 isolates of Klebsiella pneumoniae, 14 isolates of Pseudomonas aeruginosa, 12 isolates of Proteus mirabilis, and 8 isolates of Escherichia coli. The antibiotic susceptibility of all different bacterial isolates was tested against 24 different antibiotics using the disc diffusion method. The results showed that many bacterial isolates exhibited multidrug resistance (MDR). The current study indicated that a total of 22 Staphylococcus aureus isolates showed multidrug resistance to the following antibiotics: the highest resistance rate was against ofloxacin and clarithromycin (73%), while clindamycin resistance was 55%. Resistance to doxycycline and chloramphenicol was 27%, while all isolates showed 100% sensitivity to gentamicin.

The study also showed that the 26

Klebsiella pneumoniae isolates exhibited varying levels of resistance, including a high resistance rate of 100% to amoxicillin, and resistance rates of 92% to cefotaxime and cefepime. 85% of isolates showed resistance to Ciprofloxacin (31%), Tetracycline (38%), Aztreonam (54%), Amoxicillin-clavulanate (23%), Co-trimazole (15%), Levofloxacin (15%), Gentamicin (23%), Amikacin (8%), Imipenem (8%), and Levofloxacin (15%).

Fourteen Pseudomonas aeruginosa isolates showed high resistance to the antibiotics Cefepime (100%), Ceftazidime (86%), and Ticarcillin-clavulanate (71%). Bacterial isolates also showed resistance to Ciprofloxacin, Ofloxacin, and Aztreonam (57%). All isolates showed sensitivity to these antibiotics. With a resistance rate of 100% to Piperacillin/Tazobactam, while Meropenem, Imipenem, and Netilmicin showed low resistance (14%). As for Proteus mirabilis, its isolates showed high resistance (100%) to Amoxicillin, while the resistance rate was 50% for Cefepime, Amoxicillin-clavulanate, and Tetracycline. It also showed high resistance (67%) to Cefotaxime, Gentamicin, Ciprofloxacin, Aztreonam, and Amikacin (33%). Eight E. coli isolates showed 100% resistance to Amoxicillin-clavulanate, Cefepime, Cefotaxime, Amoxicillin, and Ciprofloxacin. Aztreonam, Levofloxacin, Gentamicin 75% and 50%, while the percentage was 25%. In Hassan, there was a high sensitivity (100%) to Tetracycline and Co-triazole.

Keywords: Burns; Wound Healing; Bacterial Infection; Antibiotics; Inflammatory Response.

1.1. Introduction:

Burns and wounds are among the most common public health problems worldwide. Many factors contribute to this, including heat, chemicals, electricity, and scalding. Although bacteria are present, most burns and wounds heal through natural repair mechanisms and various treatments (Rose and Chan, 2016). However, the healing process may be slowed or halted in some cases, not only due to the effects of bacteria present on the skin, but also because of the intervention of various other autoimmune causes and associated diseases, as well as uncontrolled and ineffective inflammatory responses, which significantly impact the natural healing process

of burns and wounds (Javia et al., 2018).

A wound is defined as a disruption of the skin's protective function; that is, a loss of epithelial continuity, with or without the loss of underlying connective tissue (such as muscles, bones, and nerves) (Fon and Huang, 2020). Skin injuries, especially chronic wounds, are among the most common medical problems in the developed world. Impaired angiogenesis causes numerous problems during wound healing and skin regeneration, given its vital role in tissue renewal. Therefore, stimulating or enhancing angiogenesis can be an effective strategy for accelerating wound healing (Noor et al., 2021).

Wounds are commonly infected by a variety of microbes and viruses that may be transmitted through the patient's natural bacteria or through environmental or skin bacteria present on medical staff (Varina Jr. et al., 2013). Burn patients have an increased risk of infection due to their weakened immune systems, which can lead to sepsis. There are numerous causes of infection, including skin irritation, immunodeficiency, surgical procedures performed in healthcare facilities, and prolonged hospital stays (Ahmed, 2012; Chaudhry et al., 2019).

1.2. The objectives of this study were:

1- To isolate and identify the bacterial species present in burn and wound samples using standard laboratory methods.

2- To identify the most common bacterial species in burn injuries compared to wounds.

3- To identify the most effective antibiotics against bacteria isolated from burns and wounds.

2. Literature Review

2.1. Burns and Wounds

Burns are a global public health problem, causing an estimated 180,000 deaths annually, mostly in developing countries and the WHO South-East Asia region. Compared to developed countries, the infant mortality rate in developing countries is seven times higher (Javia et al., 2018). Burns have significant economic, material, psychological, and social impacts due to the substantial annual expenditure on their treatment. Risk factors for burns include socioeconomic status, race, culture, gender, and age, as well as other factors related to place of residence and the cause of injury (Shariati et al., 2019).

Wounds, on the other hand, are simply defined as a disruption of tissue continuity at the cellular and anatomical levels (Bennett et al., 1988). They may result from physical, chemical, thermal, microbial, immunological, or viral injuries. Wound healing comprises a complex series of cellular and biochemical processes that restore the structural, histological, and functional integrity of injured tissues, along with their strength and normal state (Raina et al., 2008).

2.2 Wound Classification

Wounds can be classified in several ways, including their cause, location, type of injury or symptoms, nature, depth, tissue loss, or clinical appearance. Wounds are classified as open or closed based on their underlying cause, and as acute or chronic based on the physiology of wound healing (Sabali et al., 2012).

1. Open Wounds

In this case, blood leaks from the body, and bleeding is evident. These are further classified as: lacerations, lacerations, abrasions or superficial wounds, puncture wounds, penetrating wounds, and gunshot wounds (Sabaly et al., 2012).

2. Closed Wounds

In this case, blood leaks from the circulatory system but remains within the body. These include bruises, hematomas, and crush injuries (Sabaly et al., 2012).

3. Acute Wounds

In this case, an acute wound is a tissue injury that typically heals through a regular and timely remodeling process, resulting in a sustained restoration of anatomical and functional integrity. Acute wounds are usually caused by lacerations or surgical incisions, and the wound healing process is completed within the expected timeframe (Sabaly et al., 2012).

4. Chronic Wounds

Chronic wounds are wounds that have not healed normally, leading to a pathological inflammatory condition. These wounds either require a long time to heal or recur frequently (Javia et al., 2018).

2.3 Classification of Burns

Burns or contusions can result from heat emitted by gaseous, liquid, or solid substances, or chemicals. The duration and temperature of the burn determine the extent of the damage. Electricity and chemicals can also cause burns. Skin burns can be assessed according to the three-degree injury classification system: first, second, and third degree (Callum et al., 2017). First-degree burns are clinically characterized by redness, pain, and slight swelling of the skin. The redness is due to vasodilation, while the swelling is due to increased vascular permeability. First-degree burns do not cause necrosis; sunburn is an example of a first-degree burn (Callum et al., 2014).

Second-degree burns: Clinically, second-degree burns present as blisters, pain, and redness. Histologically, the blisters result from separation of the epidermis from the dermis. Necrosis in these burns is concentrated in the epidermis, with the dermis remaining unaffected (Callum et al., 2014). Third-degree burns: Clinically, these burns appear dry, waxy white, leathery, brown, or charred. Due to nerve damage, pain sensation is reduced or absent. Third-degree burns damage both the epidermis and dermis, leading to necrosis. Histologically, charring occurs in both the epidermis and dermis, and cell death is present. Regeneration is limited to the skin appendages, if they remain undamaged (Callum et al., 2014).

Burn and wound infections are a major cause of morbidity and mortality in the developed world (Abelgren et al., 2002). Burn patients are more susceptible to hospital-acquired infections, catheter-associated infections, thrombophlebitis, and pneumonia due to their compromised immune systems resulting from skin exposure (Mosser and Pham, 2009). Significant progress has been made in burn treatment over the past six decades, particularly in treatment procedures, infection prevention, prevention of metabolic overreactions, and strategic management (Munavo, 1992). Despite these major advances, burns remain the leading cause of death in burn centers (Sharma et al., 2006). Burn injuries are a global public health concern, as they damage the largest organ or parts of the human body—the skin—which provides homeostasis, temperature regulation, sensation, immune defense, and acts as a barrier against infection (Pujji et al., 2019).

2.5 Classification of Bacteria Infecting Burns and Wounds

Most bacteria in the laboratory are classified as Gram-positive or Gram-negative. Both have a peptidoglycan cell wall and a protein-phospholipid bilayer membrane. The thick peptidoglycan layers in the cell wall of Gram-positive bacteria stain purple, while Gram-negative bacteria have a distinctive outer membrane with a thinner peptidoglycan layer and a periplasmic space between the cell wall and membrane, which is pink. Gram-negative bacteria contain lipopolysaccharides (LPS), purine channels, and the lipoprotein murin in their outer membrane, components not found in Gram-positive bacteria (Jawetz et al., 2010).

2.6 Gram positive Bacteria

2.6.1. Staphylococcus aureus

Staphylococcus aureus is a normal flora of human skin and mucous membranes and their spread

is either endogenously or from infected skin. S.aureus is gram positive bacterium spherical bacteria, non-motile, non-spore forming, facultative anaerobe, that is catalase and coagulase positive, it oxidase negative (Bitrus et al., 2018).

They are requiring certain important amino acid and vitamins for growth and can have the ability to with stand high concentrations of salts. cell wall is made up of peptidoglycan which contains crosslinks of glycine residue that allows sensitivity towards lysostaphin(Lindsay, 2014).

Multiple antibiotic resistant Staphylococcus aureus. Methicillin-resistant Staphylococcus aureus (MRSA) have been a major public health concern in humans (Tanomsridachchai et al., 2021).

Pathogenicity of staphylococcus aureus

Staphylococcus aureus is the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. It opportunistic pathogen (Bagnoli et al., 2018). This bacteria cause skin infection, abscess formation, boils, acne and impetigo. Infections of deeper tissue and organs include pneumonia, bacteremial. Endocarditis, meningitis osteomylitis and cystitis. Sepsis, Enteritis due to enterotoxins contamination of food, toxic shock syndrome (Becker, 2018). S. aureus is a major cause of nosocomial and community acquired infections (Lacoma et al., 2021).

2.7. Gram Negative Bacteria

2.7.1 Enterobacteriaceae

Enterobacteriaceae is a family of gram negative, facultatively aerobic and anaerobic, non-spore forming rods, characteristics of this family include being motile, catalase positive, and oxidase negative and glucose fermentation with no gas production (Belluco et al., 2016).

The bacteria belonging to this family are characterized by being widespread in nature, as they are found in soil, water and plants, and it is clear from the name of this family that they live in the digestive tract of humans and animals and cause pathological injuries as they have been isolated from wounds, burns, sputum, urine and excretion, and others (Penalver et al., 2005). This family lives in the gastrointestinal tract, as is evident from the family name, Wounds, sputum, excretion, fluid, insertion and blood in addition to its presence as a natural flora, and with a high percentage in the channel for humans and animals (van Duin et al., 2013).

2.8.1.1 Klebsiella pneumoniae

Klebsiella pneumonia belongs to the Enterobacteriaceae family (Greenwood et al., 2007). Klebsiella was first described by Karl Friedlander in 1882 as a bacterium isolated from the lungs of patients who died of pneumonia (Friedländer, 1882).

Klebsiella is a genus of gram negative, non spore forming, facultative anaerobes, rod shaped bacterium measuring (0.3-1.0) μm in width and (0.6 - 6.0) μm in length, nonmotile, lactose fermentation, Klebsiella species exhibit mucoid growth K. pneumoniae organisms occur in soil and water and on plants, and some strains are considered a part of the normal flora of the human gastrointestinal tract (Melegh, 2015).

Klebsiella grows at a temperature of (12- 43)°C, and the optimum degree for their growth is about 37°C, and are killed at a temperature of 55°C for half an hour (Melegh, 2015)

Pathogenicity of Klebsiella pneumoniae

Pathogenicity is the ability of bacteria to cause disease. The diseases caused by this bacterium are diseases that are transmitted in hospitals,including urinary tract infections, respiratory tract infections, surgical wounds and meninges (Li et al., 2014). Klebsiella are the second cause of bacteremia and Septicemia in children and newborns, and acute illnesses, especially in people with weak immune systems due to diabetes, chronic heart disease, and pulmonary vasoconstriction, especially in the elderly and newborns. Childbirth, and that most hospitalized,

Klebsiella infections are highly fatal if not treated properly (Damian et al., 2009).

Klebsiella pneumoniae is an important opportunistic pathogen and a frequent cause of nosocomial infections (Struve and Krogfelt, 2004).

2.7.2 Pseudomonas aeruginosa

Pseudomonas aeruginosa was first isolated in 1882 by Scientist Gessard from cases of purulent wounds and named Psedomonas pyocyaneas, and after that it was named Pseudomonas aeruginosa (Moore and Flaws, 2011), They are belongs to the Pseudomonadaceae family (Conti et al., 2009; Holt et al., 1994).

Pseudomonas aeruginosa is gram negative rods. The cell diameter ranges between (0.5-0.8) mμ and the length is (1.5-3) mμ, where the cell appears single or in pairs or short chains (Todar, 2008), non spore forming, noncapsulate, motile by one or two pollar flagella, obligately aerobic, the optimum temperature for growth is 37°C but has the ability to grow at a temperature of 42°C (growth at 42°C is an important characteristic that distinguishes this species from the genus), and the optimum pH for growth is between (7.4-7.6) (Garbe et al.,2011). The bacteria produces a number of pigments, the most important of which are the blue green pigment (biocyanin). The culture medium of the bacteria acquires the color of biocyanins such as Muller Hinton agar and nutrient agar, which is a non-fluorescent pigment. It has great diagnostic significance for P.aeruginosa because it is the onlyspecies within this genus that has the ability to produce this pigment, the other pigment being the yellowish green pigment (Pyoverdin). It is a fluorescent dye and there are other pigments such as the red dye (Pyorubin) that is stimulated by adding the amino acid glutamate to the culture medium, and the scarcity of isolates produces the dark brown pigment (Pyomelanin) that is catalyzed by adding aromatic amino acids to the culture medium such as tyrosine (El-Fouly et al.,2015).

Pathogenicity of Pseudomonas aeruginosa

Pseudomonas aeruginosa is an increasingly prevalent human opportunistic pathogen (Diggle and Whiteley, 2020), and is one of the most common bacteria found in hospital infections responsible for hospital-acquired pneumonia, hospital-acquired urinary tract infections, surgical wound infections and bloodstream infections (De Bentzmann and Plésiat, 2011). P.aeruginosa is one of the most common and deadly pathogens, it causes wound and burn infections, and can cause a skin infections, both localized and diffuse. The common predisposing factors are breakdown of the integument which may result from burns, trauma or dermatitis, urinary tract infections, ear infection, gastrointestinal infections, eye infections (Campa et al., 2012).

2.7.3 Escherichia coli

Escherichia coli was first diagnosed by German scientist Escherich Theodore in 1885 during his study on the normal bacteria in the intestine in the feces of infants as commensal bacteria, the intestine is colonized immediately after birth. E coli are (1.1-1.6) μm in diameter and (2.0-5.0) μm in length. They are gram-negative bacilli, mobile by the flagellum of the peritoneum. The flagellum surrounds the whole body and does not form spores (Riedel et al., 2019).

Its colonies are soft, slightly convex, moist, mucous or non-mucous when possessing a capsule. Shiny pink on the MacConkey agar, green metali sheen on the Eosin Methylene Blue, while hemolysis occurs on blood agar. its growth in pH (4.4-9). The optimum temperature for its growth (36-37)°C. It is also positive for catalase, negative for oxidase and urease, and positive indole. E.coli able to fermentation a large range of carbohydrates with both acid and gas production (Hemraj et al., 2013).

Pathogenicity of E. coli

Escherichia coli, often lovingly referred to as E. coli, holds a prominent place within the family of Enterobacteriaceae (Brown et al.,2017). This bacterium, while necessary in some contexts, is primarily seen as an opportunistic invader. It is capable of causing a range of diseases, including

distressing conditions like diarrhea, meningitis, septicemia, and bacteremia. E. coli is notably common in urinary tract infections, and its presence can lead to a host of challenges for both humans and animals. Additionally, it can be responsible for respiratory infections, wound infections, and healthcare-associated infections, particularly following surgical procedures (Nolan et al., 2020). What makes E. coli particularly concerning is its ability to produce various virulence factors, which enable it to cause a significant number of enteric infections. These can present as severe conditions such as hemorrhagic colitis, dysentery, and travel-related diarrhea. In vulnerable groups, especially young children and infants, (Donnenberg and Finlay, 2013). E. coli can lead to serious diarrhea, highlighting the importance of understanding this bacterium's impact on health. Beyond gastrointestinal illnesses, E. coli is also linked to a variety of other medical issues, including pneumonia and conjunctivitis, as well as more serious conditions like endocarditis and skin and soft tissue infections. The breadth of diseases associated with this bacterium underscores the necessity for awareness and proactive management when it comes to infections caused by E. coli. (Olowe et al., 2017).

2.7.4 Proteus mirabilis

The story of Proteus mirabilis begins with the distinguished scientist Hauser, who first identified this genus in 1885. Hauser's meticulous work led to the isolation of Proteus from feces, wastewater, and organic matter. With this discovery, a new chapter in microbiology was opened, providing insights that continue to inform our understanding of this diverse and complex group of bacteria. (Dzutsev and Trinchieri, 2015).

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Pathogenicity of P. mirabilis

Proteus mirabilis is a microorganism of the Enterobacteriaceae family, due to its health problems for humans and animals. It causes infection of the digestive system and the reproductive system, and the medical importance of this bacterium comes after the bacteria E.coli and K.pneumonia

because it causes health problems and pathological infections of the reproductive system, digestive system and other systems (Armbruster et al., 2017). Also, P.mirabilis bacteria cause diarrhea in children and adults and cause skin ulcers as well (Sayal et al., 2018).

It was possible to isolate it from cases of middle ear infection and meningitis infection in new-borns, as well as Gastrointestinal Syndrome, and this bacteria was isolated from patients with various wounds and abscesses. Ear infection, meningitis, and bacteraemia, which may sometimes result from an injury to the urinary tract or as a result of performing surgical operations resulting in Systemic Inflammatory Response Syndrome, symptoms of fever and low level of white blood cells, type Neutropenia, it is believed that P. mirabilis has an important role in infection (Rheumatoid arthritis), because antibodies to these bacteria were found in people with this disease (Filipiak et al., 2020).

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Pathogenicity of P. mirabilis

Proteus mirabilis, a member of the Enterobacteriaceae family, is a microorganism that can lead to various health issues in both humans and animals. It plays a significant role in infections affecting the digestive and reproductive systems, and its medical relevance is notable, coming right after well-known bacteria like E. coli and K. pneumoniae. This little bacterium can create quite a few challenges, often leading to infections that impact not just the digestive tract but also the reproductive and other bodily systems (Armbruster et al., 2017). For instance, P. mirabilis can lead to diarrhea in both children and adults, and has been known to cause discomforting skin ulcers as well (Sayal et al., 2018). Beyond these, there have been instances where this bacterium was identified in cases of middle ear infections and meningitis in newborns. It has even been isolated from patients with diverse wounds and abscesses, highlighting its opportunistic nature. While it may seem daunting, understanding the role of such microorganisms in our health is a crucial part of caring for ourselves and our loved ones. With awareness and proper care, we can navigate these challenges more effectively. (Filipiak et al., 2020).

2.8 Multiple Drug Resistance of Bacteria

Resistance refers to the ability of bacteria to withstand the effects of antibacterial agents, preventing them from effectively disrupting reproduction or killing the bacteria. Over the years, the widespread use of antibiotics has led to the emergence of multiple drug resistance, creating significant challenges in treating these infections (Shaikh et al., 2016). The Infectious Diseases Society of America (IDSA) has identified antimicrobial resistance as one of the most pressing threats to human health globally (America, 2011). It’s a serious concern, one that impacts countless lives and the effectiveness of medical treatments. Bacteria employ several mechanisms to develop this resistance. For instance, they can alter their cell walls or modify the drugs themselves through various enzymes. There are changes like mutations in ribosomes or the methylation of 16S rRNA that further contribute to their resilience. Additionally, some bacteria may increase the production of enzymes capable of modifying or degrading antimicrobials, such as aminoglycosides and beta-lactamase. It is essential to understand these complexities, as they not only highlight the ingenuity of these microorganisms but also remind us of the importance of responsible antibiotic use and ongoing research in combating these challenges. Together, we can work towards solutions that prioritize health and well-being for everyone. (Shaikh et al., 2016).

2.9 Virulence Factors of Bacteria

Virulence factors are substances that bacteria make that encourage infection and growth in a host through a number of different processes. While some VFs can be acquired by mutation or the acquisition of mobile genetic elements (MGEs), others can be created universally by a species of bacteria (Bien et al., 2011).

2.9.1. Immune System Evasion and Host Cell Invasion

Bacteria adhere to extracellular matrix (ECM) proteins or host cell surface receptors using long, hair-like projections called pili (Webb et al., 2008). Additionally, bacteria can pass across transcellular gaps to penetrate the host's epithelial surface. Once within the host cell, they can either escape being taken up by phagosomes, actively use toxins to destroy phagosomes, stop the production of phagolysosomes, or leave phagosomes and live in the intracellular environment (Webb et al., 2008).

2.9.2. Biofilm Formation and Quorum Sensing

By secreting tiny chemicals known as autoinducers, bacteria employ quorum sensing to interact with one another. Autoinducer concentrations approach a threshold when there are enough bacteria in an environment, which causes changes in VF production and the creation of biofilms that enable bacteria to share nutrients and withstand antibiotics (Deep et al., 2011).

Biofilms are sessile colonies of microorganisms that stick to biotic and abiotic surfaces through extracellular matrix (ECM) made of proteins, polysaccharides, and extracellular DNA. Quorum sensing and the intracellular secondary messenger c-di-GMP pathway are essential for the production of biofilms (Tolker and Nielsen, 2015; Hall et al., 2017).

2.9.3. Toxins

Gram-negative bacteria's outer cell membrane contains a significant amount of the endotoxin lipopolysaccharide (LPS). In humans, lipid A binds LPS to the cell membrane and is identified by toll-like receptor 4 (TLR-4), which causes the release of proinflammatory cytokines and endotoxic shock (Ramachandran et al., 2014).

2.9.4. Super antigens

Superantigens are extremely powerful toxins that are produced by Yersinia, Streptococcus, Mycoplasma, and Staphylococcus aureus. Superantigens (SAgs) can activate T cells without the need for antigen processing and presentation by crosslinking major histocompatibility complex (MHC) class 2 molecules on antigen-presenting cells (APCs) and T cells (Krakauer et al., 2019).

3. Materials and Methods

3.1. Materials

A range of instruments and equipment were used, including an autoclave, Bunsen burner, compound light microscope, laminar air flow hood, micropipette, oven, and petri dish. A variety of chemical and biological reagents were also used, such as AB blood, Gram stain, hydrogen peroxide (H2O2) (6%), oxidase reagent, Kovacs reagent, and methyl red. Additionally, several culture media were used, including blood base agar, eosin methylene blue (EMB) agar, MacConkey agar, mannitol salt agar, methyl red-Voges Proskauer (MR-VP) broth, Simmons citrate agar, Pseudomonas agar, nutrient broth, and peptone water broth, all manufactured by HIMEDIA and originating in India. A range of antibiotics were also used. Meropenem, Imipenem, Amoxicillin-clavulanate, Piperacillin-tazobactam, Ceftazidime, Cefotaxime, Cefepime, Ticarcillin-clavulanate, Gentamicin, Amikacin, Netilmicin, and a group of other antibiotics, all in a 10 µg concentration, were manufactured by HIMEDIA and originated in India.

3.2. Methods

3.2.1 Sterilization

Reagents, Solutions, and pigments were It was sterilized using an autoclave at 121°C and under pressure 15 pound/inch2 for 15 minutes. As for Glassware sterilized dry heat in the electric oven at 180°C for 2 h, while needles and loops sterilized by burner (Harrigan and McCance, 2014).

3.2.2 Preparation of Reagents and Solutions

According to Cheesbrough (2012), the solution was made by dissolving 0.85g of sodium chloride in 90 ml of distilled water (NaCl) until the volume reached 100 mL. The mixture was then autoclaved for 15 minutes at 121°C and 15 pounds per inch. After that, it was stored at 4°C until it was needed. The solutions were made by mixing 0.5 ml of 1% barium chloride solution (BaCl2.2H2O) with 9.5 ml of 1% (v/v) pure sulfuric acid solution. This resulted in a barium sulfate solution with a density high enough to produce turbidity equivalent to 1.5 ×108 CFU/ml bacterial suspension (Benson, 2002). One gram of tetra-methyl-p-phenylene-diamin-dihydrochloride was dissolved in one hundred milliliters of distilled water to create the oxidase reagent, which was employed as an indication in the oxidase test (Mac Faddin, 2000). To identify bacteria that produce catalase, this reagent was used at a concentration of 3% H2O2 diluted in D.W. and kept in a dark container (Forbes et al., 2007). Using a water bath, dissolve 5 grams of para-dimethyl aminobenzaldehyde in 75 milliliters of isopropyl alcohol. Then, add 100 milliliters of HCl. The reagent was kept in a dark bottle in the refrigerator, and the detector had a pale yellow tint (MacFaddin, 2000). Add 200 milliliters of distilled water to get the volume to 500 milliliters after dissolving 0.1 grams of methyl red in 300 milliliters of 95% concentrated methyl alcohol (MacFaddin, 2000). Vogas-Proskaur reagent: 5% alpha nephthol: dissolve 5 grams of alpha nephthol at a 95% concentration in 100 milliliters of ethyl alcohol. 40% potassium hydroxide (KOH).

3.2.3 Cultural Media

Every commercial media used in this study was produced in accordance with the manufacturer's instructions. Other cultural media were created in the lab, according to scientific references. All of these media were autoclaved at 121° C for 15 minutes at 15 pounds per square inch.

3.2.3.1 Blood-based agar

an enhanced bacterial medium that promotes the majority of bacterial species' development. produced in accordance with the manufacturer's instructions, 3.75 mg of blood agar base was added to 100 milliliters of distilled water, covered with cotton, and autoclaved at 121 degrees Celsius for 15 pounds (Forbes et al., 2007). After cooling the medium to about 50°C, 5% fresh human blood was added, and 15ml was aseptically dispensed into sterile Petri dishes. The dishes were then sealed in plastic bags and kept between 2 and 8 °C to prevent moisture loss. The main purpose of this medium was to separate bacteria and evaluate their capacity to hydrolyze blood (MacFaddin, 2000).

3.2.3.2 Agar MacConkey

Gram-negative bacteria were identified using selective and differential media. Lactose-fermenting bacteria, like E. coli, Klebsiella, and Enterobacter, are pinkish-colored, while non-lactose-fermenting bacteria, like Proteus, pseudomonas, and Shigella, are pale or yellow. Following the manufacturer's recommendations, 5.1 grams of the medium were suspended in 100 milliliters of distilled water, boiled until the medium was completely dissolved, autoclaved at 15 pounds of pressure, cooled for 15 minutes, and thoroughly mixed before pouring.

3.2.3.3 Eosin-methylene blue agar (EMB)

The colon bacillus, E. coli, may be identified and enteric lactose fermenters and non-lactose fermenters can be distinguished using lactose and Eosin methylene blue. Because so much acid

is created, the E. coli colonies were blue-black with a metallic-green sheen. (Josephine and others, 2006).

3.2.3.4 Salt Agar Mannitol

Staphylococci species were isolated using this medium as a selective medium (MacFaddin, 2000). The manufacturing company's recommendations were followed in its preparation.

3.2.3.5 Broth infused with brain and heart

This medium was made by autoclaving 95 ml of brain heart infusion broth and 5 ml of glycerol at 121ºC for 15 minutes. For a very long time, it was employed to preserve bacterial isolates (Forbes et al., 2007). The manufacturing company's instructions were followed throughout its preparation.

3.2.3.6 Agar with Pseudomonas:

This medium was made by dissolving 48.4 g of Pseudomonas agar powder in 1000 ml of distilled water, adding 10 ml of glycerol, autoclaving at 121°C for 15 minutes at 15 pounds per inch, and then cooling at 45°C. A single CFC supplement vial was added. The medium was then transferred into sterile Petri dishes (Baron et al., 1994).

3.2.3.7 Hinton agar Muller

Muller Hinton agar medium is made in accordance with the manufacturer (Himedia) and is available for purchase as a simple powder. Due to its live aseptic preparation, this medium is utilized for sensitivity testing. Sterilization was done in an autoclave at 121°C for 15 minutes (Pfaller et al.,2004).

3.2.3.8 Agar with nutrients

The purified isolates were subcultured on the slant medium and kept at 4°C in the refrigerator.

3.2.4 Specimen collection In order to identify the most significant bacteria in burns and wounds, fifty specimens (50 from burn infections and 50 from wound infections) were collected from patients at Baquba Education Hospital in Babile between November 2025 and December 2026 under the supervision of a specialist physician.

3.2.4.1 Sample Culturing

Samples from burns and wounds were rapidly cultivated on macConkey agar and blood agar. The isolates were purified using the streaking method on mannitol salt agar, Eosin Methylene Blue, and Pseudomonas agar. After the agar plates were incubated for 24 hours at 37°C, the bacteria under investigation were subjected to biochemical and diagnostic assays (AmezquitaLopez et al., 2018).

3.2.4.2 Bacterial Identification

Bacterial cells, colonies' morphological characteristics, microscopic analysis, and biochemical testing were used to isolate and identify the bacteria (Varadi et al., 2017).

3.2.4.3 Characterization of culture

Bacterial isolation and diagnosis were made while examining the phenotypic traits of isolates based on the size, shape, color, edges, and hemolysis capacity of the colony on blood agar.

3.2.5 Bacterial Isolate Preservation

A. Temporary Preservation

After being identified on the nutrient agar slant, bacterial isolates were inoculated. The tubes were kept at 4°C for a few weeks after being incubated for 24 hours at 37°C.

B. Extended Preservation

kept bacterial isolates in 20% glycerol for a considerable amount of time—one to two years. The medium was made by adding 2 milliliters of glycerol to 8 milliliters of brain heart infusion broth. It was then put in a flask and autoclaved for 15 minutes at 121 degrees Celsius and 15 bar of pressure per inch. After cooling, tubes were injected with a pure colony and cultured for 24 hours at 37°C. Ultimately, tubes were stored in deep freeze (Crocker and Burnett, 2005).

3.2.5 Test for antibiotic susceptibility

The following steps were taken in accordance with (CLSI, 2024) for the sensitivity test procedure:

1. Isolates were separated into single bacterial colonies on culture medium. Using 0.5 of a standard MacFarland solution, which yields a density of 1.5x 108 CFU/ml, the turbidity and density of the bacterial suspension were controlled for 18–24 hours in a test tube containing 3ml Normal Saline.

2. To achieve a uniform growth, a sterile cotton swab was dipped into the tube holding the bacterial suspension and applied to the Muller Hinton ager by wiping it on the medium's surface. The dishes were then allowed to dry for ten minutes at room temperature.

3. The inoculated Muller Hinton was covered with the antibiotic disk.

3.3. Analysis of statistics

Using the statistical software (SPSS) and suitable statistical techniques, the researcher analyzed and interpreted the data as follows: First, the variables' percentages Column forms come in second (Allison, 2012).

4. Results and Discussion

4.1 Bacterial isolation and identification

4.1.1 Isolation of bacteria

The study involved collecting 100 clinical samples (wounds and burns) from patients of various ages and genders at Al-Hilla Teaching Hospital in Babylon Governorate between the beginning of November 2025 and the end of December 2025, using direct swabs taken from the patients. The samples were cultured on MacConkey agar and blood agar using the streaking method. After culture, 82 clinical isolates of different bacterial genera were obtained. The results showed that only 18 samples did not exhibit growth, as the swabs were taken from the patients. This may be attributed to several factors, including inaccuracies in the techniques used, such as insufficient swab size to represent the contaminants, and the use of antiseptics or antibiotics. Table (4-1) shows the number of samples according to their source.

Sources	Specimens No. (%)	No. of Isolates	NO Growth	Gender	
				Male No.(%)	Female No.(%)
Wounds	50	38	12	14	24
Burns	50	44	6	26	18
Total	100	82	18	40	42

Table (4-1) The number of specimens according to their sources.

4.1.2 Bacterial identification

All bacterial isolates were identified using selective and differential culture media, as well as microscopic examination and biochemical tests. The VITEC compact 2 system was used for confirmation. Table (4-2) shows that two types of bacteria, Staphylococcus aureus, were Gram-positive, isolated from wounds in 10 (26%) and from burns in 12 (27%), respectively. The other

four species belong to the Gram-negative bacteria, namely Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, and Escherichia coli, which were isolated from burns at rates of 16 (36%), 6 (13%), 4 (9%), and 6 (13%) respectively, while they were isolated from wounds at rates of 10 (26%), 8 (21%), 8 (21%), and 2 (5%) respectively.

Bacterial isolates	Burns (50)	Wounds (50)	Total (100)
Staphylococcus aureus	12 (27%)	10 (26%)	22(26%)
Klebsiella pneumoniae	16 (36%)	10(26%)	26(31%)
Pseudomonas aeruginosa	6(13%)	8(21%)	14(17%)
Proteus mirabilis	4(9%)	8(21%)	12(14%)
Escherichia coli	6 (13%)	2(5%)	8(9%)
Total	44 (98%)	38 (99%)	82(97%)

Table (4-2): bacterial isolation of burns and wounds infection

4.2 Antibiotic Susceptibility

4.2.1 Antibiotic test of S. aureus

The results of the current study shown in the figure (4-1) indicated that from the total 22 isolates of S. aureus showed resistance to the following antimicrobial agents; Gentamicin 0%,(100% Sensitive), Chloramphenicol 27% , Clindamycin 55%, Ofloxacin 73%, Clarithromycin73%, Nitrofurantoin 18%, Rifampicin 9% ,Doxycycline 27%, and Co-trimazole 9%.

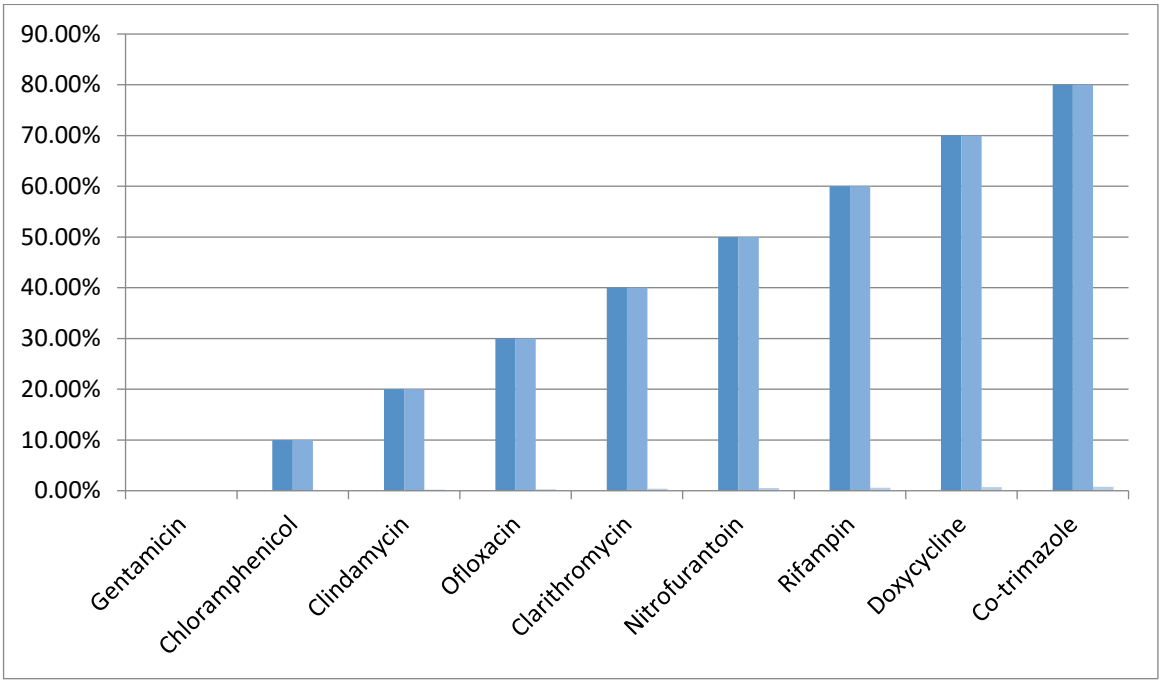

Figure (4-1) The percentages of antibiotic resistance of S. aureus

The results of resistance to antibiotic Ofloxacin 73% , agreed with the study of Al-Tamimi (2021) who showed that the resistant of S.aureus isolates to Ofloxacin was 82% ,But disagree with his results who showed the resistance to Gentamicin was 87%.

The current study of S. aureus resistance to Clindamycin close with the study of Mahmoud (2020) who showed the resistant to this antibiotic was 39%, and agreed with the results obtained by Al-Tamimi (2021) who showed the resistant to Clindamycin 41% . Chloramphenicol was resist in 27% of S. aureus isolates, this findings were a somewhat close to AL-Zengena in (2020)

and Noaman in 2020 who reported that the resistance to Chloramphenicol was 34% and 31% respectively, Clarithromycin was resist in 73% of S. aureus isolates, this result was agree with Jasim and Alzubaidy in (2022) who reported that S. aureus isolates were resist to Clarithromycin by 74%.

The resistance to Doxycycline 27% of S. aureus detected by Al-Geobory (2011) as well as Rushdy (2009) established that the resistance of S. aureus isolates were 38% these results were agreement with the current results. Staphylococci resist Doxycycline, through two mechanisms: first, active efflux of the antibiotic; second, the of the ribosome to the drug. Nitrofurantoin was resist in 18% of S. aureus isolates, Jasim and Alzubaidy in (2022) found that 100% of S. aureus isolates were resist to Nitrofurantoin.

Bacteria have three aminoglycoside mutating enzymes that are encoded by plasmids. These enzymes work by inhibiting antibiotics such as Gentamicin, as well as causing resistance to this group due to a change in the (30S) subunit to which the antibiotics were bound. Chloramphenicol inhibits protein synthesis because it is an inhibitory antibiotic that binds to the (30S) subunit. Enzymatic inhibitors are the most common resistance mechanism in bacteria. This process relies on a variety of strategies to alter the structure of antimicrobial drugs, including hydrolysis, a type of interaction that often occurs with beta-lactam antibiotics (Bhullar et al.,2012). The cause for the ongoing rise in antibiotic resistance might be due to widespread use of these antibiotics by people, which leads to the creation of novel strains with high resistance to antibiotics (Llarrull, Fisher and Mobashery, 2009).

The emergence of resistance to antibiotics in Gram-positive pathogens has become a major international problem as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria (Manandhar et al.,2019).The problem of increasing antimicrobial resistance is even more threatening when considering the very limited number of new antimicrobial agents that are in development (Labes, 2023).

4.2.2 Antibiotics Susceptibility of K. Pneumoniae

The results of the antibiotics sensitivity of K. pneumoniae isolates showed variable resistance as shown in Figure (4-2) which include: Ciprofloxacin 31%, Tetracycline 38%, Aztreonam 54%, Amoxicillin100%, Amoxicillin-clavulanate 23%, Co-trimazole 15%, Levofloxacin 15%, Gentamicin 23%, Cefotaxime 92%, Amikacin 8% ,Imipenem 8% and Cefepime 85%.

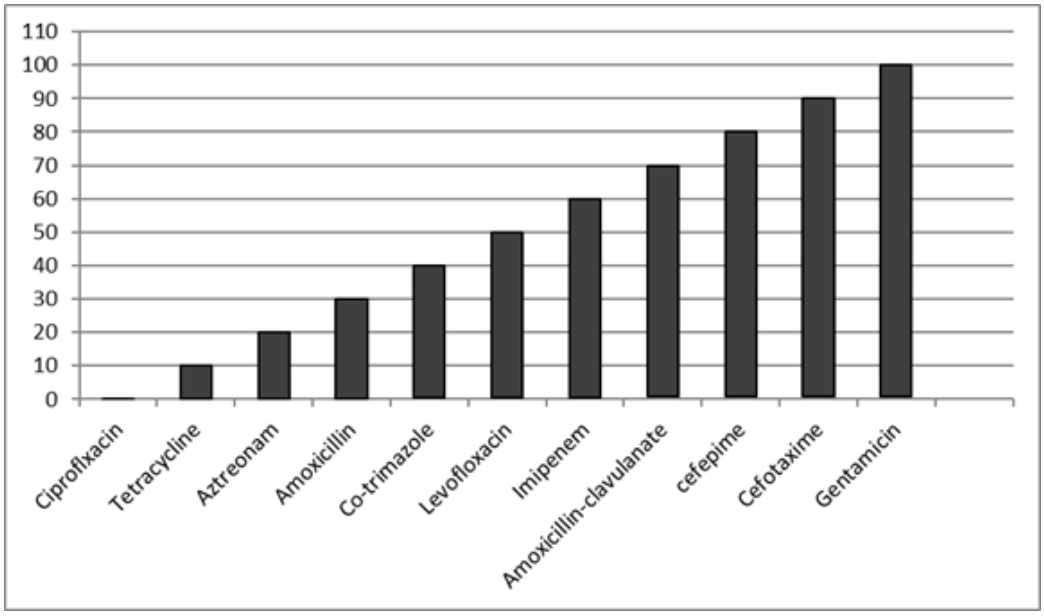

Figure (4-2) The percentages of antibiotic resistance of K. Pneumoniae

This result is agreed with Caneiras et al., (2019) in which K. pneumoniae isolates exhibited a full

antibiotic resistance that is 87.1% to cefotaxime and 41.9% of Ciprofloxacin. While high resistance to Cefotaxime 92%, it result corresponded to a study in Egypt that revealed a high resistance rate to cephalosporins, which was in agreement with this study (Al-Baz et al., 2022) that is, 96%.

The resistant isolates to antibiotic may be due to the production of extended-spectrum B-lactamases (ESBLs) that hydrolyze these antibiotics. Also, high resistance in the Cephalosporin class could be attributed to unregulated antibiotic usage, which contributed to the establishment of resistant isolates transmitted among hospitalized patients.

Alwan in (2020) done that K. pneumoniae were resistance against different types of antibiotics, Amoxicillin-clavulanate 34.78% and Tetracycllin 26%,This results agree with current study.

When comparing the results of our study with other studies found that, Bitew et al., (2017) mention the resistance of K.pneumoniae isolates to Gentamycin was 21.1% this result agree with the present study but differ with AL-Zengena (2013) was (77%) for Ciprofloxacin, Cefepime 85%. Resistance for isolates of K. pneumoniae, Gentamycin was 23% ,his finding was agree with Bitew et al., (2017) who indicated(21.1 %).

Tetracycline 38% this finding was agree with Bitew et al., (2017) who indicated 21.1%. Imipenem 8% this finding was agree with Ali and Ismael ,(2017) who indicated 0% .Gentamycin 23% this finding was agree with Bitew et al., (2017) who indicated 21.1%

K. pneumoniae resistance to Aztronam was approximately agree with the result obtained in another local study on Sarojamma and Ramakrishna (2011) who stated antibiotic resistance was 70%. Antibiotic Cefotaxime showed resistance in 92% of the isolates in the current study, as shown in Tables(4–3). The results agree with a study conducted in Iraq by Caneiras et al., (2019) , who discovered that cefotaxime had a resistance 87%.

K. pneumoniae is one of the clinically significant organisms that has attracted much public health concern. It plays an important role in spreading antimicrobial resistance genes from bacteria in the environment to clinically important bacteria (Nirwati et al., 2019). Klebsiella pneumoniae is an important multi-drug-resistant Gramn egative pathogen, associated with nosocomial (AL-Muqdadi, 2019).

4.2.3 Antibiotic Susceptibility test of Pseudomonas aeruginosa

The results of the present study shown in the figure (4-3) indicated that from all P. aeruginosa 7 isolates showed many antibiotics resistance, Piperacillin 29%, Cefepime 100%, Ceftazidime 86%, Ticarcillin-clavulanate 71%, Piperacillin /Tazobactam 0%, Aztreonam 57%, Ciprofooxacin 57%, Ofloxacin 57%, Netilmicin 14%, Imipenem, and Meropenem 14%.

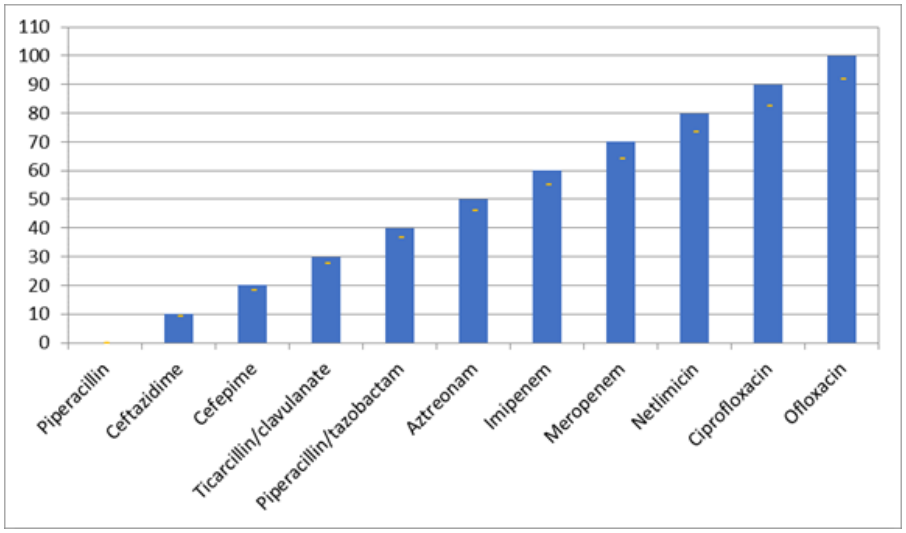

Figure (4-3) The percentages of antibiotic resistance of P. aeruginosa

P. aeruginosa showed the highest antibiotic resistance Cefepime 100%, The results showed that the proportion of bacteria resistant to 100%, these results are in agreement with Al-Janabi (2020) showed that the antibiotic resistance 100%, Other studies Alwan (2020) who indicated that resistance to Piperacillin was 26%. agree these results were consistent with the current study.

When comparing the present results with other studies; the researcher found the resistance of P. aeruginosa isolates against Ciprofloxacin and Imipenem were 26% and 13.3%, AL-Janabi (2019) which in agreement with the current study. Ceftazidime resistance rate was 86% which is similar to with Fattma et al in (2017) which was 91.49%

Other studies like, Saleh (2012) who indicated the resistance to Ciprofloxacin and Ofloxacin was 25% and 40%, these results were close to the present study but disagree with the results reported by Rana et al., (2010) when showed the rate of resistance 4% to Ciprofloxacin. Mahmoud, (2020) pointed that the resistant rates for Aztreonom and Meropenem was 45% and 15% This result was identical to present study.

The results of current study, resistance was seen Piperacillin/ Tazobactam 71%, this agree with what Elhariri et al. (2017) was 76.2%.

Finally, the resistance to Piperacillin/Tozabactam was 0% (100% sensitive) this result is consistence with Hameed finding in 2017 which was 0%.

The variable in resistance may be due to the sources of samples and ecological and test conditions. It was concluded from this study and previous studies; it was shown that P. aeruginosa resistance to antibiotics is caused by a variety of mechanisms indicated by its capacity to modify membrane permeability. It also owns efflux pumps and manufactures wide-narrow beta-lactamase enzymes and biofilm development, as well as resistance plasmids that carry various resistance genes (Hong et al., 2016).

4.2.4 Antibiotics Susceptibility test of Escherichia coli

The results shown in Table (4-3) indicated that the E. coli isolates showed resistance which include: Ciprofloxacin 25%, Aztreonam 25%, Cefotaxime 100%, Amoxicillin 100%, Cefepime 75%, Amoxicillin-clavulanate 50%, Levofloxacin 25%, Gentamicin 25%, Amikacin, Imipenem, Tetracycline and Co-triazole were resistant 0%.

Antibiotics	% Susceptibility
Ciprofloxacin	25%
Aztreonam	25%
Cefotaxime	100%
Amoxicillin	100%
Cefepime	75%
Amoxicillin-clavulanate	50%
Levofloxacin	25%
Gentamicin	25%
Amikacin, Imipenem, Tetracycline and Co-triazole	0%

Table (4-3) The percentages of antibiotic resistance of E. coli

There were many studies that indicated the resistance to antibiotics including those conducted by Ahmed (2016) who reported the resistance to Imipenem was 6% this result was close to the current study but differ with AL-Nuaeyme (2018) which mentioned the resistance to Imipenem were 40%

A study done by Ahmed (2016) detected the resistance of this bacteria to Amoxcillin was 92% was close to the present study and disagree with Mahdy (2018) who show the resistance to

Amoxcillin was 24%. In other studies, Amber et al., (2016) and Mhammad (2020) as it showed the percentage of resistance against Levofloxacin were 36.4% and 30% respectively this results close to the current study. As for β-lactam combinations, which includes Amoxicillin-clavulanate in current study resistance was 50%, this result agree with found by study Ahmed (2021) which was 60% and 76.6% to Cefepime, which corresponding with current study.

E. coli resist-lactam antibiotics by producing beta-lactamase enzymes, which are an important way to resist beta-lactam antibiotics. Most of the genera of Gram-negative bacteria possess genes that encode B- Lactamase enzymes (Chuma et al.,2013). The isolates of E. coli have more than one antibiotic-resistant mechanism, such as being able to form the biofilm, It may also be able to configure Efflux pumps and change target locations on which the antibiotic works (LIedo etal.,2009).

4.2.5 Antibiotics Susceptibility test of P. mirabilis

P. mirabilis isolates showed the variety resistance to antimicrobial agents were; Ciprofloxacin 33%, Tetracycline 50%, Aztreonam 33%, Amoxacillin 100%, Levofloxacin 17%, Imipenem 17%, Amoxicillin-clavulanate 50%, Cefepime 50%, Cefotaxime 67%, Gentamycin and Amikacin was 33%, Table (4-4).

Antibiotics	% Susceptibility
Ciprofloxacin, Gentamycin, Aztreonam and Amikacin	33%
Tetracycline	50%
Amoxacillin	100%
Levofloxacin, Imipenem	17%
Amoxicillin-clavulanate,	50%
Cefepime	50%
Cefotaxime	67%

Table (4-4) The percentages of antibiotic resistance of P.mirabilis

Al-Khateeb (2014) who pointed the resistance ratio of P.mirabilis to Gentamicin was 36% this results agree with current study.

A study done by AL-Dulaimi (2019) detected the resistance of this bacteria to Cefotaxime was 83.3% % was close to the present study.

Emergence and spread of multidrug resistant P. mirabilis isolates, including those producing ESBLs, AmpC cephalosporinases and carbapenemases, are being more and more frequently reported. This bacterial species are usually susceptible to fluoroquinolones(Girlich et al. 2020). Proteus spp. have been either inhibited by Clavulanic acid or resistant to the action of this antibiotic. They produce specific enzymes which hydrolyze narrow-spectrum Penicillins but are not active on 2nd and 3rd generations and carbapenems (Girlich et al., 2015)

The resistance of the Enterobacteriaceae family to beta-lactamase group is due to its ability to produce beta-lactamase enzymes and its resistance by several mechanisms, including reducing the permeability of the antibiotics into the cell, as well as analyzing the antibiotics by beta-lactamase enzyme, and the other reducing affinity to the enzyme Penicillin-Binding Proteins (Brooks et al.,2007). Bacterial resistance to the group of quinolones is mediated by Efflux pumps that cause multiple antibiotic resistance (Shaikh and Anis 2016)The resistance of Klebsiella spp to anti-quinolones is mediated by flow pumps that cause multiple antibiotic resistance. The high rate of bacterial resistance to this antibiotic is the wide use of this antibiotic.

5. Conclusions and Recommendations:

5.1. Conclusions

The following conclusions were drawn from the current study's findings:

1. Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis were the next most frequently isolated bacteria from burn and wound infections, after Staphylococcus aureus.

2. The majority of bacteria found in burns and wounds were resistant to multiple medicines (MDR).

5.2. Recommendations

1. Further research is needed to determine the optimal course of action for treating infection with this pathogen and to evaluate the advantages of early detection of bacteria and other virulence factors that contribute to the pathophysiology of bacterial isolate infection.

- Because these bacterial isolates have significant virulence characteristics that contribute to the development of infections, more genetic research might be done on the types of bacteria that cause burns and wound infections.

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