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Microbiological Study of Antibiotic Susceptibility Patterns in Clinical Bacterial Isolates in Ajdabiya City

Abdulhakem Almahde Kalefa Makari [1 *], Sara Saad Elgeroshi [2]

[1] Department of Biology, Faculty of Arts and Sciences/Mizdah, Gharyan University, Libya

[2] Department of Medical Laboratories, Higher Institute of Medical Sciences and and Technology, Ajdabiya, Libya

دراسة ميكروبيولوجية لنمط الحساسية للمضادات الحيوية في عزلات بكتيرية سريرية بمدينة أجدابيا

عبد الحكيم المهدي مكاري [1 *]، سارة سعد الجروشي [2]

[1] قسم الاحياء، كلية الآداب والعلوم /مزدة، جامعة غريان، ليبيا

[2] قسم المختبرات الطبية، المعهد العالي للعلوم والتقنية، اجدابيا، ليبيا

[*]Corresponding author: makarihakem@gmail.com

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Abstract:

The current microbiological study explores antibiotic resistance patterns in clinical bacterial isolates in Ajdabiya, Libya, where bacterial infections remain a leading cause of death with mortality rates reaching 30%. The study aimed to identify the most prevalent bacterial species and measure their sensitivity to a variety of common antibiotics. Diverse clinical samples, including swabs, urine, and body fluids, were collected from the laboratories of the Higher Institute of Medical Sciences and Technology. Results revealed microbial diversity including Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter spp.*, and the *Enterobacteriaceae* family, in addition to Gram-positive bacteria like *Staphylococcus aureus*. Susceptibility testing showed alarming resistance rates toward several classes of antibiotics, particularly in isolates associated with community-acquired infections. The study also demonstrated that therapeutic efficacy is concentrated in actively growing bacteria, while latent forms pose a significant medical challenge. The paper concludes that the spread of resistance genes and genetic transfer mechanisms among strains necessitates strict surveillance strategies and the development of targeted therapeutic protocols to limit the depletion of available treatment options. The study recommends the need to enhance awareness regarding the rational use of antibiotics and conduct in-depth genetic research to understand the composition of resistance genes in the local environment.

Keywords: Antibiotic Resistance, Clinical Isolates, Ajdabiya, Microbial Susceptibility, Gram-negative Bacteria, Public Health in Libya.

الملخص

تقوم الدراسة الميكروبيولوجية الحالية باستكشاف أنماط مقاومة المضادات الحيوية في العزلات البكتيرية السريرية بمدينة أجدابيا، ليبيا، حيث تظل العدوى البكتيرية سبباً رئيسياً للوفاة بنسبة تصل إلى 30%. هدفت

الدراسة إلى تحديد الأنواع البكتيرية الأكثر انتشاراً وقياس مدى حساسيتها لمجموعة متنوعة من المضادات الحيوية الشائعة. تم جمع عينات سريرية متنوعة تشمل المسحات، البول، وسوائل الجسم من مختبرات المعهد العالي للعلوم والتقنيات الطبية. أظهرت النتائج تنوعاً ميكروبياً شمل بكتيريا سالبة الجرام مثل *Pseudomonas aeruginosa* و*Acinetobacter spp* وعائلة *Enterobacteriaceae*، بالإضافة إلى بكتيريا موجبة الجرام مثل *Staphylococcus aureus*. كشفت اختبارات الحساسية عن معدلات مقاومة مقلقة تجاه عدة فئات من المضادات الحيوية، خاصة في العزلات المرتبطة بالعدوى المكتسبة من المجتمع. كما بينت الدراسة أن الفعالية العلاجية تتركز في البكتيريا النشطة نمواً، بينما تشكل الأشكال الكامنة تحدياً طبياً كبيراً. تخلص الورقة إلى أن انتشار الجينات المقاومة وآليات الانتقال الجيني بين السلالات يتطلب استراتيجيات رقابية صارمة وتطوير بروتوكولات علاجية موجهة للحد من استنزاف خيارات العلاج المتاحة. توصي الدراسة بضرورة تعزيز الوعي حول الاستخدام الرشيد للمضادات الحيوية وإجراء بحوث جينية معمقة لفهم تكوين جينات المقاومة في البيئة المحلية.

الكلمات المفتاحية: مقاومة المضادات الحيوية، العزلات السريرية، أجدابيا، الحساسية الميكروبية، البكتيريا سالبة الجرام، الصحة العامة في ليبيا.

1.INTRODUCTION

Bacterial infections remain one of the leading causes of death globally, with mortality rates from severe infections reaching approximately 30%. While antibiotic (Salem, & Salem, 2025). treatment has been shown to improve survival rates, certain Gram-negative microorganisms present significant challenges, particularly in cases of community-acquired infections. Key pathogens in this group include *Pseudomonas aeruginosa*, *Acinetobacter* spp., and the *Enterobacteriaceae* family. Over time, bacterial pathogens have developed increased resistance to commonly used antibiotics, making antimicrobial resistance a critical medical and public health concern. Such resistance often leads to treatment failure, posing serious risks, especially for critically ill patients. Among Gram-negative bacilli, antibiotic resistance is especially pronounced, with some strains exhibiting high levels of resistance to aminoglycosides, beta-lactams, and quinolones (Salem, et al 2025).

The extensive use of broad-spectrum antibiotics has contributed to the rise of antibiotic-resistant strains in many Gram-negative organisms. This resistance has developed in Gram-negative bacilli due to both their innate resistance in some species and their remarkable ability to acquire antibiotic-resistant traits from one another. Certain Gram-negative microorganisms, such as *Pseudomonas aeruginosa, Acinetobacter* species, and the *Enterobacteriaceae* family, pose especially significant challenges. As a result, newer generations of antibiotics will continue to face persistent and growing challenges from these resilient pathogens. Beta-lactamases serve as the primary defense mechanism for Gram-negative bacteria against broad-spectrum beta-lactam antibiotics. These antibiotics, which include penicillins, cephalosporins, and monobactams, rank among the most commonly prescribed treatments globally. To date, over 400 distinct types of beta-lactamases have been identified from clinical isolates. Resistance to these newer beta-lactam antibiotics due to the action of beta-lactamases developed rapidly (Bengtsson-Palme et al., 2018).

1.1 AIM OF THE STUDY

1. To isolate and identify bacteria contributing to antibiotic resistance.
2. To determine the rate of antibiotic resistance.
3. To examine antimicrobial sensitivity patterns of the isolated bacteria and assess their prevalence.

2. REVIEW OF LITERATURE 2.1 Microbial infection Clinical microbiology focuses on analyzing specimens obtained from patients suspected of having infectious diseases. Its aim is to identify changes in the type and distribution of microflora or investigate the presence of specific microorganisms that could potentially be the cause of illness. When microbes or other living agents infiltrate the body, they multiply and trigger a response known as "infection." An infection is a dynamic process involving the invasion of the body by pathogenic microorganisms and the reaction of tissues to these microorganisms and their toxins. The interaction between a host and a pathogen is inherently dynamic, as both influence and modify each other's functions and activities. The outcome of an infection is determined by the virulence of the pathogen and the host's level of resistance or susceptibility, which largely depends on the effectiveness of defense mechanisms within the host (Ourenza et al., 2020).

2.2 Antibiotics

Antibiotics are natural compounds produced by microorganisms, which, even at low concentrations, can inhibit the growth or eliminate other microorganisms. These substances have been extracted from various sources, primarily from bacteria, such as tetracyclines, bacitracin, polymyxin, chloramphenicol, and streptomycin, as well as from fungi, including cephalosporins and penicillins. Penicillin, the first antibiotic to be discovered, was identified by Sir Alexander Fleming in 1928. The onset of industrialization marked a game-changing era, leading to the development of numerous additional antibiotics (Aldred et al., 2014).

2.3 The Basic Characteristics of Antibiotics

Antibiotics serve multiple purposes, including treating and preventing infections as well as promoting growth in animals. They originate from three primary sources: molds or fungi, bacteria, and synthetic or semi-synthetic compounds. These medications can be administered internally or applied topically, functioning either by inhibiting the growth of pathogens or by killing them outright. Based on their mode of action, antibiotics are categorized into two types: bacteriostatic drugs, which slow down the growth of pathogens, and bactericidal drugs, which eliminate bacteria. That said, this classification isn't entirely rigid, as their effects can vary depending on factors such as drug concentration, bacterial species, and the stage of bacterial growth.

Antibiotics tend to be most effective against bacteria that are actively growing, rather than non-growing persisters or dormant spores. When two antibiotics are used together, their combined effects can vary, ranging from additive (where the effects simply sum up), synergistic (where they enhance each other's efficacy), or antagonistic (where one reduces the effectiveness of the other). Antibiotics are also categorized into broad-spectrum and narrow-spectrum types. For instance, tetracycline, a broad-spectrum antibiotic, is effective against Gram-positive (G+), Gram-negative (G-), and even mycobacteria.

2.4 Major antibiotic families and their mechanisms of action

Antimicrobial agents are classified as either bacteriostatic or bactericidal. Bacteriostatic agents work by inhibiting bacterial growth or reproduction, allowing the host's immune system the opportunity to eliminate the bacteria. In such cases, successful removal of the bacteria relies on the effectiveness of the immune system. Bactericidal agents, on the other hand, directly kill bacteria, ensuring their elimination regardless of the immune system's competence. Inhibition of cell wall synthesis is achieved through agents such as penicillins, cephalosporins, daptomycin, and glycopeptides. For protein synthesis, tetracyclines, aminoglycosides, oxazolidinones, streptogramins, ketolides, macrolides, and lincosamides are utilized. DNA synthesis can be inhibited by fluoroquinolones. Competitive inhibition of folic acid synthesis involves sulfonamides and trimethoprim. RNA synthesis can be inhibited using rifampin, while metronidazole falls into the category of miscellaneous agents (Allen et al., 2009).

2.5 Antibiotic resistance

Antibiotic resistance arises when bacteria undergo changes that diminish or nullify the effectiveness of drugs, chemicals, or other agents intended to treat or prevent infection. As a result, the bacteria persist and continue to multiply, leading to greater harm. The widespread use of antibiotics contributes significantly to the development and spread of antibiotic resistance (Kadak, & Salem, 2020). To assess bacterial susceptibility to antibacterial agents, scientists determine the minimum inhibitory concentration (MIC) needed to halt bacterial growth. Resistance, in turn, is characterized by bacteria that fail to be inhibited at typical systemic concentrations of a drug under standard dosing schedules or those that exceed established MIC thresholds (Holmes et al., 2016).

2.6 Main mechanisms of antimicrobial resistance

Microorganisms exhibit resistance through several key mechanisms:

1. **Drug Inactivation or Modification:** Certain bacteria enzymatically deactivate antibiotics; for example, the production of β-lactamases renders penicillin G ineffective. Bacterial cells may also deploy protective enzymes that attach acetyl or phosphate groups to the antibiotic molecule, reducing its ability to bind to bacterial ribosomes.
2. **Alteration of Target Site:** Modifications to the target site, such as the alteration of penicillin-binding proteins (PBP) in MRSA, make bacteria less susceptible. Ribosomal protection proteins can also induce conformational changes that allow protein synthesis to continue while blocking antibiotic action.
3. **Alteration of Metabolic Pathways:** Some resistant bacteria bypass the need for para-aminobenzoic acid (PABA), a precursor for folic acid targeted by sulfonamides, by utilizing preformed folic acid instead.
4. **Reduced Drug Accumulation:** This occurs through reduced drug permeability or enhanced active efflux. Specialized efflux pumps in the cellular membrane actively expel antibiotics before they can exert their effects (Miller et al., 2014).

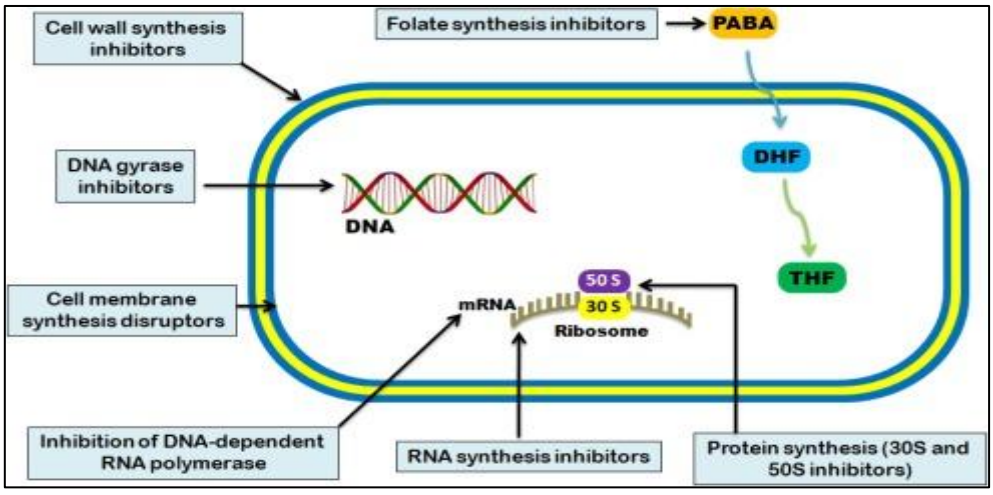

Figure (1): Antibiotic resistance in microbes.

3. MATERIALS AND METHODS

3.1 Collection of Samples and Specimens

3.1.1 Urine Samples Urine samples were collected from infected patients for culture investigations. Morning mid-stream urine samples were collected in sterile containers to maximize microbial concentration, utilizing bacterial accumulation during overnight bladder storage. Patients were provided with clear instructions on correct collection methods to reduce contamination risks. Once collected, specimens underwent aerobic incubation at 37°C for 24

to 72 hours to promote bacterial growth. Given urine's suitability as a medium for microbial proliferation, samples were promptly analyzed and cultured using specific media, including Cystine Lactose Electrolyte Deficient (C.L.E.D.) agar and MacConkey agar. Previous data indicated that methicillin-resistant *S. aureus* (MRSA) represented 5.7% of all bacterial isolates tested, while methicillin-resistant coagulase-negative *Staphylococci* occurred at a higher percentage (27.4%) than MRSA. Gram-negative bacilli were reported in 2009 at 47.1%.

3.1.2 Pus Swabs Specimens were collected from infected skin at various sites. Collections were performed under sterile conditions using sterile gloves and swabs to prevent contamination. The samples were subsequently cultured for bacterial growth and antibiotic sensitivity testing. Various types of bacterial growth media were utilized in the laboratory. Bacteria grown in a laboratory environment require a controlled supply of nutrients, water, and a suitable environment to survive and thrive. The most common isolate recovered from these cultures was *Staphylococcus aureus*.

3.2 Media Used for Isolation and Cultivation of Pathogenic Bacteria Pathogenic bacteria were isolated using culture plates prepared with the following media:

3.2.1 Nutrient Agar: A general-purpose medium designed for the cultivation of a wide range of microorganisms.

3.2.2 Blood Agar: Consists of nutrient agar (900 ml) combined with sterile whole blood (100 ml). The sterile nutrient agar is melted and cooled to 45-47ºC before animal (horse or sheep) or human blood is added aseptically to maintain sterility.

3.2.3 Chocolate Agar (Enriched Medium): A modified version of blood agar where red blood cells are lysed by gradually heating the medium to 80ºC. It is specifically used for the cultivation of fastidious organisms.

3.2.4 Cystine Lactose Electrolyte Deficient (C.L.E.D.) Agar: Recommended for diagnostic urinary bacteriology, supporting the growth of all urinary pathogens while providing clear colonial differentiation.

3.2.5 MacConkey Agar: A selective and differential medium primarily used for detecting coliform organisms such as *Escherichia coli*, as well as isolating *Salmonella* and *Shigella* species.

3.3 Microscopic Examination of Specimens 3.3.1 Gram Staining The Gram staining technique was employed to identify bacteria based on their Gram reaction (Gram-positive or Gram-negative) and morphology. The method involves the following reagents:

- Crystal violet (primary stain)
- Lugol’s iodine (mordant)
- Acetone-alcohol (decolorizing agent)
- Neutral red solution (0.1% w/v) or Safranin (counterstain)

This process is essential in differentiating bacterial species for diagnostic microbiology.

3.4 Antimicrobial Susceptibility Testing

3.4.1 Antimicrobial Agents Utilized All isolated pathogenic bacteria obtained from various specimens underwent sensitivity testing against 20 different antimicrobial agents. These agents were specifically employed for Gram-negative bacilli, while Rifampicin (RD) was used exclusively to assess susceptibility in Gram-positive cocci.

3.4.2 Disc Diffusion Susceptibility Approach (Kirby-Bauer Method) The disc diffusion technique remains the standard method for routine antimicrobial susceptibility testing. A filter paper disc, impregnated with a measured volume and optimal concentration of an antimicrobial agent, is placed onto an agar plate (typically Mueller-Hinton Agar) uniformly inoculated with the test microorganism. This creates a controlled environment to observe the zone of inhibition and assess the susceptibility of the organism.

Figure 2 (a) Media preparation

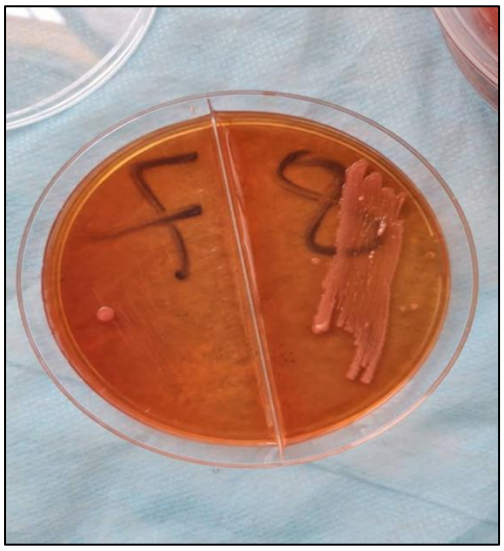

Figure 3.1 Klebsiella bacteria

Figure 3.2 Pseudomonas

Figure 3.3 E. Coli

Figure 3.4 Staph aureus

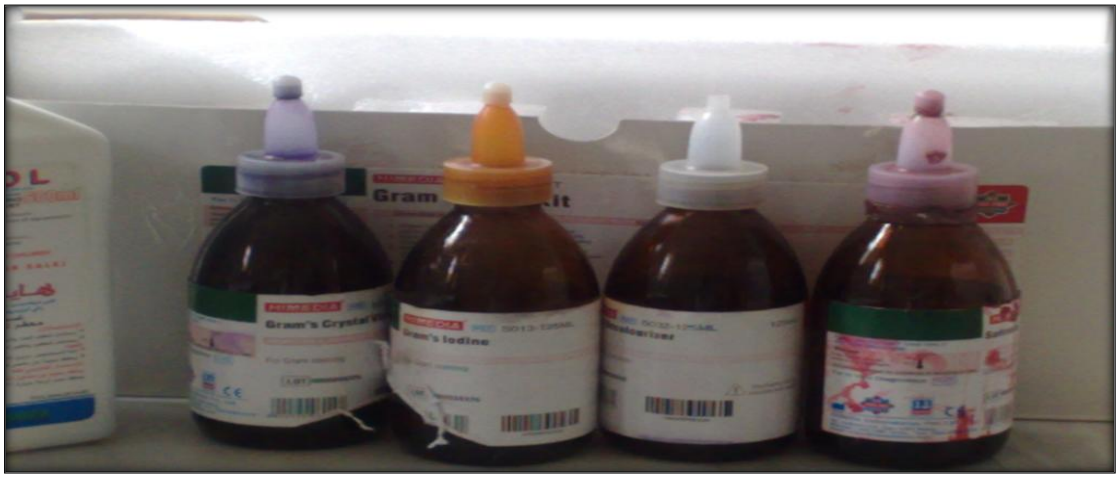

Figure 4 Gram stain

Figure 5 (a,b) Antibiotic Susceptibility Discs

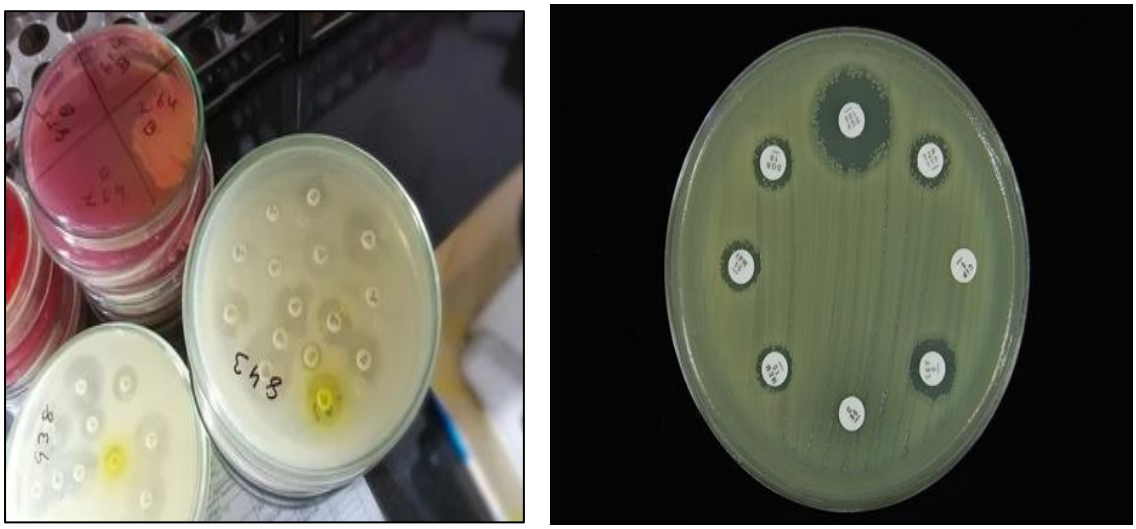

Figure 6 Results of Antibiotic Susceptibility test of bacteria staph aureus

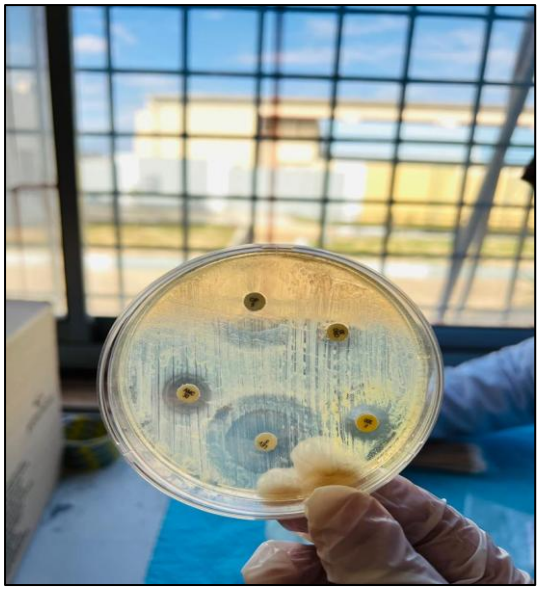

Figure 7.1 Results of Antibiotic *E. coli*

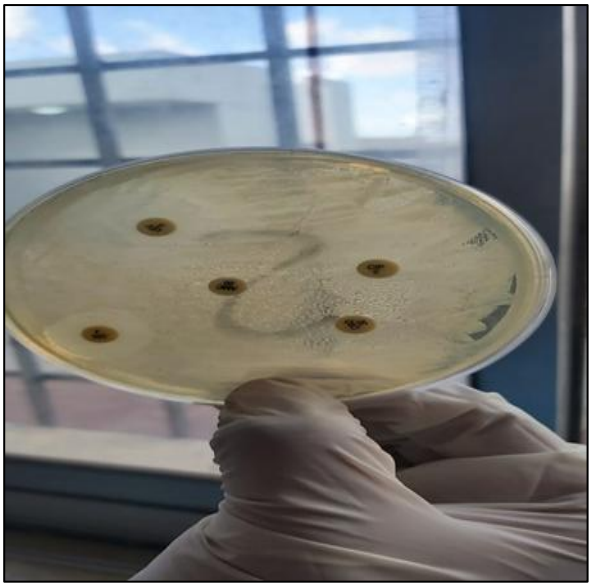

Figure 7.2 Results of Antibiotic *Klebsella*

4.RESULTS

4.1 Sample Collection and Distribution of Bacterial Isolates

Collection, isolation, and identification of pathogenic bacteria from different clinical samples and specimens were performed on samples collected from outpatients of different public and private hospitals in Ajdabiya City. The sources of the clinical samples and specimens were: (a) Body fluid: urine samples, 60 cases (55.14%); (b) Skin (pus) swabs, 40 cases (45%).

The most common bacterial isolate from the culture growth was *Staphylococcus aureus* (90%) from pus swabs. In this study, the isolated organisms were highly sensitive to colistin (100%), cefadrox (99.4%), cephalothin (96.9%), amikacin (93.9%), ciprofloxacin (90.8%), chloramphenicol (90.2%), clindamycin (89.6%), gentamicin (89.0%), and Augmentin (87.7%), respectively.

Conversely, high resistance was observed against oxacillin (42.9%), ampicillin (33.7%), penicillin (23.3%), as well as erythromycin and vancomycin equally (16.6%), respectively (Figure. 8).

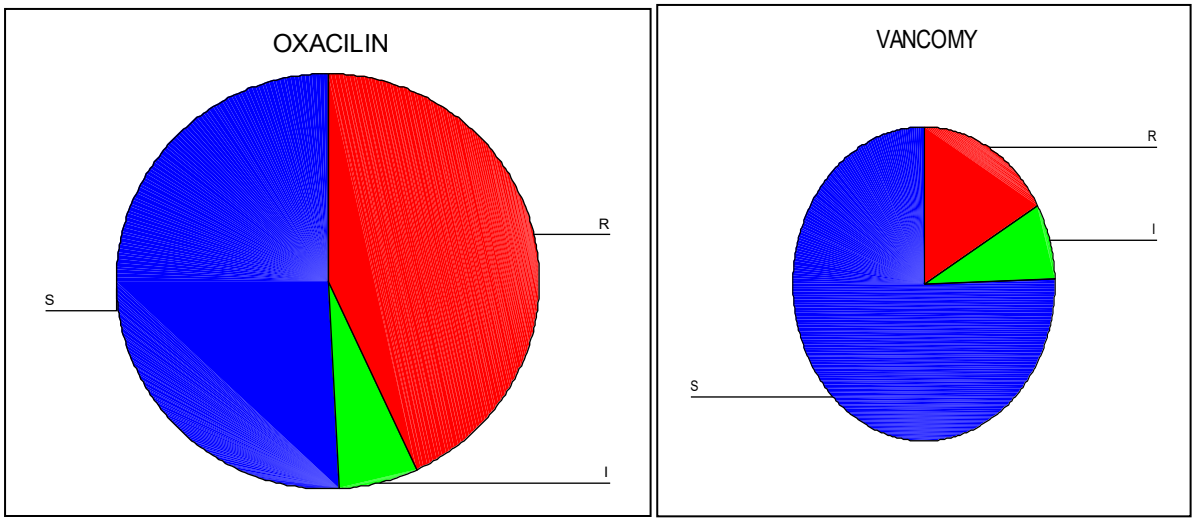

Figure 8 *Staphylococcus aureus* showed higher resistance to these antibiotics.

Table 1 Antimicrobial susceptibility of *Staphylococcus aureus* and reference values for the disc diffusion method in pus samples.

Antibiotic	R	I	S	Total
Oxacillin	(36.8%)	(4.3%)	(32.5%)	(73.6%)
Vancomycin	(11.0%)	(8.0%)	(54.6%)	(73.6%)
Ampicillin	(27.0%)	(1.2%)	(45.4%)	(73.6%)
Penicillin	28 (17.2%)	(4.3%)	(52.1%)	(73.6%)
Erythromycin	16 (9.8%)	(1.8%)	(62.0%)	(73.6%)
Gentamycin	14 (8.6%)	–	(65.0%)	(73.6%)
Augmentin	(7.4%)	(1.2%)	(65.0%)	(73.6%)
Ciprofloxacin	(5.5%)	(1.8%)	(66.3%)	(73.6%)
Clindamycin	(5.5%)	–	(68.1%)	(73.6%)
Chloramphenicol	7 (4.3%)	(0.5%)	(68.7%)	(73.6%)
Amikacin	6 (3.7%)	(1.2%)	(68.7%)	(73.6%)
Streptomycin	5 (3.1%)	–	(70.6%)	(73.6%)
Kanamycin	4 (2.5%)	–	(71.2%)	(73.6%)
Colistin	–	–	(73.6%)	(73.6%)
Cefadrox	(0.5%)	–	(73.0%)	(73.6%)
Cefoxitin	(3.7%)	–	(69.9%)	(73.6%)
Rifampicin	(3.7%)	–	(69.9%)	(73.6%)

4.2 Prevalence of Gram-positive isolates according to susceptibility to antimicrobial agents

The results indicate that rifampin demonstrated the highest antibacterial effectiveness (95.8%) against Gram-positive isolates, followed by teicoplanin (91.6%), ciprofloxacin (90.2%), vancomycin (87.4%), trimethoprim-sulfamethoxazole (86.0%), linezolid (84.6%), gentamicin synergy (69.2%), daptomycin (65.0%), tetracycline (59.4%), clindamycin (47.6%), erythromycin (45.5%), Augmentin (35.0%), imipenem (25.2%), oxacillin (20.3%), gentamicin (18.9%), cefoxitin (17.5%), and ampicillin with the lowest effectiveness at 0.7%.

4.3 Prevalence of Gram-negative isolates according to susceptibility to antimicrobial agents

The antibacterial effectiveness was observed as follows: imipenem demonstrated the highest activity at 92.0%, followed by meropenem at 90.3%. Amikacin showed 77.9% effectiveness, while trimethoprim-sulfamethoxazole stood at 49.6%. Piperacillin/tazobactam exhibited 47.8% activity, cefoxitin showed 46.9%, ciprofloxacin had 44.2%, and colistin was effective at 43.4%. Subsequently, ceftazidime achieved 37.2%, gentamicin presented 36.8%, with aztreonam and tetracycline both at 35.4%. Cefepime displayed 31.9% effectiveness, followed by cefotaxime at 30.1% and cefuroxime at 17.7%. Piperacillin showed 16.8%, Augmentin reached 15.0%, ampicillin had an effectiveness of 8.0%, and cephalothin ranked the lowest at 6.2%.

The prevalence of methicillin resistance was notably high among coagulase-negative staphylococci (CNS), with a resistance rate of 55.2%. This aligns with findings reported in a French general hospital, where the rate of methicillin-resistant *Staphylococcus aureus* (MRSA) reached 46%. Similarly, a study conducted by Allen HK, et al. (2009) revealed that 65% of bloodstream *S. aureus* isolates were resistant to methicillin. Furthermore, this research underscores that methicillin resistance among coagulase-negative staphylococci remains significant, with 53% of CNS isolates exhibiting resistance. In addition, Gram-negative rods demonstrated high resistance to the majority of antimicrobial agents (Salem, 2024).

This trend could be attributed to the delayed implementation of infection control measures in the studied hospital and the substantial presence of extended-spectrum beta-lactamases (ESBLs) among Gram-negative bacilli, which was observed at a rate of 30.8% in Martyr Mohammed Al-Magariaf Hospital. Such ESBLs considerably restrict therapeutic options for infections caused by these strains, primarily due to two contributing factors: cross-resistance (e.g., resistance to aminoglycosides or fluoroquinolones) and the potent hydrolytic impact of these enzymes.

Table 2 Prevalence of Gram-positive isolates according to susceptibility to antimicrobial agents (urine sample).

Antimicrobial	Susceptible	Intermediate	Resistant
Gentamicin synergy	%69.2	%4.2	%26.6
Gentamicin	%18.9	–	%81.1
Imipenem	%25.2	–	%74.8
Cefoxitin	%17.5	–	%82.5
Cefotaxime	%3.5	%2.1	%94.4
Ampicillin	%0.7	–	%99.3
Penicillin	%6.3	–	%93.7
Oxacillin	%20.3	–	%79.7
Augmentin	%35.0	–	%65.0
Daptomycin	%65.0	–	%35.0
Trimethoprim-Sulfamethoxazole	%86.0	%2.1	%11.9
Teicoplanin	%91.6	%3.5	%4.9
Vancomycin	%87.4	%2.1	%10.5
Clindamycin	%47.6	%4.2	%10.5
Erythromycin	%45.5	%2.1	%52.4
Linezolid	%84.6	%3.5	%11.9
Ciprofloxacin	%90.2	%2.8	%7.0
Rifampin	%95.8	%0.7	%3.5
Tetracycline	%59.4	%0.7	%39.9

Table 3 Prevalence of Gram-negative isolates according to susceptibility to antimicrobial agents (urine sample).

Antimicrobial	Susceptible	Intermediate	Resistant
Amikacin	77.9%	–	%22.1
Gentamicin	%36.8	%0.9	%62.8
Imipenem	%92.0	%3.5	%4.4
Meropenem	90.3%	–	%9.7
Cephalothin	%6.2	%4.4	%89.4
Cefuroxime	%17.7	%3.5	%78.8
Cefoxitin	46.9%	%6.2	%46.9
Ceftazidime	%37.2	%0.9	%61.9
Cefotaxime	%30.1	%1.8	%68.1
Cefepime	%31.9	%2.7	%65.5
Aztreonam	%35.4	%0.9	%63.7
Ampicillin	8.0%	%3.5	92.0%
Piperacillin	16.8%	%0.9	%82.3
Augmentin	15.0%	11.5%	73.5%
Piperacillin-Tazobactam	47.8%	%8.8	%43.4

Colistin	43.4%	1.8%	54.9%
Trimethoprim-Sulfamethoxazole	49.6%	–	%50.4
Ciprofloxacin	44.2%	2.7%	53.1%
Tetracycline	%35.4	%3.5	%61.1

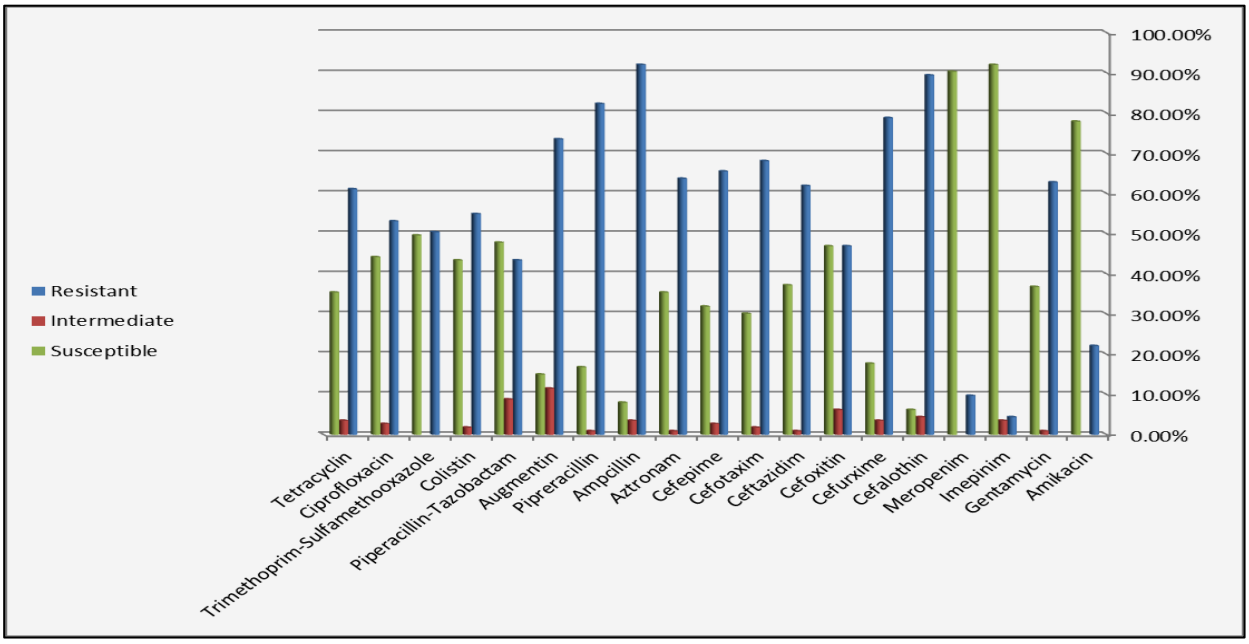

Figure 9 Prevalence of Gram-negative isolates according to susceptibility to antimicrobial agents.

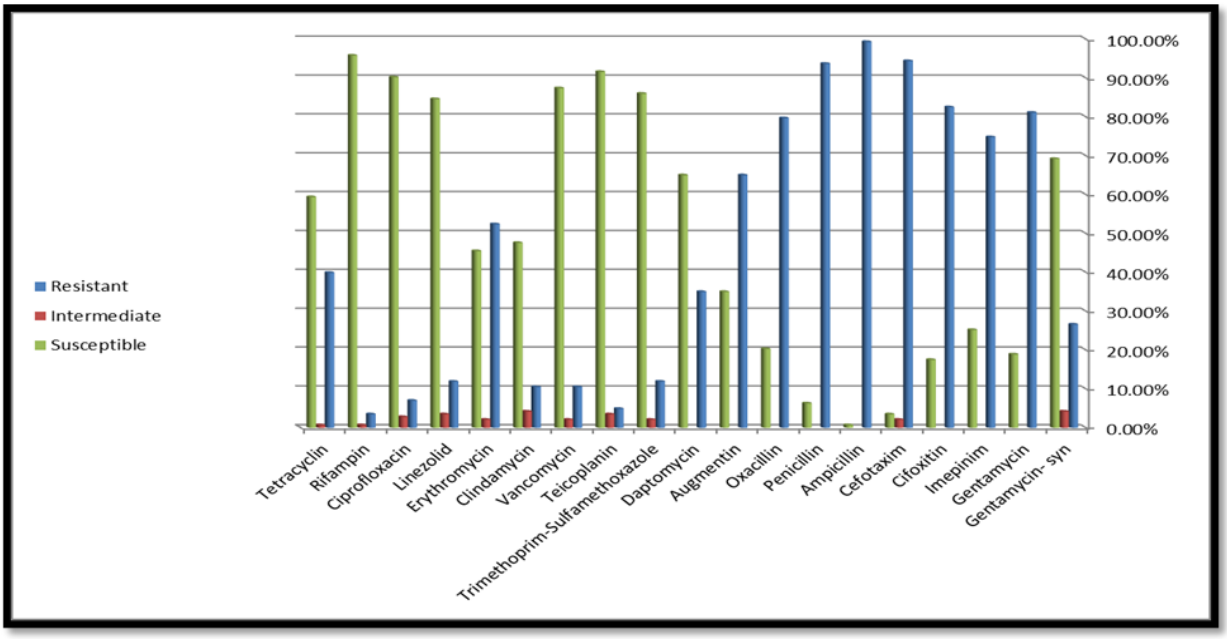

Figure 10 Prevalence of Gram-positive isolates according to susceptibility to antimicrobial agents.

5.CONCLUSION

The extensive use of antibiotics has undeniably played a major role in the worldwide increase in antimicrobial resistance. In certain species, this resistance has reached such alarming levels

that no existing clinical treatments are effective anymore. Tackling this issue requires comprehensive strategies that blend epidemiological and behavioral measures with research aimed at uncovering the core mechanisms of drug resistance. A key aspect of this research involves studying the genetic composition of resistance genes, their locations, and variations, which are essential for identifying the factors fueling resistance. Additionally, investigating genetic connections among markers and examining potential transfer mechanisms is equally important for advancing our understanding and developing targeted solutions.

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