Alqalam Journal of Medical and Applied Sciences. 2025;8(1):345-351
https://doi.org/10.54361/ajmas.258150

Original article

Antimicrobial Resistance Patterns of *Escherichia coli* Among Visitors of Pathology Centers in Gharyan

Hala Almshawit[1]*, Retaj Shagshog[1] Hana Aldib[1] Sarah Areebi[1], Weam Tahar[2]

[1]*Department of Zoology, Faculty of Sciences, University of Gharyan, Gharyan, Libya*
[2]*Department of English Language, Faculty of Arts, University of Gharyan, Gharyan, Libya*
Corresponding Email. hala.almshawit@gmail.com

Abstract
The collection of comprehensive data is crucial for monitoring antimicrobial resistance (AMR) patterns and implementing targeted interventions. However, there is a lack of data regarding the AMR status of pathogens in Libya, hinders the efforts to address the problem effectively. This retrospective study evaluates the prevalence of *Escherichia coli* AMR isolates to gentamycin, chloramphenicol, cefoxitin (or) ceftriaxone, meropenem, moxifloxacin (Mox), amoxicillin/clavulanate (AUG) or ampicillin-sulbactam (UNZ) among the visitors to the main pathology laboratories in Gharyan city. Reports from 107 AMR tests demonstrate that *E. coli* was mostly isolated from Urinary Tract Infections (UTIs, 85%) followed by vaginal infections (8.4%) and wound infections (6.5%). More than half of the isolates were multi-drug resistant which is a clear indicator of the need for continuous monitoring. The overall pattern demonstrates a high resistance rate to cephalosporins, and combinations of penicillins with beta-lactamase inhibitors (UNZ or AUG), followed by high resistance rates to Mox. In contrast, *E. coli* isolates showed high sensitivity to meropenem followed by gentamicin and chloramphenicol. However, the isolation site seems to affect the resistance patterns, most of the wound isolates were resistant to gentamycin. In contrast, all vaginal isolates were resistant to UNZ or AUG, yet both types showed high susceptibility to meropenem.
Keywords: *Escherichia coli*, Antimicrobial Resistance, Urinary Tract Infections, Gharyan, Libya.

Introduction

Antimicrobials resistance AMR is a term used when bacteria, fungi, viruses, and parasites no longer respond to the antimicrobial medicines that were previously effective, making infections harder to treat. Consequently, this leads to an increased risk of severe disease transmission and higher death rates [1]. Natural resistance might be intrinsic, defined as a feature that is common within a bacterial species, that is independent of previous antibiotic exposure, and not related to horizontal gene transfer. Additionally, bacteria can also acquire resistance genes from other related organisms with the type of resistance varying based on the species and the genes acquired [2].

The resistance to the antibacterial compounds can occur through several mechanisms. For example, limiting drug uptake because of the structure and composition of the cell wall layers. The structure could be the absence or presence of LPS layer, high levels of lipids as in *Mycobacteria,* or complete absence of cell wall such as in *Mycoplasma* which can be a target of the antibiotic. Living within biofilms significantly reduces the uptake of antibiotics. Another mechanism of AMR is drug inactivation which can occur through the degradation of the antibiotic, or by adding a chemical group to the drug. For instance, hydrolysis of β-lactam antibiotics by β-lactamase. In addition, antibiotic target site modification is a well-known strategy to avoid antimicrobial agents. Efflux pumps is another effective mechanism to eliminate the penetrated antibiotics, with many of these pumps transporting a variety of compounds (multi-drug [MDR] efflux pumps) [3].

A comprehensive analysis of the burden of AMR, published by the Institute for Health Metrics and Evaluation has estimated that AMR was associated with approximately 4.9 million deaths in 2019 globally, including an average of 1·27 million fatalities attributable to bacterial AMR [4]. AMR is considered to be the third cause of death worldwide, after ischemic heart disease and stroke, and the fourth cause of death in Libya. In Libya, 557 fatalities attributable to AMR and 2,200 deaths associated with AMR were reported in 2019 [5].

Medical professionals can be guided to choose the appropriate treatment after diagnosing the antibiotics susceptibility category to the isolate. Antimicrobial Susceptibility Testing (AST) is the gold standard test for diagnosing susceptibility. Susceptibility and resistance are usually measured as a function of the minimum inhibitory concentration (MIC). When it comes to bacteria, MIC is the interpretive category determinations of susceptible (S), increased exposure (I) and resistant (R) strains to the antibiotic. The breakpoints which are used to define these categories correspond to the standard clinical dosing regimen [6, 7].

Escherichia coli is a gram-negative bacterium and a well-known member of Enterobacteriaceae family. Normally *E. coli* is a gastrointestinal microflora, but may cause infections in case of overgrowth or extra-intestinal translocation. *E. coli* can be found in soil, water, undercooked meats, vegetables, and on the surfaces of hospitals and long-term care facilities. *E. coli* is considered as a major cause of nosocomial infections, including ventilator-associated pneumonia (VAP) and catheter-associated urinary tract infections

***Received**: 25-12-2024 - **Accepted**: 16-02-2025 - **Published**: 24-02-2025*

(UTIs). Generally talking, pathogenic strains may cause UTIs, bacteraemia, pneumonia and peritonitis [8]. The CDC guidelines of *E. coli* infections treatment, demonstrate that most *E. coli* infection cases recover without using antibiotics. In some cases, such as STEC infection the usage of antibiotics can worsen the problem. However, antibiotics are sometimes recommended to treat severe intestinal illness and people at risk for severe illness [9]. Miss-prescription of antibiotics and the availability of over-counter antibiotics in some countries, especially the developing ones, render the spread of AMR strains beyond the acceptable range. The overall burden of AMR in Libya as a developing country is not well understood due to poor documentation and inadequate data collection. Based on this need, this study aims to report AMR rates of *E. coli* clinical isolates in Gharyan city, a city located at the north-western district of Libya.

Methods

Study population and sampling technique

This cross-sectional descriptive study was conducted in Gharyan, a city located 80 km south of Tripoli, in Al-Jabal al Gharbi District at the north-western of Libya. This study used routine recovered *E. coli* isolates which were collected from the main four clinical laboratories in the region. The bacterial cultures were collected occasionally from April 2023 to March 2024. The isolates were transferred as bacterial cultures on Petri dishes to the laboratories of Gharyan College of Sciences. The bacterial isolates were subcultured on nutrient agar and incubated at 35 ºC overnight before testing their antibiotics susceptibility.

Antibiotics Susceptibility Testing

The bacterial isolates were subjected against a panel of antibiotics for susceptibility testing. Antimicrobial susceptibility tests of the isolates were performed using Kirby–Bauer disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing guidelines [10]. Briefly, an overnight subculture was used to prepare standardized bacterial suspension, which then was swabbed onto the surface of Mueller-Hinton agar (Liofilchem). Commercial disks of chloramphenicol (CHL), gentamicin (GEN), cefoxitin (or) ceftriaxone, meropenem (MER), moxifloxacin (MOX), amoxicillin/clavulanate (AUG) or ampicillin- sulbactam (UNZ) (all from CondaLab) were placed on the surface of 90mm plate aseptically with no more than six disks on each plate. The concentration of each antibacterial agent was chosen according to the recommended values by EUCAST guidelines [11]. The diameter of the inhibition zone around the disk was measured after overnight incubation at 35 ºC. The measurements were interpreted using the breakpoint tables for interpretation of MICs and zone diameters published by EUCAST [10]. *E. coli* ATCC25922 was used for quality control purposes.

Statistical analysis

All data were analysed using GraphPad Prism 10 software (San Diego, CA, USA). Graphs were used to show the prevalence and distribution of the isolated bacteria and their antibacterial susceptibility expressed in percentages. Additionally, frequency tables were used to display the susceptibility patterns of the isolates against the used antibiotics.

Results

Distribution of E. coli in the samples

A total of 107 *E. coli* isolates have been collected randomly from pathology centres in the city of Gharyan. Based on the tests conducted at their facilities, the highest number of *E. coli* isolates came from urine (92 isolates), while vaginal and wound isolates being the lowest (8 and 9 respectively) (Figure 1).

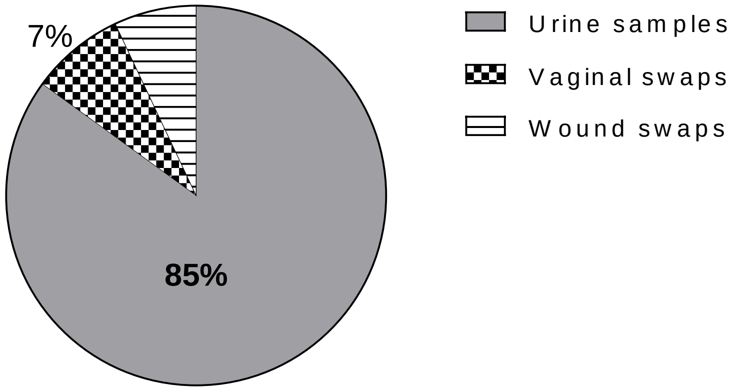

Figure 1. Distribution of E. coli among the clinical specimens

***Received**: 25-12-2024 - **Accepted**: 16-02-2025 - **Published**: 24-02-2025*

https://doi.org/10.54361/ajmas.258150

Frequency of Multi-drug resistance isolates

The isolates have been classified as susceptible (S), resistant (R) or increased exposure (I) based on the breakpoint tables for interpretation of MICs and zone diameters published by EUCAST [11]. From 107 *E. coli* isolates, 52% were resistant to at least two antibiotics (Fig. 2, Supplementary Table S1). Three of the isolates were resistant to at least 5 antibiotics.

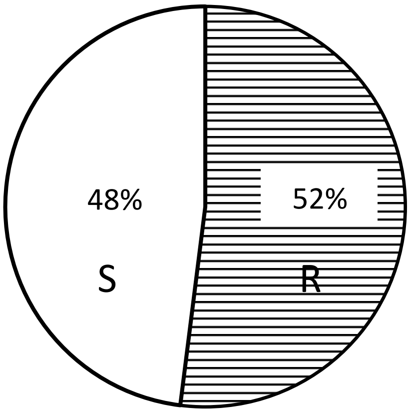

Figure 2: Frequency of Multi-drug resistance E. coli isolates; Resistant (R), Susceptible (S)

Antibiotic resistance patterns

Not every culture has results for its susceptibility to the six groups of antibiotics. That was because of unavailability of the antibiotic at the time of the test or the results were not readable. In total 107, 108, 100 *E. coli* isolates were tested for their susceptibility to CHL, MER and cephalosporins respectively. In addition, 76 isolates were tested to GEN, 85 to MOX, 35 to the AUG or UNZ. The general antibacterial susceptibility pattern of *E. coli* isolates from different specimen type is summarised in figure 3. This group was dominated by isolates from urine, and the overall pattern showed remarkable resistance to cephalosporins, the combinations of penicillins and beta-lactamase inhibitors (UNZ and AUG), followed by high resistance rates to MOX. On the other hand, *E. coli* isolates showed high sensitivity and relatively low resistance to meropenem followed by gentamicin and chloramphenicol. This pattern represents the AMR rates of urine isolates as they are dominant.

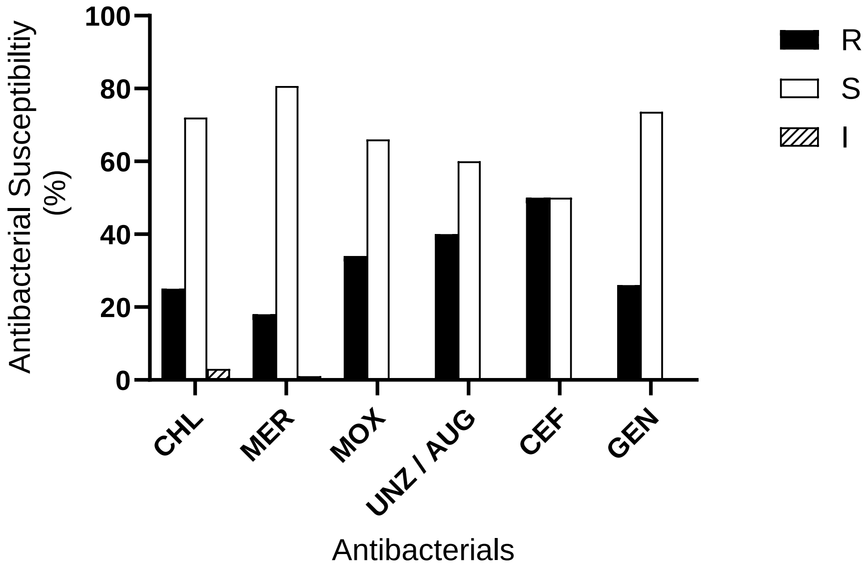

Figure 3. Antibacterial susceptibility pattern of E. coli isolates from different specimen types.

The isolates classified as susceptible (S), resistant (R) or increased exposure (I) based on the breakpoint tables for interpretation of MICs and zone diameters published by EUCAST (EUCAST, 2024a). Chloramphenicol (CHL), gentamicin (GEN), cefoxitin or ceftriaxone (CEF), meropenem (MER), moxifloxacin (MOX), amoxicillin/clavulanate (AUG) or ampicillin-sulbactam (UNZ).

***Received**: 25-12-2024 - **Accepted**: 16-02-2025 - **Published**: 24-02-2025*

Interestingly, analysing the susceptibility of *E. coli* isolates from wounds separately demonstrates a different pattern of resistance. The gentamicin resistance rate was the highest (70%) in these isolates, followed by CHL and the combinations of penicillins and beta-lactamase inhibitors (UNZ and AUG). In contrast, the sensitivity rate was three times higher than resistance when meropenem was used, whereas the sensitivity rate was double the resistance to MOX (Figure 4).
Analysing the resistance rates of vaginal isolates to the antibiotics also showed different pattern (Fig. 5). The highest resistance rate was for UNZ and AUG with no sensitive strains found, followed by cephalosporins and CHL. The isolates showed equal ratio of resistance and susceptibility to GEN (1:1) with a slight increase in resistance rate to MOX.

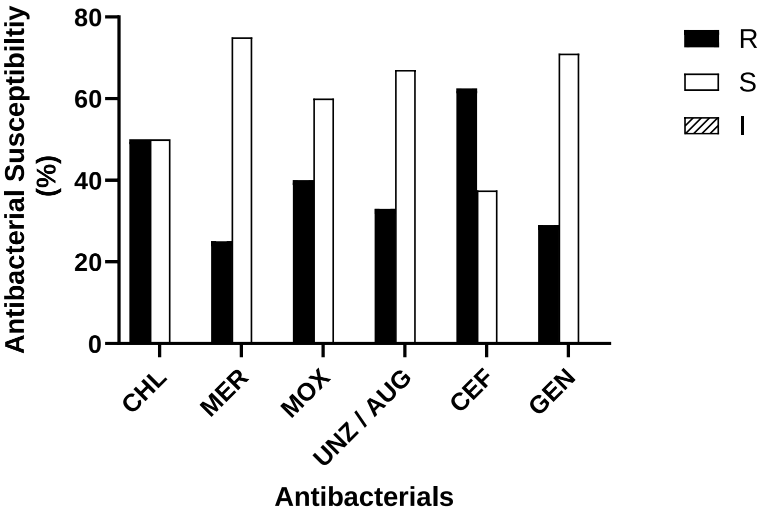

Figure 4. Susceptibility of E. coli isolates from wound specimens. The isolates classified as susceptible (S), resistant (R) or increased exposure (I) based on the breakpoint tables for interpretation of MICs and zone diameters published by EUCAST. Chloramphenicol (CHL), gentamicin (GEN), cefoxitin or ceftriaxone (CEF), meropenem (MER), moxifloxacin (MOX), amoxicillin/clavulanate (AUG) or ampicillin-sulbactam (UNZ).

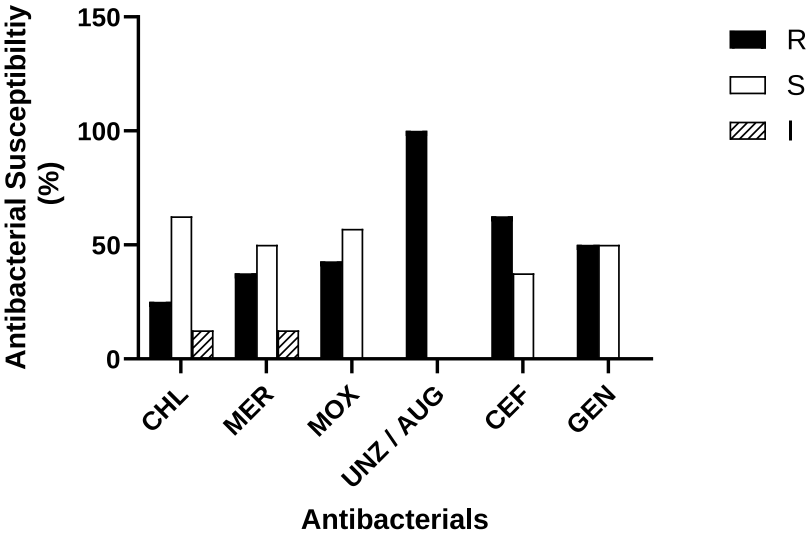

Figure 5. Susceptibility of E. coli isolates from vaginal specimens. The isolates classified as susceptible (S), resistant (R) or increased exposure (I) based on the breakpoint tables for interpretation of MICs and zone diameters published by EUCAST. chloramphenicol (CHL), gentamicin (GEN), cefoxitin or ceftriaxone (CEF), meropenem (MER), moxifloxacin (MOX), amoxicillin/clavulanate (AUG) or ampicillin-sulbactam (UNZ).

Discussion

The first-line available options for treating serious infections caused by Enterobacteriaceae include penicillins, cephalosporins, monobactams, carbapenems, fluroquinolones, and in certain situations, aminoglycosides. Unfortunately, the frequency of resistance to these agents has been increasing globally during the last few decades [12]. The traditional clinical analysis does not reflect the true value of antibiotics, because they often exclude suspected drug-resistant patients and adopt a non-inferiority design [13]. Therefore, statistical reports are important to portray the exact rates of antibiotic resistance within the

Received*: 25-12-2024 -* ***Accepted****: 16-02-2025 -* ***Published****: 24-02-2025*

society to warn healthcare professionals for the need to control infectious diseases.
There is no doubt that the massive lack of regular reports on the distribution of AMR in Libya hinder efforts to plan for suitable responses to address the problem effectively. The current study aimed to investigate the prevalence and patterns of AMR among *E. coli* isolates from various clinical specimens in Gharyan City. The findings highlight significant insights into the distribution of *E. coli* across different sample types, the extent of multi-drug resistance, and offer details of resistance patterns that could inform treatment strategies and public health interventions.
The results showed that *E. coli* was most frequently isolated from urine specimens (85%), followed by wounds (~ 8%) and vaginal samples (~ 7%). A systematic review analysed AMR reports over the past 20 years in Libya showed that UTIs were the most common reported clinical diagnosis in the country [14]. This distribution also aligns with the known clinical prevalence of *E. coli* as a common pathogen in urinary tract infections (UTIs) in Libya [15, 16]. The low proportion of isolation rates from vaginal and wound specimens may also suggest that, while *E. coli* can be present in these locations, it might not be the dominant pathogen compared to UTI. In contrast, Atia and his team [17] demonstrate that *E. coli* was the dominant Gram-negative bacteria isolate from vaginal infections, while Gram positive bacteria isolates were the main bacterial isolates from this site.
The ongoing increase of multidrug resistance among the Enterobacteriaceae is a major public health problem. The results of this study indicate that 52% of the *E. coli* isolates exhibited resistance to at least two antibiotics, with 3 out of 107 isolates showing resistance to five or more antibiotics from the different antibiotic classes. Multidrug resistance among *E. coli* from patients with urinary tract infections has been reported for a long time in North-western region of Libya (18, 19, 20). However the rate of prevalence was quite lower (33% [18]; 22% [20]). The notable increase of multi-drug resistance rate is of concern as it emphasises the growing challenge of treating infections caused by *E. coli* as it limits the therapeutic options. The high prevalence rate of MDR isolates highlights the necessity for ongoing surveillance and stewardship programs to manage and moderate resistance.
It is known that the resistance rate of Enterobacteriaceae to many antibiotics is relatively high in Libya [4]. The resistance patterns observed in this study are reflective of global trends but also present unique local characteristics. About 40% resistance rate has been reported towards cefoxitin the second-generation cephalosporin and ceftriaxone the 3rd generation cephalosporin. This rate is similar to what has been reported by Ghengesh et al. 2003 in Tripoli, where 49% of *E. coli* isolates were resistant to cephaloridine [21]. On another hand, it is almost four times higher than the reported resistance rate to ceftriaxone in 2017 [22]. This significant increase of resistance rate to cephalosporins in the region is a warning indicator of inefficient therapeutic outcomes using this group of antibacterial in UTI.
Importantly, according to EUCAST experts guidelines [22] a warning report regarding uncertain therapeutic outcome for infections other than urinary tract infections should be issued for isolates which are resistant to ceftriaxone and might be susceptible to other third generation (cefotaxime, ceftazidime) or fourth generation (cefepime) cephalosporin. Moreover, the significant resistance to the combinations of penicillins and beta-lactamase inhibitors (UNZ and AUG) among urine isolates and other isolates suggests a possible spread of beta-lactamase producing strains, which are known to hydrolyse cephalosporins and penicillins thus render them ineffective. The high resistance to MOX which present a different group of antibiotics (Fluoroquinolones), further complicates treatment options. Fortunately, the resistance rate to MER, a carbapenem does not exceed 15%. However, this low percentage should not be ignored as these strains can cause outbreaks in healthcare settings [24]. Carbapenems are considered as the drugs of choice for ESBL and AmpC producers and in case of invasive infections caused by carbapenems-producing Enterobacteriaceae, combined therapy is recommended in the treatment [25].
Generally, low resistance rates to gentamycin have been demonstrated (26%) which is equal to what had been reported by Elsyah et al. 2017 in Tripoli [22]. Another study also conducted in 2017 showed lower rate of *E. coli* resistance to GEN (16%) [20]. Interestingly, analysing the AMR rate of wound and vaginal isolates separately represents a different pattern from the general one. Despite the small number of *E. coli* wound and vaginal isolates (9 and 8 respectively) which can be statistically unreliable, some indicators can be concluded with caution. The results indicate that the isolation site affects the susceptibility of the microorganism to certain antibiotic. Analysing the AMR rate of wound isolates in separate showed a high rate of resistance to gentamycin, along with substantial resistance to CHL and UNZ/AUG. In addition, this observation suggests an adaptation of *E. coli* to these environments, possibly due to localized selective pressures or previous exposure to disinfectants or/ and antibiotics. In contrast, the relatively higher sensitivity to MER and MOX in wounds highlights these agents as potential treatment options in these sites.
The *E. coli* vaginal isolates showed the highest resistance to UNZ and AUG, with no susceptible strains identified. This result may reflect a hospital-specific or local issue, such as the misuse of these antibiotics in preventing or treating infections. The equal ratio of resistance and susceptibility to gentamicin, combined with increased resistance to MOX, suggests a diverse resistance profile in vaginal isolates that may require well planned treatment approaches.

Received*: 25-12-2024 -* ***Accepted****: 16-02-2025 -* ***Published****: 24-02-2025*

In conclusion, this study demonstrates that high rate of MDR *E. coli* isolates is a significant issue in Gharyan. The patterns of the prevalence of antimicrobials resistance vary depending on the site of infection and the antibiotic type. The results highlight both common and unique resistance issues. Addressing these challenges will require a multi-approach, including enhanced society awareness and surveillance on wise antibiotic use, and continued research to identify and eliminate factors contributing to resistance which inform more effective treatment protocols.

Acknowledgments
The authors would like to thank Mr. Melad Khalefa from Alnahda pathology Lab. and Miss Hana from Alhekma pathology Lab for their generous support.

Conflicts of Interest
The authors declare no conflicts of interest.

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***Received**: 25-12-2024 - **Accepted**: 16-02-2025 - **Published**: 24-02-2025*

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المستخلص

تجميع بيانات عن معدلات انتشار مقاومة المضادات الميكروبية في المجتمع يُعتبر أمرًا بالغ الأهمية في مواجهة التحديات الصحية، وذلك عن طريق المراقبة وتنفيذ التدخلات المطلوبة. هناك نقص في البيانات المتعلقة بمعدلات مقاومة مضادات الميكروبات لمسببات الأمراض في ليبيا، مما يعيق الجهود الرامية إلى معالجة المشكلة بشكل فعال. تُقيّم هذه الدراسة معدل انتشار مقاومة عزلات من الإشريشية القولونية لمجموعة من المضادات الحيوية بين زوار أهم مختبرات التحاليل الطبية في منطقة غريان. المضادات الحيوية التي تضمنتها الدراسة هي الجنتاميسين، والكلورامفينيكول، والسيفوكسيتين (أو) السيفترياكسون، والميروبينيم، والأمبيسيلين-سولباكتام (أو) الأموكسيسيلين- كلافولانات، والموكسيفلوكساسين. أظهرت نتائج 107 اختبار حساسية للمضادات الحيوية أن الإشريشية القولونية كانت معزولة في الغالب من عينات البول (85%)، تليها العينات المهبلية (8.4%) ومسحات الجروح (6.5%). كما أنه أكثر من نصف العزلات أظهرت مقاومة متعددة للأدوية، وهذا مؤشر مهم عن الحاجة إلى المراقبة المستمرة. يوضح النمط العام للنتائج معدلات مقاومة عالية للسيفالوسبورينات ومجموعة البنسلينات المدعمة بمثبطات البيتا لاكتاميز، تليها معدلات مقاومة مرتفعة للموكسيفلوكساسين. بالمقابل أظهرت نتائج الاختبارات حساسية العزلات للميروبينيم تليها الجنتاميسين والكلورامفينيكول. كما أن موقع العزل يبدو أنه عامل مُؤثر على أنماط المقاومة، فمعظم عزلات الجروح مقاومة للجنتاميسين بينما العزلات المهبلية كانت مقاومة للبنسلينات المدعومة بمثبطات البيتالاكتاميز. في حين كلاهما أظهر حساسية عالية للميروبينيم.

Received*: 25-12-2024 -* ***Accepted****: 16-02-2025 -* ***Published****: 24-02-2025*