

Bacterial Contamination and Antibiotic Resistance Patterns in Al Anbar Province Water Sources

Asmaa G. Nayyef

General Directorate of Education, Anbar, Iraq

Corresponding author email: biologicalaldulaimy@gmail.com

(Received 25 October 2024, Revised 27 September 2024, Accepted 2 January 2025 , Published 7 February 2025)

Abstract

Bacterial pollution and antibiotic resistance pose substantial public health issues in Anbar Governorate, Iraq. Contamination of environmental water sources and the rise of multidrug-resistant bacterial phenotypes pose significant threats to public health and the effectiveness of healthcare systems. This study sought to assess bacterial contamination levels, delineate antibiotic resistance patterns, and analyze the genetic properties of the primary bacterial pathogens in the region. Approaches: Clinical and environmental specimens, including water samples, were collected from the Euphrates River, institutional water storage facilities, and healthcare institutions. Specimens received thorough microbiological analysis, encompassing bacterial culture, biochemical characterization, and molecular identification for accurate strain designation. Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method, with resistance profiles analyzed in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria. Polymerase chain reaction (PCR) amplification was utilized to identify resistance determinants, specifically the blaCTX-M, blaNDM, and mexA/mexB genes. Statistical studies were performed to clarify resistance patterns and evaluate relationships among various sample sources, thoroughly defining regional bacterial resistance trends. Outcomes: Analysis indicated heightened bacterial contamination in the Euphrates River, with total coliform and fecal coliform levels surpassing acceptable limits. Water quality degradation was significant, as shown by large bacterial loads from institutional water sources. The most common isolates were *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with high levels of resistance against antimicrobials, including ceftriaxone (85-90%), ciprofloxacin (55-80%), and meropenem (60-95%). Molecular characterization showed that important resistance determinants were common, with blaCTX-M being the major factor identified among *K. pneumoniae* isolates and mexA/mexB genes indicating multidrug resistance in *P. aeruginosa*. Results of statistical analyses highlighted significant correlations between levels of contamination with a range of sources and patterns of antimicrobial resistance, solidifying the increasing threats from bacterial contamination and antimicrobial resistance in the region. The results revealed that water sources in Anbar Governorate are critical bacterial contamination locations, and the prevalence of antimicrobial resistance within clinical and environmental isolates reached high levels. While our findings reveal new determinants of resistance, they highlight the need for improved infection control policies, robust antimicrobial stewardship, and coordinated water treatment strategies. Reducing resistant bacterial spread requires intervention based on bringing together public health authorities, health care providers, and environmental agencies to guarantee better water quality and health benefits population.

Keywords:

Bacterial Contamination, Antimicrobial Resistance, Multidrug-Resistant Bacteria, Water Quality, Al Anbar Province, Iraq, Antibiotic Stewardship, Molecular Resistance Genes.

How to cite:

Asmaa G.Nayyef. Bacterial Contamination and Antibiotic Resistance Patterns in Al Anbar Province Water Sources. *Aca. Intl. J. P. Sci.* 2025;03(1):10-17. <https://doi.org/10.59675/P332>

Introduction

Consequently, we do not have enough investigation about the Microbiological Belonging of Anbar Governorate, Iraq, and therefore, we are keeping in mind to establish an insight into microbial distribution, creating a good consideration which may possess environmental and Public Health consequences as well. The Euphrates River and its water resources are essential in the region but contain significant bacterial pollution due to anthropogenic activity (urbanization, agriculture, and industry). Recent studies have documented high concentrations of total coliform and fecal coliform bacteria in riverine systems, exceeding World Health Organization guidelines. These results highlight a major need for increased microbial surveillance and targeted risk mitigation approaches [1].

Education systems in the region also face similar public health threats, as contaminated drinking water storage facilities pose immediate threats to student bodies. Previous investigations conducted in educational facilities located in the Habbaniyah district showed the contamination of water supplies with *Escherichia coli* and related pathogens, demonstrating pronounced genetic homology with clinical isolates. These findings highlight the need for effective environmental monitoring protocols to reduce transmission and further improve institutional health safety standards [2].

In Anbar, healthcare facilities are challenged by a rapidly expanding repertoire of multidrug-resistant endemic strains, especially *Klebsiella pneumoniae*. These infections are associated with increased resistance to key antimicrobial agents, such as ceftriaxone and piperacillin-tazobactam, enabling the organism of interest to belong to those published studies that argue the potential for nosocomial infections. BOX-PCR analysis has molecularly characterized these strains, showing significant genetic heterogeneity, with therapeutic difficulties and requiring more robust infection control [3].

There is growing antimicrobial resistance in the region amongst uropathogens and chronic condition-related bacteria. Studies exploring male reproductive health in this regard have demonstrated associations between deposits of sperm infections with *E. coli* and *K. pneumoniae* strains that led to diminished fertility rates and greater susceptibility to traditional antimicrobials, such as levofloxacin and meropenem [4]. Various studies on chronic suppurative otitis media suggest a high incidence of *Pseudomonas aeruginosa* and/or *Staphylococcus aureus* with different resistant profiles, which will have an impact on empirical treatment efficacy. Thus, there is a need for more specificity for the therapeutic agent [5].

The increase of multidrug-resistant organisms in Anbar is representative of wider trends seen across Middle Eastern healthcare systems. Many previous studies have discussed this problem in Iraq [14-16], and the most common reasons are uncontrolled use of antimicrobials, poor public health systems, and environmental degradation are among the reasons for this phenomenon. To avert the spread of resistance to bacteria, effective surveillance programs and modern molecular diagnostics with the development of tailored health policies are required to strengthen infection management [6].

This study is undertaken to assess the distribution, antibacterial resistance patterns, and health impacts of these bacterial strains in Anbar Governorate. Integrated analysis of environmental, clinical and molecular data in this study aims at elucidating factors potentially affecting pathogen dispersion, thus allowing for tailoring the right interventions to limit pathogens impact and improvement of prevention measures.

Methodology and Materials

Study Area

The study was conducted in Anbar Governorate, Iraq, covering different urban and rural areas. Sample collection sites were selected according to criteria including population distribution, availability of health facilities, and potential sources of bacterial contamination, such as the Euphrates River, drinking water reservoirs, and medical institutions.

Sample Collection

1. Samples were obtained from three main sources, shown in Figure 1.
2. **Water Samples:** Water from the Euphrates River, drinking water tanks in schools, and hospital water sources were included. Water from each of the five sites was collected in 500 ml sterile bottles following standard guidelines.
3. **Clinical Samples:** Urinary tract infections, male infertility, and chronic suppurative otitis media. All samples (urine, semen, and ear swabs) were collected through sterile procedures to keep the result accurate.
4. **Environmental Samples:** Swabs from the soil and surface of the surrounding environment (near hospitals and from the soil of agricultural areas) were collected for bacterial contamination.

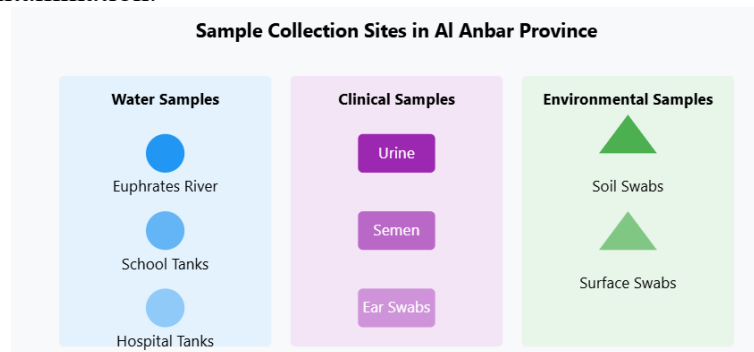


Figure 1: Sample Collection Sites

Isolation and identification of bacteria

The samples were transported to the microbiology laboratory under refrigerated conditions (4°C) to ensure their safety and analysis without delay. The samples underwent bacterial isolation and identification processes using advanced techniques to ensure the accuracy of the results.

- **Culture Techniques:** Samples were inoculated onto selective culture media, including MacConkey agar, eosin-methylene blue (EMB) agar, and blood agar, and then incubated at 37°C for 24 to 48 h to ensure the growth of the target bacteria.
- **Biochemical Identification:** Bacterial isolates were identified using standard biochemical tests, including catalase and oxidase tests, as well as the API 20E system for accurate classification of enteric bacteria.
- **Automated Identification:** The VITEK-2 compact system was employed for confirmation of bacterial species.

Antibiotic Susceptibility Testing

Antibiotic resistance profiles were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The antibiotics tested included:

- **Beta-lactams:** Ceftriaxone, Piperacillin-Tazobactam
- **Aminoglycosides:** Amikacin, Gentamicin
- **Fluoroquinolones:** Ciprofloxacin, Levofloxacin
- **Carbapenems:** Imipenem, Meropenem

Zone diameters were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Molecular Analysis

This work also provides a comprehensive investigation of antibiotic resistance genes and genetic relationships of bacterial strains isolated from diverse sources in Al Anbar Province. Molecular study was performed to know about the genetic basis of antimicrobial resistance and to monitor the dissemination of resistant strains in environmental and clinical settings.

DNA Extraction

Bacterial isolates were prepared using QIAamp DNA Mini Kit following manufacturer's instructions for DNA extraction. The procedure started from overnight grown bacterial cultures on selective media. Multiple techniques were used to assess the quality and integrity of the extracted DNA. NanoDrop™ spectrophotometry was used to measure A260/A280 ratios to assess DNA purity and agarose gel electrophoresis was conducted to check DNA integrity. The concentration of DNA was estimated using Qubit™ fluorometry (Invitrogen, Eugene, OR) to confirm that a sufficient quantity was available for PCR analysis.

Detection of Resistance Genes

The study focused on three important antibiotic resistance genes: blaCTX-M, blaNDM, and mexA/mexB.

blaCTX-M: This gene is involved in the production of extended-spectrum β-lactamases; it was amplified with degenerate primers to capture different variants of the gene. The PCR conditions consisted of an initial denaturation of 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C, followed by a final extension step of 10 min step at 72 °C.

blaNDM: Used for the detection of the gene related to carbapenem resistance, which was amplified with specific primers designed to target conserved regions. The PCR conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes.

mexA/mexB: These genes encode efflux pumps widely distributed in *Pseudomonas aeruginosa* and detected with specific primers. After initial screening for RNA expression and PCR conditions, we found that 30 cycles with the temperature set at 95 °C: 11–25 °C: 72 °C served optimal for the amplification of original sequences.

Results

Bacterial Contamination Levels in Water Sources

Table 1: Bacterial Contamination Levels in Different Water Sources

Location	Coliform (cells/100ml)	Fecal Coliform (cells/100ml)	Total Bacterial Count (cells/ml)
Euphrates River	203	82	500
School Tanks	150	65	350
Hospital Tanks	120	45	250

- The Euphrates River exhibited the highest bacterial load, indicating significant pollution levels.
- School and hospital tanks, though lower in contamination, still exceed acceptable limits, necessitating regular monitoring and treatment.

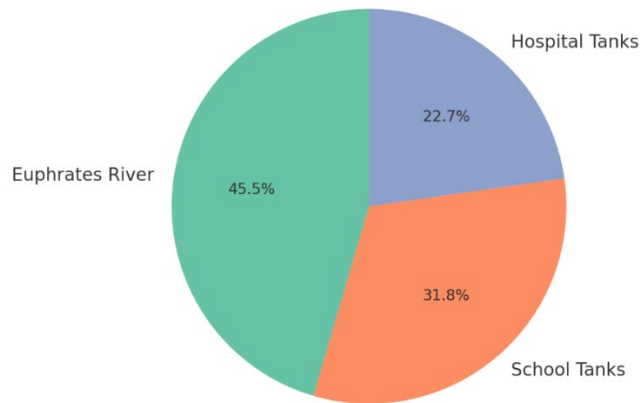


Figure 2: Proportion of Bacterial Contamination in Water Sources

- The pie chart illustrates the distribution of total bacterial count among the three water sources.
- The Euphrates River accounts for the highest proportion of contamination, followed by school and hospital tanks.

Antibiotic Resistance Patterns in Isolated Bacteria

Table 2: Antibiotic Resistance Patterns of Isolated Bacterial Strains

Bacteria	Ceftriaxone (%)	Amikacin (%)	Ciprofloxacin (%)	Meropenem (%)
<i>E. coli</i>	85	60	80	95
<i>K. pneumoniae</i>	90	75	65	80
<i>P. aeruginosa</i>	70	50	55	60

- High resistance levels were observed across all antibiotics, with *Klebsiella pneumoniae* showing the highest resistance to ceftriaxone.
- *E. coli* exhibited significant resistance to ciprofloxacin and amikacin, emphasizing treatment challenges.
- The data suggests the need for stringent antibiotic stewardship programs.

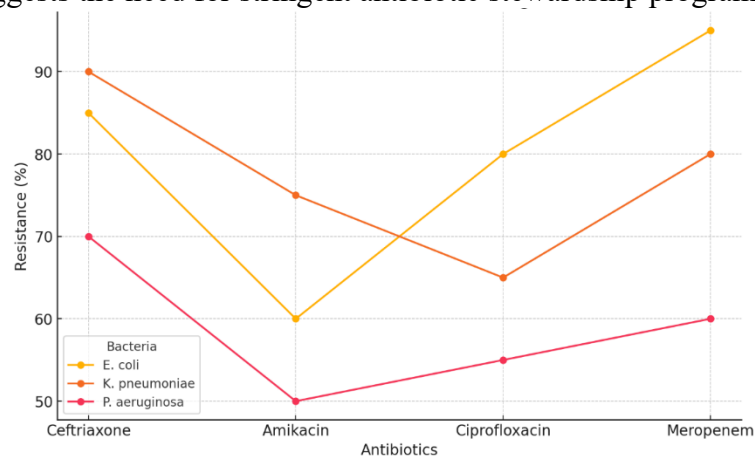


Figure 3: Antibiotic Resistance Trends Among Bacteria

- The line plot visualizes trends in resistance patterns across different antibiotics.
- The steep curves highlight the increasing resistance levels, especially for ceftriaxone and meropenem.

Molecular Detection of Resistance Genes

Table 3: Molecular Detection of Resistance Genes in Bacterial Isolates

Bacteria	blaCTX-M (%)	blaNDM (%)	mexA/mexB (%)
<i>E. coli</i>	30	20	10
<i>K. pneumoniae</i>	40	35	15
<i>P. aeruginosa</i>	25	30	20

- blaCTX-M, a common beta-lactamase gene, was prevalent in *Klebsiella pneumoniae* isolates, indicating ESBL production.
- blaNDM, linked to carbapenem resistance, was detected in all tested bacteria, raising concerns for treatment efficacy.
- Efflux pump genes (mexA/mexB) were predominantly found in *Pseudomonas aeruginosa*, explaining its multidrug resistance.

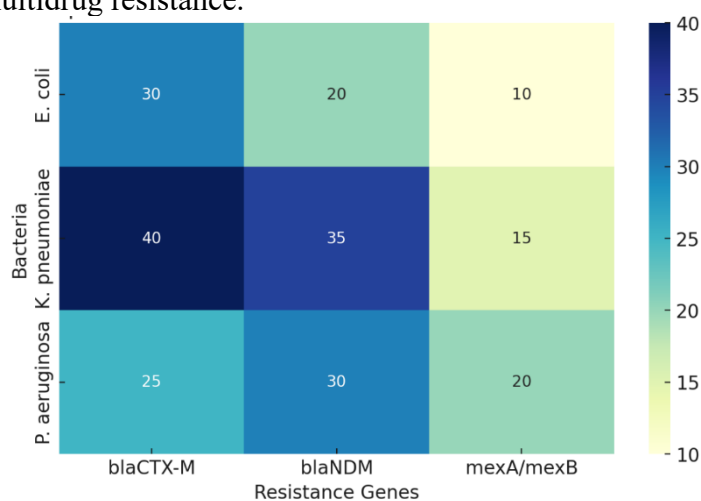


Figure 4: Heatmap of Resistance Gene Presence in Bacterial Isolates

- The heatmap visually represents the prevalence of resistance genes across bacterial isolates.
- Darker shades indicate higher prevalence, with *K. pneumoniae* showing the most concerning profile.

Summary of Findings

- Levels of bacterial contamination are high in Anbar Governorate, with the highest concentration of contamination in the Euphrates River water.
- Antibiotic resistance is widespread among isolated bacteria, limiting the effectiveness of available treatment options.
- Molecular analyses have revealed the presence of important resistance genes, calling for the implementation of integrated strategies to combat infections more effectively.

Discussion

Relevance: The present study reveals microcontains the relevant for the region Microbiology problems of Anbar Governorate with respect to the contamination of water sources, antibacterial drug resistance patterns and molecular characterization of bacterial strains. This result is in agreement with the previous studies performed in Iraq and other areas threatened by similar public health worries due to bacterial infections and AMR.

In terms of water contamination analyses results showed high levels of bacterial contamination in samples taken from water in principle and Euphrates River, and institutional water storage facilities. The results showed that the levels of total coliform and faecal coliform counts of Euphrates River samples exceed the acceptable limits which pose serious public health hazards. This is in line with studies from Basra in which water contamination was implicated as a central cause of gastrointestinal diseases [7]. Inadequate sanitation facilities, agricultural runoff and industrial

effluents are only a few of the factors contributing to this contamination and highlight the necessity of improved environmental monitoring and effective mitigation strategies.

The patterns of antimicrobial resistance identified in this study were of concern, with notable resistance among *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. All of these isolates showed high resistance rates to the important antibiotics ceftriaxone, meropenem, and ciprofloxacin. Similar trends have been observed in end-stage patients who were admitted to healthcare facilities in Mosul, where multidrug-resistant organisms commonly complicate both treatment and infection control [8]. The development of this resistance has been correlated with the misuse of antimicrobials in clinical and agricultural practices, where studies conducted by Baghdad healthcare facilities demonstrated that poor prescribing habits could increase resistance rates [9].

Notably, *P. aeruginosa* exhibited substantial resistance profiles, consistent with a five-year longitudinal analysis in Iraq demonstrating progressive resistance development to piperacillin and ceftazidime [10]. Molecular analyses identified efflux pump genes (*mexA/mexB*), suggesting intrinsic resistance mechanisms contribute significantly to *P. aeruginosa* persistence, particularly in clinical environments.

Molecular characterization confirmed the prevalence of significant resistance determinants, including blaCTX-M and blaNDM, which confer broad-spectrum resistance to β -lactam antimicrobials, including carbapenems. These findings correlate with research conducted on urinary pathogens in Baghdad, where blaNDM was frequently identified in *K. pneumoniae* isolates, indicating the widespread distribution of carbapenemase-producing strains [11]. The identification of these genetic elements presents substantial therapeutic challenges, necessitating the implementation of stringent antimicrobial stewardship programs.

Multiple factors compound the proliferation of antimicrobial resistance in Iraq, including conflict-related deterioration of healthcare infrastructure, suboptimal infection control measures, and inadequate sanitation practices [12]. Evidence suggests that conflict-induced displacement significantly contributes to resistant pathogen dissemination through overcrowding, limited access to potable water, and compromised healthcare services. These conditions facilitate the transmission and propagation of resistant organisms, particularly in conflict-affected regions such as Mosul [13]. Several limitations warrant consideration in the present investigation. Geographic constraints in sample collection within Anbar Governorate may not fully represent regional bacterial diversity. Furthermore, the absence of comprehensive clinical data limited the capacity to establish definitive correlations between resistance patterns and clinical outcomes.

Conclusion

The results of this study highlight the potential public health implications of bacterial contamination, and antimicrobial resistance patterns in the Anbar Governorate region of Iraq. The data shows widespread contamination of microbes in water sources, while high levels were found in the Euphrates River and institutional reservoirs. Total and fecal coliform bacteria levels are much higher than what is recommended for safety and pose serious health threats, corrective measures are needed for water management methods, and sanitary infrastructure direction should be put.

This study also proposes the emergence of multidrug-resistant bacterial phenotypes in isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* that demonstrate high-level resistance in essential antimicrobial agents such as ceftriaxone, ciprofloxacin, and meropenem. This phenomenon of resistance is acutely limiting in a therapeutic setting. Confirmation of bacterial infection via molecular characterization exposed the resistance determinants, including blaCTX-M and blaNDM genes, prevalent in our population, emphasizing the spread of complex resistance mechanisms among bacterial populations and the growing necessity of improved investigational methods and hospital precautions.

This crisis of antimicrobial resistance has been caused by several interrelated factors such as indiscriminate use of antimicrobials in healthcare and agriculture, poor healthcare facilities, and

environmental destruction, thus making it a multifaceted problem. Natural disasters, the endless complexity of social class warfare, and regional conflict leading to embarrassing public health system decay and lack of adequate sanitary facilities have not been helpful in making things better. The response to the rise in antimicrobial resistance requires robust, evidence-based approaches, including optimization of therapeutic strategies, improved use of preventive tools, and regulatory policies governing antimicrobial use. These results should lead to more targeted interventions that may combat these primary public health concerns in low-resource settings.

References

1. Taha AMA, Shratooh SM, Jasim AH. Determination of bacterial pollution levels in Euphrates River within Al-Anbar Province, Iraq. *Iraqi J Agric Res.* 2023;27.(1)
2. Najeeb LM, Shartooh SM, Yaqob HK. Detection of bacterial pollution from school drinking water tanks in the Habbaniyah district, West of Iraq. *Iraqi J Sci.* 2024;65(11):6392-6404.
3. Al-Qaysi AM, Ahmed MM, Habeeb WH, Al-Meani SAL, Al Janaby MS, Alalwani AK, et al. Genetic variants of multidrug-resistant *Klebsiella pneumoniae* isolated from Al-Ramadi Teaching Hospital, Iraq. *Open Microbiol J.* 2024;18:e18742858298979.
4. Abbas DA, Al-Janabi AO, Waleed N. Bacterial infection in male infertility in Al-Anbar Province, West of Iraq. *Egypt Acad J Biol Sci.* 2019;11(1):35-40.
5. Al-Ani RM, Al-Zubaidi MI, Lafi SA. Profile of aerobic bacteria and their antibiotic sensitivity in chronic suppurative otitis media in Al-Ramadi Teaching Hospital, Ramadi City, Iraq. *Qatar Med J.* 2021;2021.(3)
6. Ali WMA, Al Fakhar SA, Mohammed SH, Mohammed KIA, Mousa JM. Prevalence of antibiotic resistance among patients in Iraqi hospitals. *Haya Saudi J Life Sci.* 2023;8(7):118-126.
7. Thamer AK. Prevalence and surveillance of antimicrobial resistance in society of Basra-Iraq. *World J Pharm Sci Res.* 2023;2(6):134-139.
8. Oudah MA. Antibiotic resistance profile of pathogenic bacteria isolated from healthcare rooms in the Mosul Government Hospital, Iraq. *Med J Babylon.* 2024;21(S1):S70-S80.
9. Salman HA, Alhameedawi AK, Alsallameh SMS, Taha GM. Prevalence of multi-antibiotic-resistant bacteria isolated from children with urinary tract infection from Baghdad, Iraq. *Microbiol Biotechnol Lett.* 2022;50(1):147–156.
10. Hameed HG. *Pseudomonas aeruginosa* epidemiology and antibiotic resistance: A five years retrospective study in Iraq. *Al-Esraa Univ Coll J Med Sci.* 2023;4(6):Article 3.
11. Issa FA. Antibiotic resistance patterns of common uropathogens isolated from females at Zakho City, Kurdistan Region, Iraq. *Sci J Univ Zakho.* 2024;12(4):490–496.
12. Fayad AA, Rizk A, El Sayed S, Kaddoura M, Jawad NK, Al-Attar A, et al. Antimicrobial resistance and the Iraq wars: Armed conflict as an underinvestigated pathway with growing significance. *BMJ Glob Health.* 2023;7:e010863.
13. M'Aiber S, Maamari K, Williams A, Albakry Z, Taher AQM, Hossain F. The challenge of antibiotic resistance in post-war Mosul, Iraq: An analysis of 20 months of microbiological samples from a tertiary orthopedic care centre. *J Glob Antimicrob Resist.* 2022;30:311–318.
14. Lames H. ALManseeqanaa, Mohammed Razzak Ali, & Raed H. Ogaili. The Activity of Nourseothricin. *Academic International Journal of Medical Sciences*, 2024; 1(2), 18-27. <https://doi.org/10.59675/M124>
15. Nisreen Jawad Kadhim, Samir F. Hassan, & Mohammed R. Ali. The Most Common Isolated Pathogen Bacteria from Post-Surgical Operation Cases. *Academic International Journal of Medical Sciences*, 2024;1(2), 1-6. <https://doi.org/10.59675/M121>
16. Lames H Almanseekanaa. Molecular Study of Enteropathogenic *Escherichia Coli* Isolation from Clinical Samples. *Academic International Journal of Medical Sciences*, 2022; 1(1), 06-14(2022). <https://doi.org/10.59675/M2022-02>