Egyptian Journal of Medical Microbiology Volume 34 / No.2 / April 2025 49-55 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Prevalence of Multidrug-resistant *Acinetobacter* Species in Pediatric Patients in AL Najaf Province

[1]Liqaa S. Zghair, [1]Zahraa Y. Motaweq*, [2]Zahraa M.M. Alrufaie, [3]Yusra Z. Salman, [4]Makki A. Muslim A. Alzahra, [1]Mohauman M. Majeed

[1]Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq
[2]Jabr Ibn Hayan University, College of Medicine, Pediatric Department
[3]Senior pediatrician Al Najaf health directorate, Iraq
[4]Kufa University, College of Medicine, Pediatric Department

Key words:
Multidrug-resistant, Acinetobacter species, pediatric patients, AL Najaf Province

Corresponding Author:
Zahraa Yosif Motaweq
Department of Biology, Faculty of Science, University of Kufa, Najaf, Iraq
zahraa.mutawak@uokufa.edu.iq

ABSTRACT

Background: *Acinetobacter baumannii is the cause of opportunistic nosocomial infection and one of the six major multidrug-resistant bacteria in hospitals worldwide.* ***Objectives:*** *Blood stream infections and ventilator-associated pneumonia are the most frequent diseases brought on by this human bacteria, and mortality can reach 35%.* ***Methodology:*** *In this study, 77 blood and urine samples were collected from children who had been receiving treatment for Acinetobacter infection at AL-Zahra teaching hospital in Al-Najaf, Iraq, for two years. In contrast to the 24 Acinetobacter spp. recovered in 2021–2022, only 53 Acinetobacter spp. were found in 2020–2021.* ***Results****: The findings showed that Acinetobacter isolates were divided into the following species: In 2020–2021, Acinetobacter spp. cause 9.43% of the isolates, 1.8% of the isolates were Acinetobacter lwoffii, and 88.6% of the population was Acinetobacter baumannii. The isolates of Acinetobacter spp. that were used in this study were resistant to a variety of antibiotics, including CIP (73.3%), CAZ (89.13%), MEM (68.2%), CTX (89.4%), IPM (83.3%), LEV (75%), TOB (62.5%), and SXT (70.27%). This is in contrast to the isolates that were collected in 2020–2021, which had higher resistance percentages toCIP(57. 8%), CAZ (83.3%), MEM (45.45%), CTX (90.90%), IPM (44.44%), LEV (41.6%), TOB (20%), SXT (55.5%) for isolates that collected in 2021-2022.* ***Conclusions*** *Acinetobacter baumanni isolates from blood and urine. have been recognized as an important pathogen that causes various infection in Najaf City. High antibacterial agent's resistance among Acinetobacter spp. and MAR index was a concern among these isolates. There are increasing in the antibiotic resistance of Acinetobacter spp for COL, TOD, TIG and POLB in 2021-2022 period*

INTRODUCTION

Acinetobacter bacteria is a pleomorphic aerobic gram-negative bacillus commonly isolated from the hospital environment and hospitalized patients. The Acinetobacter has more than 50 species, most of which are nonpathogenic environmental organisms. The most common infection-causing species is *Acinetobacter baumannii*, *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* [1]. This type of *Acinetobacter* can cause a variety of infections in people, including: blood infections (fever, Chills, vomiting, confusion), urinary tract infections (frequency, urgency, dysuria, hematuria, cloudy urine, foul smelling urine), pneumonia and wound infection[2]. Healthcare facilities all over the world struggle with *A. baumannii's* in famous tendency to create and gain resistance to almost every antibiotic in our arsenal[3]. However, the most hazardous conditions that *A. baumannii* can cause include bacteremia and ventilator-associated pneumonia (VAP). This risk is greatly heightened for patients in critical care units (ICUs), where fatality rates can exceed 40%[4] . Despite the fact that it was acknowledged that the number of reports published from low-income countries (LIC) may be less than that of high-income ones, information about the *A. baumannii* epidemic is flowing from all over the world. This was explained as being due to a lack of resources for pathogen detection, identification, and even reporting results rather than the low prevalence of *A. baumannii* in these situations[5]. An emerging approach to deal with the problems of *A. baumannii* antibiotic resistance is to look for new ways to identify effective, long-lasting treatment options [6].

The prevalence of *A. baumannii* infections varies around the globe; for example, in the USA, it was anticipated that there will be 8500 cases annually in 2019, with an 8% fatality rate. However, it has also been associated with community-acquired infections in Asia and Australia, as well as infections related to war

and natural disasters[7]. Eastern and southern European countries saw more illnesses than northern and western European countries, with the prevalence of infections in Europe on the other side of the Atlantic Ocean ranging from 2 to 8%[8]. The fact that *A. baumannii*, and notably CRAB, is spreading throughout the African population is clear despite the lack of information Similar patterns can be seen in South east Asia, where *A. baumannii* was blamed for 10.7 to 23.3% of hospital-acquired infections (HAIs)[9].An increasing public health concern in the Caribbean and Latin America is CRAB[10].

A *baumannii* is able to withstand the bactericidal and bacteriostatic effects of antibiotics thanks to a variety of resistance mechanisms shared with other Gram-negative bacteria. These include producing hydrolytic enzymes like carbapenemases and extended spectrum b-lactamases (ESBLs), altering the outer membrane protein porin permeability, and amplifying efflux pumps that expel antibiotics from the cell (efflux mechanisms)[11] . This study aimed to identify the antibiotic resistance of bacterial isolates by isolation and diagnosing *Acinetobacter* spp, which causes severe nosocomial infections.

METHODOLOGY

Diagnosis of *Acinetobacter* spp

Different differential, selective, and enrichment media were used to grow the bacteria found in blood and urine specimens taken from young patients who were infected with *Acinetobacter* spp. during the 2020–2021 and 2021–2022 study periods at AL-Zahra teaching Hospital/Al-Najaf, Iraq. Each urine sample was collected, centrifuged at 3000 rpm for 5 minutes, and then the precipitate was cultured on selective medium. Identification of *Acinetobacter* was done by biochemical reactions and Vitek-2 compact system.

Antibiotics Susceptibility Test (Disk Spread Technique):

The Muller Hinton agar medium (India) was used to cultivate each genus of bacteria. Each plate received an antibiotic disc, and 0.1 ml of the bacterial suspension (instead of 0.5 from the MacFrland tube) was added to the agar surfaces. Following a 24-hour incubation period at 37°C, the antibiotic discs were gently pressed down to guarantee thorough contact with the bacteria-inoculated agar. The inhibition zone was then measured in millimeters (mm). The results led to the conclusion that they were either resistant or sensitive[12].

PCR:

Using the boiling technique, genomic DNA was successfully recovered from *Acinetobacter* isolates. The RNA/DNA spectrophotometer (Biodrop) instrument directly evaluated the concentration and purity of extracted DNA; extracted DNA purity ranged between (1.8-2). Gel electrophoresis was used to confirm and analyze the extracted DNA[13].

Primer type of pml:

Primer *CTX-M* Sequence was 5´-3´ F: AACCGTCACGCTGTTGTTAG, R: TTGAGGCGTGGTGAAGTAAG in the Product size 512 bp[8] and *AmpC* sequence was 5´-3´ F: TGCTCGGCATCTCTTGCTCT, R: CAGCTTGAGCGGCTTAAGGA in the product size 558 bp[7].The condition of the primers were included: Initial Denaturation 94\2m, followed 35 cycles of (Denaturation 95\30 sec., Annealing 56\45 sec and Extension 72 \30 sec), finally the Final Extension was 72\7 min. The reaction mixture was held at 4°C until use while the final extension step took place at 72°C for about 10 minutes. Each and every PCR amplification was performed using a Verity Thermal Cycler (Agilent, UK). Then, 1% agarose gel electrophoresis was used to analyze all of the PCR products, and they were all stained with red ethidium bromide dye. Finally, the gel documentation system was used to identify the electrophoresis results.

RESULTS

Isolation and identification of bacterial isolates from patients

In this investigation, pediatric patients' blood and urine samples were collected over a two-year period in order to isolate and identify *Acinetobacter* spp. as the primary source of these infections; however, only 77 *Acinetobacter* spp. were isolated in these specimens. Only 53 *Acinetobacter* spp. were detected in all specimens in 2020–2021; in contrast, 24 *Acinetobacter* spp. were found in all specimens in 2021–2022. The following is a list of bacteria according to species: In 2020–2021, there were 9.43% *Acinetobacter* spp., 1.88% *A. lwoffii*, and 88.6% *A. baumannii*, as opposed to 100% *A. baumannii* in 2021–2022.

The study's findings, as shown in Table (1) and Figure (1), indicated that the infection rate among children receiving medical care and whose ages range from 0 to 5 years is higher when compared to children whose ages (6 to 10 years) and (11 to 15 years) are two years older.

Table 1: Prevalence of bacteria during 2020-2022

Age Group	Total No. (%) in 2020-2021	Total No. (%) in 2021-2022
(0-5) year	48 (90.5)	18(75)
(6-10) year	3(5.66)	4(16.66)
(11-15) year	2(3.77)	2(8.33)
Total	**53(100)**	**24(100)**

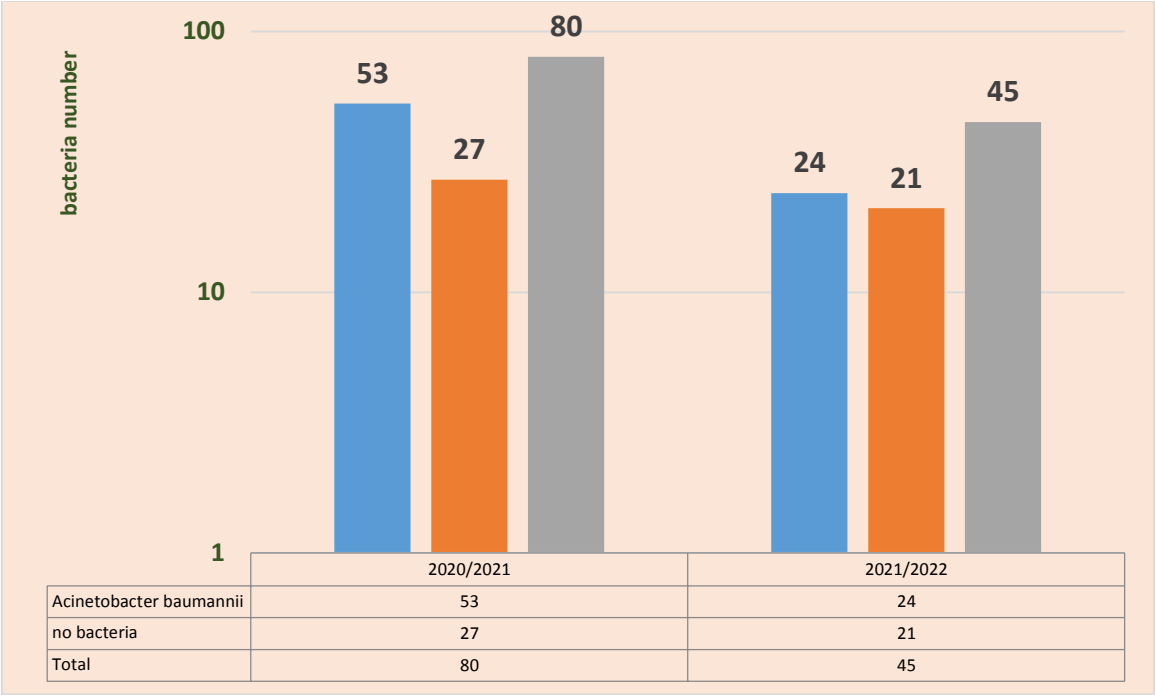

Fig. 1: Distributions of bacteria isolated during (2020-2022).

Antibiotic susceptibility test

The results of the disk diffusion method antibacterial susceptibility test for 77 isolates against 16 commonly used antibacterial agents revealed that all *A. baumannii* isolates were resistant to many antibiotics used to treat bacterial infections, including: Imipenem, Ceftazidime, Meropenem, Levofloxacin, Ceftriaxone, Amikacin, Pipracillin, Ciprofloxacin, Trimethoprim, Colstin[14].

Table 2: Susceptibility tests for *Acinetobacter* spp to many antibiotics.

Antibiotics	2020-2021		2021-2022	
	% R	% S	% R	% S
CIP	73.3	26.6	47.3	57.89
MEM	68.2	31.7	54.5	45.45
CAZ	89.13	8.69	16.66	83.33
PRL	90.9	9.09	15.38	84.61
CTX	89.4	10.52	9.09	90.90
CRO	92.3	7.69	7.69	92.30
SXT	70.27	29.7	44.4	55.5
IPM	83.3	16.66	55.5	44.4
COL	5.88	94.11	88.8	11.11
TOB	62.5	37.3	80	20
TIG	50	50	100	0
AK	73.68	26.3	83.33	16.66
SAM	80	20	20	80
LEV	75	25	58.3	41.66
Polymyxin B	0	100	100	0
FEP	87.5	12.5	20	80

S: Sensitive, R: Resistance.

Molecular screening of *AmpC* gene

Of the 20 isolates 24/40 (60%) gave positive result of present *AmpC* gene, AmpC-β-lactamase producing *Acinetobacter* spp. isolates identified using the PCR technique (Figure 2).

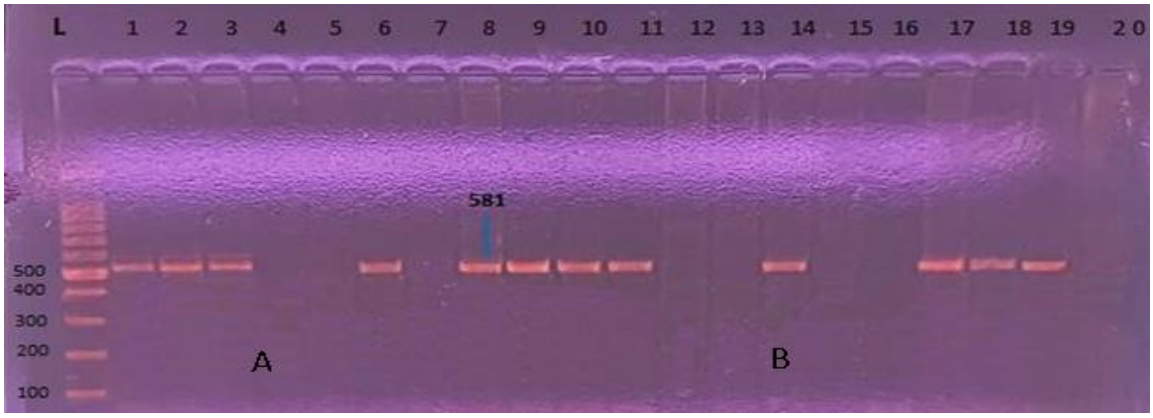

Fig. 2: Gel electrophoresis of PCR amplified product of Ampc gene with product 558 bp of *Acenitobacter* spp. isolates, (A:1-10 isolates in 2020-2021 and B: isolates in 2021-2022). (L), DNA molecular size marker (100-4000 bp ladder), products migrated at 85 volt for 90 minutes and stained with ethidium bromide.

Expression of AmpC is regulated by AmpR, a transcriptional regulator, coded by the *AmpR* gene located next to AmpC and divergently transcribed. The AmpR mutant was more susceptible to β-lactams than the wild-type strain and lost the ability to induce the transcription of AmpC. Transcriptomic analysis also showed that AmpR was constitutively transcribed both in the wild-type strain and in all the isogenic mutants, confirming that the activity of AmpR depends on the interaction with co-regulators rather than an over- or under-expression of AmpR. This is in accordance with the current model where, in the absence of β-lactams, AmpR represses AmpC transcription and when exposed to antibiotics, cells accumulate peptidoglycan catabolites (i.e. 1,6-anhydroMurNAc-peptides), changing it to an activator of AmpC transcription. AmpR plays a role in physiological processes and influences the expression of over 500 genes, some of which encoding virulence genes and other transcriptional regulators[15,16].

One of the goals of this study was to count and categorize the number and frequency of ESBL and Inducible AmpC-producing *Acinetobecter* spp. isolates. This step is critical to avoiding the wrong treatment option. The resistant to multiple commercial drugs among genus of Enterobacteriaceae are often related to the production of extended-spectrum β-lactamases (ESBLs) and represent an increasing global threat. The first report of ESBL-producing isolates from vegetables and fruit was reported in 2014 in The Netherlands[17].

Molecular screening of *CTX-M* Genes

A total of 40 *Acinetobacter* spp. isolates from clinical cases were molecularly examined for the presence of the *CTX-M* gene. The current study pointed out that *CTX-M* gene present among 22/40 (55%) of isolates, Figure (3).

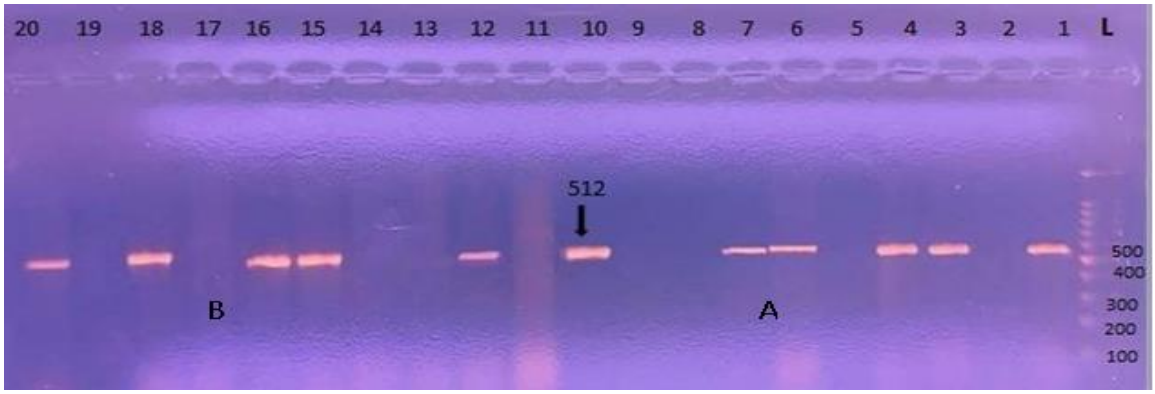

Fig. 3: Gel electrophoresis of PCR amplified product of *CTX-M* gene with product 512 bp, (A:1-10 isolates in 2020-2021 and B: 11-20 isolatesin 2021-2022), L: DNA molecular size marker (100-4000 bp ladder), products migrated at 85 volt for 90 minutes and stained with ethidium bromide.

DISCUSSION

The results of the current investigation concurred with those, who noted a marked rise in the prevalence of *Acinetobacter* species in the healthcare industry[14,18]. It is still the most common organism linked to common infections of the skin, urinary system, blood, and other soft tissues[11]. The outcomes of the current investigation were consistent with Al-Mayahi FSA[19]. It was noted that the high elevation in drug resistance to all classes that CLSI recommended, (2021), and this High resistance to most of the drugs is recommended by CLSI, such as piperacillin-tazobactam, cefepime, imipenem, gentamicin, and ceftazidime, respectively, while 87% of isolates were resistance to carbapenem drugs (Imipenem and Meropenem), and this constitutes a source of concern. Also, the results of this test showed that all Gram negative bacteria was a resistance to Tigcyclin, Polymyxin B, Colstin, Tobromycin while most *A. baumannii* was sensitive for Ceftriaxone, Ceftazidime, Pipracillin, ampicillin-sulbactam, Cefepime, Cefotaxime while other antibiotic showed variety in their activity against gram negative bacteria in 2021-2022 (Table 2). The study found that many of the isolated bacteria were multidrug-resistant (MDR), which makes treating infections challenging. An major issue in global health care is the rise in antimicrobial resistance to potent medications[20].

The synthesis of enzymes that cleave drug structures is one of the pathogen's many defense mechanisms against the effects of antibiotics. Over the past ten years, antibiotic resistance among *Acinetobacter* species has rapidly grown. This may be in part due to the organism's relatively impermeable outer membrane and multidrug efflux pumps[21].

This information is consistent with a recent study conducted in Iraq who reported that 87% of isolates were resistant to carbapenem drugs (imipenem and meropenem), while 96.7%, 93.5%, 80.6%, and 71% of isolates were resistant to piperacillin-tazobactam[22]. Another recent study reported that *A. baumannii* recorded a resistance rate reached 74%, for piperacillin, ceftazidime and ciprofloxacin, while 32%, 70%, 88%, and 98% for imipenem, meropenem, cefotaxime and ceftriaxone respectively, as well as 62%, 68%, and 76% of isolates were resistance to levofloxacin, amikacin and gentamicin and finally 98% of isolates were observed resistance to sulfa drug. As Iraq regard as low resources country and poor antibiotics control policy many studies agreed with us for rapid raising of antimicrobial resistant[23-25].

Several studies, both local and global, have found that the bla/CTX-M type (one type of ESBL) is emerging in a number of countries around the world. Similarly, several global studies have been conducted on the abilities of ESBL-producing isolates[26]. The *CTX-M* gene encodes enzymes from the Ambler class A beta-lactamase family. These enzymes are becoming increasingly common in Enterobacteriaceae all over the world. This study found a high prevalence of CTX-M ESBL-producing *Acinetobacter* spp. As a result, tracking and monitoring the global spread of *Acinetobacter* spp. that produce CTX-M ESBLs in community settings is critical from a public health standpoint[27]. The results of the current investigation concurred with those[13], who noted a marked rise in the prevalence of *Acinetobacter* species in the healthcare industry. It is still the most common organism linked to common infections of the skin, urinary system, blood, and other soft tissues[11].

CONCLUSION

Depending on the results, we conclude that: *Acinetobacter baumanni* isolates from blood and urine, have been recognized as an important pathogen that causes various infection in Najaf City. High antibacterial agent's resistance among *Acinetobacter spp*. and MAR index was a concern among these isolates. There is an increasing in the antibiotic résistance of *Acinetobacter* spp for COL, TOD, TIG and POLB in 2021-2022.

Recommendations

This dangerous raising of resistance to many microorganisms against many antimicrobial drugs should be concerned about, which may be due to randomized and poor antibiotic prescribing policies, especially in low-resource and developing countries like Iraq, so we need frequent data collection and study.

Assignment

All the participants provided informed consents for inclusion in the study and were assured that all the informations provided would be used solely for the purposes of this study and treated confidentially.

Ethical Approval Declaration

The procedures followed in this study were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki). In addition, each participant provided written consent following a concise overview of the project.

Acknowledgements

We would like to thank College of Science, Department of Pathological Analysis, Clinical sites, and the patients for their participation.

Competing interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

This manuscript has not been previously published and is not under consideration in another journal.

Funding: Authors did not receive any grants from funding agencies

REFERENCES

1. Wong D, Nielsen TB, Bonomo RA. Clinical and Pathophysiological Overview of *Acinetobacter* Infections: a Century of Challenges. Clinical Microbiology Reviews 2017 January, 2017; 30(1) : 409-447.
2. Majeed HT, Motaweq ZY. Phylogenetic Group of Escherichia coli Isolated from Inflammatory Bowel Disease in Al Najaf province, Egyptian Journal of Medical Microbiology (Egypt), 2024; 33(4): 129–134.
3. Vázquez-López R, Solano-Gálvez SG, Vignon-Whaley J. *Acinetobacter baumannii* resistance: A real challenge for clinicians, Antibiotics, 2020; 9:205-210.
4. Asif M, Alvi IA, Rehman SU. Insight into *Acinetobacter baumannii*: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities, Infect. Drug Resist, 2018;11:1249-1255.
5. Rizk SS, Elwakil WH, Attia AS. Antibiotic-Resistant Acinetobacter baumannii in Low-Income Countries (2000–2020): Twenty- One Years and Still below the Radar, Is It Not There or Can They Not Afford to Look for It?, Antibiotics 2021; 10: 764-766.
6. Vivas R., Barbosa AAT, Dolabela SS, Jain S. Multidrug-resistant bacteria and alternative methods to control them: An overview, Microbe. Drug Resist, 2019; 25:,890–908.
7. Zeina A, Kanafani, MD, Missoula S, Kanj MD. Acinetobacter infection: Epidemiology, microbiology, pathogenesis, clinical features, and diagnosis 2023, Antibiotics, 2022; 9(3) : 254-260.
8. Ayobami O, Willrich N, Harder T. The incidence and prevalence of hospital acquired (carbapenem-resistant) Acinetobacter baumannii in Europe, Eastern Mediterranean and Africa: A systematic review and meta-analysis. Emerg, Microbes Infect, 2019; 8:1747–1759.
9. Ling M.L, Apisarnthanarak A, Madriaga, G. The burden of healthcare-associated infections in Southeast Asia: A systematic literature review and meta-analysis, Clin. Infect. Dis., 2015; 60:690–1699.
10. Yu H, Ezpeleta-Lobato, G, Han X. Carbapenamase-Producing *Acinetobacter* baumannii in China, Latin America and the Caribbean: A Systematic Review and Meta-Analysis, MEDICC Rev, 2022;24 :59–69.
11. Iohannis K, Scilit P. *Acinetobacter* baumannii Antibiotic Resistance Mechanisms, MDPI, 2021 ;10(3):373-279.
12. James S. Lewis S. Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing, Microbes Infect , 2023; (33) :402-405.
13. Jawade HA, Motaweq ZY, Rasool HD Hussain FH. Study of Antibiotic Resistance in ESKAPE Bacteria Using β-lactamase and ESBL Genes, Journal of Angiotherapy, 2024;8(3) : 9618-9622.
14. Al-Ghazaly NFZ. Molecular investigation of AmpC-Family and Sulfa Drugs Resistance Genes among Acinetobacter Spp. Isolated from Different Clinical Sources in Al–Najaf City, Thesis/Faculty of science-Kufa University, 2021:101–106.
15. Hassan AA, Motaweq ZY. Phenotypic and genotypic study of biofilm formation in multi-drug resistance Enterobacter species in Al-Najaf province, Iraq, Microbial Biosystems, 2024: 9(2),83-91.
16. Taha HKA, Al-Rufaie MM., Motaweq ZY. Utility of n-bromo succinamide for the spectrophotometric determination of phenylephrine hydrochloride in pharmaceutical formulations, Latin American Journal of Pharmacy,2021;40(Special Issue) :27–33.
17. Witwit INK, Motaweq ZY, Mubark HM. Synthesis, characterization, and biological efficacy on new mixed ligand complexes based from azo dye of 8-hydroxy quinolone as a primary ligand and imidazole as a secondary ligand with some of transition metal ions,Journal of Pharmaceutical Sciences and Research, 2018; 10(12) :3074–3083.
18. Murugaiyan J, Kumar PA, Rao GS, Iskandar K, Hawser S, Hays JP, van Dongen MB. Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics, Antibiotics, 2022*;* 11(2) :200-205.
19. Al-Mayahi FSA, Ali RH. Preliminary study of emergence MDR of pro videncia spp. isolates producing ESBL, AmpC and MBL among patients with RTI and in wastewater in AL-Diywaniyah city, Iraq, Biochem. cell. Arch, 2018; Supplement 1 :1357-1368.
20. Paauw A, Caspers MP, Schuren FH, Leverstein-van Hall MA, Delétoile A, Montijn RC, Fluit AC. Genomic diversity within the Enterobacter cloacae complex, PLoS one,2008;3(8):e3018.
21. Guérin F, Isnard, C, Cattoir V, Giard JC. Complex regulation pathways of AmpC-mediated β-lactam resistance in Enterobacter cloacae complex,

Antimicrobial agents and chemotherapy, 2015;59(12) :7753-7761.

22. Roshdan MA, Abeer AA, Mona MS, Hasnaa SA. Evaluation of Colistin and Tigecycline Susceptibility Testing Methods for *Klebsiella pneumoniae* and *Acinetobacter baumannii* Clinical Isolates. Egyptian Journal of Medical Microbiology (Egypt), 2021; 30(2):35-45.
23. Balasubramanian D, Kumari H, Jaric M , Fernandez M, Turne KH, Dove SL, Narasimhan G, Lory S, Mathee K. Deep sequencing analyses expands the Pseudomonas aeruginosa AmpR regulon to include small RNA- mediated regulation of iron acquisition, heat shock and oxidative stress response, Nucleic Acids Res., 2014; 42:979-998.
24. Bassetti M, Peghin M, Pecori D. The management of multidrug- resistant Enterobacteriaceae, Current Opinion in Infectious Diseases, 2016 ;29(6) :583-594.
25. Chelaghma W, Loucif L, Bendahou M , Rolain JM. Vegetables and Fruit as a Reservoir of β-Lactam and Colistin-Resistant Gram-Negative Bacteria: A Review, Microorganisms,2021; 9(12) : 2534-2535.
26. SophieB, Francois-Xavier W, Axel C, Martine V, Eric M. Karine P. Clonal Emergence of Extended-Spectrum β-Lactamase (CTX-M-2)- Producing Salmonella enterica Serovar Virchow Isolates with reduced susceptibilities to ciproß oxacin among poultry and humans in Belgium and France (2000 to 2003), J C M , 2006 ;44: 903-906.
27. Abdulla NY, Motaweq ZY, Alrufaie ZMM, Zghair LS. Phenotypic and Genotypic Study of Biofilm Formation in Multidrug Resistance Bacteria Isolated from Urinary Tract Infection from Diabetes Patients, AIP Conference Proceedings,2024; 3092(1) : 1-9.
28. Kareman AE, Rowida RI, Radwa ME, Asmaa FA, Radwa AE. Phenotypic Methods and Molecular Metallo-β-lactamases Genes Detection in Different Clinical Carbapenems Resistant Acinetobacter Isolates from Tanta University Hospitals. Egyptian Journal of Medical Microbiology (Egypt), 2021; 30(3): 89-95.