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Original article

Phenotypic Investigation of Beta-Lactamase Enzymes in Multidrug Resistant *Klebsiella Pneumoniae* Clinically Isolated in Diyala Governorate /Iraq

Zainab M. Taha Al-Ezzi* and Saba J. Jawad Al Zubaidi

1- Department of Science Biology, College of Education for Pure Sciences, Diyala University, Iraq.

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ABSTRACT

Background: *Klebsiella pneumoniae* is one of the main causes of many opportunistic infections, and it is highly resistant to antibiotics, due to the many virulence factors including beta-lactamase enzymes that enable it to cause pneumonia, urinary tract infection, wound infections and burns, bacteremia and meningitis. **Methods:** 132 samples were obtained from different clinical sources from Baquba Teaching Hospital and Al-Batool Maternity and Children Hospital in Diyala Governorate /Iraq during the period from (September to December 2023). *K. pneumoniae* isolates were identified based on phenotypic diagnosis and biochemical tests, confirmation of the results by VITEK2 system. The disc diffusion method was used to determine the ability of the bacterial isolates to resist antibiotics using 12 antibiotics and production of beta-lactamase enzymes. **Results:** Out of a total of 132 samples, 50 samples (37.87%) were *K. pneumoniae*. The results of antibiotic sensitivity showed that resistant isolates were 100% resistant to of Ampicillin, 70% Aztreonam, 74% ceftazidime, 92% ceftriaxone, 48% Imipenem and Meropenem, 48% Amikacin, 38% Gentamicin, 42% Tobramycin, 46% Levofloxacin and Ciprofloxacin, 68% Trimethoprim/sulfamethoxazole. The isolates showed multiple and different antibiotic resistance, 30 isolates by 60% with multiple resistance (MDR). 14 (28%) with Extensive drug resistance (XDR). The phenotypic detection of beta-lactamase enzymes was performed for 20 of the MDR isolates. The results showed that the percentage of the presence Extended spectrum β -Lactamase enzymes ESBLs was 80%, Metallo β -Lactamase enzymes MBLs was 100% and AmpC enzymes 25%. **Conclusion:** We need to develop valuable strategies for the prevention and control of infections.

Introduction

Klebsiella pneumoniae is an opportunistic pathogen that infects different parts of the body, causing urinary tract infection, respiratory tract infection (pneumonia), bacterial meningitis, purulent liver abscess, wound inflammation, burns and bacteremia, so it can be isolated from different clinical sources [1]. It is found as a normal flora in different parts of the body, where it is found on the

surface of the mucous membrane of the digestive system and a few of it in the nasopharynx [2]. *K. pneumoniae* has become a major cause of health care associated infection and a risk factor for severe community-acquired infections as it is transmitted from person to person through contact or direct contact with infected patients, or the use of contaminated patient tools and contaminated blood transfusions [3]. The phenomenon of increased

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* Corresponding author: Taha AL-Ezzi, Zainab Muhammad

E-mail address: zainabmtaha99@gmail.com

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beta-lactam antibiotics resistance in *Klebsiella pneumoniae* is particularly alarming ,because it leads to an almost untreatable community-acquired infection excessive use of antibiotics leads to the emergence of new more resistant strains [4]. Antibiotics are products from some microorganisms, capable of inhibiting the growth of other microorganisms in certain concentrations without affecting the cells of the product's body. *K. pneumoniae* bacteria can be multi-resistant, meaning that they resist more than one type or more than one group of antibiotics and are called Multidrug Resistant MDR [5]. Antibiotics have a major role in the decrease in the rate of infection with *K. pneumoniae*, but the effectiveness of antibiotics has decreased continuously with the increasing ability of bacteria to develop their defenses and resistance to the action of these antibiotics and the transmission of resistance between strains with the increase in the indiscriminate use of antibiotics, which is more common in developing countries [6]. Beta-lactamase enzymes are known as proteins responsible for resisting a wide range of antibiotics associated with beta-lactam, because they inhibit the effectiveness of beta-lactam antibiotics, which is one of the most common means of resistance, beta-lactamase enzymes are found in all bacteria Gram positive and negative, *K. pneumoniae* bacteria have a lot of enzymes that make them resistant to antibiotics, the most important of which are Extended-spectrum beta-lactamase enzymes (ESBLs), Metallo beta-lactamase enzymes (MBLs), and Ampicillinase Class C Beta-Lactamase (AmpC) enzymes [7].

Materials And Methods

Bacterial Specimens collection

132 samples were collected from different clinical sources for patients from Baqubah Teaching Hospital, Al-Batool Maternity and Children's Hospital, and educational laboratories in Diyala Governorate after examining the specialist doctor for both sexes and all ages. For the period from September to December 2023. The samples were 68 urine, 21 sputum, 16 wound swabs, 16 burn, 9 blood and 2 spinal fluid). The samples were grown on different media (Blood agar, MacConkey agar, methylene Eosin blue) and then incubated for 24 hours at a temperature of 37 ° C for the purpose of isolating the bacteria and their initial diagnosis.

Bacterial isolation and identification

The samples were cultured on MacConkey agar medium and methylene Eosin blue medium, then incubated for 24 hours at a temperature of 37 ° C. Isolates were identified based on morphological traits [8], Biochemical identification [9-10]. VITEK2 system (bioMérieux, Marcy-l'Etoile, France) is used for confirming diagnosis and antibiotic susceptibility.

Antibiotic sensitivity Test

The susceptibility of 50 isolates to antimicrobial was tested by using the disks diffusion method [6], The antibiotics used are {Ampicillin (10 µg), Aztreonam (30 µg), Cefazidime (30 µg), Ceftriaxone (30 µg), (10 µg) Imipenem, Meropenem (10 µg), Amikacin (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), and Trimethoprim / sulfamethoxazole (1.25/23.75 µg). Confirmation of the results by VITEK2 system.

Phenotypic detection of β – Lactamase enzymes

The ability of bacterial isolates under study to production of Broad-spectrum β – Lactamase enzymes using the double disc synergy test, as a widening of the inhibition region occurred between the disc of the central antigen, which is Amoxicillin–Clavulanic acid with a group of antibiotics, including (Aztreonam, Ceftriaxone and Cefotaxime) is a positive result depending on the method [11]. Metallo β – Lactamase Enzymes using the antibiotic Imipenem and EDTA+ Imipenem combination method an increase in the inhibition area of more than 7 ml around the Imipenem-EDTA disc compared to the inhibition area around the Imipenem disc is a positive result depending on the method [12]. AmpC enzymes using Disk Antagonism test for AmpC enzymes as a widening of the inhibition region occurred between the disc of the central antigen, which is Cefoxitin with a group of antibiotics, including (Ceftriaxone and Cefotaxime) is a positive result [13-14].

RESULTS AND DISCUSSION

Isolation and Identification *Klebsiella pneumoniae*

The results of the current study showed that out of a total of 132 samples, 50 samples (37.87%) were *Klebsiella pneumoniae*, while 82 (62.12%) samples were bacteria other than *Klebsiella pneumoniae*. The results of the current study were close to results study by [15] as the percentage of *K. pneumoniae* bacteria was 39.4%. The results of this

study came higher than the percentage obtained by the researcher [16] as the percentage of *K. pneumoniae* was 33.78%. On contrast, the percentage is less than that obtained by [17] where the percentage of *K. pneumoniae* was 92.6%. The difference in the diversity of values for the distribution of *K. pneumoniae* isolates may be due to the different environments from which the isolation was collected, the health conditions of the patients, the time of taking the sample, the method, and other variables.

Antibiotic susceptibility tests for bacterial isolates *K. pneumoniae*

The sensitivity of the *K. pneumoniae* bacterial isolates was tested to 12 antibiotics are (Ampicillin, Aztreonam, Ceftazidime, Ceftriaxone, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Levofloxacin, Ciprofloxacin, Trimethoprim/ sulfamethoxazole), Which are most commonly used to treat bacterial infections. The bacterial isolates were resistant to Ampicillin (100%), Aztreonam (70%), Ceftazidime (74%), Ceftriaxone (92%), Imipenem and Meropenem (48%), Amikacin (48%), Gentamicin (38%), Tobramycin (42%), Levofloxacin and Ciprofloxacin (46%), Trimethoprim/sulfamethoxazole (68%). These results were close with the results reached by [18], where it was found that the percentage of Ampicillin was 97%, Aztreonam 78%, Ceftriaxone 84%, Ceftazidime 74%. The results of the current study were consistent with study by [19], where the percentage of resistance to Ceftazidime was 73%, Meropenem and Imipenem was 48%. The study conducted in Iran by [20] did not agree with the results current, Meropenem, and Imipenem was 13.7%. The reason for the resistance to a group of beta- lactam antibiotics in *K. pneumoniae* bacteria is the production of Extended-spectrum beta-lactamase enzymes, increased resistance of *K. pneumoniae* to beta-lactam antibiotics (Carbapenem) lies in their ability to produce the Metallo beta-lactamase enzymes, Also Increased resistance of *K. pneumoniae* to beta-lactam antibiotics (Cephalosporins) lies in their ability to produce the AmpC enzymes, which include the enzymes Penicillinase Carbapenemase and Cephalosporinase, as these enzymes break the β -lactam ring, leading to inhibition of the action of antibiotics belonging to the Penicillin, Carbapenem and Cephalosporins groups [21]. The results of current study were consistent with the findings of [15], where the percentage of resistance to

aminoglycoside, (Gentamicin was 36.23%, Amikacin 47.82% and Tobramycin 43.47%). While it was not consistent with the findings of [22] since all isolates were sensitive. The reason for the resistance to aminoglycosides in *K. pneumoniae* can be explained by their ability to produce the aminoglycoside-modifying enzymes, or the modification of the ribosome by the enzyme Methyltransferase, as previous studies showed a common presence of mechanisms of resistance to aminoglycoside-bet-lactams in isolates of *K. pneumoniae* [23]. The results of current study were consistent with the findings of [17], where the resistance to Levofloxacin was 43.90%, and Ciprofloxacin 46.34%. In contrast. It was not consistent with the study of [24], as the resistance rate in this study was lower (Levofloxacin 13.5% and Ciprofloxacin 7.7%). The results of the present study are consistent with the findings of [15] where the resistance of Trimethoprim/Sulfamethoxazole was 65.22%, on the other hand it was not agree with the findings of [25], where the resistance rate to Trimethoprim/Sulfamethoxazole was 38.2%.

K. pneumoniae isolates showed multiple, and different resistance to the antibiotics used in this study. 30 isolates by 60% with multiple resistance MDR. 14 (28%) with Extensive drug resistance (XDR). The antibiotic sensitivity test showed 6 isolates of the bacteria *K. pneumoniae* by 12% were sensitive to most of the antibiotics used in the study Multidrug sensitive (MDS). 20 isolates (MDR) of *K. pneumoniae* bacteria were selected that resisted most of the antibiotics used in the study for the purpose of phenotypic screening for beta-lactase enzymes. 20 isolates were selected from *K. pneumoniae* isolates that resisted most of the antibiotics used in the study for phenotypic detection of β - Lactamase enzymes.

Phenotypic detection of Extended Spectrum β -Lactamase (ESBLs)

All 20 isolates of *K. pneumoniae* (MDR) under study were subjected to phenotypic detection for the purpose of investigating the production of Extended spectrum β - Lactamase enzymes (ESBLs) using the double disc synergy test, as a widening of the inhibition region occurred between the disc of the central antigen, which is Amoxicillin–Clavulanic acid with a group of antibiotics, including (Aztreonam, Ceftriaxone and Cefotaxime) is a positive result. The results of the current study showed that the number of *K. pneumoniae* that are capable of producing broad-spectrum beta-

lactamase enzymes were 16 isolates out of a total of 20 isolates with a rate 80%, while 4 isolates with a rate of 20% did not produce ESBLs. The results of the current study were almost convergent with the result reached by [15] in the city of Baquba, where the percentage of *K. pneumoniae* isolates producing broad-spectrum beta-lactamase enzymes was 83.32%. It did not agree with the findings of [26], where *K. pneumoniae* produced 35.7%. The reason for the difference in the production of ESBLs between this study and other studies is the difference in isolates, and the different use of antibiotics in each country, and the volume of antibiotic resistance to pathogens in the study area.

Phenotypic detection of Metallo β - Lactamase (MBLs)

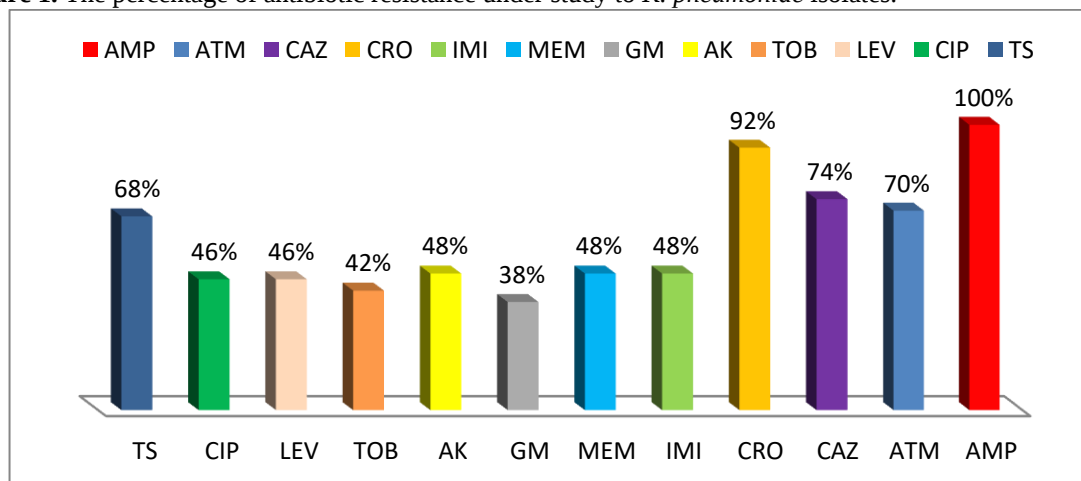
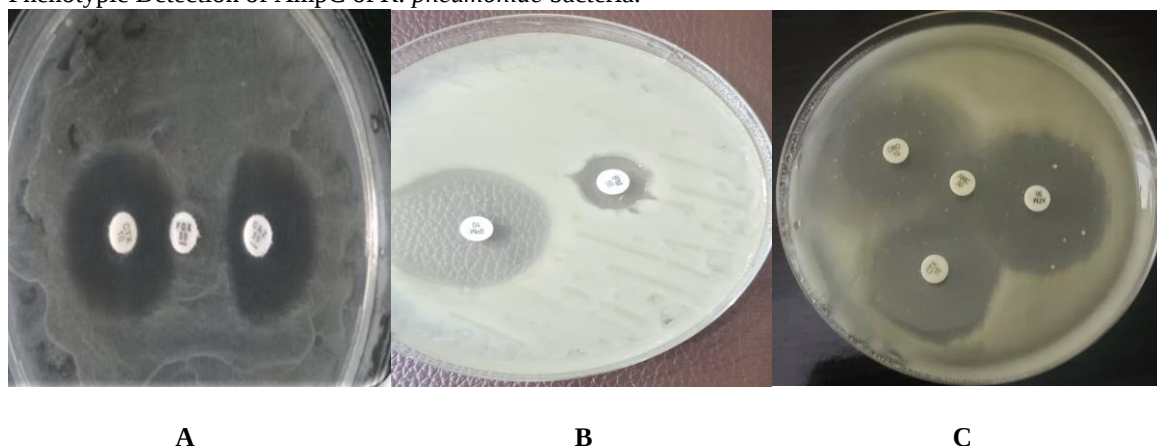
All 20 isolates of *K. pneumoniae* (MDR) under study were subjected to phenotypic detection for the purpose of investigating the production of Metallo β - Lactamase enzymes (MBLs), using the antibiotic Imipenem and EDTA+ Imipenem combination method. The results of the current study indicated that the number of isolates of *K. pneumoniae* producing the Metallo beta-lactamase enzymes was all 20 isolates by 100%. The results of my study came in agreement with the results of the study conducted by [27], her study showed that 38 isolates out of a total of 38 gave a positive result for testing the production MBLs with a rate 100%. On the other hand It did not agree with the results of [28] as the production of *K. pneumoniae* for the MBLs was 15%.The difference in the production rates of MBLs enzymes in many studies, may be due to the difference in the sources of sample collection, the methods used to detect these enzymes, and may be caused by the insensitivity and non-specificity of the phenotype and the presence of other genes, and that the production of bacteria for MBLs enzymes makes bacteria resistant to a wide range of beta-lactamase antagonists, thus making these antibiotics ineffective to eliminate bacteria .

Phenotypic detection of AmpC enzymes: Disk Antagonism test for AmpC enzymes

All 20 isolates of *K. pneumoniae* (MDR) under study were subjected to phenotypic detection for the purpose of investigating the production of Ampicillinase Class C β - Lactamase (AmpC), using Disk Antagonism test for AmpC enzymes as a widening of the inhibition region occurred between the disc of the central antigen, which is Cefoxitin with a group of antibiotics, including (Ceftriaxone and Cefotaxime) is a positive result. The results of the current study showed, that the number of *K. pneumoniae* producing AmpC in this way were 5 isolates of a total 20 isolates with a rate of 25%, while 15 isolates with a rate 75% did not produce AmpC. The results of this study are consistent with a local study conducted by the researcher [29], where its study showed that the percentage of *K. pneumoniae* producing these enzymes reached by 26.66%. The results of my study was close to the results of the researcher [16] in Diyala Governorate, where its study showed that the percentage of *K. pneumoniae* bacteria producing these enzymes reached by 22%. It did not consistent with the study conducted by [30], the percentage of *K. pneumoniae* producing these enzymes reached by 75%. The appearance of a small percentage of positive isolates of AmpC enzymes may be due to the possibility of ESBLs associated with AmpC enzymes in the same isolation and expressed equally, leading to blocking one of the other when screening for AmpC enzymes, which pose a serious threat to the diagnosis and treatment of pathogenic bacteria producing these enzymes [31].

Table 1. Extended-Spectrum β -Lactamase (ESBLs), Metallo β -lactmase (MBLs), Ambler class C -lactamase AmpC of *K. pneumoniae* isolates. (n=20).

β -lactamase enzymes	Produce enzyme n(%)	Non produce enzyme n(%)
ESBLs	16 (80%)	4 (20%)
MBLs,	20 (100%)	0
AmpC	5 (25%)	15 (75%)

Figure 1. The percentage of antibiotic resistance under study to *K. pneumoniae* isolates.**Figure 2.** A. Positive phenotypic detection of (ESBLs). B. Positive phenotypic detection of (MBLs) C. Positive Phenotypic Detection of AmpC of *K. pneumoniae* bacteria.

The results of the phenotypic detection of beta-lactamase enzymes (ESBLs, MBLs, AmpC). Showed that 5 isolates out of a total of 20 isolates by 25% produce all enzymes. These results were close with the results reached by [16] where the percentage of *K. pneumoniae* isolates producing all beta-lactamase enzymes was 22%. The ability of *K. pneumoniae* bacteria to produce beta-lactamase enzymes of all kinds makes them dangerous, and resistant to a wide range of antibiotics [32]. Most beta-lactamase genes are present on chromosome or mobile genetic elements such as plasmid, resistant strains spread rapidly, leading to increased mortality, pathogenicity, and health care costs, and the combined gene expression of many beta-lactamases genes in the organism may complicate the problem of drug resistance and thus limit treatment options [33].

Conclusion

The present study highlights spread *Klebsiella pneumoniae* strains that produced beta-lactamase enzymes in is worrisome which plays an important role in conferring resistance of *K. pneumoniae* to β -Lactam antibiotics, we need to develop valuable strategies for the prevention and control of infections.

Conflict of interest

These authors declare that above-submitted work was not funded by any governmental or private funding source nor supported by any financial projects. The authors declare that they have no conflict of interest.

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None

Data availability

All data generated or analyzed during this study are included in this published article.

Authors' contribution

All authors made significant contributions to the work presented, including study design, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

References

- 1- Flaih ON, Najeb LM, Mohammad RK. Molecular Detection of Serotypes K1 and K2 of *Klebsiella pneumoniae* Isolated from wound and Burn Infection. *Al- Anbar Sci* 2016; 9 (1): 311 – 317.
- 2- Martin Rebekah M, and Michael A. Bachman. "Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*." *Frontiers in cellular and infection microbiology* 8 (2018): 4.
- 3- Chew Ka Lip, Raymond TP Lin, and Jeanette WP Teo. "*Klebsiella pneumoniae* in Singapore: hypervirulent infections and the carbapenemase threat." *Frontiers in Cellular and Infection Microbiology* 7 (2017): 515.
- 4- Gharrah Mustafa Muhammad, Areej Mostafa El-Mahdy, and Rasha Fathy Barwa. "Association between Virulence Factors and Extended Spectrum Beta-Lactamase Producing *Klebsiella pneumoniae* Compared to Nonproducing Isolates." *Interdisciplinary Perspectives on Infectious Diseases* 2017.1 (2017): 7279830.
- 5- Deschamps C, Fournier E, Uriot O, Lajoie F, Verdier C, Comtet-Marre S, Thomas M, Kapel N, Cherbuy C, Alric M, and Almeida M. Comparative methods for fecal sample storage to preserve gut microbial structure and function in an in vitro model of the human colon. *Applied Microbiology and Biotechnology*. 2020; 104(23), pp.10233-10247.
- 6- CLSI. Performance standards for antimicrobial susceptibility testing, 28th ed. CLSI supplement M100. Wayne, PA. clinical and laboratory standards institute. 2018. 38(3):102-122
- 7- Saremi M, Saremi L, Feizy F, Vafaei S, Lashkari A, Saltanatpour Z, & Nazari R N. The prevalence of VIM, IMP, and NDM-1 metallo-beta-lactamase genes in clinical isolates of *Klebsiella pneumoniae* in Qom Province, Iran. *Journal of Medical Microbiology and Infectious Diseases* 2020; 8(1), 34-39.
- 8- Wanger A, Chavez V, Huang R S P, Wahed A, Actor J K, and Dasgupta A. *Microbiology and Molecular Diagnosis in Pathology*. Elsevier Inc 2017; All Rights Reserved.300pp
- 9- Brown A E. *Microbiological Application Laboratory Manual in General Microbiology*. McGraw – Hill. 2005; New York.
- 10- Macfaddin J. *Biochemical Tests of Medical Bacteria* (3rd ed) Lippincott William and Wilkins. 2000; USA.
- 11- National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing twelfth informational supplement. 2019.
- 12- Supriya U, lay R, and Amitabha B. Presence of different B-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC – lactamase enzyme. *Journal of Infection in Developing countries* 2010; 44: 239 – 242.
- 13- Parveen M, Harish R, Parija SC. (2010). AmpC Beta Lactamases among Gram negative clinical Isolates from tertiary Hospital, South India. *Brazi. J. Microbiol* 2010; 41, 596-602.
- 14- Al-Saleem S L, and Sharif A Y. Investigation of AmpC enzymes in some types of Gram-negative bacteria by phenotypic and molecular

- method. Al-Rafidain Journal of Science 2021; 26-16:(3)30.
- 15-AL-Zubaidi S J J. Genetic diversity of the capsule in multiple antibiotic-resistant bacteria *K. pneumoniae* and testing the effect of zinc nanoparticles oxide on them. PhD thesis, College of Education for Pure Sciences, University of Diyala.2020.
 - 16-AL-Shabender G A I. Molecular diversity in some isolates *Klebsiella pneumoniae* form patients in Baquba city. M.Sc. University of Diyala, College of Science Department of Biology. 2023.
 - 17-Al-Lihibi A A I. Molecular sequencing of a complete genome of multidrug-resistant *Klebsiella pneumoniae* bacteria using Next generation sequence technique. Ph.D. thesis, Diyala University, College of Education for Pure Sciences.2023.
 - 18-Alobadi T H Z. Molecular Identification of *Klebsiella pneumoniae* using capsule Genes. thesis. M.Sc. Biotechnology. University of Al – Nahrain, the College of science .2014.
 - 19-Al-aajem M B. Molecular Detection of some Virulence factors and Impact of Probiotic *Lactobacillus* sp. Against Multidrug Resistance *Klebsiella pneumoniae* Isolated from Urinary Tract Infections. M.Sc. thesis. University of Diyala, Biology/ Microbiology, College of Sciences. 2021.
 - 20-Hosseinzadeh Z, Ebrahim–Saraie H S, Sarvari J, Mardaneh J, Dehghani B, Rokni – Hosseini S M H, and Motamedifar M. Emerge of blaNDM1 and bla OXA – 48 – like harboring carbapenem – resistant *Klebsiella pneumoniae* isolates from hospitalized patient , in southwestern Iran , Journal of the Chinese Medical Association 2018; 81 (6) : 536 – 540 .
 - 21-Zhou Q, Tang M, Zhang X, Lu J, Tang X, & Gao Y. Detection of AmpC β -lactamases in gram-negative bacteria. *Heliyon*. 2022; 8(12).
 - 22-Al-Dulaimi T H K. Study of antibiotic resistance of *Klebsiella pneumoniae* bacteria using Vitek2 device isolated from clinical samples. *Journal of the University of Babylon/Pure and Applied Sciences*. 2017; 5 (4): 1298-1305.
 - 23-Mancini S, Poirel L, Tritten M L, Lienhard R, Bassi C, & Nordmann P. Emergence of an MDR *Klebsiella pneumoniae* ST231 producing OXA-232 and RmtF in Switzerland. *Journal of Antimicrobial Chemotherapy* 2018; 73(3), 821-823.
 - 24-Seyedpour S M, and Eftekhari F. Quinolone Susceptibility and detection of qnr acc (6)–Ib–cr geno in community isolates of *Klebsiella pneumoniae* Jundishapur.J.Microbiol. 2019; 7(7): 1.
 - 25-Mohsen S M Y, Hamzah H A, Imad Al-Deen M M, and Baharudin R. Antimicrobial Susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* with Extended – Spectrum β -lactamase associated genes in Hospital Tengku Ampuan Afzan , Kuantan , Pahang . Malays, J. Med. Sci 2016; 23 (2): 14 – 20.
 - 26-Al-Taai H R R. Bacteriological comparison between *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from different infectious sources. Diyala. J. Pure. Sci 2016; 12 (1): 162 – 182.
 - 27-Khudair M M. Molecular detection of some antibiotic resistance genes in *Klebsiella pneumoniae* bacteria isolated from different clinical sources. M.Sc. Thesis. College of Education for Pure Sciences, Ibn Al-Haytham. 2019.
 - 28-Devi L S, Broor S, Rautela R S, Grover S S, Chakravarti A, & Chattopadhyaya D. Increasing

- prevalence of *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M-type extended-spectrum beta-lactamase, carbapenemase, and NDM-1 in patients from a rural community with community acquired infections: A 3-year study. *International Journal of Applied and Basic Medical Research* 2020; 10(3), 156-163.
- 29-Mohamed I Q. Molecular investigation of some biofilms and quorum sensing genes in *Klebsiella pneumoniae* isolated from different clinical cases. B.Sc. Thesis. University of Diyala, the College of Science. 2022.
- 30-Moghadam M T, Shariati A, Mirkalantari S, and Karmostaji A. The complex genetic region conferring transferable antibiotic resistance in multi-drug resistant and extremely drug-resistant *Klebsiella pneumoniae* clinical isolates", *New microbes and new infections* 2020; 36, p. 100693.
- 31-Ogefere H O, Osikobia J G, Omoregie R. Prevalence of AmpC β -lactamase among Gram-negative bacteria recovered from clinical specimens in Benin City, Nigeria. *Tropic J Pharmaceut Res.* 2016; 15:1947–1953. doi:10.4314/tjpr. v15i9.20
- 32-Padmini N, Ajilda A A K, Sivakumar N, & Selvakumar G. Extended spectrum β -lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: critical tools for antibiotic resistance pattern. *Journal of basic microbiology* 2017; 57(6), 460-470.
- 33-Dhungana K, Awal B K, Dhungel B, Sharma S, Banjara M R, and Rijal K R. Detection of *Klebsiella pneumoniae* carbapenemase (KPC) and metallo betalactamae (MBL) producing Gram negative bacteria isolated from different clinical samples in a Transplant Center, Kathmandu, Nepal. *Nepal. ASMI* 2019; 2(12), pp.60-69.