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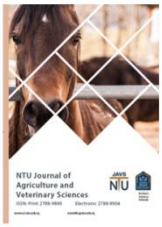

Studying the prevalence of multidrug resistant *Klebsiella pneumoniae* in Kirkuk city

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A B S T R A C T

Klebsiella pneumonia is an opportunistic pathogen causes several diseases including sepsis, pneumonia, and wound infections. There are two pathotypes of *Klebsiella pneumonia*: classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumonia* (hvkp), which is an emerging variant of (ckp), clinically distinguished by invasive and multiple site infections. A total of 150 samples were collected from different hospitals in Kirkuk city during the period between November 2021 to June 2022. The age of patients ranged between (20– 60) years old of both sexes. These samples were highly recovered from females with a rate 66.67% compared to the males 33.33%. Thirty (20%) *K. pneumonia* was recovered from different clinical specimens including urine, sputum, burn and wound swabs. The most common age group infected with *K. pneumoniae* was (20-40) with a rate of 63.33% and commonly recovered from inpatients 53.33% rather than outpatients (46.67%). The capability of *K. pneumoniae* isolates to form biofilm was also examined by using tissue culture plate (TCP) and Congo red agar (CRA) methods. The results indicate that biofilm production by TCP method was 70% (46.67% strong biofilm producer, 23.33 % moderate and 30% were negative), while Congo red agar (CRA) method showed 60% positivity for biofilm and 40% was negative. Antibiotic susceptibility test was conducted to all isolates by using disc diffusion test towards 8 antimicrobial agents. *Klebsiella pneumoniae* isolates showed multiple resistance against 3 or more of different antibiotic groups (gentamicin 93%, ampicillin 96%, amoxicillin – clavulanate 90%, cefotaxime 83%, ceftazidime 96%, meropenem 36%, levofloxacin 76 % and gentamicin 93 %. *K. pneumoniae* isolated from inpatients and from sputum samples were more resistant to antibiotics.

Introduction

Klebsiella pneumoniae is a non-motile Gram-negative bacterium belongs to the Enterobacteriaceae family [1]. *K. pneumoniae* is considered as one of the opportunistic nosocomial pathogens [2]. This microorganism causes a variety of diseases such as bacteremia, pneumonia and urinary tract infections. Recently , *K. pneumoniae* has attracted the attention of researchers around the world due to its disease severity, resistance against several antibiotics and the difficulty of treatment [2]. The increased prevalence of multidrug-resistant (MDR) of *K. pneumoniae* strains in recent years is more likely due to overuse of conventional antimicrobial agents [3]. *K. pneumoniae* has developed several mechanisms for resistance towards different antimicrobials [4]. One of the most important mechanisms for developing the MDR is efflux pump systems and biofilm formation capacity[5]. Efflux pumps are protein-based structures that are capable to extrude the different toxic substances out of cells [6]. Biofilm formation allow *K. pneumoniae* to escape from the host immune response and antibiotics [7]. another study found that 80% of biofilm-forming isolates from 100 clinical samples showed an MDR phenotype[8]. Resistance of pathogenic bacteria to different antibiotics has become a serious worldwide problem because of fatal outcome of defective treatment and the difficulty to find optimal treatments [9]. K*lebsiella pneumoniae* has been revealed to have the ability to acquire resistance to many antibiotics, especially third generation cephalosporins. Beta - lactam antibiotics are one of the most commonly used antibiotics in the treatment of bacterial infections and the production of β- lactamase enzymes are the most common bacterial resistance mechanisms [10]. The Extended Spectrum Beta Lactamase (ESBL) producer *K. pneumoniae* have increased all over the world. The ESBLs are divided to several groups; the main groups are TEM, CTX, and SHV derivatives [11]. The present study has focused on isolation of *Klebsiella pneumoniae* from different clinical samples and studying their antibiotic resistance profile against different groups of antibiotics and risk factors associated with *K. pneumoniae* infections.

Materials and Methods

Bacterial Strains

One hundred and fifty clinical samples (urine, sputum, blood, wound and burn swabs) were collected from patients attending hospitals in Kirkuk city during the period (November 2021 to June 2022). These patients were in different ages ranged from 20-60 years of both sexes. *K. pneumoniae* isolates were identified by Gram staining, cultural and biochemical tests, and further confirmed by using VITEK-2 compact system.

Antimicrobial susceptibility test

Antibiotic susceptibility test (Kirby- Bauer disk diffusion) was used to detect *K. pneumoniae* strains resistance towards selected antibiotics according to Clinical and Laboratory Standards Institute (CLSI) guidelines [5,12]. Antimicrobial susceptibility assay of 8 antibiotics were performed using commercially available antibiotics including Ampicillin(10µg), Azithromycin(15µg), ceftazidime (30 µg), cefotaxime (30µg), gentamicin (10 µg), Amoxicillin/Clavulanic acid (AMC, 20/10 µg), Levofloxacin (5 µg), meropenem (10 µg). Bacterial inoculum was prepared by mixing three or four colonies with normal saline. The bacterial suspension's turbidity was compared to McFarland turbidity standards corresponds to 1.5×10^8 CFU/ml. Bacterial suspension were swabbed on Muller Hinton plate surface in three directions ensures that the inoculum is evenly distributed across the entire surface. The plates were left for incubation for 15 min. After incubation, antibiotic discs were placed on inoculated plates and left for overnight incubation at 37 °C. Following the incubation, the inhibition zone were measurred and recorded in millimeter. The results were interpretated based on CLSI. Furthermore, multidrug-resistant (MDR) isolates were detected based on their resistance to at least three or more antimicrobial classes.

Biofilm formation

Biofilm formation of *K. pneumoniae* isolates was detected using Congo red agar and microtiter plate assay. By Congo red agar (CRA), *K. pneumoniae* isolates were cultured on BHI broth supplemented with 5% sucrose and Congo red dye. *Klebsiella pneumoniae* isolates capable to form biofilm on Congo red agar appear as dry dark crystalline colonies while the non- biofilm producers appear as red colonies [13].

Biofilm formation was confirmed by using microtiter plate. Hundred and eighty microliters of overnight *K. pneumoniae* culture were placed into microtiter 96 wells and incubated for 24 hr at 37℃. After incubation, each well was washed with Phosphate buffer saline and stained with crystal violet for 15 min. Finally, the bacterial stained cells were dissolved in ethanol and the absorbance was measured at 570 nm. LB broth without bacteria was used as a control. The examined isolates were classified into three categories (non-biofilm producers ($OD \leq ODc$), moderate biofilm former ($2\times ODc < OD \leq 4\times ODc$) and strong biofilm producers ($4\times ODc < OD$) [5].

Results and Discussions

Prevalence of *K. pneumonia* among clinical samples:
Out of 150 clinical samples, 30 (20%) were positive for *K. pneumoniae* and the remaining were negative. Bacterial isolates were initially identified depending on cultural, microscopical, and biochemical tests. That was confirmed by using Vitek 2-compact system [14] as seen in Figure1.

The results also showed that 33.33% of *K. pneumoniae* was isolated from sputum, 30% from urine samples, 20% from burn swabs and 16.67% from wound infections. Blood samples were free from *K.pneumoniae*, as shown in Table 2. Partially in agreement with results of epidemiological study conducted by [15] reported that *Klebsiella pneumoniae* was dominant among clinical samples isolated from urine and blood.

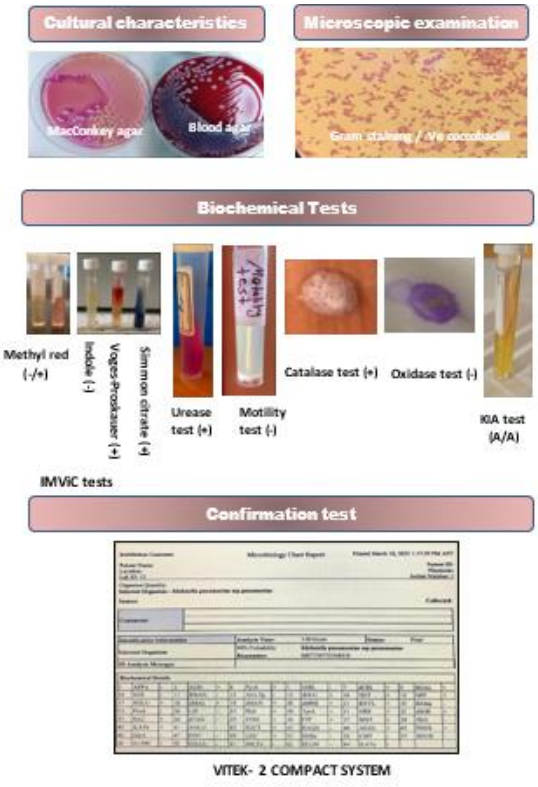

Figure 1: Diagram showing cultural and biochemical profile of *Klebisella pneumoniae*.

These findings agree with [16] reported that out of 268 clinical samples, 36 (13.4 %) *K. pneumoniae* isolates were recovered. In contrast, [17] found that *Klebsiella* spp. formed 54.16 % of total isolates from clinical specimens and 79.12 % was identified as *K. pneumoniae*. [18] also revealed the highest percentage of *K. pneumoniae* (74.4%), which is higher than that reported in the current study.

According to the results of the present study, the prevalence of *K. pneumoniae* among sputum samples 10(33.33%) and urine samples 9(30%) were found to be considerably higher than that in other clinical samples (wound and burn swabs) (Table 1). Similar to our findings, [15] reported that *Klebsiella pneumoniae* was dominant among all clinical samples isolated from urine. Similarly, [19] showed that *K. pneumoniae* isolated from urinary tract infection cases were found to yield the highest number when compared to other cases.

Another study done by [13] found that out of 468 different clinical samples, 61 (13.03%) isolates were identified as *K. pneumoniae* and urine showed the highest percentage (50.8%) followed by wound swab (24.6%) and (13.1%) of blood samples. These results are supported by a previous local study done by [20] in Diyala province reported that *K. pneumonia* (42.9%) was the dominant among urine samples. Similarly, [21] showed that 44% of *K. pneumoniae* strains were isolated from urine. The latter study also recorded that 8% of *K. pneumoniae* recovered from sputum, 8% burn swabs and 4% wound swabs, these numbers however are lower than that reported in the recent study. In contrast, [22] reported the lowest percentage of *Klebsiella* spp. isolated from the UTI (2.1%).

Table 1: prevalence of *Klebsiella pneumoniae* isolated from different clinical samples

Clinical samples	Total	Positive	Negative
		No (%)	No (%)
Blood	20	0	20(16.67)
Burn swab	20	6(20)	14(11.67)
Sputum	40	10(33.33)	30(25)
Urine	50	9(30)	41(34.17)
Wound swab	20	5(16.67)	15(12.5)
Total	150	30(20)	120(80)

Distribution of *K. pneumoniae* isolates according to the age and sex

The results of recent study showed that 63.33% of patients with *K. pneumonia* infection were in the age group (20- 40), 26.67% of patients within the age group 40-60 years while the lowest rate was < 60 age group as show in Table (2). The current results disagreed with those demonstrated by [23] stated higher rate of *K. pneumoniae* infections among elderly patients, mainly in the age group ≥ 60 years. They referred that male patients above the age of 60 years were subjected to greater frequency of *K. pneumoniae* infections. The high percentage of infections was recorded in the age group of 20-40 which may be due to certain reasons comorbidities of some disorders like diabetes or collection of a significant number of samples in this age group compared to the samples of other age

groups. The prevalence of infections in recent study are in disagreement with those recorded by [23]found that *K. pneumoniae* were mainly recovered from patients with the age group less than 10 years. Our results also demonstrated that 66.67 % of *K. pneumoniae* was females and 33.33% was males. These results referred that females were more susceptible to get *K. pneumoniae* infections than males. Similarly, [23] stated that the infection in female was higher (54.5 %) than male (45.6 %). In the study of [24] viewed that 209 cases were males and 995 cases were females. [18] also showed that out of 50 *K. pneumoniae* isolates from UTI, female infections were more than males. On the other hand, these results were in contrary to those recorded by [19] showed that males were more susceptible to *Klebsiella* infections than females. Another study done by [25]also showed that all *K. pneumoniae* infections an apparent bias toward male predominance and to affect older adults. Although there is no significant difference in percentage of origin of samples.

Table 2: Distribution of *K. pneumonia* isolates according to age and sex.

Age groups (years)	No.	%
20-40	19	63.33
40-60	8	26.67
60 >	3	10
Total	30	100
Sex		
Male	10	33.33
Female	20	66.67
Total	30	100

Antimicrobial Resistance

Agar disc diffusion test (Kirby-Bauer method) was used in accordance to Clinical Laboratory Standards Institute (CLSI) standards (2020) to assess antimicrobial susceptibility test for eight antibiotics as seen in Figure (2). The isolates showed high levels of resistance towards antibiotics used in this study as seen in Figure (3). The 29 (96.67%) of *K. pneumoniae* isolates were resistant to ampicillin, 29 (96.67%) resistant to ceftazidime, 26 (93.33%) resistant to gentamicin, 27(90%) resistant to azithromycin, 27(90%) resistant to amoxicillin-clavulanate, 25(83.33%) resistance to cefotaxime and 23(76.67%) resistance to levofloxacin. Although, meropenem is one of the most effective antibiotics against *K. pneumoniae*, while the isolates of current study showed moderate resistance 11 (36.67%) to meropenem. Thirty of (100%) of *K. pneumoniae* isolates showed multidrug resistant (resistant to 3 or more of antibiotics used in this study).

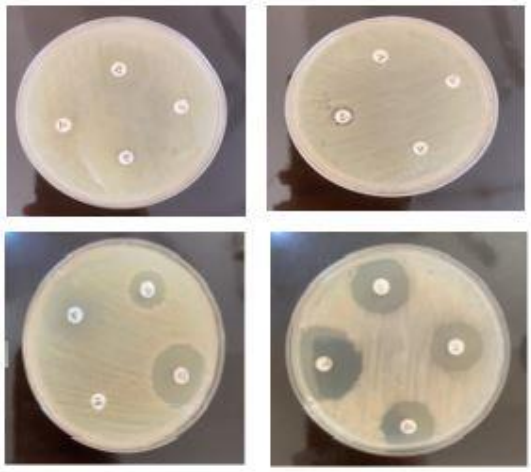

Figure 2: Disc diffusion test of *K. pneumoniae* isolates using Kirby-Bauer method

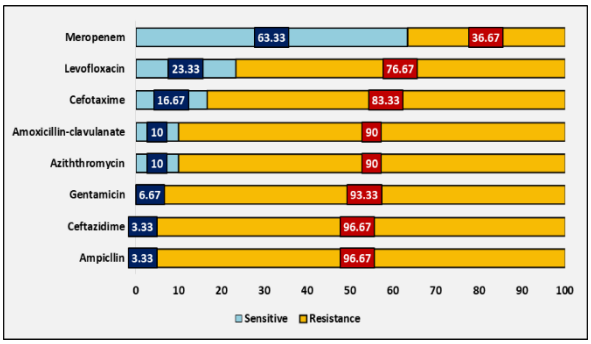

Figure 3: Antibiotic susceptibility test of *K. pneumonia* isolates.

Through the possess mechanisms of resistance to carbapenems include production of lactamases and mutations that alter the expression and/or function of porins and PBPs [26, 27]. Combinations of these mechanisms can cause high levels of resistance to carbapenems in *K. Pneumoniae*[28] . It is very important for public healthcare to monitor and report the changes in antimicrobial-resistant isolates[29]. The current study agreed with a study done by [30]who mentioned that *Klebsiella pneumoniae* was highly resistant to ampicillin by producing β-lactamases that render these isolates resistant to most β-lactam antibiotics. The results were also consistent with those of [31]who found that resistance percentage of *K. pneumoniae* against β-lactam antibiotics include ampicillin (97.6%) and cefotaxime (66.1%). A Study done by [32] revealed the emergence of efflux pump-mediated drug resistance in MDR *K. pneumoniae* bacteria in Iraq. Another study done by [33] for a total of (107) clinical *K. pneumoniae* isolates showed that all isolates were MDR to minimum 6 and maximum 14 antibiotics out of 17.

Biofilm production

The results revealed that (60%) of *K. pneumoniae* isolates was biofilm producer by appearance of black colonies on Congo red agar, while the other (40%) of isolates were formed red colonies indicating no biofilm formation as seen in Figure 4.

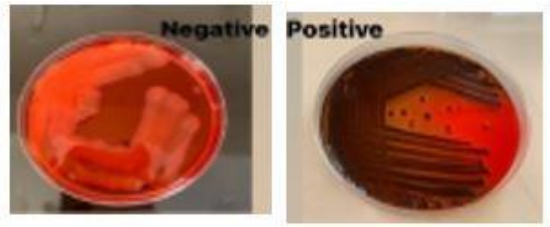

Figure 4: Biofilm formation of *K. pneumonia* on Congo red agar

Additionally, the virulence-related genes, such as the yersiniabactin biosynthesis gene (*ybts*), aerobactin synthase gene (*iucC*), and mucoid phenotype A (*rmpA*) gene, play an important role in the production of biofilms. Biofilm formation was further quantified using microtiter plate test as seen in Figure 5.

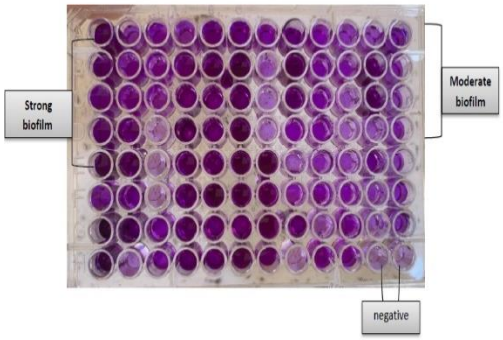

Figure 5: Biofilm formation in Microtiter plate assay

The results revealed that *Klebsiella pneumoniae* isolates have capability to form biofilm with different patterns. Thus, it was categorized into three types: strong biofilm producer (46.67%), (23.33%) moderate biofilm former and (30 %) of isolates categorized as non-producer biofilm as seen in Table 3.

Table 3: Identification of biofilm by using Congo red agar and Tissue culture methods

Biofilm formation		No.	%	Total
Congo red agar	Positive	18	60	30 (100%)
	Negative	12	40	

Tissue culture method			
Strong biofilm former	14	46.67	30 (100%)
Moderate biofilm former	7	23.33	
Non- biofilm former	9	30	

The results of current study showed that 70% of *K. pneumoniae* isolates had capability to form biofilm in tissue culture plate assay. Similar to our result, [34] how quickly biofilms formed on different *K. pneumoniae* strains, and their findings demonstrated that 85.63% of the strains were biofilm-forming. The hvKp strains also showed form much more biofilm than cKp strains [35].

Conclusion

In this study, we demonstrated the high prevalence of *Klebsiella pneumoniae* among patients in Kirkuk city with a rate of 20%, mainly recovered from young people with the age group ranged from 20 to 40 years. *K. pneumoniae* isolates were commonly recovered from sputum samples followed by urine, wound and burn swabs. Furthermore, females were more susceptible to *Klebsiella pneumoniae* than males. Most of *K. pneumoniae* bacteria infected hospitalized patients and were more resistant to antibiotics and formed biofilm.

Competing Interests

There are no competing interests.

References

[1] José A, Bengoechea., Joana Sa, Pessoa. (2019). *Klebsiella pneumoniae* infection biology: living to counteract host defenses. FEMS Microbial Rev 43(2): 123–144.

[2] Ranjbar, R, Fatahian K. A., Chehelgerdi, M. (2019). Molecular characterization, serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the *Klebsiella pneumoniae* strains isolated from different types of hospital-acquired infections. Infect Drug Resist 12(1):603–611.

[3] Fuzi, M., Rodriguez Baño, J., Toth, A. (2020). Global evolution of pathogenic bacteria with extensive use of fluoroquinolone agents. Front Microbiol11(1): 271.

[4] Wanjiang, Z., Yao, Z., Changzhen, W., Wenyu, L., Ruichao, L., Fuguang, C., Tian, L., Yanhe, Zh., Stefan, S., Siguo, L. (2019). Characterization of a multi drug resistant porcine Klebsiella pneumoniae sequence type 11 strain coharboring blaKPC-2 and fosA3 on two novel hybrid plasmids. mSphere 4(5): e00590-19.

[5] Soady, N. R., Karomi, A. S. (2022). Assessment Of Biofilm Production of Obligatory Aerobic and Anaerobic Bacteria for Acne Patients In Kirkuk–Iraq. Journal of Pharmaceutical Negative Results, 161-164.

[6] Montazeri A, Salehzadeh A, Zamani H (2020) Effect of silver nanoparticles conjugated to thiosemicarbazide on biofilm formation and expression of intercellular adhesion molecule genes, icaAD, in *Staphylococcus aureus*. Folia Microbial (Praha) 65:153–160.

[7] Sundaramoorthy, N.S, Thothathri S, Bhaskaran M, Kumar A, Prasad G, Nagarajan S (2021). Phages from Ganges River curtail in vitro biofilms and planktonic growth of drug resistant Klebsiella pneumoniae in a zebrafish infection model. AMB Expr 11:27.

[8] Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S (2012). Antibiotic resistance pattern of biofilm-forming uropathogens isolated from catheterized patients in Pondicherry, India. Australas Med J 5:344–348.

[9] Wedley, A. L., Dawson, S., Maddox, T. W., Coyne, K. P., Pinchbeck, G. L., Clegg, P., ... & Williams, N. J. (2017). Carriage of antimicrobial resistant *Escherichia coli* in dogs: Prevalence, associated risk factors and molecular characteristics. Veterinary microbiology, 199, 23-30.

[10] Vasaikar, S., Obi, L., Morobe, I., & Bisi-Johnson, M. (2017). Molecular characteristics and antibiotic resistance profiles of *Klebsiella* isolates in Mthatha, Eastern Cape province, South Africa. International journal of microbiology.

[11] Manoharan, A., Premalatha, K., Chatterjee, S., Mathai, D., & SARI Study Group. (2011). Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. Indian journal of medical microbiology, 29(2): 161-164.

[12] Abdullah, I. T. (2013). The study of resistance pattern of some bacteria isolated from child blood to two aminoglycoside antibiotics. Kirkuk University Journal Scientific studies. 8(1):15-26.

[13] Hasani, A., Soltani, E., Ahangarzadeh Rezaee, M., Pirzadeh, T., Ahangar Oskouee, M. et al., (2020). Serotyping of *Klebsiella pneumoniae* and its relation with capsule-associated virulence genes, antimicrobial resistance pattern, and clinical infections: a descriptive study in medical practice. Infection and Drug Resistance: 1971-1980.

[14] Zain AL- Abdeen, S. S & Al-Talabany, B. A (2017). Comparative diagnostic study of *Klebsiella* usingApi20E System and the device vitek2 and PCR. Kirkuk University Journal-Scientific Studies, 12(1): 81-94.

[15] Ajimuda, O. E., Sanmi-Kayode, I., Adeniyi, O. O., Alaka, O. O., & Onipede, A. (2022). Prevalence of extended spectrum Beta-Lactamase producing *Klebsiella* species from patients' specimens in a tertiary teaching hospital in Ile-Ife, Southwest Nigeria. African Health Sciences, 22(2): 146-155.

[16] Al-Obadi, T. H. Z. (2014). Molecular Identification of *Klebsiella pneumoniae* Using Capsule Genes. Doctoral dissertation, M. Sc. Thesis. College of Science, Al-Nahrain University, Baghdad, Iraq.

[17] Omar-Zahid, L. A. (2009). Extraction and Purification of the Klebocin from Clinically Isolated *Klebsiella* and Studying Its Biochemical and Biological Characteristics (Doctoral dissertation, Msc. thesis. College of Science. University of Baghdad).

[18] Jasim, S. A., Abdulrazzaq, S. A., & Saleh, R. O. (2020). Virulence Factors of *Klebsiella pneumoniae* Isolates from Iraqi Patients. Systematic Reviews in Pharmacy, 11(6).

[19] Vandhana, V., Saralaya, K. V., Bhat, S., Shenoy Mulki, S., & Bhat, A. (2022). Characterization of Hypervirulent *Klebsiella pneumoniae* (Hv-Kp): Correlation of Virulence with Antimicrobial Susceptibility. International Journal of Microbiology.

[20] Razooqi Al-Aajem, B. M., Saleem, A. J., & Jasim, H. M. (2021). detection of extended spectrum beta-lactamase genes (bla-ctx and bla-shv) and same virulent genes in *k. pneumoniae* isolated from urinary tract infection. Biochemical & Cellular Archives, 21(2).

[21] Zaki, N. H., Alwan, A. H., & Abas, S. M. (2016). New Natural Medium Using Vitis vinirfera for Siderophore Production from Clinical Isolates of *Klebsiella pneumonia.* Appli Micro Open Access.

[22] Alsaimary, I. E., Tossonian, V. H., Al-Nahi, L. M., Al-Abass, M. N., Al-hilfi, H. A., & Albaldawi, I. E. (2014). Occurrence of Multidrug Resistant Bacteria (Mdrb) among Operating Theatres in Various Hospitals of Al-Basrah Province. Donn. J. Micro. Biotech. Res, 1(2), 035-041.

[23] Khan, E.; Ejaz, M.; Zafar, A.; Jabeen, K.; Shakoor, S.; Inayat, R. and Hasan, R. (2010). Increased isolation of ESBL producing *Klebsiella pneumoniae* with emergence of carbapenem resistant isolates in Pakistan: Report from a tertiary care hospital. J. Pak Med Assoc, 60(3): 186-190.

[24] Magliano, E., Grazioli, V., Deflorio, L., Leuci, A. I., Mattina, R., Romano, P., & Cocuzza, C. E. (2012). Gender and age-dependent etiology of community-acquired urinary tract infections. The scientific world journal, 2012.

[25] Parrott, A. M., Shi, J., Aaron, J., Green, D. A., Whittier, S., & Wu, F. (2021). Detection of multiple hypervirulent *Klebsiella pneumoniae* strains in a New York City hospital through screening of virulence genes. Clinical microbiology and infection, 27(4): 583-589.

[26] Walsh, T. R. (2010). Emerging carbapenemases: a global perspective. International journal of antimicrobial agents, 36: S8-S14.

[27] Bleriot, I., Blasco, L., Delgado-Valverde, M., Gual-de-Torrella, A., Ambroa, A., Fernandez-Garcia, L et al., (2020). Mechanisms of tolerance and resistance to chlorhexidine in clinical strains of *Klebsiella pneumoniae* producers of carbapenemase: role of new type II toxin-antitoxin system, PemIK. Toxins, 12(9): 566.

[28] Suay-García, B., & Pérez-Gracia, M. T. (2019). Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. Antibiotics, 8(3): 122.

[29] Effah, C. Y., Sun, T., Liu, S., & Wu, Y. (2020). *Klebsiella pneumoniae*: an increasing threat to public health. Annals of clinical microbiology and antimicrobials, 19(1): 1-9.

[30] Namikawa, H., Yamada, K., Sakiyama, A., Imoto, W., Yamairi, K., Shibata, W., et al., (2019). Clinical characteristics of bacteremia caused by hypermucoviscous *Klebsiella pneumoniae* at a tertiary hospital. Diagnostic Microbiology and Infectious Disease, 95(1): 84-88.

[31] Rastegar, S., Moradi, M., Kalantar-Neyestanaki, D., & Hosseini-Nave, H. (2019). Virulence factors, capsular serotypes and antimicrobial resistance of hypervirulent *Klebsiella pneumoniae* and classical *Klebsiella pneumoniae* in Southeast Iran. Infection & chemotherapy, 51.

[32] Kareem, S. M., Al-Kadmy, I. M., Kazaal, S. S., Mohammed Ali, A. N., Aziz, S. N., Makharita, R. R., ... & Hetta, H. F. (2021). Detection of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in Klebsiella pneumoniae. Infection and drug resistance: 555-563.

[33] Fatima, S., Liaqat, F., Akbar, A., Sahfee, M., Samad, A., Anwar, M., ... & Khan, A. (2021). Virulent and multidrug-resistant *Klebsiella pneumoniae* from clinical samples in Balochistan. International Wound Journal, 18(4): 510-518.

[34] Karimi, K., Zarei, O., Sedighi, P., Taheri, M., Doosti-Irani, A., & Shokoohizadeh, L. (2021). Investigation of antibiotic resistance and biofilm formation in clinical isolates of *Klebsiella pneumoniae*. International Journal of Microbiology, 2021.

[35] Hefzy, E. M., M Taha, R., Abd El Salam, S., Abdelmoktader, A., & AF Khalil, M. (2023). Hypervirulent *Klebsiella pneumoniae*: Epidemiology, virulence factors, and antibiotic resistance. Novel Research in Microbiology Journal, 7(1): 1857-1872.