Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 2, 33-44

Molecular Detection of Some Virulence Genes in *K. pneumonia* Coinfection with COVID -19 Iraq Infection

[1]Zainab F. Shubrem , [2]Wathiq A. Hatite

[1,2] Institute of Genetic Engineering and Biotechnology for post graduate studies, University of Baghdad, Baghdad, Iraq.

Received: February 4, 2024 / Accepted: March 28, 2024 / Published: July 5, 2025

Abstract: The coronavirus pandemic of 2020 was caused by the coronavirus disease 2019 (COVID-19), an infectious disease with severe acute respiratory syndrome that was first discovered in Wuhan, China. Since then, it has spread throughout the world. Co-infections between viruses and bacteria are among the deadliest medical conditions, with greater fatality rate. Research on bacterial superinfections in patients with coronavirus disease 2019 (COVID-19) is scarce. COVID-19 patients are at risk for colonization with *K. pneumonia* and hospital-acquired infections. Therefore, Infections with K. pneumonia, particularly those caused by extremely virulent strains, may make COVID-19 more difficult to treat. Fifty sample of COVID-19 infection collected and detected positive by (RT) PCR with 50 apparently control healthy sample than we cultured 50 sputum and nasal pharyngeal swabs for all COVID19 positive samples ,fifty sample of coinfection bacterial in the COVID19 patients identified by A- Vietk-2system ,B-confirmed by molecular detection PCR , The fifty-isolate distributed as 27 (54 %) *k. pneumonia*, 6 (12 %) *E. coli*, *P. aeruginosa* 15 (30%), other bacteria 2(4%)the *k. pneumonia* is the largest number of coinfection . The results of the current study demonstrated a positive correlation between the presence of certain virulence genes and the ability of bacterial isolates to resist antibiotics. Certain virulence genes (rmpA, mrkA, and iuCc, ybts, magA,) have been found by molecular means. our study was done by conventional PCR technique. we found *Klebsiella pneumonia* isolates which was found rmpA18(66%) of this isolates at bp 535, then 7(25%)) isolate were positive for *mrkA* gene at 115bp,whereas *iucC* gene was recorded in 15(55%) isolates at bp 1075 , magA found in 3 (11%) at 1283, and ybts found in 20(74%) at 242 bp.

Keywords: magA mucoviscosity-associated gene A**,** *iucC* gene iron siderophores aerobactin synthase gene**,** rmpA (Regulator of mucoid phenotype gene)**,** ybts yersiniabactin (YbtS), *mrk*A (Mannose resistant *Klebsiella* polypeptide A) gene**.**

Corresponding author: (E-mail: zainabfayadhsh.phd@gmail.com).

Introduction

COVID -19 with bacterial coinfection

Coronavirus disease of 2019 (COVID-19) spread and killed a lot of people (1,2). is a global emergency due of its quick According to data that is currently available from the World Health Organization(WHO), there were more than 6.3 million deaths linked to SARS-CoV-2 and its variations globally between the beginning of COVID-19 outbreaks and 2022, with over 574 million confirmed cases (3).The well-known pathogen *Klebesilla pneumonia*, which is recognized as an enteric bacterium, belongs to the Enterobacteriaceae family, which is defined and depicted as non-motile and Gram-negative (G-ve). This well-known pathogenic agent is linked to hospital-

associated illnesses and is responsible for about one-third of G. Even though everyone is working to stop the COVID-19 outbreak (4). The immunization against COVID-19 is still administered correctly, although the exact distribution of this vaccination is still unclear worldwide. Co-infection by SARS-CoV-2 and other respiratory illnesses that cause the virus has also emerged as a real concern for COVID-19 patients' care. (5). Microbial co-infection generally increases the risk of serious illness (6). One acknowledged contemporary microbial infection is COVID-19. One of the major recovery concerns is the occurrence of microbial co-infections, especially viral–bacterial co-infections, which are becoming more common and pose a greater risk of death (7). As previously mentioned in past study studies, complications from bacterial infections are thought to be one of the main reasons for influenza-related deaths. (8,9,10). In relation to immune system failure, (11) found that the lymphocytes, specifically the T, B, and NK cells, were harmed as a result of an infection caused by SARS-CoV-2. A reduction in the lymphocytes and human immunity activity, according to (12, 13) may be the greatest cause of co-infection. The treatment protocol for the SARS-CoV-2 infection includes corticosteroid compounds, as suggested by the WHO.

In Present research we confirmed the coinfection in COVID-19 by PCR technique based on the earlier report by (14) found the rate of bacterial secondary infections was confirmed in 1055 COVID-19 patients via polymerase chain reaction (PCR) tests, demonstrating *K. pneumonia* was among the other detected bacteria (14), additional study by (15) evaluated the effect of illnesses caused by multidrug-resistant (MDR) *K. pneumonia* in COVID-19-infected cases and recommended that maximizing infection management measures is a crucial strategy for preventing *K. pneumonia* and other multidrug-resistant bacteria in COVID-infected patients (16). Certain signs of pneumonia induced by SARS-Cov-2 and *K. pneumonia* were found to be similar.

Virulence factors of bacteria

Yersiniabactin biosynthesis gene (ybts), mucoviscosity-associated gene A (magA), iron siderophores aerobactin synthase gene (iucC), and regulators of mucoid phenotype A (rmpA) are only a few of the virulence-associated genes that are essential to the pathogenicity of *K. pneumoniae* strains (17). It has been discovered that a variety of virulence factors, including adhesion factors and capsular formation, mediate *K. pneumonia* infections, which spread to every part of the body. The elements of virulence include outer membrane Iron transport systems and porin efflux pumps are mediated by proteins (OMPs). Although other virulence factors like as fimbriae, siderophores, and O antigen are present, a small number of genetic variables are responsible for the production of capsule K. pneumonia. In K1 serotype, the chromosomal gene magA_ is in charge of capsule formation. Regarding the lack of magA, pathogenic strains with hyper-mucoviscosity are attributed to the rmpA Regulator of Mucoid Phenotype gene. Two genes, the regulator of mucoid phenotype A (rmpA) and magA, were initially linked to invasive infections (18,19). It is now well known that the capsular polymerase that is encoded by magA is located within the gene cluster that designates *K. pneumonia* capsular

serotype K1 (20). The rmpA gene is an extracellular regulatory plasmid-borne gene. showed that the hypermucoviscous phenotype and the clinical condition caused by invasive strains were associated with strains containing the rmpA gene. The ability to produce siderophores like aerobactin, enterobactin, and yersiniabactin, as well as genes involved in ferric iron uptake, allantoin metabolism, and biofilm formation, are among the several virulence factors of yersiniabactin YbtS, a phenolate-type siderophore that is structurally distinct from Ent *K. pneumonia* (21). *K. pneumoniae* strains that are able to generate excessive amounts of siderophores are referred to as hypervirulent. (22). The strains which do not secrete siderophores have dropped virulence and hence are less efficient at colonization and infection. (23), demonstrated that siderophores are crucial for pathogenic K. pneumonia to survive in low level of iron (24). According to the study (25,26), the usage of broad-spectrum antibiotics was the main factor driving the increase in antimicrobial resistance during the COVID-19 pandemic (27).

Materials and methods

The samples were collected with based on clinical examination, in accordance with the WHO criteria (3), Fifty sample of COVID-19 infection collected and detected positive by RT PCR with 50 appertenately control healthy sample than we cultured 50 sputum and nasal pharyngeal swabs for all COVID19 positive samples fifty sample of coinfection bacterial in the COVID19 patients identified by A-Vitek-2 system .B-confirmed by molecular detection PCR , The culture media included: Blood base Agar MacConkey agar, Mueller Hinton agar, Nutrient Agar Simmon's citrate agar (HiMedia India) were used in this study, shown (Figure1) positive string test result for *Klebsiella pneumonia* isolate from Sputum of patient with Coronavirus disease. The antimicrobial susceptibility test by antibiotic disc to COVID 19 patients. The primers were created using Primer 3plus, V4, and their reference sequences were verified twice by the University Code of Student Conduct (UCSC) programs and the National Center for Biotechnology Information (NCBI) database. Alpha DNA Ltd. manufactured and lyophilized them in Canada. (Table1) lists all primer sequences that were used. in this study's assays.

Table (1): The study's designed primers of genes

rmpA (Foreword)	ACT GGG CTA CCT CTG CTT CA
rmpA (Reverse)	CTT GCA TGA GCC ATC TTT CA
mrkA (Foreword)	ACGTCTCTAACTGCCAGGC
mrkA (Reverse)	TAGCCCTGTTGTTTGCTGGT
iucC (Foreword)	TGGATTGATGCTCAAACTCTG
iucC (Reverse)	TGCATCGCTCATTGACAGTA
ybtS (Foreword)	GACGGAAACAGCACGGTAAA
ybtS (Reverse)	GAGCATAATAAGGCGAA
16S rRNA	AGAAGCCGACCTGAGAGGGTGA
magA(Foreword)	GGTGCTCTTTACATCATTGC
magA(Reverse)	GCAATGGCCATTTGCGTTAG

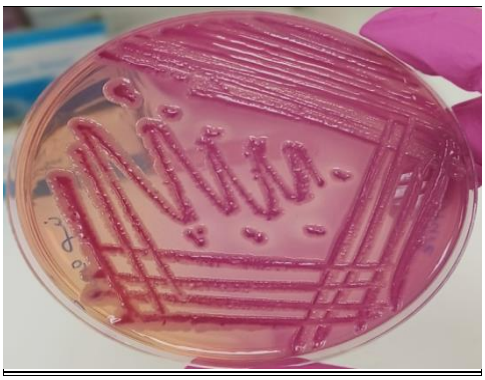

Figure (1): Positive string test result for *Klebsiella pneumonia* isolate from Sputum of patient with Coronavirus disease

Molecular identification of *K. Pneumonia*

Extraction of genomic DNA

After genomic DNA was extracted using the EasyPure® Bacteria Genomic DNA Kit (TRANS/China), concentration could be determined using a UV Spectrophotometer, which shows that the nucleic acids absorb most light at 260 nm.

Conventional PCR reaction

A partial sequence was chosen for this study to evaluate the gene's association with coinfection bacteria - covid19 in Iraqi sample. To start the PCR, the reaction show (Table 2) was tuned by testing four annealing temperatures: 56, 58, 60, and 62°C. The annealing temperature of 58 °C was the optimum for producing clear and sharp bands in agarose gel, hence it was used in the current study. This protocol employs 2xEasyTaq® PCR SuperMix (TRANS/China) . All PCR reactions were carried out in a 25 µl final volume and according to the manufacturer's instructions. The components and volumes of PCR reaction 12.512.5µl Forward primer1 µl, Revers primer1 µl DNA4µl, Nuclease free water 6.5µl.

Table (2): PCR program

Step	Temperature	Time	cycle
1-Denaturation	94 ºC	5 min.	(1)
2-Denaturation	94 ºC	30 sec.	35
3-Annealing	58 ºC	30 sec.	
4-Extension	72 ºC	30 sec.	
5-Extension	72 ºC	5 min.	(1)

Agarose gel electrophoresis

The extracted DNA and amplified PCR fragments were separated on an agarose gel and then seen under UV light after ethidium bromide staining, DNA loading and electrophoresis The gel's wells were loaded with a mixture of a combination (3µl of loading dye and 7µl of extracted genomic DNA (or product PCR)). Following the loading of all wells, the electrical power was turned on for 60 minutes at 100 volts (5V/cm2). This caused DNA with a negative charge to migrate from the cathode (-) to the anode (+) poles. Agarose Gel staining and UV visualization after staining electrophoresis gels with ethidium bromide, which was made by adding 70µl of the 10 mg/ml ethidium bromide to 300 ml of D.W.; the gel

was stained by soaking in the solution for 20-30 minutes, and then the gel was placed into the gel documentation system to view the DNA bands at a 365 nm wavelength. Special software was utilized to save the photos captured by the device on the computer (28).

DNA sequencing

The amplified (PCR) fragments were subjected to Sanger sequencing using an automated DNA sequencer (ABI3730XL, Macrogen Corporation), Korea, Genious prime software showed the genotypes after aligning with a main reference sequence in the Gene Bank.Its shareware licensing, efficient up-to-date modules and speedy ability to provide findings make it one of the most popular applications among molecular biologists today (29).

Statistical analysis

The IBM SPSS Statistics 26 program's statistical analysis was utilized to determine how different factors affected the study's parameters. The T-test and one-way ANOVA were utilized to compare the means statistically.

Results and discussion

Isolation of bacterial coinfection from COVID- 19 patients

Fifty isolates of *bacterial* spp. were collected during this study during **2023** from different hospitals in Baghdad city: Yarmouk Teaching Hospital, Al -Wasti Teaching Hospital, and Teaching laboratories of Medical City hospital. The fifty-isolate distributed as 27 (54 %) *k. pneumonia*, 6 (12 %) *E. coli*, *P. aeruginosa* 15 (30%), other bacteria staphylococcus aureus 2(4%) the *k. pneumonia* is the largest number of coinfection. (Figure 2) shows distribution of coinfection bacteria isolates.

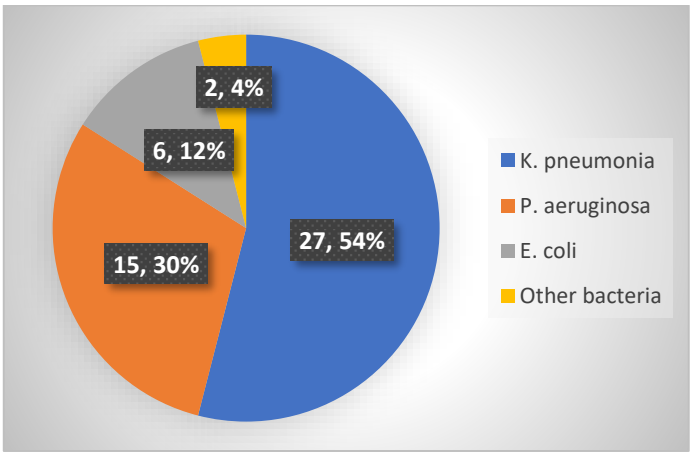

Figure (2): Dispersal of isolated coinfection bacteria.

It has been shown that COVID-19 patients with bacterial coinfection have varying prevalence and resistance profiles. Following 1380 COVID-19 patients, the most common G-ve isolates in Bahrain were *K. pneumoniae* (23.8%), P. aeruginosa (23.2%), *A. baumannii* (22.0%), and *E. coli* (17.1%). (26). Furthermore, among them 1495 COVID-19, *A. baumannii* (35.6%) and *K. pneumoniae* (30.8%) were the two most often isolated big bacteria found in the 19 patients with bacterial coinfections who were hospitalized in Wuhan (30). Thus, it's critical to keep an eye on bacterial coinfection in COVID-19 patients. hospital infections, especially those caused by bacteria resistant to many

drugs COVID-19's prior outcome (4) revealed that COVID-19 patients admitted to the intensive care unit (ICU) had greater rates of bacterial coinfection than patients who were not in the ICU. Most bacterial strains that were recovered from sputum were gram-negative bacteria, including *A. baumannii and K. pneumoniae.* Gram-negative bacteria isolated from COVID-19 patients showed a high level of resistance, particularly in ICU patients (13). The study's findings indicate that, in order to control the pandemic both locally and globally, it is crucial to continuously monitor bacterial coinfection and resistance patterns in addition to improving infection control measures. in another previous study by Bahceci *et al*. (14). Furthermore, polymerase chain reaction (PCR) testing were used to confirm the rate of bacterial co-infections in 1055 COVID-19 patients, showing that K. pneumonia was one of the additional bacteria found. The most frequently The bacteria that were found were Escherichia coli and *Klebsiella pneumonia,* which is frequently linked to ventilator-associated pneumonia and hospital-acquired pneumonia. In the early stages of infection, three to seven days after infection, the majority of the bacteria detected in the study by (31) were *Klebsiella pneumoniae, Acinetobacter baumannii*, and Enterococcus species. After seven days from admission, the same bacteria persisted in the late infection stage, to which Escherichia coli was added in the same ratio as *Acinetobacter baumannii* and Enterococcus species. After seven days from admission, *Klebsiella pneumoniae* predominated.

Identification of *K. pneumonia*

Identification of *K. pneumonia* was done depending on cultural, microscopic, and biochemical characterization, and confirmed by Vietk-2 System. For isolation and utilization in our investigation, the swabs were grown on MacConkey, Blood, Nutrient, and Chrom agar orientations. Different biochemical tests were performed for characterization of *K. pneumonia,* the results are summarized that the bacterial isolates were negative for oxidase, while results positive catalase, urease and citrate utilization, Indole test and VP tests.

VP: Voges-Proskauer.

Molecular study of *K. Pneumonia*

genomic DNA extraction

Genomic DNA was successfully extracted from 27 isolates of *K. pneumonia* as shows (Figure 3) the genomic DNA analyzed by agarose gel electrophoresis.

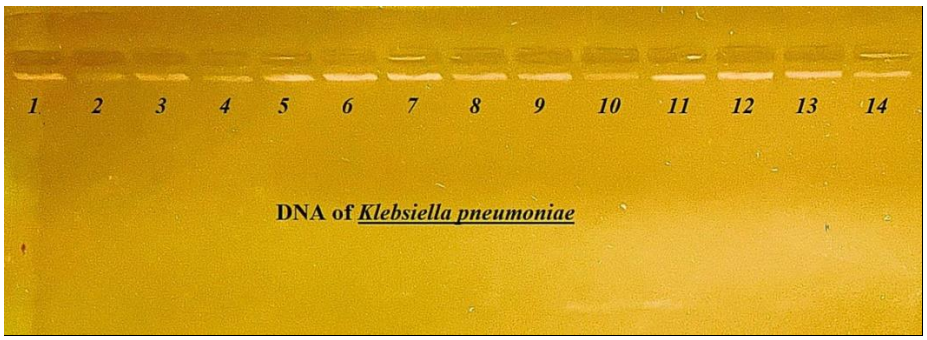

Figure (3): Gel electrophoresis for extraction DNA from *K.pneumonia*, agarose 1%, at 100 voltsfor 60min.). Visualized under u.v. light after staining with using ethidium bromide

Conventional PCR for detection *K. Pneumonia* by used 16SrRNA

A partial sequence was chosen for this study to evaluate the *16sRNA* gene's association with bacterial coinfection with COVID 19 as show (Figure 4). To start the PCR, the reaction was tuned by testing four annealing temperatures: 56, 58, 60, and 62°C. The annealing temperature of 58 °C was the optimum for producing clear and sharp bands in agarose gel, hence it was used in the current study shown (Table 3). This protocol employs 2xEasyTaq® PCR SuperMix. All PCR reactions were conducted in a final volume of 25 µl. volume and according to the manufacturer's instructions.

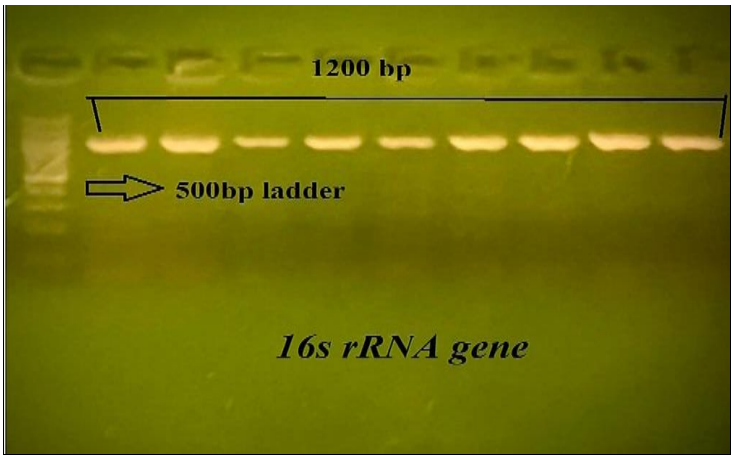

Figure (4): Gel electrophoresis for PCR product of 16S rRNA 1200 bp primer) shows 1200 bpPrimer TM at (58 C), (Agarose 2%, in 70 volts and 60min, so visualized by using u.v. light after staining by ethidium bromide.

The components and volumes of PCR reaction

2xEasy*Taq*® PCR SuperMix 12.5ul, forward primer 1ul, reverse primer 1ul, DNA 4 ul, Nuclease free water 6.5 ul.

Table (3): PCR program

Step	Temperature (ºC)	Time	Cycle
-Denaturation	94**(ºC)**	5 min.	(1)
-Denaturation	94**(ºC)**	30 sec.	35
-annealing	58**(ºC)**	30 sec.	
-extension	72**(ºC)**	30 sec.	
-extension	72**(ºC)**	5 min.	(1)

Conventional PCR results

Strong methods that can provide important details about the K. pneumonia infection are virulence analysis and molecular typing. In our present work we showed that the studied genes (*rmpA, mrkA,iucC,ybts* and *magA*) were found in 27 isolates according to paper by Hosoda *et al*(32). (Figures 5,6,7,8,9) illustrated shine bands of positive results of previous genes as compared with DNA ladder (1500 pb). K. pneumonia strains 27 (54%) have been identified from our hospitalized infection samples using established microbiological methods. Nosocomial infections are caused by the multidrug-resistant (MDR)

opportunistic bacteria *Klebsiella pneumonia*. Strong techniques like virulence analysis and molecular typing can yield pertinent data on *K. pneumonia* infections. We found K. pneumonia antibiotic resistance and virulence-associated gene analysis in our search. our result *k. pneumonia* is more resistance to 97% Ceftazidime , 85% Gentamicin , 92%Ciproflocacin , 96% levofloxacin and sensitive to Imipenem 50% . Previous result by (33**)** The highest prevalence of resistance was observed against ciprofloxacin (75%), trimethoprim–sulfamethoxazole (73%) and nitrofurantoin (68%). The study by (34) 2016 *K. pneumonia* more resistinse to Augmentin 100%, cefotaxime 100, and more sensitive to Impeneme 100%, when (35) found *K. pneumonia* coinfection with COVID 19 (97.7%) resistance against Cefixime, ciprofloxacin, penicillin- Tazobactam, ciprofloxacin, so increased resistance to Levofloxacin, Gentamycin and Amikacin.

Virulence gene screening results

The current study demonstrated a positive association between a capacity of bacteria to resistant antibiotics and the presence of certain virulence genes, using molecular analysis to identify the virulence genes rmpA, mrkA, and iuCc, ybts, and magA. The present study used the standard PCR method to conduct our investigation. *Klebsiella pneumonia* isolates were discovered to have rmpA 18 (66%) for these isolates at bp 535, as shown in (Figure 5), then 7(25%) isolate were positive for *mrkA* gene at 115bp show Figure(6),whereas *iucC* gene was recorded in 15(55%) isolates at bp 1075 show (Figure 7), ybts found in 20(74%) at 242 bp) as show (Figure 8) and magA found in 3 (11%) at 1283 as show (Figure 9).

Previous study by (36) used PCR amplification for detecting virulence genes of *K. pneumoniae* iucC, rmpA and magA. Among 30 isolates of *K. pneumonia*, 17 (56.66%) have the PCR response results for the (iucC). Furthermore, the PCR reaction findings did not reveal any isolate of *K. pneumonia* that carried the rmpA and magA genes..

The previous study by (37) was done by (PCR) technique. It was discovered that isolates of *K. pneumonia* were detected in 26 (78%) of the isolates at 1071 bp, followed by 17 (17%) isolates that tested positive for the rmpA gene at 967 bp and 5 (15.15%) isolates that tested positive for the mrkA gene at 862 bp. as soon as the earlier research by (33) indicated that 75%, 5%, 30%, and 48% of the isolates had the virulence-associated genes ybts, magA, iucC, and rmpA, respectively. For every studied isolate, the frequency of biofilm-associated genes, including mrkA, was 88%. A follow-up study by used the repetitive element sequence-based PCR rep-PCR method to separate the *K. pneumoniae* strains into 11 distinct genetic patterns. Because of the elevated frequency of resistance present of various virulence factors, high levels of biofilm gene expression in a variety of clones of *K. pneumonia* strains pose a significant health risk in clinical settings (33). PCR was used to identify the genes linked with mrkA biofilm and revealed the frequency of rmpA virulence genes. The most frequently occurring gene is rmpA, which has been linked to *K. pneumonia* hypermucoviscosity and high pathogenicity (18). The rmpA gene, which is a potent virulence plasmid with 180 kilobases on a multicopy

plasmid, is essential for the production of the mucoid phenotype. in relation to *K. pneumoniae.* The earlier study by (37) demonstrated a positive association between the presence of some virulence genes and the ability of bacterial isolates to withstand antibiotic resistance the virulence genes (rmpA and mrkA) were identified molecularly using a standard PCR method. It was discovered that the prevalence of isolates of *K. pneumonia* was much higher than anticipated. Five isolates (15.15%) tested positive for the mrkA gene at 862 bp, whereas 17 isolates (51% of the isolates) tested positive for the rmpA gene at 967 bp.

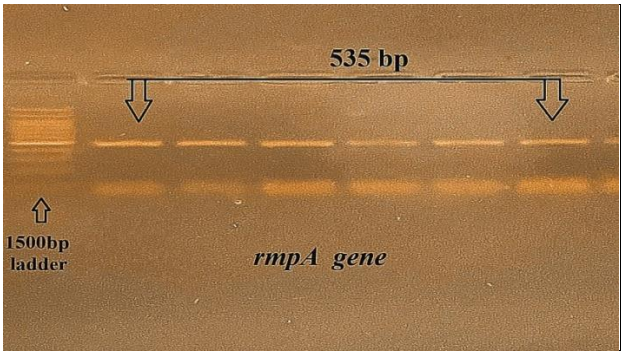

Figure (5): Gel Electrophoresis for pcr of (*rmpA* primer) shows rmpA 535 bp primer tm. at (C), (Agarose 2%, 70 volts at 60min**). Visualized under u.v light after staining by using ethidium bromide.**

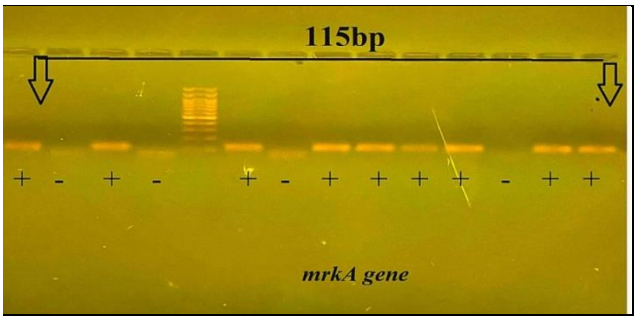

Figure (6): Gel electrophoresis for pcr product (*mrkA,*) primer) shows mrkA 115 bpPrimer TM at (58 C), agarose 2%, 70 60min. visualized by u.v. light after staining by ethidium bromide.

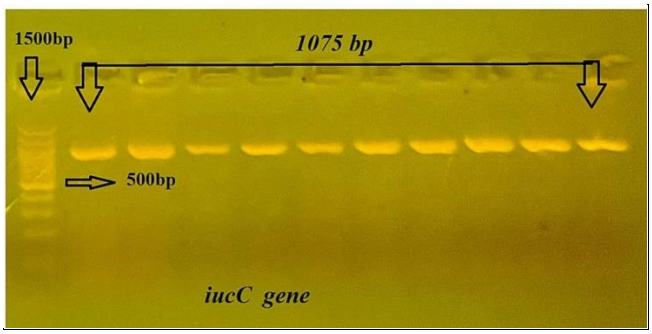

Figure (7): Gel electrophoresis for PCR product of (,*iucC,*) primer) shows 1075bp Primer TM at (58 C), agarose 2%, 70 volts at 60min.). visualized under u.v. after staining by EB.

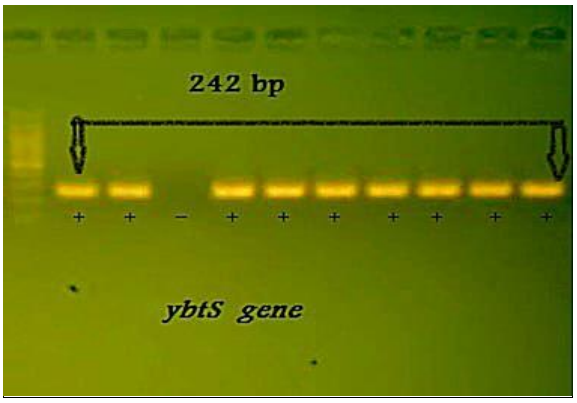

Figure (8): Gel electrophoresis pcr product of (*ybts*) primer shows 242 bp primer tm at (58 C), use agarose about 2%in 70 volts at 60min.). visualized under U.V light after staining with ethidium bromide.

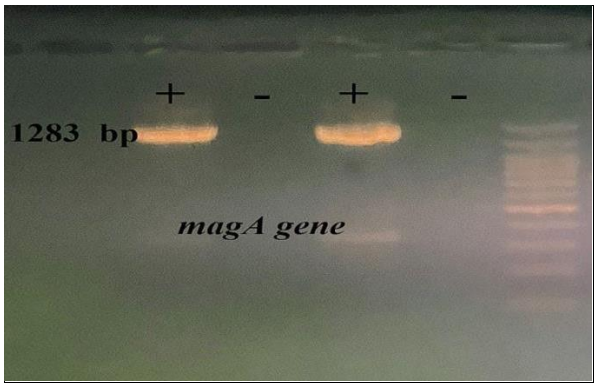

Figure (9): Gel electrophoresis pcr product of (*magA*) primer shows 1283 bp primer tm at (58 C), agarose 2%,70 volts at 60min. Visualized under u.v. light ,staining with EB

References

1. Farah, H. and Ali, I. A. (2023). Molecular Detection of Candida spp. Isolated from Female Patients Infected with COVID-19 in Baghdad City. Iraqi Journal of Biotechnology, 22 (1): 238-244
2. Zhu, X.; Ge, Y.; Wu, T.; Zhao, K.; Chen, Y.; Wu, B., *et al* Cui, L. (2020). Co-infection with respiratory pathogens among COVID-2019 cases. Virus research, 285, 198005.
3. World Health organization (2020).Novel Coronavirus(2019-nCoV):situation report,36.Bazaid, A. S.; Barnawi, H.; Qanash, H.; Alsaif, G.; Aldarhami, A.; Gattan, H., *et al*. (2022). Bacterial coinfection and antibiotic resistance profiles among hospitalised COVID19patients. Microorganisms, 10(3): 495.
4. Almashhadani, A. D. and AL-Thwani, A. N. (2022). Determination of Angiotensin-Converting Enzyme 2 (ACE2) Receptor Level in Samples of Iraqi Patients Infected with COVID-19, Iraqi Journal of Biotechnology, 21(2): 178-182.
5. Lansbury, L.; Lim, B.; Baskaran, V. and Lim, W. S. (2020). Co-infections in people with COVID-19: a systematic review and meta-analysis. Journal of infection, 81(2): 266-275.
6. Khudhr, E. N. and Shehab, Z. H. (2022). Rapid Identification of some typical and atypical Pneumonia co-infections associated with COVID-19 patients by a real -time PCR assay Iraqi Journal of Biotechnology, 21(2): 331-340.
7. Maki, F. M. AL-Thwani, A. N. and Jiad, K. S. (2022). Interleukin-1 Beta (IL-1β) Persistence in Post SARS-CoV-2 Infection and Vaccination: A Double Case Control Study, Iraqi Journal of Biotechnology, 21(2): 561-567.
8. Massey, B. W.; Jayathilake, K. and Meltzer, H. Y. (2020). Respiratory microbial co-infection with SARS-CoV-2. Frontiers in Microbiology, 11: 2079.

9. Touny, A.; Rageh, F.; Riad, E.; Sakr, M. A.; Abdelhady, S. A.; Elgamal, R., *et al.* (2023). Incidence of Co-infection and its Impact on COVID-19 Patients admitted in the Intensive Care Unit. Egyptian Journal of Anaesthesia, 39(1): 141-148.
10. Wagner C.; Griesel, M.; Mikolajewska, A.; Mueller, A.; Nothacker, M.; Kley, K., *et al*. (2021). Systemic corticosteroids for the treatment of COVID-19. Cochrane Database of Systematic Reviews, 8: CD014963.
11. Luo, Y.; Xie, Y.; Zhang, W.; Lin, Q.; Tang, G.; Wu, S., *et al*. (2019). Combination of lymphocyte number and function in evaluating host immunity. Aging (Albany NY), 11(24): 12685.
12. Yusof, R. C.; Norhayati, M. N. and Azman, Y. M. (2023). Bacterial coinfection and antibiotic resistance in hospitalized COVID-19 patients: a systematic review and meta-analysis. PeerJ, 11: e15265.
13. Bahceci, I.; Yildiz, I. E.; Duran, O. F.; Soztanaci, U. S.; Harbawi, Z. K.; Senol, F. F., *et al*. (2022). Secondary bacterial infection rates among patients with COVID-19. Cureus, 14(2).
14. García-Meniño, I.; Forcelledo, L.; Rosete, Y.; García-Prieto, E.; Escudero, D. and Fernández, J. (2021). Spread of OXA-48-producing *Klebsiella pneumonia*e among COVID-19-infected patients: The storm after the storm. Journal of infection and public health, 14(1): 50-52.
15. Montrucchio, G.; Corcione, S.; Sales, G.; Curtoni, A.; De Rosa, F. G. and Brazzi, L. (2020). Carbapenem-resistant *Klebsiella pneumonia*e in ICU-admitted COVID-19 patients: Keep an eye on the ball. Journal of global antimicrobial resistance, 23, 398-400.
16. Highsmith, A. K. and Jarvis, W. R. (1985). *Klebsiella pneumonia*e: selected virulence factors that contribute to pathogenicity. Infection Control & Hospital Epidemiology, 6(2): 75-77.
17. Chang, L.; Bastian, I. and Warner, M. (2013). Survey of *Klebsiella pneumonia*e bacteraemia in two South Australian hospitals and detection of hypermucoviscous phenotype and magA/rmpA genotypes in *K. pneumoniae* isolates. Infection, 41: 559-563.
18. Yu, W. L.; Ko, W. C.; Cheng, K. C.; Lee, H. C.; Ke, D. S.; Lee, C. C., *et al*. (2006). Association between rmpA and magA genes and clinical syndromes caused by *Klebsiella pneumonia*e in Taiwan. Clinical infectious diseases, 42(10): 1351-1358.
19. Yeh, K. M.; Lin, J. C.; Yin, F. Y.; Fung, C. P.; Hung, H. C.; Siu, L. K., *et al*. (2010). Revisiting the importance of virulence determinant magA and its surrounding genes in *Klebsiella pneumonia*e causing pyogenic liver abscesses: exact role in serotype K1 capsule formation. The Journal of infectious diseases, 201(8): 1259-1267.
20. Nassrf, X.; Honore, N.; Vasselon, T.; Cole, S. T. and Sansonetti, P. J. (1989). Positive control of colanic acid synthesis in Escherichia coli by rmpA and rmpB, two virulence-plasmid genes of *Kiebsiella pneumoniae*. Molecularmicrobiology, 3(1): 1349-1359.
21. Yu, W. L.; Fung, C. P.; Ko, W. C. and Chuang, Y. C. (2007). Polymerase chain reaction analysis for detecting capsule serotypes K1 and K2 of *Klebsiella pneumonia*e causing abscesses of the liver and other sites. The Journal of infectious diseases, 195(8): 1235-1236.
22. Sahly, H.; Navon-Venezia, S.; Roesler, L.; Hay, A.; Carmeli, Y.; Podschun, R., *et al*. (2008). Extended-spectrum β-lactamase production is associated with an increase in cell invasion and expression of fimbrial adhesins in *Klebsiella pneumonia*e. Antimicrobial agents and chemotherapy, 52(9): 3029-3034.
23. Diancourt, L.; Passet, V.; Verhoef, J.; Grimont, P. A. and Brisse, S. (2005). Multilocus sequence typing of *Klebsiella pneumonia*e nosocomial isolates. Journal of clinical microbiology, 43(8): 4178-4182.
24. Magnasco, L.; Mikulska, M.; Giacobbe, D. R.; Taramasso, L.; Vena, A.; Dentone, C., *et al*. (2021). Spread of carbapenem-resistant Gram-negatives and Candida auris during the COVID-19 pandemic in critically ill patients: one step back in antimicrobialstewardship?. Microorganisms, 9(1): 95.
25. Saeed, N. K.; Al-Khawaja, S.; Alsalman, J.; Almusawi, S.; Albalooshi, N. A. and Al-Biltagi, M. (2021). Bacterial co-infection in patients with SARS-CoV-2 in

the Kingdom of Bahrain. World journal of virology, 10(4): 168-181.
26. Said, K. B.; Alsolami, A.; Moussa, S.; Alfouzan, F.; Bashir, A. I.; Rashidi, M., *et al*. (2022). COVID-19 clinical profiles and fatality rates in hospitalized patients reveal case aggravation and selective co-infection by limited gram-negative bacteria. International journal of environmental research and public health, 19(9): 5270.
27. Coleman, W. B. and Tsongalis, G. J. (Eds.). (2006). Molecular diagnostics: for the clinical laboratorian. Springer Science & Business Media.
28. AL-zohairy, A. (2011). BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences. 2(1): 60-61.
29. Li, J.; Wang, J.; Yang, Y.; Cai, P.; Cao, J.; Cai, X., *et al*. (2020). Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: a retrospective analysis. Antimicrobial Resistance and Infection Control, 9(1): 1-7.
30. Susan, M.; Susan, R.; Lazar, V.; Bagiu, I. C.; Mihu, A. G.; Bagiu, R. V., *et al*. (2023). COVID-19 association with multidrug-resistant bacteria superinfections: Lessons for future challenges. Experimental and Therapeutic Medicine, 25(6): 1-7.
31. Hosoda, T.; Harada, S.; Okamoto, K.; Ishino, S.; Kaneko, M.; Suzuki, M., *et al*. (2021). COVID-19 and fatal sepsis caused by hypervirulent *Klebsiella pneumonia*e, Japan, 2020. Emerging infectious diseases, 27(2): 556-259.
32. Mirzaie, A. and Ranjbar, R. (2021). Antibiotic resistance, virulence-associated genes analysis and molecular typing of *Klebsiella pneumonia*e strains recovered from clinical samples. AMB Express, 11(1): 1-11.
33. Hamdi, N. M. and Najeeb, L. M. (2016). Isolation and diagnosis of Klebsiella spp from different environmental sites and a comparative study of it's susceptibility against some antibiotics. Journal of university of Anbar for Pure science, 10(1): 1-6.
34. Mobarak-Qamsari, M.; Jenaghi, B.; Sahebi, L.; Norouzi-Shadehi, M.; Salehi, M. R.; Shakoori-Farahani, A., *et al*. (2023). Evaluation of Acinetobacter baumannii, *Klebsiella pneumonia*e, and Staphylococcus aureus respiratory tract superinfections among patients with COVID-19 at a tertiary-care hospital in Tehran, Iran. European Journal of Medical Research, 28(1): 314.
35. Al-Aajem B.; Jasim H. and Saleem A. (2021). Detection of Virulent genes Khe, iuc, rmp, magA in *Klebsiella pneumonia*e Isolated from Urinary Tract Infection. International Journal of Drug Delivery Technology, 11(4): 1470-1473.
36. Nihad, J.; Hayder, S. and Mohanad, J. (2020). Molecular detection of Virulence Factors genes Associated with Immune Resistance in *Klebsiella pneumonia* Medico-legal Update, 20(3): 1459.