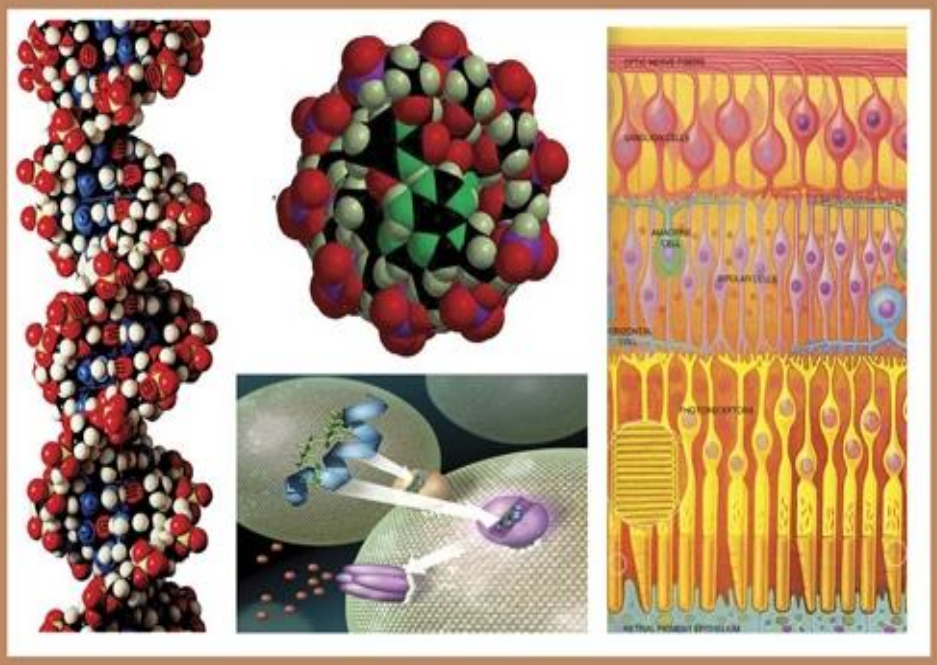
EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
PHYSIOLOGY & MOLECULARBIOLOGY

C



ISSN
2090-0767

WWW.EAJBS.EG.NET

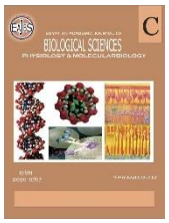
Vol. 15 No. 1 (2023)

.

Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp59-65 (2023)*
DOI: 10.21608/EAJBSC.2023.309053

Egypt. Acad. J. Biolog. Sci., 15(1):59-65 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Studying Several Virulence Factors Phenotypically and Genetically to Isolate *Klebsiella Pneumonia* from Diabetic Patients

Sarah A. Mohammed and Nibras Y. Abdulla
Department of Biology, Faculty of Science, Kufa University
***E-mail: nibras.alsallami@uokufa.edu.iq**

ARTICLE INFO
Article History
Received:10/6/2022
Accepted:20/7/2023
Available:26/7/2023

Keywords: *Klebsiella pneumonia,* diabetic foot ulcer, virulence factors.
.

ABSTRACT

Klebsiella pneumoniae is the most frequent cause of nosocomial and community-acquired infections. The virulence factors help *Klebsiella pneumoniae* establish an infection in the human body by contributing to its ability to survive in a variety of environmental circumstances. 42 *K. pneumoniae* clinical specimens were isolated from diabetic patients suffering from urinary tract infection and diabetic foot ulcer in this study. *K.pneumoniae* virulence factors are currently being identified using phenotypic and genotypic approaches. The *K. pneumoniae* isolates that were positive for several virulence factors included 33 (78.6%) capsule, 17 (40%) hypermucoviscosity development, 14 (33.3%) proteases, and none were positive for hemolysis .

K. pneumoniae isolates had a virulence gene that was 15% wcaG, 60% entB, and negative for rmpA.

INTRODUCTION

K. pneumonia is a gram-negative, capsulated, nonmotile bacilli, lactose fermenting, facultative anaerobic bacteria of the Enterobacteraceae family.*K. pneumoniae* colonizes the epidermis, oral, breathing, and digestive flora asymptomatically. *K. pneumoniae* is a recognized pathogen of infections acquired in hospitals and the general population (Martin and Bachman, 2018). They include pneumonia of the lower lungs, bacteremia, septicaemia, urinary tract disease, wound infections, neonatal septicemia, liver abscess, meningitis, endophthalmitis, ventilator-associated pneumonia (VAP), and central line-associated bloodstream infection (CLABSI). (Ko *et al*., 2002).

K. pneumoniae pathogenicity is mostly due to different virulence factors that allow it to overcome innate host immunity and maintain infection in a mammalian host (Bengoechea and Sa Pessoa,2019). These virulence factors aid in its survival in different environments and hence aid in establishment the disease in the human organism (Paczosa and Mecsas, 2016). *K. pneumoniae* pathogenicity is influenced by a number of virulence factors, such as fimbrial adhesins, lipopolysaccharides, capsule and siderophores, biofilm growth, lipase, lecithinase, haemagglutination, protease, gelatinase, hemolysis, and hypermucoviscosity. All of these virulence factors have the ability to infect both hospitalized patients and the general public with a wide spectrum of diseases (Piperaki *et al.,* 2017; Ramakrishnan *et al.,* 2019). Additionally, it creates hemolysin protein, a exotoxin that lyses blood cells, allowing bacteria to proliferate (Esmaeel and Sadeq, 2018).

Klebsiella pneumoniae has three strains: classical *K. pneumoniae* (cKP), hypervirulent *K. pneumoniae* (hvKP), and multidrug-resistance K. pneumoniae (MDR-hvKP) (Lin *et al.*,2020; Zhu *et al.*,2021).

Classical *K. pneumoniae* (cKp) infections are more common (Esmaeel and Sadeq, 2018). These bacteria survive in hospital settings and cause illnesses in elderly individuals. These cKp strains are not the same as hypervirulent *K.pneumoniae* (hvKp) strains (.Choby *et al.*,2020).

Although these bacteria have virulence genes that may encode capsules (magA, k2A, wcaG), hypermucoviscosity (magA, rpmA), adhesins (fimH, mrkD, kpn), lipopolysaccharides (wabG, uge, ycfM), and iron acquisition systems (iutA, iroN, entB.

MATERIALS AND METHODS

Bacteria:

Different enrichment, differential, and selective media were employed to culture bacteria from diabetic patients with diabetic foot ulcers and urinary tract infections who visited the diabetes center at AL-Sader Hospital in Al Najaf Province. A biochemical test was conducted to identify the bacterium after it had grown in the medium

Virulence Factors Detection:

Hemolysis:

At 37°C, Hemolysis was seen after *K. pneumonia* colonies were inoculated on standard sheep blood agar. (Ramakrishnan *et al*.,2019).

Capsule Identification:

A *K. pneumonia* colony that had been incubated overnight was placed on a clean slide, stained for two minutes with methylene blue, and then rinsed with tap water. Observed a capsule encircling the organism (Collee et al., 1996). *Klebsiella pneumoniae* isolate was inoculated on regular sheep blood agar media and incubated at 37°C for 24 hours using the

Hyper Mucoviscosity Detection by String Test:

The string test was then demonstrated using a normal inoculation loop. When the produced string stretched longer than 5 mm, it indicated an HMV phenotype (Li *et al.*, 2014).

Protease Detection:

K. pneumonia was injected onto freshly made milk agar and incubated for 72 hours at 37°C. hours, resulting in the formation of hallo surrounding the colonies, indicating protease synthesis (Piperaki *et al.*, 2017).

Genomic DNA Extraction:

Boiling was used to process the DNA of the isolated bacterium. In 300 microliters of sterile TE buffer, many bacterial colonies were added and cooked for 15 minutes in a water bath at 100 degrees Celsius, quickly cooled to -20 degrees Celsius for 15 minutes, centrifuged, and the supernatant was saved for later amplification operations (Shah et al., 2017).

Gene Amplification:

The following three primers were used to amplify the genes of bacterial isolate DNA extracts: wcaG, entB, and rmpA. 8 L of master mix, 5 L of DNA template, 1.5 L for each primer, and 4 L of deionized water for PCR were added. The protocol was followed in accordance with the manufacturer's instructions for Promega Biosystem. The following thermal cycling conditions were used for amplification: Pre-denaturation at 95°C for 5 minutes, followed by 30 cycles of 1 minute at 94°C, 1 minute at 58°C, 1 minute at 72°C, and 10 minutes of final elongation at 72°C.

Table 1: lists the oligonucleotide primers used in this research.

Gene	Oligonucleotide Sequence	PCR product size	Reference
wcaG	F: 5'-GGTTGGKTCAGCAATCGTA-3' R: 5'-ACTATTCCGCCAACTTTTGC-3'	169	Turton *et al.,* 2010
rmpA	F: 5'-ACTGGGCTACCTCTGCTTCA-3' R: 5'-CTTGCATGAGCCATCTTTCA-3'	516	Turton *et al*., 2010
entB	F: 5'-ATTTCCTCAACTTCTGGGGC-3' R: 5'-AGCATCGGTGGCGGTGGTCA-3'	371	El Fertas-Aissani *et al*., 2013

RESULTS AND DISCUSSION

Detection of several *K. pneumonia* virulence factors using phenotypic techniques

In this investigation, we discovered that 33 (78.6%) of *K. pneumoniae* contain capsule (Table 2 and Fig. 1). Our findings contradict those of (Hullur *et al.,* 2022), who found that the percentage of capsule generation by *Klebsiella pneumonia* was 100%. The capsule is a significant virulence factor that participates in at least two harmful pathways, including direct inhibition of the host immune response and phagocytosis resistance. (Kang *et al*., 2015).

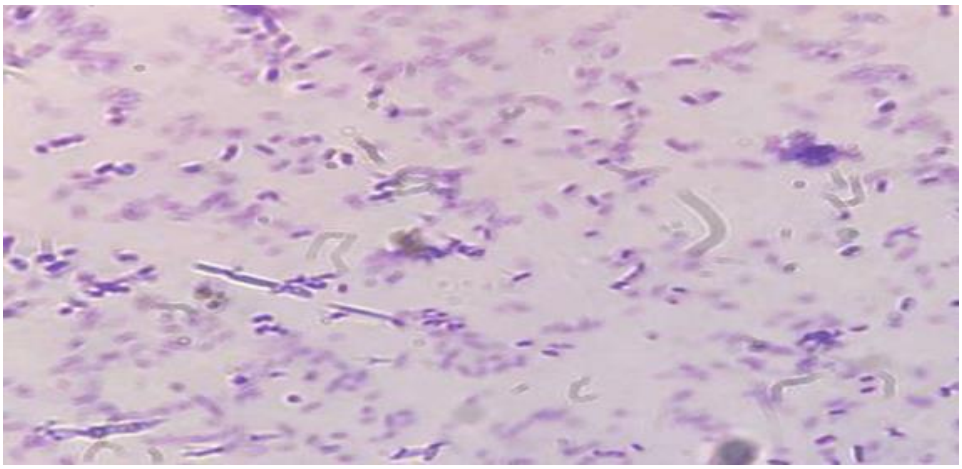


Fig. 1: Capsule production by *K. pneumonia.*

Our hypermucoviscosity analysis revealed that 17 (40%) of the *Klebsiella* tested positive for the string test, creating a viscous thread-like string of more than 5 mm in length and so identifying positive for the hmv phenotype (Fig. 2). Our findings were consistent with (Cerón *et al.,* 2022), who found the ratio to be 53.3%, and agree with (Hullur et al,2022; Osama *et al.*,2023), who found the ratio to be 44% and 42%, respectively, and disagree with (Liu et al,2019), who found 16% of *K. pneumonia* to be positive. Surface polysaccharide synthesis is thought to protect *K. pneumonia* against phagocytosis and fatal serum immune factors (Russo and Marr,2019).

Fig. 2: Hypermucoviscosity test by *K. pneumonia.*

The ability of *K. pneumoniae* to make protease was studied by streaking on nutritional agar with skim milk and found that only 14 (33.3%) of *K. pneumoniae* could produce protease (Fig. 3). Our findings agreed with those of (Ramakrishnan *et al.*,2019), who found that 44% of *K. pneumonia* was positive for protease. However, our findings disagreed with those of (Hullur *et al.*,2022), who found that 90% of *K. pneumonia* was positive for protease production. Microbial proteases operate as virulence factors in many diseases caused by bacteria (protein breakdown). These enzymes are known to cause damage to the host organism's defensive proteins. Protease has been shown to antimicrobial peptide (Anbu, 2016).

Our result showed that all *K. pneumonia* isolates were negative for hemolysis test on blood agar

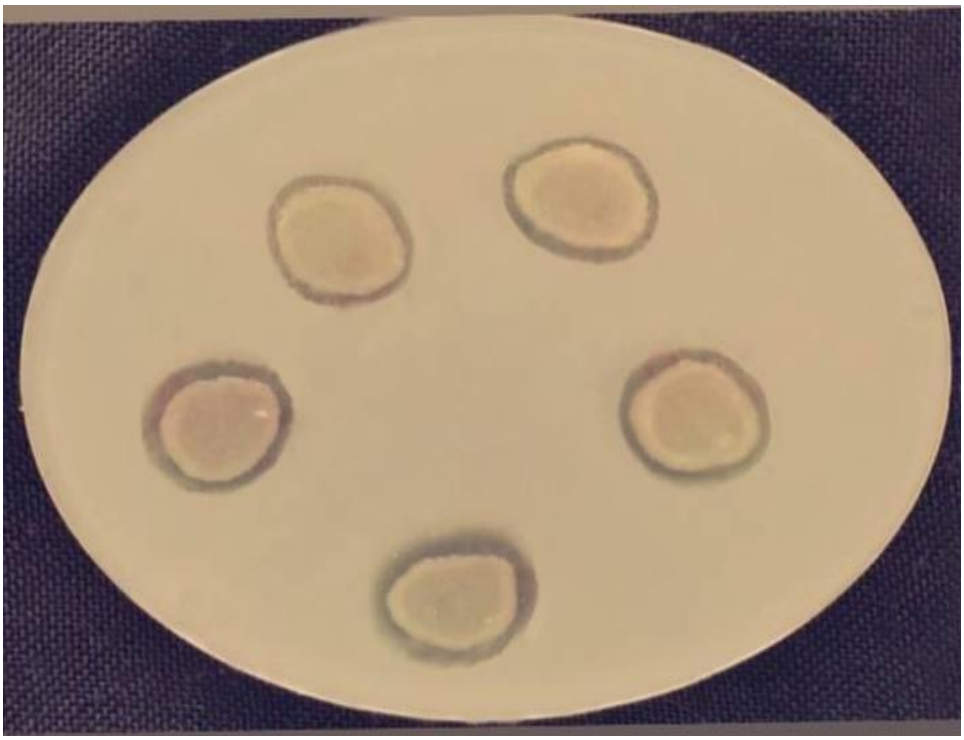


Fig. 3: Protease production by *K. pneumonia.*

Table 2: Some Virulence Factors Produced by K. pneumonia, Broken Down by Percentage and Number**.**

Capsule	33 (78.6%)	9 (21.4%)
Hypermucoviscosity	17(40 %)	25(60%)
Protease	14 (33.3 %)	28(66.6%)
Hemolysin	0(0%)	42(100%)

Detection of Several *K. Pneumonia* Virulence Factors Using Genotypic Techniques:

In the current investigation, we chose 20 isolated *K. pneumoniae* and employed three genes of virulence factors. Among the three identified virulence genes, the siderophore gene entB (enterobactin) was the most common (12%) (Fig. 4). Although enterobactin is the principal siderophore employed by *K. pneumoniae*, our findings contradict those of (Remya *et al*.,2020) who found that 90% of *K. pneumoniae* isolates had the *entB* gene.

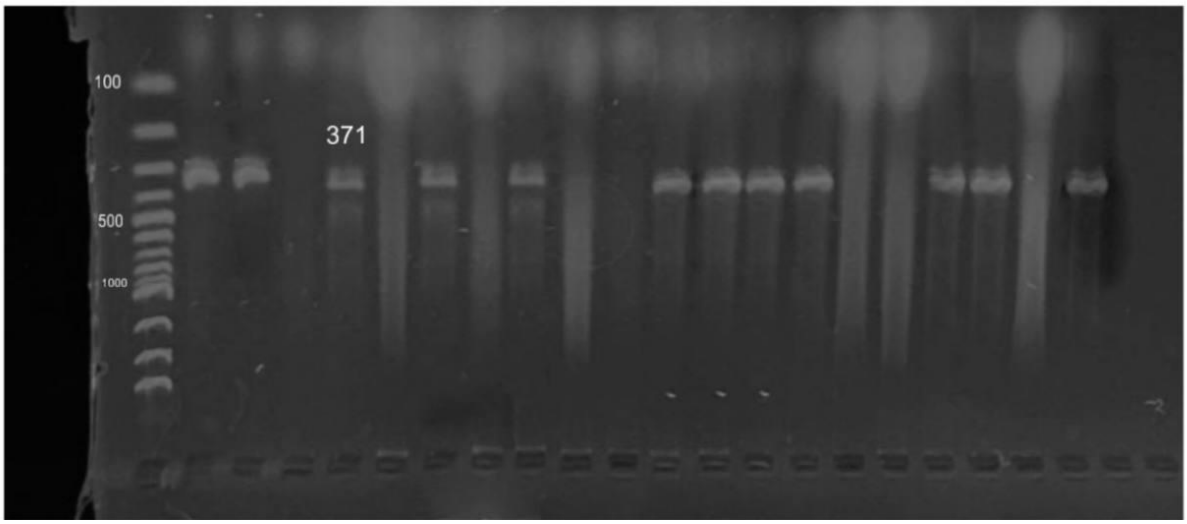


Fig. 4: Determined *entB* gene in *K. pneumoniae*.

The wcaG gene was found in 3 (15%) of the *K. pneumonia* samples (Fig. 5). Our findings were corroborated by (Soltan *et al.*,2020), who discovered wcaG in 27.9% of the isolates. Resistance to phagocytosis is promoted by capsule genes (wabG) (Cortés *et al.*, 2002).

While *rmp A* gene was absent in all isolates. This result disagreement with (Soltan *et al.*,2020) who showed that 21.3% of *K. pneumoniae* isolates were positive for *rmpA.*

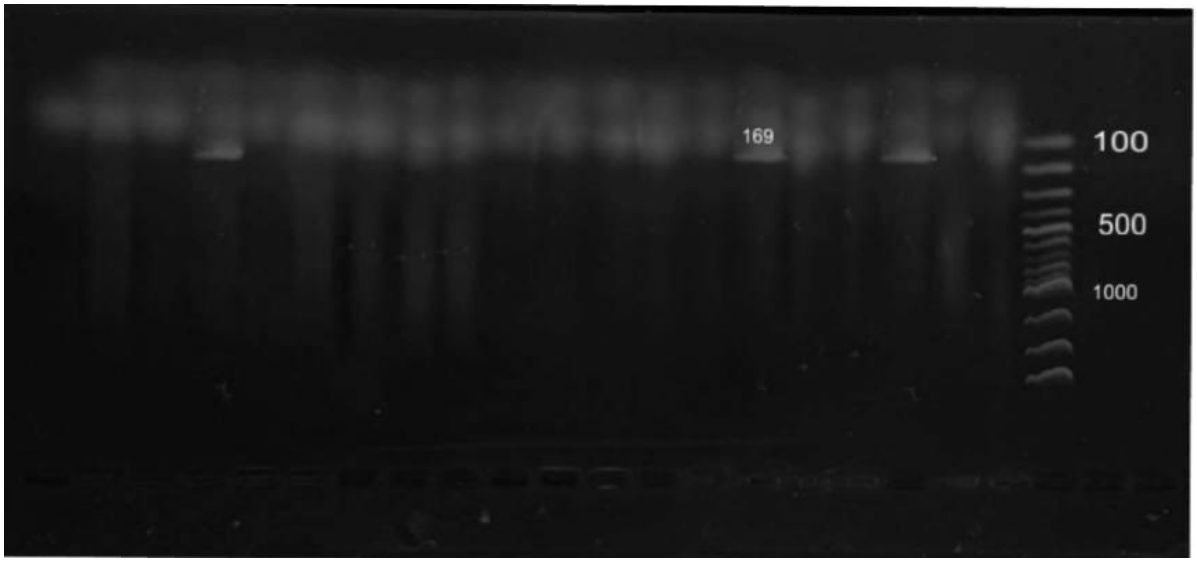


Fig. 5: Determined *wcaG* gene in *K. pneumoniae*.

REFERENCES

Anbu , P.(2016). Enhanced production and organic solvent stability of a protease from Brevibacillus laterosporus strain PAP04. *Brazilian Journal of Medical Biological Research*, 49: 1-7.

Bengoechea JA, Sa Pessoa J. (2019). *Klebsiella pneumoniae* infection biology: living to counteract host defences. *FEMS Microbiology Review*;43(2):123-144.

Cerón,A.B.; Pérez,E.M.; Cortés,L.R.G.; Jiménez, E.A.R.; Paniagua,F.V. and Contreras, G. L. P. (2022). Hypervirulence and Multiresistance to Antibiotics in *Klebsiella pneumoniae* Strains Isolated from

Patients with Hospital- and Community-Acquired Infections in a Mexican Medical Center. *Microorganisms*, 10, 2043.

Choby JE, Howard-Anderson J, Weiss DS. (2020). Hypervirulent Klebsiella pneumoniae – clinical and molecular perspectives. *Journal of Internal Medicine;*287(3):283-300.

Collee, J. G.; Fraser, A. G.; Marmion B. P. and Simmon, A. (1996) . Pactical Medical Microbiology. 14th Ed. Churchill Living Stone, USA Collins, C. H. and Lyne, P. M. Microbiological Methods. 14th Ed., Butterworth and Co. (Pubilisher).

Cortés G, Borrell N, de Astorza B, Gómez C, Sauleda J, Albertí S. (2002) Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *Klebsiella pneumoniae* in a murine model of pneumonia. *Infection and Immunity,* 70: 2583–2590.

El Fertas-Aissani R, Messai Y, Alouache S, Bakour R (2013). Virulence profiles and antibiotic susceptibility patterns of *Klebsiella pneumoniae* strains isolated from different clinical specimens. *Pathologie Biologie* ,61: 209– 216.

Esmaeel JR, Sadeq JN. (2018). Hemolysin gene detection in some isolates of *Klebsiella pneumonia* by PCR. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 17(2):49-52**.**

Hullur **,**M.S.; Natarajan ,A. and Sreeramulu, P.N. (2022). Phenotypic Characterization of Virulence Factors and Antibiogram of *Klebsiella pneumoniae* Isolates from Various Clinical Samples – A Cross Sectional Study. *Journal of Pure and Applied Microbiology*,16(3):1783-1791.

Kang Y. F., Tian P. F., Tan T. W. (2015). Research progress on virulence factors of *Klebsiella pneumoniae*. *Acta Microbiologica Sinica,* 55, 1245–1252.

Ko**,** WC, Paterson DL, Sagnimeni AJ. (2002). Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerging Infectious Diseases*, 8(2):160-166**.**

Li**,** W., Sun G., Yu Y., Li N., Chen M., Jin R. (2014). Increasing occurrence of antimicrobial-resistant hypervirulent (hypermucoviscous) *Klebsiella pneumoniae* isolates in China. *Clinical Infectious Diseases, 58(2):225-32.*

Lin, Z.**,** Zheng J., Bai B. (2020). Characteristics of Hypervirulent *Klebsiella pneumoniae*: Does Low Expression of *rmpA* Contribute to the Absence of Hypervirulence? *Front Microbiology*; 11:436.

Liu,X.; Zhang,J.; Li,Y.; Shen,Q.; Jiang,W.; Zhao,K.; He,Y.; Dai,P.; Nie,Z.; Xu,X. and Zhou,Y.(2019). Diversity and frequency of resistance and virulence genes in blaKPC and blaNDM co-producing *Klebsiella pneumoniae* strains from China. *Infection Drug Resistance,* 12: 2819–2826.

Martin RM, Bachman MA. (2018). Colonization, Infection, and the Accessory Genome of *Klebsiella pneumoniae. Frontiers in Cellular and Infection Microbiology*, 2018;8:4.

Osama, D.M.; Zaki, B. M.; Khalaf, W.S.; Mohamed M.Y.A.; Tawfick M.M. and Amin, H.A. (2023). Occurrence and Molecular Study of Hypermucoviscous/ Hypervirulence Trait in Gut Commensal K. pneumoniae from Healthy Subjects. *Microorganisms*, *11*(3), 704.

Paczosa MK, Mecsas J. (2016). *Klebsiella pneumoniae*: Going on the Offense with a Strong Defense, *Microbiology and Molecular Biology Reviews.* ;80(3):629-661**.**

Piperaki ET, Syrogiannopoulos GA, Tzouvelekis LS and Daikos GL. (2017). *Klebsiella pneumoniae:* virulence, biofilm and antimicrobial resistance. *The Pediatric Infectious Disease Journal* ,36 (10):1002-1005.

Ramakrishnan,K.; Johnsi, P.J. and Seetha, K.S.(2019). Clinical isolates of *Klebsiella pneumoniae* and its virulence factors from a tertiary care hospital. *Indian Journal of Microbiology Research,* 6(2):109-112.

Remya, P.A.; Shanthi, M. and Sekar,U.(2020). Characterisation of Virulence Genes Associated with Pathogenicity in *Klebsiella pneumonia*. *Indian Journal of Medical Microbiology,* 37 (2).

Russo, T.A.; Marr, C.M. (2019). Hypervirulent *Klebsiella pneumonia. Clinical Microbiology Reviews*, 32(3).

Shah, R. K.; Ni, Z. H.; Sun, X. Y.; Wang, G. Q. and Li, F. (2017). The determination and correlation of various virulence genes, ESBL, serum bactericidal effect and biofilm formation of clinical isolated classical Klebsiella pneumoniae and hypervirulent *Klebsiella pneumoniae f*rom respiratory tract infected patients. *Polish journal of microbiology,* 66(4): 501- 508.

Soltani, E.; Hasani, A.; Rezaee, M. A.; Pirzadeh, T.; Oskouee, M.A.; Hasani, A.; Oskouie, A. N. and Binesh, N. (2020). Virulence characterization of *Klebsiella pneumoniae* and its relation with ESBL and AmpC beta-lactamase associated resistance. *Iranian Journal of Microbiology;* 12(2): 98–106.

Turton JF, Perry C, Elgohari S, Hampton CV (2010). PCR characterization and typing of *Klebsiella pneumoniae* using capsular type-specific, variable number tandem repeat and virulence gene targets. *Journal of Medical Microbiology,* 9: 541–547.

Zhu J, Wang T, Chen L, Du H. (2021). Virulence Factors in Hypervirulent *Klebsiella pneumoniae. Frontiers in Microbiology,* 2021;12:642484**.**