RJNMSR
Review Journal of Neurological & Medical Sciences Review
Volume 3, Issue 4, 2025

ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM FRESH MEAT

Raid Ullah[1], Shah Naeem[2], Hurmat Bibi[3], Inzimamul Haq[4], Aizaz Ali Shah[5], Abida BiBi[6], Amna Waqar[7], Saifullah Khan[8*]

[1]*Department of Microbiology, Abdull Wali Khan University, Mardan Pakistan*
[2]*Faculty of Life Sciences, Centre for Biotechnology and Microbiology University of Swat*
[3]*Faculty of Life Sciences Centre for Biotechnology and Microbiology, University of Swat*
[4]*Faculty of Life Sciences, Centre for Biotechnology and Microbiology University of Swat*
[5]*Department of Animal Husbandry Anhui Agricultural University Hefei, China*
[6]*Faculty of Life Sciences, Centre for Animal Sciences and Fisheries University of Swat*
[7]*Department of Microbiology Abbottabad University of Science and Technology*
[*8]*Department Microbiology Faculty of Life Sciences Centre for Biotechnology and Microbiology University of Swat*
[1]khanraid.1122@gmail.com, [2]shahnaeem311@gmail.com, [3]huraiqbal1@gmail.com, [4]uinzimam72@gmail.com, [5]aizazsyed.62@gmail.com, [6]abidabibiswat16@gmail.com, [7]amnawaqarahmed24@gmail.com, [*8]saifullahkhanuos@gmail.com

Corresponding Authors: *
Saifullah Khan

DOI: https://doi.org/

Received	Accepted	Published
28 July, 2025	29th Aug, 2025	31 Aug, 2025

ABSTRACT

A significant multidrug-resistant (MDR) human pathogen, Klebsiella pneumoniae is a leading cause of hospital infections linked to high morbidity and mortality because there are few effective treatment options. Antibiotic resistance is a major global health concern due to the quick emergence of multidrug-resistant (MDR) strains of Klebsiella pneumoniae in clinical and animal-derived settings. Klebsiella pneumoniae isolated from fresh meat is the main purpose of the current investigation, which aims to identify its molecular characteristics and antimicrobial resistance. During the study meat samples were collected from various butcher's shops of district Abbottabad. The samples were processed and culture on the surface of MacConkey media for K. pneumonia strains isolation. One K. pneumonia strains were isolated from fresh meat by giving pink colonies on the surface of MacConkey agar media. Biochemical characterization was conducted to characterize isolates of pathogens. The biochemical test results show that isolate was positive towards catalase test result while negative towards indole and motility test results. After that, the bacterial growth was processed for testing for antibiotic susceptibility. The antibiotics employed in this investigation were amoxicillin, ceftazidime, ciprofloxacin, gentamicin, amikacin, meropenem, colistin, and tigecycline. The isolate was identified as a strain of Klebsiella pneumoniae by the analysis of the 16S rRNA gene sequence and the construction of phylogenetic trees. The isolate seemed to have a close evolutionary relationship with other strains of K. pneumoniae. K. pneumonia is still common in many regions of the world, including Pakistan, according to findings; thus, effective management and treatment are necessary to eradicate this infection. Precautionary steps, such as avoiding self-medication and inappropriate antibiotic usage, can help lower this resistance.

Keywords: *K. Pneumonia, Antimicrobial Resistance, Molecular Characterization, ESBL*

INTRODUCTION

Klebsiella pneumoniae is a gram-negative, enveloped, non-motile bacterium that is a member of the Enterobacteriaceae family (Wang *et al.*, 2017). For *K. pneumoniae*, humans are the main reservoir. Between 1% and 6% of people in the general population have the bacterium in their nasopharynx, and between 5% and 38% have it in their feces (Manges, 2015). Hospital staff members' hands and the patient's digestive tract are the primary sources of infection. Antibiotic resistance and infection can result from a variety of variables that contribute to the bacterium's virulence (Rodríguez-Medina *et al.*, 2019). As the most significant virulence element, the polysaccharide capsule of the organism enables the bacteria to avoid opsonophagocytosis and serum fatal injuries by the host organism (Abbas *et al.*, 2024). There are now 77 varieties of capsular bacteria that have been investigated, and the less virulent Klebsiella species that do not have a capsule are generally less common. Gram-negative bacteria's outer surface is coated in lipopolysaccharides, which are a second virulence factor (Paczosa & Mecsas, 2016). One of the main causes of the sequelae in sepsis and septic shock is the host organism's inflammatory cascade, which is triggered by the detection of lipopolysaccharides. Fimbriae, another virulence element, enable the bacterium to adhere to host cells. Another virulence element required by the bacterium to infect hosts is siderophores. In order for the infecting organism to spread, siderophores must get iron from the host (Paczosa & Mecsas, 2016).

The emergence of multidrug-resistant (MDR) *Klebsiella pneumonia* in animal gut microbiota is a serious public health and veterinary health problem. This Gram-negative opportunistic pathogen has become more prevalent in cattle, where it distributes antibiotic resistance genes (ARGs) through zoonotic and environmental pathways. It is one of the main blood-borne pathogens that cause nosocomial infections (Puspanadan *et al.*, 2012). MDR *K. pneumoniae,* which are thought to be involved in the horizontal transmission of plasmids containing ESBLs and carbapenemases, are found in the stomach of animals that produce food Alternative strategies, such phage (bacteriophage) biocontrol, must be used to stop the emergence of resistance as traditional antibiotic treatments are no longer effective (Walter *et al.*, 2018).

Certain bacteria, including *Klebsiella pneumoniae*, are currently exhibiting a high rate of antibiotic resistance as a result of changes to the organism's core genome. Gram-negative organisms' resistance to beta-lactam medicines was initially identified by Alexander Fleming in 1929 (Kutter & Sulakvelidze, 2004). Since then, *K. pneumoniae* has been thoroughly investigated and it has been demonstrated that it produces a beta-lactamase that hydrolyzes the beta-lactam ring in antibiotics. In 1983, extended-spectrum beta-lactamase (ESBL) *K. pneumoniae* was discovered in Europe, and in 1989, it was discovered in the US. Third-generation cephalosporins are rendered ineffectual against therapy by ESBLs' ability to hydrolyze oxyimino cephalosporins (Paczosa & Mecsas, 2016). Carbapenems were introduced as a therapy option for ESBL as a result of this resistance. However, *K. pneumoniae* was responsible for over 80% of the 9000 illnesses caused by carbapenem-resistant Enterobacteriaceae that were reported to the Centers for Disease Control and Prevention (CDC) in 2013. The organism's enhanced synthesis of ESBL enzymes, changes to the outer membrane, and up-regulation of efflux pumps have all been connected to carbapenem resistance (Valsamatzi-Panagiotou *et al.*, 2021).

A powerful method for tracking the dynamics of MDR *K. pneumoniae* development and transmission in both human and animal populations is genomic monitoring. Resistance markers, virulence genes, or phylogenetic relationships between veterinary and human isolates can be found by whole genome sequencing (WGS) (Paczosa & Mecsas, 2016). Recent research has highlighted the convergence of livestock-associated *K. pneumoniae*, such as clones of sequence types (ST) 11 and 307, on a worldwide AMR issue. The function of gut microbial communities in resistance gene persistence and transmission, however, remains little understood (Liu *et al.*, 2023).

MATERIALS AND METHODS

SAMPLES COLLECTION AND PROCESSING

Fresh meat samples were taken from several butchers stores in Abbottabad, Hazara Division, Khyber Pakhtunkhwa, Pakistan. After collection

samples immediately transported Abbottabad University of Science and Technology Microbiology Lab. Samples were carefully cleaned to remove surface contaminants and particle debris using distilled water or sterile phosphate-buffered saline (PBS). Using a tissue grinder or sterile mortar and pestle, the cleaned specimens were further physically homogenized to a fine particle fineness in order to ensure equal microbial dispersion. Samples were streaked onto MacConkey agar plates, which were then incubated at 37°C for the whole night. Both Gram staining and microscopy were used to identify the bacteria. Bacterial samples were subcultured before to each experiment, and cultures were used for eight hours. The viability and integrity of the culture were maintained by regular subculturing.

MORPHOLOGY BASED CHARACTERIZATION OF ISOLATED *K. PNEUMONIA*

GRAM STAINING

A small amount of distilled water was added to a clear slide in order to carry out the Gram staining. On the slide, a little quantity of pure culture was applied using a sterile needle. With the needle, the culture was dispersed uniformly throughout the surface. To the slide smear, a drop of crystal violet was applied using sterile water. A consistent dispersion was obtained by swirling in the crystal violet and letting it dry for around 30 seconds. The slide was meticulously washed with sterile distilled water after the crystal violet stain was applied. To get rid of any remaining crystal violet color, a droplet of Lugol's iodine was added to the smear after the slide had been thoroughly cleaned with pure distilled water. The stain is held in place by the combination of crystal violet and Lugol's iodine. Acetone was used to clean the slide following the application of Lugol's iodine. Acetone is a decolorizer that helps get rid of extra stains on the slide. A drop of safranin was applied to the slide to hide the smear. Gram-negative bacteria get their characteristic hue from the counterstained safranin stain. The slide was eroded clean after being properly cleaned with water to remove any last traces of safranin. The extra liquid was carefully removed from the slide using blotting paper. To preserve the discolored smear, a drop of mounting agent Canada balsam was applied to the slide. The slide with the plated smear was examined using a 100X magnification microscope (Greenwood *et al.*, 2012).

Biochemical Characterization

Biochemical assays, including catalase, indole test and motility test were carried out. Briefly described as follow:

CATALASE TEST

The catalase enzyme, which causes hydrogen peroxide (H2O2) to release more oxygen, is detected by the test. It is used to differentiate between various bacteria that generate the catalase enzyme. On a sterile surface, one colony was gently mixed with hydrogen peroxide to perform the *K. pneumonia* strain catalase test. The test was successful, as evidenced by the formation of gas bubbles on the culture material's surface (Reiner, 2010).

INDOLE PRODUCTION TEST

To find out if an organism can convert tryptophan to indole, an indole test is utilized. To conduct the Indole test, a culture of the bacterial strains was injected into a tube containing tryptophan broth and maintained at 37 °C for 24 to 48 hours. Gently stir in 0.5 ml (5 drops) of Kovac's regent. Examine the liquid's highest layer; if purple or red rings show up there, a successful outcome is displayed; if yellow rings show up, an unsuccessful outcome is displayed (KOMAL, 2019).

MOTILITY TEST

This test is used to find out if an organism can move by using its flagella. The location of the flagella depends on the kind of bacterium. Once the semisolid agar has been prepared, place it in test tubes to perform the *K. pneumonia* strain motility test. Attach a straight needle to the colony once the culture has grown on nutrient agar medium for 18 to 24 hours. Once you are in the middle of the tube, only make a 1/3 to ½ inch deep puncture. Make sure the needle travels in the same direction as it exits the medium. Incubate at 35° to 37°C for up to seven days to find out if a diffuse growth zone has formed from the inoculation line (Shields & Cathcart, 2011).

DISK DIFFUSION SUSCEPTIBILITY TESTING

This is employed to culture facultative anaerobic and pathogenic aerobic bacteria on Mueller-Hinton agar covered with various antibacterial filter paper disks. Through the determination of the bacteria's sensitivity or resistance to different antibiotic medications, the disk diffusion

susceptibility test helps physicians choose therapeutic choices for their patients. It is possible to deduce indirectly from the growth around the disks that the medicine has the power to suppress that organism (Hudzicki, 2009). 0.5 McFarland was used to create bacterial suspensions. Following the application of the suspension, antibiotic disks were positioned on the Mueller-Hinton agar plate surface. The plates' susceptibility to antibiotics was assessed by incubating them for 16–18 hours at 37°C. We then measured the inhibitory zones in millimetres (Hudzicki, 2009).

DNA EXTRACTION

The Qiagen RTU kit was used to extract the whole genomic DNA of the tested bacterial culture. To determine the spore concentration needed for extraction, 1 mL of the culture containing 108 cfu/mL was centrifuged. The extraction tube (2.5 mL) was filled with 250 µL of proteinase K to remove any potential proteins and lysis buffer AL. After centrifuging the suspension, the supernatant was disposed of. To get rid of the particles, 95% ethanol was added to the lysate. After passing the cleaned lysate through a purification micro spin column, AE buffer was used to elute the column. AW1 and AW2 were the washing buffers that were utilized. Quantification of the isolated DNA was done with the NanoDrop spectrophotometer NS1020. For subsequent downstream analysis, the isolated DNA was kept at -20 °C. 1% agarose gel electrophoresis was used to evaluate the isolated DNA's purity (Vilain *et al.*, 2009).

PCR AMPLIFICATION AND SANGER SEQUENCING

The isolated DNA was amplified with F/R primers specific to 16S. 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) and 27F 5' (AGA GTT TGA TCM TGG CTC AG) are the sequences. The PCR product was predicted to be between 1.4 and 1.6 kb. Exonuclease I and SAP enzymes were used for enzymatic digestion in order to sequence the PCR product. The PCR product from agarose gel electrophoresis was then run through a purification column and elution buffer. Sanger sequencing was performed on the cleaned PCR product using primers 785F 5' (GGA TTA GAT ACC CTG GTA) and 907R 5' (CCG TCA ATT CMT TTR AGT TT) (Chen & Tsen, 2002) (Table 1).

Stage	PCR Protocol	Temperature (°C)	Time (min.)
1st	Initial Denaturation	94	5.0
2nd (35 Cycles)	Denaturing	94	0.5
	Annealing	52.7	0.5
	Extension	72	2.0
3rd	Final Extension	72	5.0
4th	Hold	4	∞

BIOINFORMATICS ANALYSIS

Chromas and BioEdit tools were used to evaluate the sequence in order to determine the bacterial strain's evolutionary connection. The sequence was edited for low-quality and superfluous amplifications, and the peaks were adjusted. The highly matched sequences from the databank were obtained using the NCBI's basic local alignment search tool (BLASTn). The Clustal Omega bioinformatics program was used to perform multiple sequence alignment of the chosen BLASTn returned sequences before the phylogenetic tree developing process. The tree was constructed and examined after the MSA in order to determine *Klebsiella pneumoniae's* evolutionary connection to other bacterial species. Using the sequenced bacterial strain and MEGAX software, the evolutionary connection with other species was analyzed to create a phylogenetic tree. For the creation of phylogenetic trees, the Fast Minimum Evolution Method and Max Sequence Difference 0.75 were employed.

RESULTS

MORPHOLOGICAL CHARACTERIZATION

K. pneumoniae is a rod-shaped, facultatively anaerobic, encapsulated, lactose-fermenting, gram-negative bacteria that is not mobile. Lactose fermentation caused the *K. pneumoniae* colonies to become pink on MacConkey agar medium (Figure 1).

.

FIGURE 1: MORPHOLOGICAL CHARACTERIZATION OF BACTERIAL ISOLATE

GRAM STAINING RESULTS

Using an isolated strain of *K. pneumoniae* cultured for a whole night, Gram staining identified the organism are Gram-negative rod shape (Figure 2).

FIGURE 2: MICROSCOPY OF BACTERIAL ISOLATE

4.3. CATALASE TEST RESULTS

The isolated bacteria generated gas bubbles on a glass slide after being treated with a few drops of 3% H2O2, indicating that the catalase test was positive. The catalase test result showed that all the *K. pneumoniae* bacterial strain were positive (Figure 3).

RJNMSR Review Journal of Neurological & Medical Sciences Review

FIGURE 3: CATALASE TEST RESULTS OF BACTERIAL ISOLATE

INDOLE TEST

A reddish-colored ring appeared on the glass tube surface as soon as the kovac's reaction was injected, signifying a successful indole test. Indole negativity is indicated by yellow or no color. The bacterium *K. pneumoniae* gave a negative indole test by adding 5-6 drops of kovac's reagent (Figure 4).

FIGURE 4: INDOLE TEST RESULTS FOR BACTERIAL ISOLATE

MOTILITY TEST

In this test semisolid agar substrate was used to determine bacterial motility. A diffusive zone of growth from the inoculation line indicates bacterial motility. All strains of *K. pneumoniae* are non motile (Figure 5).

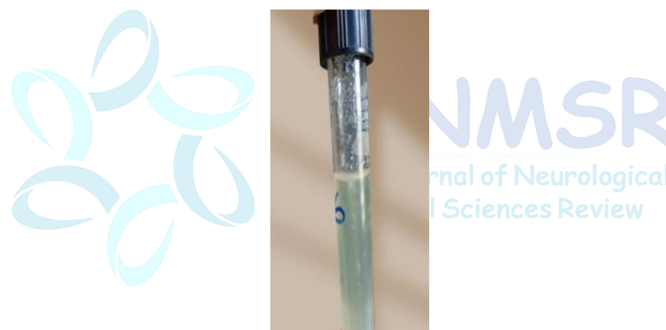

FIGURE 5: MOTILITY TEST RESULTS FOR BACTERIAL ISOLATE

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The study reveals high resistance rates to various antibiotics. High resistance (>70%) Amoxicillin (94.3%), Ceftazidime (88.5%), Ciprofloxacin (76.9%), Moderate resistance (30-70%): Gentamicin (63.3%), Amikacin (41.4%), Meropenem (32.2%), Low resistance (<10%):, Colistin (8.0%), Tigecycline (5.7%). These findings suggest that Colistin and Tigecycline may be effective treatment options. Aminoglycosides (Gentamicin, Amikacin) and Fluoroquinolones (Ciprofloxacin) show moderate to high resistance.

Carbapenems (Meropenem) exhibit moderate resistance (figure 6).

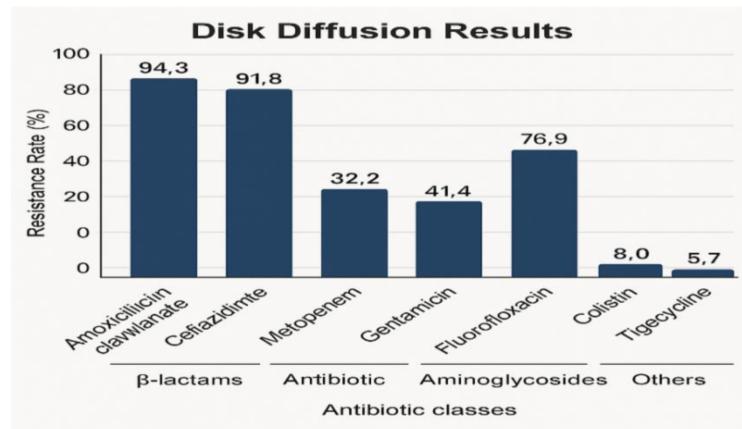

FIGURE 6: ANTIMICROBIAL SUSCEPTIBILITY RESULT FOR BACTERIAL ISOLATE

16S RRNA GENE SEQUENCE ANALYSIS

K. pneumonia strain (Accession No. MT379476.1) and the 16S rRNA gene sequence were 98.37% identical, according to the BLASTn analysis. The evolutionary link between the isolate and other Bacilli species was displayed in the phylogenetic tree created with MEGA X software. The isolate grouped with *K. pneumonia* strains, according to the tree, which supported the findings of the BLAST analysis. The isolate and other Klebsella species were clearly separated by the distance-based tree, suggesting a different phylogenetic position. (Table 2).

TABLE 2: MICROBIAL INFORMATION EXTRACTED FROM BLASTN RESULTS.

	Accession No.	CP154197.1
Subject	Description	*Klebsiella pneumoniae*
	Length (b)	1275
	Start	1
	End	1275
	Coverage	100
Score	Bit	2305
	E-value	0.0
Identities	Match/Total	1266/1275
	Percentage (%)	99.29

PHYLOGENETIC ANALYSIS

The isolate was determined to be a strain of *Klebsiella pneumoniae* based on phylogenetic tree building and examination of the 16S rRNA gene sequence. The isolate and other *K. pneumoniae* strains appear to have a tight evolutionary connection, according to the data. These results shed important light on the evolutionary linkages and genetic diversity of Klebsiella species. *K. pneumoniae* is a rod-shaped, Gram-positive bacterium. Studying *K. pneumoniae* can yield important information about its survival strategies and possible applications in biotechnology, including bioremediation and biocontrol. Because of its capacity to adapt to harsh conditions, it is an important study topic with potential uses in a number of biotechnological domains. Given its role in pathogenicity and antibiotic resistance, *K. pneumoniae* is an important bacterium. Pneumonia, UTIs, and sepsis are the main illnesses caused by the bacterium, especially in hospitalized or immunocompromised patients. It is regarded as an MDR (multidrug-resistant) pathogen. Given the serious danger to public health posed by carbapenem-resistant *K.*

pneumoniae (CR-KP), pathogenic control in public hospitals is a major issue (Table 3 Figure 7).

TABLE 3: TOP 10 BLASTN RESULTS

Scientific Name	Max Score	Total Score	Query Cover (%)	E-value	Per. Ident (%)	Acc. Len (b)	NCBI Accession N0.
Klebsiella pneumoniae	2313	18322	99%	0	99.14	5383757	CP154197.1
Klebsiella pneumoniae	2309	18200	99%	0	99.06	5462803	CP036320.1
Klebsiella pneumoniae	2309	2309	99%	0	99.06	1415	MT707441.1
Klebsiella variicola	2309	2309	99%	0	99.06	1435	PQ182199.1
Klebsiella pneumoniae	2307	18460	99%	0	99.06	5426972	CP146145.1
Klebsiella pneumoniae	2307	18316	99%	0	99.06	5302499	CP154231.1
Klebsiella pneumoniae	2307	18239	99%	0	99.06	5651566	CP152580.1
Klebsiella pneumoniae	2307	18316	99%	0	99.06	5475219	CP129694.1
Klebsiella pneumoniae	2307	18261	99%	0	99.06	5243745	CP132670.1
Klebsiella pneumoniae	2307	18228	99%	0	99.06	5423858	CP125201.1

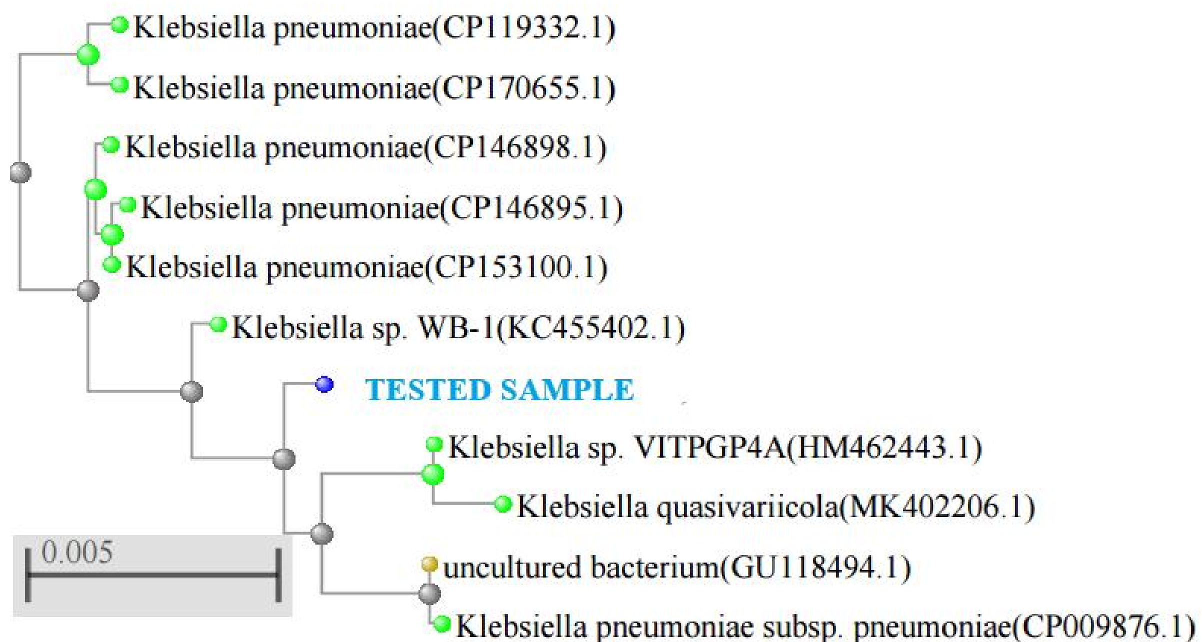

FIGURE 7: PHYLONETIC TREE RESULTS

DISCUSSION

The widely recognized microbe *K. pneumoniae* is responsible for persistent lung infections in alcoholics and immunocompromised people. One of the most prevalent eight pathogens responsible for nosocomial infections is *K. pneumoniae.* Patients' gastrointestinal tracts and healthcare providers' hands are the main sources of Klebsiella-associated infections (Campos *et al.*, 2016). Since carbapenems are effective against Klebsiella that is resistant to the ESBL, extended

spectrum beta lactamases are the enzymes that exhibit resistance to the antibiotics that act on the beta lactam ring. Where the ESBL failed, carbapenems like imipenem and Meropenem were vulnerable to Klebsiella. A strain of *K. pneumoniae* has been discovered that exhibits carbapenem resistance for the first time (Gasink *et al.*, 2009).

The current study was designed to evulated the Antimicrobial Resistance and Molecular Characterization of *Klebsiella pneumoniae* isolated from fresh meat. Samples of fresh meat was taken from butcher's shops in district Abbottabad. Meat samples was processed for bacterial strains isolation. Samples were streaked onto MacConkey agar plates, which were then incubated at 37°C for the whole night. *K. pneumoniae* produces pink colonies on MacConkey agar medium. The biochemical characterization was another aspect of study. In the current study test for catalase, indole and motility was carried out. Biochemical test results show that *K. pneumonia* was positive for catalase while negative for indole and motility test results. Similar study was repored by Aziz *et al.*, (2005) which shows *K. pneumonia* shows positive results towards positive results for catalase test while gave a negative results e for indole and motility tests.

To determine the pattern of antibiotic sensitivity in the isolated isolates, sensitivity testing was conducted. Amoxicillin (94.3%), Ceftazidime (88.5%), and Ciprofloxacin (76.9%) are instances of drugs with high resistance (>70%). Gentamicin (63.3%), Amikacin (41.4%), and Meropenem (32.2%) are instance of moderate resistance (30–70%). Tigecycline (5.7%) and Colistin (8.0%) are examples of low resistance (<10%). These results imply that tigecycline and colistin might be useful therapeutic agents. Resistance to fluoroquinolones (Ciprofloxacin) and aminoglycosides (Gentamicin, Amikacin) ranges from mild to high. Meropenem and other carbapenems show modest resistance. Alike study by Sharif *et al.*, (2016) shows that amikacin and ciprofloxacin were the most effective antimicrobial medicines against *K. pneumonia* infections with high sensitivity rate (81%–100%). In contrast, isolated bacterial pathogen showed complete resistance to both tobramycin and kanamycin.

The BLASTn analysis revealed that the 16S rRNA gene sequence and the *K. pneumonia* strain (Accession No. MT379476.1) were 98.37% identical. The phylogenetic tree produced by MEGA X program showed the evolutionary relationship between the isolate and other Bacilli species. The results of the BLAST analysis were corroborated by the tree, which showed that the isolate clustered with K. pneumonia strains. Astal *et al.*, (2020) conducted a comparable study in which they used the results of 16S rRNA gene sequencing to construct a phylogenetic tree that demonstrated an evolutionary relationship to *K. pneumonia* species.

CONCLUSION

Important multidrug-resistant (MDR) bacteria *Klebsiella pneumoniae* can infect hospitalized patients with a variety of illnesses. The increased prevalence of MDR *K. pneumoniae* due to the increased usage of antibiotics presents more challenges and barriers in clinical treatment. *K. pneumoniae's* antibiotic resistance and mechanism are important resources for gaining a thorough understanding of the bacterium and for providing the theoretical underpinnings for therapeutic prevention of infections caused by it. The mechanisms by which *K. pneumoniae* develops resistance to antibiotics are numerous and intricate. In order to battle this significant infection, we should offer insights into feasible solutions.

REFERENCES

Abbas, R., Chakkour, M., Zein El Dine, H., Obaseki, E. F., Obeid, S. T., Jezzini, A., Ghssein, G., & Ezzeddine, Z. (2024). General Overview of Klebsiella pneumonia: Epidemiology and the Role of Siderophores in Its Pathogenicity. *Biology, 13*(2), 78.

Campos, A. C., Albiero, J., Ecker, A. B., Kuroda, C. M., Meirelles, L. E., Polato, A., Tognim, M. C., Wingeter, M. A., & Teixeira, J. J. (2016). Outbreak of Klebsiella pneumoniae carbapenemase–producing K pneumoniae: A systematic review. *American Journal of Infection Control, 44*(11), 1374-1380.

Chen, M., & Tsen, H. (2002). Discrimination of Bacillus cereus and Bacillus thuringiensis with 16S rRNA and gyrB gene based PCR primers and sequencing of their

annealing sites. *Journal of applied microbiology, 92*(5), 912-919.

Gasink, L. B., Edelstein, P. H., Lautenbach, E., Synnestvedt, M., & Fishman, N. O. (2009). Risk factors and clinical impact of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. *Infection Control & Hospital Epidemiology, 30*(12), 1180-1185.

Greenwood, D., Slack, R. C., Barer, M. R., & Irving, W. L. (2012). *Medical microbiology e-book: A guide to microbial infections: Pathogenesis, immunity, laboratory diagnosis and control. with STUDENT CONSULT online access*. Elsevier Health Sciences.

Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology, 15*(1), 1-23.

KOMAL, G. (2019). OXIDASE TEST.

Kutter, E., & Sulakvelidze, A. (2004). *Bacteriophages: biology and applications*. Crc press.

Paczosa, M. K., & Mecsas, J. (2016). Klebsiella pneumoniae: going on the offense with a strong defense. *Microbiology and molecular biology reviews, 80*(3), 629-661.

Puspanadan, S., Afsah-Hejri, L., Loo, Y., Nillian, E., Kuan, C., Goh, S., Chang, W., Lye, Y., John, Y., & Rukayadi, Y. (2012). Detection of Klebsiella pneumoniae in raw vegetables using most probable number-polymerase chain reaction (MPN-PCR). *International Food Research Journal, 19*(4), 1757.

Reiner, K. (2010). Catalase test protocol. *American society for microbiology*, 1-6.

Rodríguez-Medina, N., Barrios-Camacho, H., Duran-Bedolla, J., & Garza-Ramos, U. (2019). Klebsiella variicola: an emerging pathogen in humans. *Emerging microbes & infections, 8*(1), 973-988.

Shields, P., & Cathcart, L. (2011). Motility test medium protocol. *American society for microbiology*.

Valsamatzi-Panagiotou, A., Popova, K. B., & Penchovsky, R. (2021). Methods for prevention and constraint of antimicrobial resistance: a review. *Environ. Chem. Lett.*, 1-8.

Vilain, S., Pretorius, J. M., Theron, J., & Brözel, V. S. (2009). DNA as an adhesin: Bacillus cereus requires extracellular DNA to form biofilms. *Applied and environmental microbiology, 75*(9), 2861-2868.

Walter, J., Haller, S., Quinten, C., Kärki, T., Zacher, B., Eckmanns, T., Sin, M. A., Plachouras, D., Kinross, P., & Suetens, C. (2018). Healthcare-associated pneumonia in acute care hospitals in European Union/European Economic Area countries: an analysis of data from a point prevalence survey, 2011 to 2012. *Eurosurveillance, 23*(32), 1700843.

Wang, S., Xu, F., & Zhan, J. (2017). Introduction of natural pigments from microorganisms. *Biopigmentation and Biotechnological Implementations*, 1-22.

RJNMSR Review Journal of Neurological & Medical Sciences Review