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# Phenotypic Antimicrobial Susceptibility and β-Lactamase Gene Detection in Clinical Klebsiella pneumoniae: Evidence of Genotype-Phenotype Discordance

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**Research Article**

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# Phenotypic Antimicrobial Susceptibility and β-Lactamase Gene Detection in Clinical Klebsiella pneumoniae: Evidence of Genotype-Phenotype Discordance

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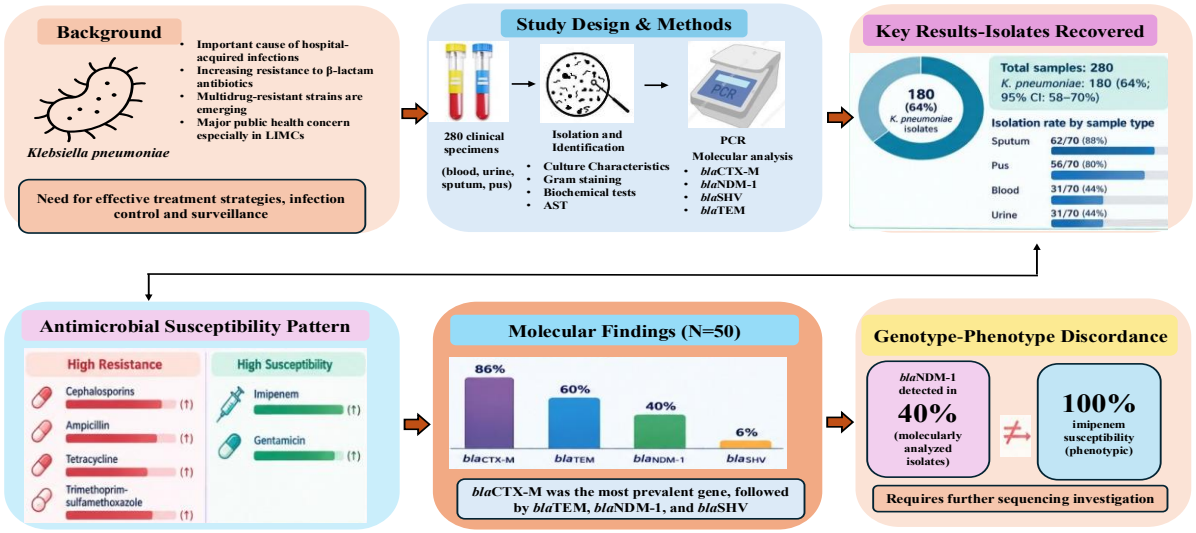
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## Graphical abstract




## Abstract

*Klebsiella pneumoniae* is an important cause of healthcare-associated infections, with antimicrobial resistance posing a significant challenge to effective treatment. This study characterized the phenotypic antimicrobial susceptibility patterns of clinical *K. pneumoniae* isolates and investigated the occurrence of selected β-lactamase and carbapenemase genes, with particular attention to genotype-phenotype discordance. A total of 280 clinical specimens, including sputum, blood, urine, and pus, were processed using standard microbiological methods. Bacterial isolates were characterized using culture characteristics, staining, biochemical tests, and

antimicrobial susceptibility testing. Polymerase chain reaction (PCR) was performed to detect selected resistance genes in 50 isolates exhibiting reduced susceptibility to third-generation cephalosporins. Overall, *K. pneumoniae* was recovered from 180/280 specimens (64%; 95% confidence interval [CI]: 58-70%), with the highest isolation rates observed in sputum (88%) and pus (80%). High resistance was observed against cephalosporins, ampicillin, tetracycline, and trimethoprim–sulfamethoxazole, whereas all isolates were phenotypically susceptible to imipenem and susceptibility to gentamicin was high. Among the 50 isolates analyzed by PCR, *bla*CTX-M was the most frequently detected gene (86%), followed by *bla*TEM (60%), *bla*NDM-1 (40%), and *bla*SHV (6%). Notably, *bla*NDM-1 was detected in 40% of these isolates despite 100% phenotypic susceptibility to imipenem, revealing a genotype-phenotype discrepancy that requires further investigation. These findings demonstrate the value of integrating phenotypic susceptibility testing with molecular detection of resistance determinants and support confirmatory carbapenemase testing, molecular surveillance, and evidence-based antimicrobial stewardship in clinical settings.

**Keywords**

*Klebsiella pneumoniae*; Antimicrobial resistance, *bla*CTX-M; ESBL; Clinical

## Introduction

Antimicrobial resistance (AMR) is recognized as one of the top ten global public health threats facing humanity, as highlighted in the most recent World Health Organization (WHO) global action reports.[1] The WHO has designated *Klebsiella pneumoniae* (*K. pneumoniae*) as an AMR bacterium of concern because it demonstrated propensity to develop resistance to various β-lactam antibiotic classes, including cephalosporins, penicillin's, and quinolones, which are frequently used to treat *K. pneumoniae* infections.[1 2]

*K. pneumoniae* is a highly infectious human bacterium that causes a number of disorders, including respiratory and urinary tract infections, blood infections, and liver abscesses.[2]These infections are frequently caused by multidrug-resistant strains carrying ESBL and carbapenemase genes, which drastically limit therapeutic options and increase morbidity and mortality globally.[3]

*K. pneumoniae* strains are broadly categorized into two main groups: hypervirulent strains (hvKP) and classical strains (cKP). Classical *K. pneumoniae* (cKP) is typically associated with nosocomial infections in immunocompromised patients or those undergoing invasive procedures, such as the

use of intravascular devices, endotracheal tubes, or surgical interventions. In contrast, hypervirulent *K. pneumoniae* (hvKP) can cause severe, disseminated infections even in immunocompetent individuals and is characterized by enhanced virulence.[4]

Within healthcare environments, *K. pneumoniae* can be transmitted through patient-to-patient contact or, less frequently, through contaminated environmental surfaces. *K. pneumoniae* is not transmitted through air.[5]

CTX-M type enzymes are now the most common kind of ESBL globally, replacing TEM- and SHV-type β-lactamases.[6] These enzymes are linked to multidrug-resistant phenotypes and provide resistance to third-generation cephalosporins.[7] The occurrence and spread of *bla*CTX-M harboring *K. pneumoniae* have been largely reported in South Asia, including Pakistan due to unchecked antibiotic usage and inadequate molecular surveillance. Region-specific data integrating molecular characterization and phenotypic resistance patterns remain limited.[8]

Since carbapenems are regarded as last-line treatment drugs, the emergence of *K. pneumoniae* strains that produce carbapenemase, especially those that have *bla*NDM-1 gene, poses serious threat to public health.[9] Treatment choices and infection control strategies must be directed by the early identification and molecular characterization of these resistance genes.[10]

Antimicrobial resistance among *K. pneumoniae* strains has emerged and spread quickly, highlighting the significance of surveillance for both antimicrobial susceptibility profiles and molecular detection of resistance determinants, which can help infection prevention and control measures. Molecular surveillance facilitates the early detection of high-risk clones and aids in directing targeted treatments including improved antimicrobial stewardship policy, isolation precautions, and improved hygiene practices.[11]

Despite multiple regional reports, published molecular studies on antibiotic resistance in *K. pneumoniae* from Faisalabad, Pakistan is still limited. The rapid dissemination of *bla*CTX-M and *bla*NDM-1 genes has been associated with mobile genetic elements and horizontal gene transfer in previous studies, highlighting the importance of molecular surveillance of these determinants.[12]

The main objective of this study was to identify β-lactamase and carbapenemase resistance genes among selected *K. pneumoniae* isolates by examining the antibiotic resistance patterns of *K. pneumoniae* isolated from clinical human samples from 10 tertiary care hospitals in Faisalabad, Pakistan. To promote antimicrobial stewardship initiatives and stop the development of AMR, such

studies help to close the knowledge gap on antibiotic resistance that currently exists in Faisalabad local health care systems.

## Materials and methods

### Ethical Consent

The study was permitted by Graduate Studies Research Board of the University of Agriculture Faisalabad (Approval No. DGS 21249-52 dated 08-05-2024) which is authorized to review and approve research involving human participants. Informed consent was obtained from the participants in accordance with the approved study protocol. Participants' confidentiality was maintained throughout the study.

### Study Design and Sample Collection

This cross-sectional study was done at the Institute of Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, from August 2025 to January 2026. Clinical samples (blood, sputum, urine and pus) were taken from patients having clinical bacterial infection from the tertiary care hospitals in Faisalabad. A total of 10 tertiary care hospitals, including both public and private healthcare facilities, were involved in the study. A total of 280 clinical specimens (70 sputum, 70 blood, 70 urine, and 70 pus samples) were collected using aseptic techniques. Samples were collected in ice boxes and immediately processed upon receipt in the laboratory. Inclusion criteria comprised clinical specimens submitted for routine microbiological analysis from patients presenting signs and symptoms of infection. Only the first isolate from each patient was included, resulting in 180 isolates from 180 unique patients and minimizing potential duplication bias. Samples with insufficient volume or showed evidence of contamination were excluded. The sampling strategy allowed for the evaluation of antimicrobial resistance patterns at various anatomical sites such as bloodstream infection, respiratory tract infection, urinary tract and soft tissue infections.

### Isolation and Identification of *K. pneumoniae*

All these samples were streaked across MacConkey and Blood agar. On MacConkey agar, *K. pneumoniae* colonies are often big, mucoid, and pink. *K. pneumoniae* forms very small, grey, moist colonies on blood plates which are typically mucoid and nonhemolytic. Universal 16S rDNA PCR was performed as a molecular confirmation of bacterial identity at the genus level in the presumptive *K. pneumoniae* isolates. Universal bacterial primers were used: Forward: 5'-AGAGTTTGATCTGGCTCAG-3', Reverse: 5' - AAGGAGGTGWTCCACC-3' which amplifies

a ~1500 bp fragment.[13] The results were viewed on the 1.5% agarose gel stained with ethidium bromide, and the presence of the expected size band was an indication of successful amplification of the product.

**Antibiotic susceptibility testing:**

For the determination of antimicrobial susceptibility of *K. pneumoniae* following antibiotic discs (Oxoid, UK) were used, Amoxicillin (10 µg), Tetracycline (30 µg), Piperacillin (100 µg), Ciprofloxacin (5 µg) Gentamicin (10 µg), Imipenem (10 µg), Cefotaxime (30 µg), trimethoprim-sulphamethoxazole (1.25µg/23.75 µg), Chloramphenicol (30 µg). Zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition, 2024 guidelines and measured using a calibrated scale. Isolates exhibiting reduced susceptibility to third-generation cephalosporins, including cefotaxime according to CLSI M100, 34$^{th}$ edition, 2024 criteria, were considered eligible for subsequent molecular analysis. To further resolve the observed phenotypic-molecular discordance, minimum inhibitory concentration (MIC) determination for imipenem and meropenem (by broth microdilution or E-test) and phenotypic carbapenemase confirmation using modified carbapenem inactivation method (mCIM) and EDTA-carbapenem inactivation method (eCIM) are recommended as additional confirmatory steps in future studies.

**Molecular Detection of β-Lactamase and Carbapenemase Genes**

Genomic DNA was extracted from 50 *K. pneumoniae* isolates exhibiting reduced susceptibility to third-generation cephalosporin cefotaxime according to CLSI M100, 34$^{th}$ edition, 2024 criteria. These isolates were selected from the total 180 *K. pneumoniae* isolates using stratified sampling across the four clinical specimen types (blood, sputum, urine, and pus). Polymerase chain reaction (PCR) was performed for the detection of β-lactamase and carbapenemase genes, including *bla*CTX-M, *bla*TEM, *bla*SHV, *bla*NDM-1, *bla*KPC, *bla*IMP, *bla*VIM, and *bla*GIM, using previously published and validated primers (Table 1). PCR was carried out using a standard thermal cycling method. Each PCR reaction was performed in a 25 µL reaction mixture containing template DNA (2µL), forward and reverse primers (1µL each, 10pmol), 2× master mix DreamTaq PCR Master Mix (Thermo Fisher Scientific, USA), and nuclease-free water. Applied Biosystems VeritiTM 96-Well Thermal Cycler, USA was used for thermal cycling. The amplification protocol included an initial denaturation step, followed by 30–35 cycles of denaturation, annealing (based

on primer-specific temperatures), and extension, with a final extension step. Positive and negative controls were used in every PCR run to verify the accuracy and reliability of PCR results.

Table 1. Primer sequences, PCR conditions, and amplicon sizes of antimicrobial resistance genes detected in clinical *K. pneumoniae* isolates from 10 tertiary care hospitals in Faisalabad.

| Gene | Primer Sequence | Annealing temp (°C) | Amplicon size (bp) | References |
|---|---|---|---|---|
| 16S rDNA | Forward: 5′-AGAGTTTGATCTGGCTCAG-3′<br>Reverse: 5′-AAGGAGGTGWTCCACC-3′ | 51 | 1500 | [13] |
| *bla*CTX-M | Forward:5′-GGATATCGTTGGTGGTGCCATA-3′<br>Reverse: 5′-TTTGCGATGTGCAGTACCAGTAA-3′ | 57 | 544 | [14] |
| *bla*TEM | Forward: 5-TCAACATTTCCGTGTCG-3′<br>Reverse: 5-CTGACAGTTACCAATGCTTA-3′ | 56 | 860 | [15] |
| *bla*SHV | Forward: 5′-ATGCGTTATATTCGCCTGTG-3′<br>Reverse: 5′-AGATAAATCACCACAATGCGC-3′ | 56 | 896 | [16] |
| *bla*NDM-1 | Forward: 5′-TGCCCAATATTATGCACCCGG-3′<br>Reverse: 5′-CGAAACCCGGCATGTCGAGA-3′ | 60 | 292 | [17] |
| *bla*KPC | Forward: 5′-TGCAGAGCCCAGTGTCAGTTT-3′<br>Reverse: 5′-CGCTCTATCGGCGATACCA-3′ | 52 | 880 | [18] |
| *blaI*MP | Forward: 5′-GGAATAGAGTGGCTTAATTCTC-3′<br>Reverse: 5′-CCAAACCACTACGTTATC-3′ | 55 | 624 | [19] |
| *bla*VIM | Forward: 5′-GATGGTGTTTGGTCGCATA-3′<br>Reverse: 5′-CGAATGCGCAGCACCAG-3′ | 52 | 390 | [20] |
| *bla*GIM | Forward: 5′-TCGACACACCTTGGTCTGAA-3′<br>Reverse: 5′-AACTTCCAACTTTGCCATGC-3′ | 52 | 477 | [21] |

## Data Analysis and Statistical Considerations

SPSS v26.0 was used for data analysis. Categorical variables, including resistance and prevalence percentages, were summarized as frequencies and percentages. Comparisons between categorical variables were performed using the Chi-square test or Fisher's exact test, as appropriate. A P-value $< 0.05$ was considered statistically significant. As the study variables were categorical, assessment of data normality was not applicable. The prevalence of *K. pneumoniae* and *bla*CTX-M positive isolates among the molecularly analyzed isolates was reported with 95% confidence intervals (CI) using Wilson score method. *K. pneumoniae* isolates exhibiting reduced susceptibility to cefotaxime was selected by stratified sampling from four clinical specimen types. A total of 280 clinical specimens were collected for phenotypic analysis.

## Quality Control and Biosafety

DNA was extracted using Thermo Scientific GeneJET Genomic DNA Purification Kit, according to the manufacturer's instructions. The purity and concentration of DNA was measured by Nanodrop spectrophotometry, and A260/A280 ranges from 1.8-2.0 were deemed suitable for further processing for PCR analysis. All laboratory procedures were carried out following Biosafety Level 2 (BSL-2) guidelines. The culture media was tested for sterility prior to use. The standard reference strains *K. pneumoniae* (ATCC 70060) and *Escherichia. coli* (ATCC 25922) was used as quality control organisms for antimicrobial susceptibility testing (AST). All lab staff received training in safe handling of pathogenic microorganisms, waste disposal, and infection control. The data of the participants were anonymized for analysis, and all records are kept according to the institutional data management guidelines.

# Results

## Occurrence of *K. pneumoniae* from clinical isolates

Of the 280 clinical samples, 180 were recognized as *K. pneumoniae*, using morphological and biochemical characteristics. Universal 16S rDNA PCR was subsequently performed as a molecular confirmation of bacterial identity, which accounted for 64% (180/280) of the clinical samples. The

prevalence varied across sample types: sputum (n=62/70, 88%), pus (n=56/70, 80%), blood (n=31/70, 44%), and urine (n=31/70, 44) (Figure 1).

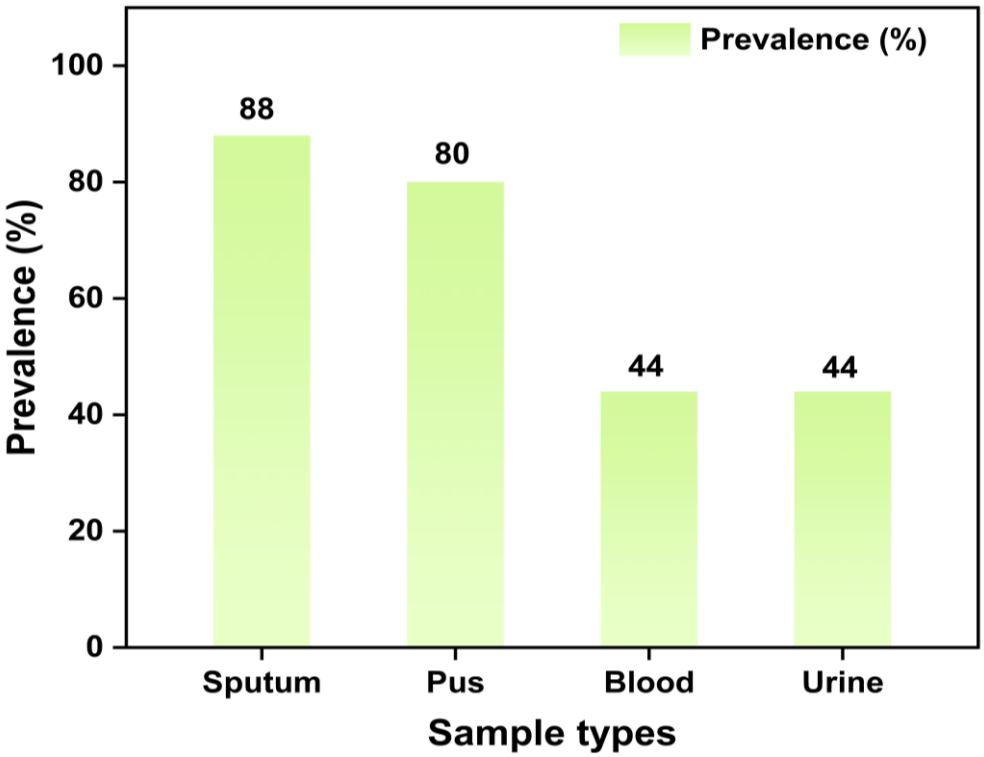



Fig.1. Prevalence of *K. pneumoniae* in different clinical samples collected from tertiary care hospitals in Faisalabad

*K. pneumoniae* produced creamy, white, round, smooth, raised, mucoid colonies measuring approximately 2–3 mm in diameter on nutrient agar (Figure 2A). On MacConkey agar, the isolates produced large, pink, mucoid, lactose-fermenting colonies. (Figure 2B-C). On the blood agar, the colonies were smooth, grayish-white, mucoid, and non-hemolytic (Figure 2D).

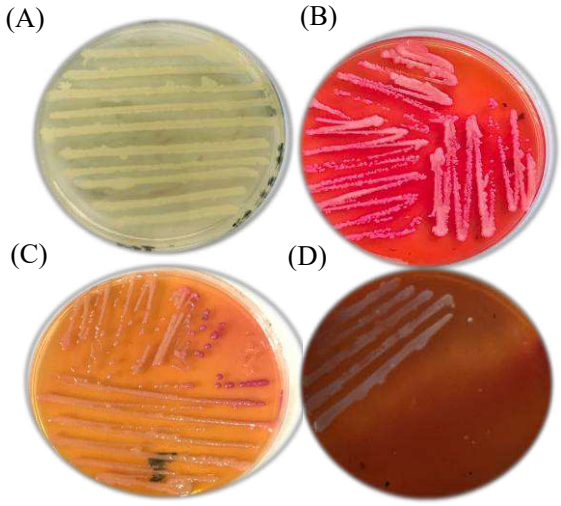



Fig. 2. (A) *K. pneumoniae* produced creamy-white, round, smooth, raised, mucoid colonies on nutrient agar. (B) large, pink, mucoid lactose-fermenting colonies on MacConkey agar. (C) mucoid colonies on MacConkey agar. (D) smooth, grayish-white, mucoid, non-hemolytic colonies on Blood agar.

## Antimicrobial Susceptibility Pattern of *K. pneumoniae* from clinical isolates

The results of antimicrobial susceptibility testing showed high levels of resistance for ampicillin, tetracycline, cefotaxime and trimethoprim–sulfamethoxazole in all sample types. In contrast, all isolates exhibited 100% susceptibility to imipenem and a high susceptibility to gentamicin. Statistical analysis using chi-square test demonstrated a significant association between sample type and antimicrobial resistance patterns for ciprofloxacin, with sputum isolates showing significantly higher resistance compared to urine and blood isolates. A significant association was also observed for chloramphenicol resistance across sample types ($P<0.05$). For remaining antibiotics including ampicillin, tetracycline, cefotaxime, trimethoprim-sulfamethoxazole, and imipenem resistance or susceptibility patterns were uniform across all sample types and statistical comparison was not applicable. Variable susceptibility patterns were observed for ciprofloxacin, chloramphenicol, and piperacillin depending on the sample type (Figure 3A–B). Overall, piperacillin demonstrated 44% resistance among the 50 molecularly analyzed *K. pneumoniae* isolates, whereas ciprofloxacin and chloramphenicol exhibited intermediate levels of resistance.

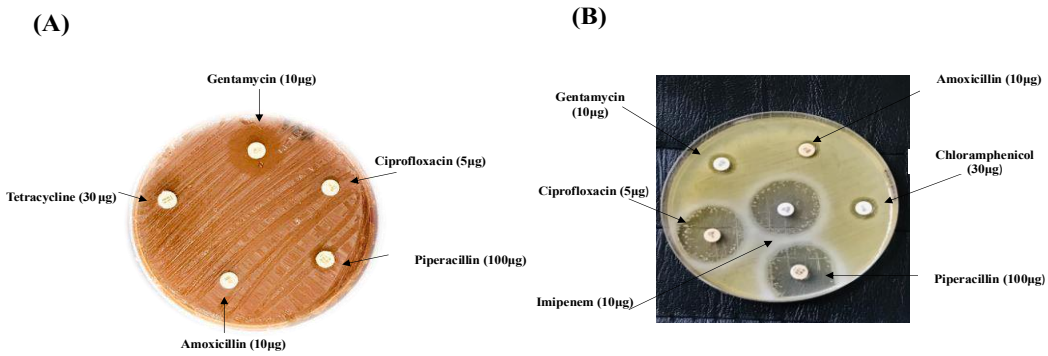



Fig. 3. (A) Zones of inhibition produced by *K. pneumoniae* isolates against gentamicin, ciprofloxacin, piperacillin, amoxicillin, tetracycline. (B) Zones of inhibition produced by *K. pneumoniae* isolates against amoxicillin, chloramphenicol, piperacillin, imipenem, ciprofloxacin, gentamycin.

Of the 180 *K. pneumoniae* strains, 50 isolates exhibiting reduced susceptibility to the third-generation cephalosporin cefotaxime were selected for molecular analysis. These results are summarized in (Figure 4).

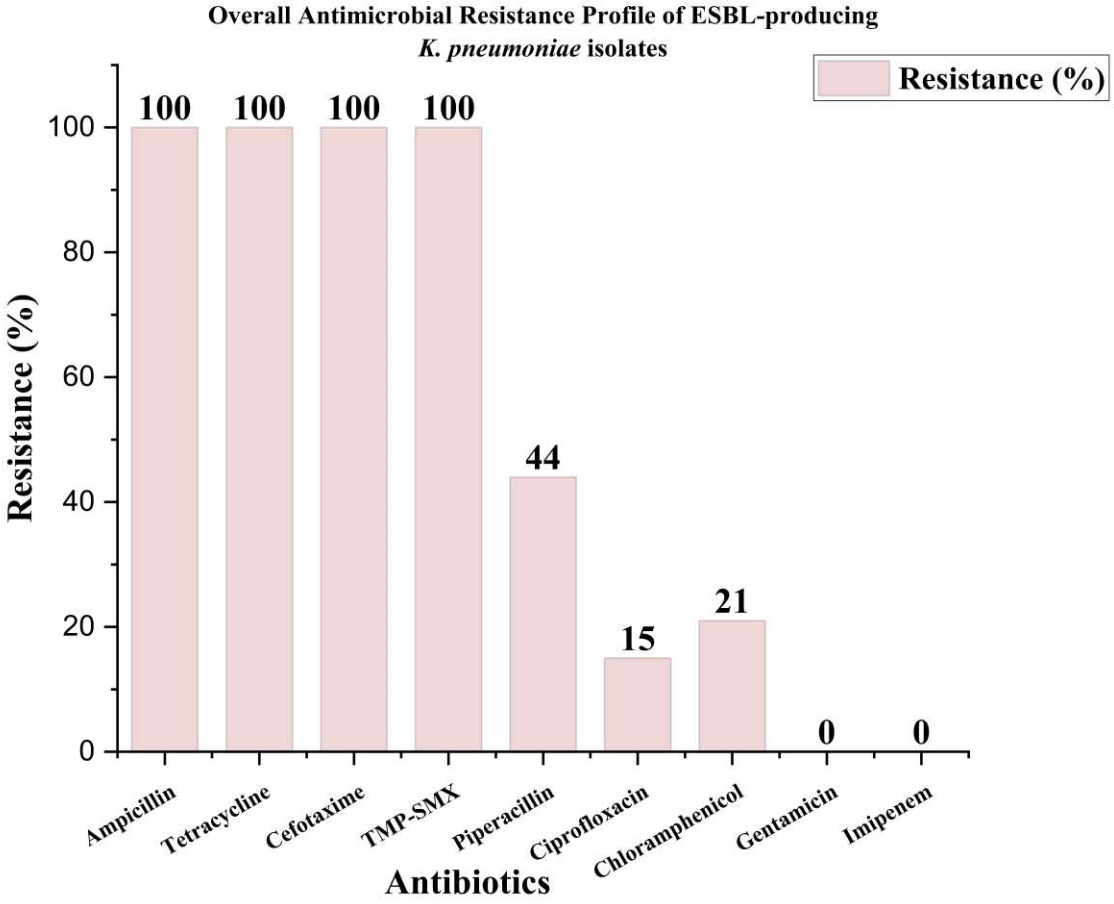


Fig. 4. Overall antimicrobial resistance profile of ESBL-producing *K. pneumoniae* isolates (n=50). Complete resistance was observed against ampicillin, tetracycline, cefotaxime, and trimethoprim-sulfamethoxazole. Piperacillin exhibited 44% resistance, while chloramphenicol (21%) and ciprofloxacin (15%) showed moderate resistance rates. No phenotypic resistance was detected against gentamicin or imipenem.

**Molecular Detection of Resistance Genes of *K. pneumoniae* from tertiary care hospital**

A total of 50 molecularly analyzed isolates were subjected to PCR analysis for the detection of β-lactamase and carbapenemase gene. Of these isolates, *bla*CTX-M was the most common gene, detected in 43/50 (86%) isolates, followed by *bla*TEM 30/50 (60%), *bla*NDM-1 20/50 (40%), and *bla*SHV 03/50 (6%) (Figure 5). No amplification was observed for *bla*KPC, *bla*IMP, *bla*VIM, or *bla*GIM. Despite the detection of *bla*NDM-1 in 40% molecular analyzed isolates by PCR, phenotypic resistance to imipenem by disk diffusion was not observed in this study. This discordance between genotypic detection and phenotypic susceptibility warrants further investigation using quantitative MIC testing and sequence verification. The overall prevalence of *K. pneumoniae* was 64% (95% CI: 58–70%). The frequency of *bla*CTX-M among the molecularly analyzed isolates was 86% (95% CI: 73–94%).

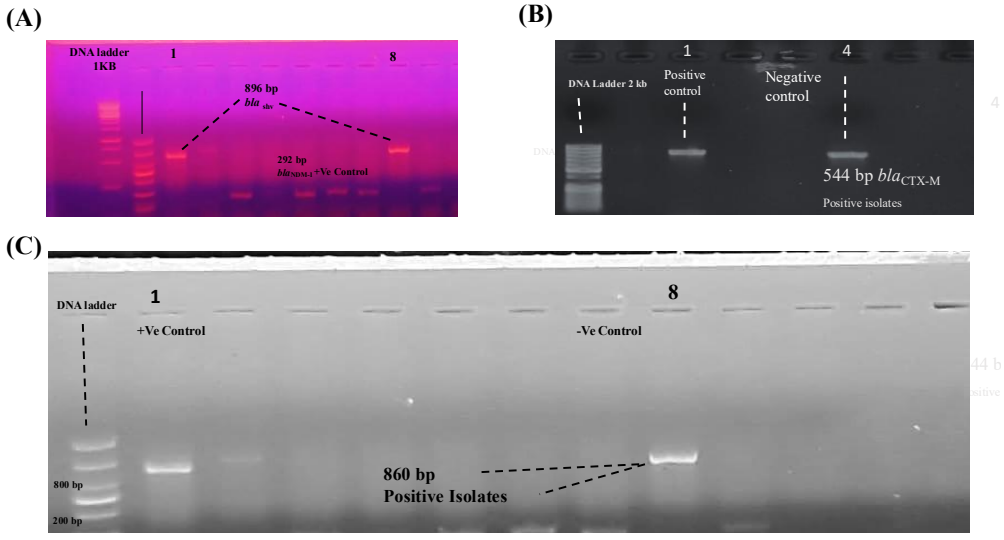



Fig. 5. Agarose gel electrophoresis images showing PCR amplification of β-lactamase and carbapenemase genes in clinical *K. pneumoniae* isolates. (A) Amplification of *bla*SHV gene showing PCR product of 896 bp and

*bla*NDM-1 gene showing PCR product of 292 bp in positive isolates. Lanes 1-6 represent positive clinical isolates; M: 1kb DNA ladder (Thermo Fisher™) for *bla*SHV and *bla*NDM-1; N: negative control. (B) Amplification of *bla*CTX-M gene showing a PCR product of 544bp in positive isolates; M:2 kb DNA ladder. (C) Amplification of *bla*TEM gene showing PCR products of 860 bp in positive isolates; M: 1kb DNA ladder Lanes 1–6 represent positive clinical isolates, followed by the negative control (N)

## Discussion

AMR is a global health problem linked with rising morbidity and mortality rates and financial burden on the health care system. It is also an important root cause of therapeutic failure in infectious diseases. AMR is estimated to result in more than 700,000 deaths every year.[22]

*Klebsiella* infections are typically caused by *K. pneumoniae* and arise in sick patients undergoing treatment for various ailments resulting in antibiotic resistance, and several cases of nosocomial infections have been reported.[23] During their hospital stay, patients are frequently exposed to a variety of pathogenic agents, increasing their risk of bacterial colonization and their chance of contracting infections from several multidrug-resistant bacteria.[24] A major opportunistic pathogen, *K. pneumoniae* causes a variety of clinical illnesses, such as septicemia in immunocompromised patients, infections of the skin and soft tissues, blood, digestive tract, and urinary tract, as well as pneumonia contracted in hospitals.[25]

In this present study, *K. pneumoniae* clinical isolates from 10 tertiary care hospitals in Faisalabad exhibited high resistance to commonly used β-lactam antibiotics, which was similar with the regional and global data of ESBL mediated resistance. Similar reports from clinical settings in Pakistan and surrounding regions, where ESBL-producing *K. pneumoniae* has been linked to multidrug-resistance and reduced susceptibility to first-line therapies, including widespread resistance to cefotaxime, ampicillin, tetracycline, and trimethoprim-sulfamethoxazole.[26]

All 50 *K. pneumoniae* isolates exhibiting reduced susceptibility to cefotaxime were molecularly characterized, with the most prevalent ESBL gene, *bla*CTX-M, being found in 86% of the isolates. This finding is consistent with results from a larger surveillance investigation conducted in Sindh, Pakistan, where 60% of isolates harbored the CTX-M-15 genotype and no carbapenem resistance was observed.[8] *bla*CTX-M was the most frequently detected β-lactamase gene among the molecularly analyzed clinical *K. pneumoniae*, as evidenced by its high incidence, in line with broader epidemiological patterns seen in the Middle East and Asia.[27, 28]

The detection of *bla*TEM and *bla*SHV among the molecularly analyzed clinical isolates further indicate the presence of multiple β-lactamase determinants in *K. pneumoniae,* commonly reported

in Enterobacteriaceae, which can make empirical treatment decisions difficult and create complex resistant profiles.[7, 29]

The *bla*NDM-1 gene was detected in 40% (20/50) of the molecularly analyzed *K. pneumoniae* isolates; however, all isolates remained susceptible to imipenem by disk diffusion. The observed genotype–phenotype discordance should be interpreted cautiously because imipenem susceptibility was assessed by disk diffusion only. MIC determination and sequence verification were not performed in the present study. These methods are therefore recommended in future investigations to resolve the observed phenotypic–molecular discordance. The detection of *bla*NDM-1 in this study is consistent with previous reports of NDM-1-producing *K. pneumoniae* in Pakistan.[30, 31] The detection of *bla*NDM-1 despite imipenem susceptibility highlights the need for further confirmatory investigation of genotype–phenotype discordance.

Recent studies on environmental and clinical surveillance have shown that MDR organisms that produce carbapenemase are common in clinical wards and can spread through contaminated surfaces and healthcare personnel. This emphasizes the importance of strict infection control procedures in preventing cross transmission.[32]

Moreover, it has been shown that combining rapid molecular diagnostics with antimicrobial stewardship programs can drastically shorten the time for optimal and successful treatment of bloodstream infections caused by ESBL and carbapenemase producing Enterobacterales such as *K. pneumoniae*. This emphasizes the importance of strict infection control procedures in preventing cross transmission.[33]

Some interventions aimed at combating carbapenem resistant *K. pneumoniae* (CRKP) which have been shown to reduce transmission and improve outcomes in hospital settings include improved hand hygiene, patient cohorting, education, surveillance and implementation of antimicrobial policies.[34]

The severe β-lactam and multidrug resistance profiles observed among our human clinical isolates in Faisalabad are mirrored in the veterinary sector [35, 36].The high prevalence of resistance genes in our study underscores the evolutionary adaptability of *K. pneumoniae*. This adaptability is not limited to human hosts; rather it represents a one health challenge which spans around environmental and animal interfaces as well.

Universal 16S rDNA PCR was used to assist bacterial identification at the genus level, whereas species-level identification was primarily based on conventional microbiological and biochemical

methods. Although higher-resolution sequencing approaches may provide greater taxonomic resolution, the techniques employed in this study are in accordance with general practice in many clinical microbiology laboratories.

Although this study was strong in several aspects such as having a relatively large sample size and combining molecular and phenotypic analyses, some limitations must be noted. The molecular epidemiology was performed on all four specimen types (sputum, blood, urine, and pus) but molecular characterization was conducted only on a subset of 50 *K. pneumoniae* isolates exhibiting reduced susceptibility to cefotaxime, which may restrain the generalizability of the genetic findings. Also, it is not possible to evaluate temporal trends in antimicrobial resistance in a cross-sectional design. The results underscore the importance of implementing regular molecular monitoring for resistances, evidence-based infection control measures and improved antimicrobial stewardship programs to prevent the spread of MDR *K. pneumoniae* in hospitals.

## Conclusion

This study demonstrates a high frequency of reduced susceptibility to cefotaxime among the clinical *K. pneumoniae* isolates investigated. Among the 50 molecularly analyzed isolates, the *bla*CTX-M gene was the most common resistance determinant (86%), while *bla*TEM (60%), *bla*NDM-1 (40%) and *bla*SHV (6%) were less frequent. The antimicrobial stewardship policies and susceptibility testing should guide empirical therapy and surveillance of carbapenemase resistance in clinical settings. Whole genome sequencing, plasmid replicon typing, and MLST should be included in the future studies to complete the characterization of the molecular epidemiology of *K. pneumoniae* in this area. Sustained antimicrobial stewardship programs, enhanced molecular surveillance, and strengthened infection prevention measures are essential to curtail the spread of MDR *K. pneumoniae* in Pakistani healthcare settings.

## Declarations


### Acknowledgement

The authors would like to thank the patients and tertiary care hospitals for their support in this study.


### Ethics Approval and Consent to Participate

The study was approved by the Graduate Studies Research Board of the University of Agriculture Faisalabad, which is authorized to review and approve research involving human participants

(Approval No. DGS 21249-52, dated 8 May 2024). Informed consent was obtained from participants before clinical specimen collection in accordance with the approved study protocol with the help of physicians.

**Data Availability**

The datasets generated and/or analyzed during the current study are available from the corresponding author on request.

**Consent for publication**

Not applicable

**Competing Interests**

The authors declare no competing of financial conflict of interest.

**Author's Contribution**

• Bushra Mumtaz: Methodology, Sampling, Lab analysis, Writing-Original Draft

•Abdul Basit: Investigation, Data Analysis, Sampling

• Muhammad Shoaib: Conceptualization, Study design, Writing-Original Draft, Supervision manuscript.

**References:**


1. Organization WH. Tackling antimicrobial resistance (AMR) together: working paper 5.0: enhancing the focus on gender and equity. World Health Organization, 2018.
2. Paczosa MK and Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiology and molecular biology reviews*. 2016; 80: 629-661, doi: 10.1128/MMBR.00078-15.
3. Salawudeen A, Raji YE, Jibo GG, et al. Epidemiology of multidrug-resistant *Klebsiella pneumoniae* infection in clinical setting in South-Eastern Asia: a systematic review and meta-analysis. *Antimicrobial Resistance & Infection Control*. 2023; 12: 142, doi: 10.1186/s13756-023-01346-5.
4. Kochan TJ, Nozick SH, Valdes A, et al. *Klebsiella pneumoniae* clinical isolates with features of both multidrug-resistance and hypervirulence have unexpectedly low virulence. *Nat Commun*. 2023; 14: 7962 10.1038/s41467-023-43802-1
5. Tsioutis C, Eichel VM and Mutters NT. Transmission of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae*: the role of infection control. *Journal of Antimicrobial Chemotherapy*. 2021; 76: 4-11 doi: 10.1093/jac/dkaa492.
6. Rangama S, Lidbury IDEA, Holden JM, et al. Mechanisms Involved in the Active Secretion of CTX-M-15 Lactamase by Pathogenic Escherichia coli ST131. *Antimicrobial Agents and Chemotherapy*. 2021; 65: doi: 10.1128/AAC.00663-21.
7. Castanheira M, Simner PJ and Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC-Antimicrobial Resistance*. 2021; 3. doi: 10.1093/jacamr/dlab092.

8. Muzaheed, Sattar Shaikh N, Sattar Shaikh S, et al. Molecular Epidemiological Surveillance of CTX-M-15-producing *Klebsiella pneumoniae* from the patients of a teaching hospital in Sindh, Pakistan. *F1000Research*. 2021; 10: 444. 10.12688/f1000research.53221.3

9. Ding L, Yang Z and Sun B. Understanding *bla*NDM-1 gene regulation in CRKP infections: toward novel antimicrobial strategies for hospital-acquired pneumonia. *Molecular medicine (Cambridge, Mass)*. 2024; 30: 29. doi: 10.1186/s10020-024-00794-y.

10. Acman M, Wang R, van Dorp L, et al. Role of mobile genetic elements in the global dissemination of the carbapenem resistance gene *bla*NDM. *Nature Communications*. 2022; 13: 1131. doi: 10.1038/s41467-022-28819-2.

11. Wyres KL, Nguyen TNT, Lam MMC, et al. Genomic surveillance for hypervirulence and multi-drug resistance in invasive *Klebsiella pneumoniae* from South and Southeast Asia. *Genome Med*. 2020; 12: 11. doi: 10.1186/s13073-019-0706-y.

12. Aslam B, Arshad MI and Aljasir SF. Transmission Dynamics of Antimicrobial Resistance from Farm to ForkPakistan Veterinary Journal. *Pakistan Veterinary Journal*. 2026; 46: 759-76710.29261/pakvetj/2026.075.

13. Woo PC, Lau SK, Teng JL, Tse H and Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clin Microbiol Infect*. 2008; 14: 908-34. DOI: 10.1111/j.1469-0691.2008.02070.x

14. Son TV, Manh ND, Trung NT, et al. Molecular detection of *bla*(CTX-M) gene to predict phenotypic cephalosporin resistance and clinical outcome of *Escherichia coli* bloodstream infections in Vietnam. *Annals of clinical microbiology and antimicrobials*. 2021; 20: 60. DOI: 10.1186/s12941-021-00466-3

15. Idrees MM, Rimsha R, Idrees MD and Saeed A. Antimicrobial Susceptibility and Genetic Prevalence of Extended-Spectrum β-Lactamases in Gram-Negative Rods Isolated from Clinical Specimens in Pakistan. *Antibiotics (Basel, Switzerland)*. 2022; 12. DOI: 10.3390/antibiotics12010029

16. Chaudhry TH, Aslam B, Arshad MI, Nawaz Z and Waseem M. Occurrence of ESBL-producing *Klebsiella pneumoniae* in hospital settings and waste. *Pakistan journal of pharmaceutical sciences*. 2019; 32: 773-8.

17. Xiang T, Chen C, Wen J, et al. Resistance of Klebsiella pneumoniae Strains Carrying *bla* (NDM-1) Gene and the Genetic Environment of *bla* (NDM-1). *Frontiers in microbiology*. 2020; 11: 700. doi: 10.3389/fmicb.2020.00700

18. Boralli C, Paganini JA, Meneses RS, et al. Characterization of *bla*(KPC-2) and *bla*(NDM-1) Plasmids of a *K. pneumoniae* ST11 Outbreak Clone. *Antibiotics (Basel, Switzerland)*. 2023; 12. DOI: 10.3390/antibiotics12050926

19. Usman M, Rasool MH, Khurshid M, Aslam B and Baloch Z. Co-Occurrence of mcr-1 and Carbapenem Resistance in Avian Pathogenic *E. coli* Serogroup O78 ST95 from Colibacillosis-Infected Broiler Chickens. *Antibiotics (Basel, Switzerland)*. 2023; 12. DOI: 10.3390/antibiotics12050812

20. Bedenić B, Luxner J, Zarfel G, et al. Characterization of Klebsiella pneumoniae Isolates Resistant to Cefiderocol from Hospitals and Outpatient Settings in Croatia. *Antibiotics (Basel, Switzerland)*. 2025; 14. https://doi.org/10.3390/antibiotics14020154

21. Ahmadi N, Salimizand H, Zomorodi AR, et al. Genomic diversity of β-lactamase producing Pseudomonas aeruginosa in Iran; the impact of global high-risk clones. *Annals of clinical microbiology and antimicrobials*. 2024; 23: 5. DOI: 10.1186/s12941-024-00668-5

22. Endale H, Mathewos M and Abdeta D. Potential causes of spread of antimicrobial resistance and preventive measures in one health perspective-a review. *Infection and drug resistance*. 2023: 7515-45. DOI: 10.2147/IDR.S428837

23. Ahmed SK, Hussein S, Qurbani K, et al. Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*. 2024; 2: 100081. 10.1016/j.glmedi.2024.100081

24. Lam MM, Wick RR, Watts SC, Cerdeira LT, Wyres KL and Holt KE. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. *Nature communications*. 2021; 12: 4188. DOI: 10.1038/s41467-021-24448-3

25. Meatherall BL, Gregson D, Ross T, Pitout JD and Laupland KB. Incidence, risk factors, and outcomes of *Klebsiella pneumoniae* bacteremia. *The American journal of medicine*. 2009; 122: 866-73. DOI: 10.1016/j.amjmed.2009.03.034

26. Irfan S, Azhar A, Bashir A, Ahmed S and Haque A. High frequency of simultaneous presence of ESBL and carbapenemase producers among nosocomial coliform isolates in Faisalabad, Pakistan. *Pakistan journal of medical sciences*. 2021; 37: 34-9 DOI: 10.12669/pjms.37.1.3192
27. Barguigua A, El Otmani F, Talmi M, et al. Prevalence and genotypic analysis of plasmid-mediated β-lactamases among urinary *Klebsiella pneumoniae* isolates in Moroccan community. *The Journal of Antibiotics*. 2013; 66: 11-6. DOI: 10.1038/ja.2012.91
28. Bevan ER, Jones AM and Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy*. 2017; 72: 2145-55. DOI: 10.1093/jac/dkx146
29. Ugbo EN, Anyamene CO, Moses IB, et al. Prevalence of *bla*TEM, *bla*SHV, and *bla*CTX-M genes among extended spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* of clinical origin. *Gene Reports*. 2020; 21: 100909. doi: 10.4084/MJHID.2019.041
30. Huang J, Deng S, Ren J, Tu J, Ye M and Wang M. Characterization of a *bla*NDM-1-harboring plasmid from a Salmonella enterica clinical isolate in China. *Molecular medicine reports*. 2017; 16: 1087-92 doi: 10.3892/mmr.2017.6733
31. Wailan AM, Sartor AL, Zowawi HM, Perry JD, Paterson DL and Sidjabat HE. Genetic Contexts of *bla*NDM-1 in Patients Carrying Multiple NDM-Producing Strains. *Antimicrobial Agents and Chemotherapy*. 2015; 59: 7405-10 DOI: 10.1128/AAC.01319-15
32. Acolatse JEE, Portal EAR, Boostrom I, et al. Environmental surveillance of ESBL and carbapenemase-producing gram-negative bacteria in a Ghanaian Tertiary Hospital. *Antimicrobial Resistance & Infection Control*. 2022; 11: 49 doi: 10.1186/s13756-022-01090-2.
33. Kunz Coyne AJ, Casapao AM, Isache C, Morales J, McCarter YS and Jankowski CA. Influence of Antimicrobial Stewardship and Molecular Rapid Diagnostic Tests on Antimicrobial Prescribing for Extended-Spectrum Beta-Lactamase- and Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in Bloodstream Infection. *Microbiology spectrum*. 2021; 9: e0046421. doi:10.1128/Spectrum.00464-21
34. Okeah BO, Morrison V and Huws JC. Antimicrobial stewardship and infection prevention interventions targeting healthcare-associated Clostridioides difficile and carbapenem-resistant *Klebsiella pneumoniae* infections: a scoping review. *BMJ open*. 2021; 11: e051983. doi: 10.1136/bmjopen-2021-051983.
35. Shehata AA, Abd-Elfatah EB, Elsheik HE, Salman MB, Khater ASI and El-Emam MMA. Epidemiological Features, Biochemical Indices, Antibiogram Susceptibility Profile and Biofilm Factor Genes of *Klebsiella pneumoniae* Isolated from Bovine Clinical Mastitis Cases. *Pakistan Veterinary Journal*. 2024; 44. DOI:10.29261/pakvetj/2024.130
36. Arafa AA and Kandil MM. Antimicrobial Activity of Zinc Oxide Nanoparticles against ESBL Producing *Klebsiella pneumoniae* Isolated from Equines in Egypt. *Pakistan Veterinary Journal*. 2024; 44. DOI: 10.29261/pakvetj/2023.121