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Phenotypic and Antimicrobial Resistance Landscape of Klebsiella pneumoniae: Hypermucoviscosity, Biofilm Formation and Drug-Resistant Phenotypes.

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ABSTRACT

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is an important healthcare-associated pathogen in which antimicrobial resistance (AMR) may coexist with phenotypes that enhance persistence and complicate clinical management. This study characterized the AMR, hypermucoviscous, and biofilm-forming profiles of *K. pneumoniae* isolates from tertiary-care hospitals in Peshawar, Pakistan.

Methods: A total of 152 *K. pneumoniae* isolates collected from urine, pus, blood, sputum, wound swabs, and body fluids were phenotypically characterized. We assessed isolates for antimicrobial susceptibility against selected antibiotics and categorized them according to multi-drug-resistant/extensively drug-resistant (MDR/XDR) phenotypes. We evaluated hypermucoviscosity using the string test and determined biofilm-forming capacity using a standardized phenotypic assay.

Results: Of the 152 isolates, 81 (53.3%) were obtained from males and 71 (46.7%) from females. Urine was the predominant specimen source (52, 34.2%), followed by pus (33, 21.7%) and blood (26, 17.1%). Resistance was particularly high to cefazolin (90.1%), amoxicillin–clavulanate (87.5%), cefoxitin (84.2%), ceftriaxone (80.9%), and ceftazidime (79.6%). Carbapenem resistance was 20.4% for ertapenem and 19.1% for meropenem, whereas colistin resistance was 11.8%. Overall, 57 (37.5%) isolates were MDR and 36 (23.7%) were XDR. Hypermucoviscosity was identified in 114 (75.0%) isolates. Biofilm formation was heterogeneous, with 32 (21.1%) strong, 47 (30.9%) moderate, 35 (23.0%) weak, and 38 (25.0%) non-biofilm-forming isolates.

Conclusions: The frequent occurrence of antimicrobial resistance, MDR/XDR phenotypes, hypermucoviscosity, and biofilm formation demonstrates a substantial phenotypic burden of *K. pneumoniae*. Integrated antimicrobial stewardship, phenotypic surveillance, and strengthened infection-prevention strategies are warranted to mitigate its clinical impact.

INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is a major opportunistic Gram-negative pathogen responsible for a broad spectrum of infections, including urinary tract infections, pneumonia, bloodstream infections, wound infections, and infections associated with invasive medical procedures. Its clinical importance has increased substantially because of the rapid accumulation and dissemination of antimicrobial-resistance (AMR) determinants, particularly among healthcare-associated strains (1). In the 2024 Bacterial Priority Pathogens List, the World Health Organization (WHO) identified third-generation-cephalosporin- and carbapenem-resistant *Enterobacterales* as critical-priority threats, reflecting the limited therapeutic options and substantial public-health consequences associated with these organisms (2). The challenge posed by *K. pneumoniae*, however, extends beyond AMR. The organism can express several phenotypic traits that contribute to survival, persistence, and infection. Among these, biofilm formation is particularly important because bacteria embedded within biofilms can exhibit reduced susceptibility to antimicrobial agents and increased tolerance to host immune defenses, thereby promoting persistent infections, particularly on tissues and medical devices (3). The relationship between biofilm formation and antimicrobial resistance is complex. It may vary among strains and clinical settings, making simultaneous assessment of both characteristics important for understanding the clinical phenotype of *K. pneumoniae (4)*. Another clinically relevant phenotype is hypermucoviscosity, traditionally assessed by the string test. Increased mucoviscosity has been associated with enhanced capsular properties and, in some strains, invasive disease. However, hypermucoviscosity should not be considered synonymous with hypervirulence, as its predictive value for hypervirulent *K. pneumoniae* is limited when assessed without additional virulence markers or genomic evidence (5). Recent evidence indicates that hypermucoviscosity can occur across diverse *K. pneumoniae* lineages, including antimicrobial-resistant backgrounds. Of particular concern is the potential convergence of AMR with persistence- and pathogenicity-associated phenotypes. The emergence of strains carrying substantial resistance while retaining characteristics such as hypermucoviscosity and biofilm-forming capacity could create infections that are both difficult to treat and capable of prolonged persistence (6). This convergence has become increasingly relevant in Pakistan, where studies have documented substantial antimicrobial resistance and the presence of hypermucoviscous and biofilm-forming *K. pneumoniae* in clinical settings (7). Despite this growing concern, phenotypic data integrating antimicrobial susceptibility, MDR/XDR classification, hypermucoviscosity, and biofilm formation remain limited from tertiary-care hospitals in Peshawar, Pakistan. Such integrated characterization is important because antimicrobial susceptibility alone does not fully describe the clinical potential of *K. pneumoniae (8)*. Therefore, this study aimed to comprehensively characterize K. pneumoniae isolates from tertiary-care hospitals in Peshawar, Pakistan, with particular emphasis on their AMR profiles, MDR/XDR phenotypes, hypermucoviscosity, and biofilm-forming capacity. By examining these traits together, this study provides a more complete phenotypic picture of the organism. It generates locally relevant evidence to support antimicrobial stewardship, laboratory surveillance, and infection-prevention strategies.

MATERIALS AND METHODS

2.1. Study Design and Sample Collection

A cross-sectional laboratory-based study was conducted from January 2025 to December 2025 at three major tertiary-care hospitals in Peshawar, Khyber Pakhtunkhwa, Pakistan: Khyber Teaching Hospital (9), Hayatabad Medical Complex (HMC), and Lady Reading Hospital (LRH). Clinical specimens were collected from patients with suspected bacterial infections according to institutional standard operating procedures (SOPs) and established microbiological protocols. We collected a total of 1,286 clinical specimens during the study period. Specimens included urine, blood, sputum, pus, wound swabs, and body fluids. All specimens were transported to the respective microbiology laboratories of each hospital under appropriate conditions and processed using standard bacteriological procedures. Samples were inoculated onto MacConkey agar, cystine–lactose–electrolyte-deficient (CLED) agar and 5% sheep blood agar, as appropriate for specimen type, and incubated aerobically at 37 °C for 18–24 h. Colonies exhibiting characteristics consistent with *K. pneumoniae*, particularly large, mucoid, lactose-fermenting colonies, were selected for further identification and purified by subculture on eosin methylene blue (EMB) agar (1). Of the 896 culture-positive specimens, 152 isolates were identified as *K. pneumoniae* and included in the phenotypic analyses.

2.2. Phenotypic Identification and Species Confirmation

Presumptive *K. pneumoniae* isolates were initially characterized by Gram staining, colony morphology, and conventional biochemical testing. The biochemical profile included citrate utilization, urease production, indole production, methyl red, Voges–Proskauer, oxidase, catalase, and motility tests. Species-level identification was subsequently confirmed using the API 20E identification system (bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions. Confirmed isolates were maintained in Luria–Bertani broth supplemented with 40% glycerol and stored at −80 °C until further phenotypic investigations (10).

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (Oxoid, UK) (8), following the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition (10). The antimicrobial panel comprised agents representing major classes used in the treatment of *K. pneumoniae* infections: amoxicillin–clavulanate (20/10 µg), piperacillin–tazobactam (100/10 µg), cefazolin (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), ertapenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg), trimethoprim–sulfamethoxazole (1.25/23.75 µg)and tigecycline (15 µg). For urinary isolates, nitrofurantoin (300 µg) was tested exclusively because of its clinical relevance to urinary

tract infections (11). Following inoculation, plates were incubated aerobically at 37 °C for 18–24 hours, and inhibition-zone diameters were measured to the nearest millimeter. Susceptibility categories were assigned according to the applicable CLSI breakpoints.

Because disk diffusion is not considered an appropriate method for determining colistin susceptibility, colistin minimum inhibitory concentrations (MICs) were determined using broth microdilution with the ComASP™ Colistin assay (Liofilchem, Italy), covering concentrations of 0.25–16 µg/mL (12). Results were interpreted according to the applicable contemporary CLSI/EUCAST interpretive criteria (1).

2.4. Classification of MDR and XDR Phenotypes

AMR phenotypes were classified according to the internationally recognized framework proposed by the European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). MDR isolates were defined as those exhibiting acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes. In contrast, XDR isolates were defined as those remaining susceptible to agents in only two or fewer antimicrobial classes. Classification was based on the antimicrobial classes represented in the AST panel rather than on the number of individual antimicrobial agents (13).

2.5. Assessment of Hypermucoviscosity

The hypermucoviscous phenotype was assessed using the standard string test. Briefly, a bacterial colony grown on blood agar was touched with a sterile inoculation loop and gently lifted from the agar surface. Formation of a viscous filament measuring ≥5 mm between the colony and inoculation loop was interpreted as a positive string test and classified as a hypermucoviscous phenotype. Isolates that failed to produce a ≥5-mm string were classified as string-test negative (14). Importantly, the string test was used specifically to characterize hypermucoviscosity and was not used as a standalone surrogate for hypervirulence (15).

2.6. Hemolysis Assay

Hemolytic activity was assessed by inoculating confirmed isolates onto 5% sheep blood agar followed by aerobic incubation at 37 °C for 24–48 h. Following incubation, plates were examined for visible zones of erythrocyte lysis surrounding bacterial growth. Hemolytic activity was recorded according to the observed hemolysis pattern (16).

2.7. Quantitative Assessment of Biofilm Formation

Biofilm-forming capacity was quantified using a microtiter plate crystal violet assay. Fresh bacterial colonies were inoculated into trypticase soy broth supplemented with 1% glucose and incubated aerobically at 37 °C for 24 h. Cultures were diluted 1:100 in fresh broth, and 200 µL of each diluted suspension was transferred into sterile 96-well polystyrene microtiter plates; wells containing sterile culture medium without bacteria served as negative controls.

The plates were incubated statically at 37 °C for a further 24 h to permit biofilm development. Following incubation, planktonic cells were removed, and wells were washed three to four times with phosphate-buffered saline (PBS) to remove non-adherent cells. The remaining adherent biofilm was fixed with 2% sodium acetate and stained with 0.1% (w/v) crystal violet. Excess stain was removed by washing with deionized water, after which the plates were air-dried. The retained crystal violet was subsequently solubilized with 95% ethanol, and absorbance was measured at 630 nm using a microplate reader. The optical-density cut-off (ODc) was calculated from the negative controls as the mean OD plus two standard deviations. Isolates were categorized according to their measured OD values as follows: non-biofilm producers, OD ≤ ODc; weak biofilm producers, ODc < OD ≤ 2×ODc; moderate biofilm producers, 2×ODc < OD ≤ 4×ODc; and strong biofilm producers, OD > 4×ODc (17).

2.8. Statistical Analysis

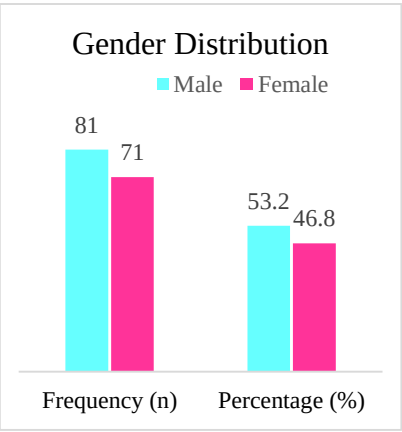
Statistical analyses were performed using Stata version 16. Descriptive statistics summarized demographic, clinical, and microbiological variables (18). Data visualization and supplementary analyses were conducted using Microsoft Excel.

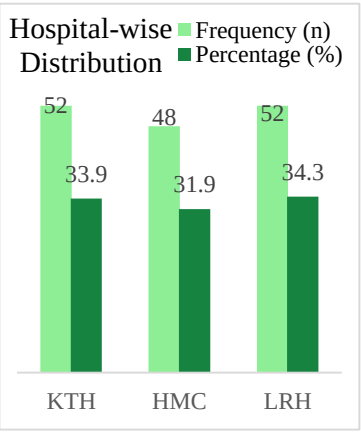
Results

3.1. Epidemiological and Clinical Distribution of *K. pneumoniae* Isolates

The final analysis included 152 confirmed K. pneumoniae isolates. The isolates were relatively evenly distributed by sex, with 81 (53.2%) recovered from male patients and 71 (46.8%) from female patients. The isolates were obtained from three major tertiary-care hospitals: 52 (34.2%) from KTH (9), 48 (31.6%) from HMC, and 52 (34.2%) from LRH.

Urine represented the predominant specimen source, accounting for 52 (34.2%) isolates, followed by pus 33 (21.7%), blood 26 (17.1%), sputum 20 (13.2%), wound swabs 15 (9.9%), and body fluids 6 (3.9%). Geographically, isolates were most frequently associated with Mardan (47; 30.9%), followed by Peshawar (38; 25.0%), Charsadda (32; 21.1%), Swabi (21; 13.8%), and other districts (14; 9.2%) (**Figure 1**).





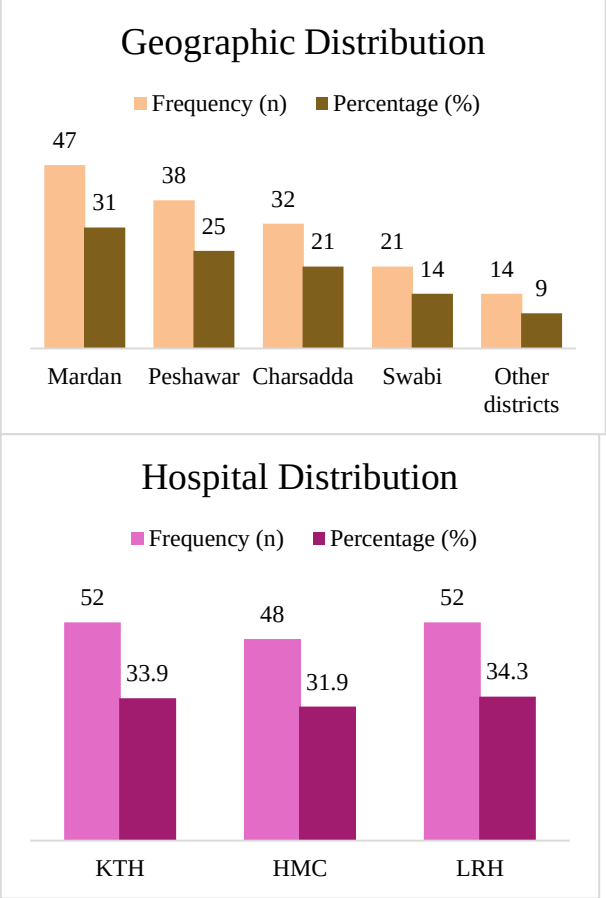


Figure 1. Demographic, clinical, institutional and geographic distribution of *Klebsiella pneumoniae* isolates in the study population (n=152).

3.2. Antimicrobial Susceptibility Profile

The AST profile demonstrated a pronounced burden of resistance among the clinical *K. pneumoniae* isolates (**Figure 2**). Resistance was particularly high to the commonly used β-lactam agents. The highest resistance rates were observed for cefazolin (137/152; 90.1%), amoxicillin–clavulanate (133/152; 87.5%), cefoxitin (128/152; 84.2%), ceftriaxone (123/152; 80.9%), ceftazidime (121/152; 79.6%) and piperacillin–tazobactam (117/152; 77.0%). Resistance to cefepime was also substantial, affecting 115/152 (75.7%) isolates.

Fluoroquinolone resistance was similarly prominent, with 110/152 (72.4%) isolates resistant to ciprofloxacin and 102/152 (67.1%) resistant to levofloxacin. Among aminoglycosides, resistance was observed in 90/152 (59.2%) isolates for amikacin, 105/152 (69.1%) for tobramycin, and 100/152 (65.8%) for gentamicin. Resistance to tetracycline and trimethoprim–sulfamethoxazole was 97/152 (63.8%) and 113/152 (74.3%), respectively.

In contrast, carbapenems retained comparatively greater activity, although resistance remained clinically important: 31/152 (20.4%) isolates were resistant to ertapenem and 29/152 (19.1%) to meropenem. Colistin demonstrated the highest activity among the agents tested, with 134/152 (88.2%) isolates susceptible and 18/152 (11.8%) resistant. Nitrofurantoin, tested only among urinary isolates, showed susceptibility in 32/52 (61.5%) isolates,

while 20 (38.5%) were resistant. Tigecycline showed susceptibility in 139/152 (91.4%) isolates, with resistance detected in 13 (8.6%).

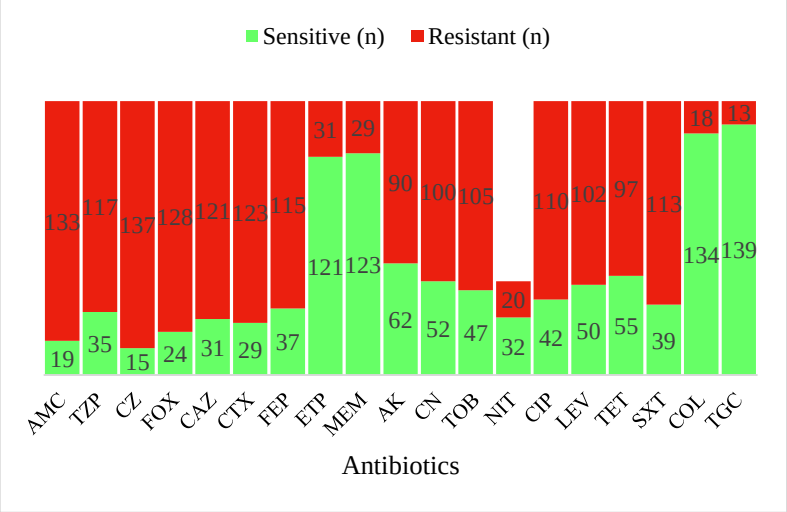


Figure 2: AST profile of *K. pneumoniae* isolates across tested antibiotic classes (n=152)

3.3. Distribution of MDR and XDR Phenotypes

The isolates demonstrated substantial heterogeneity in their overall antimicrobial-resistance profiles. Of the 152 isolates, 13 (8.6%) were fully susceptible, 46 (30.3%) were resistant to one or two antimicrobial classes, 57 (37.5%) fulfilled the criteria for MDR, and 36 (23.7%) were classified as extensively drug-resistant (XDR. Collectively, MDR and XDR isolates accounted for 93/152 (61.2%) of all isolates, indicating a considerable burden of clinically difficult-to-treat phenotypes.

Resistance patterns varied according to specimen source. Among urinary isolates, 24/52 (46.2%) were MDR and 16/52 (30.8%) were XDR, whereas blood isolates comprised 8/26 (30.8%) MDR and 3/26 (11.5%) XDR isolates. Among sputum isolates, 7/20 (35.0%) were MDR and 4/20 (20.0%) were XDR. Pus isolates demonstrated a particularly high resistance burden, with 16/33 (48.5%) MDR and 11/33 (33.3%) XDR isolates. Wound swabs yielded 2/15 (13.3%) MDR and 2/15 (13.3%) XDR isolates, whereas none of the six isolates recovered from sterile body fluids were classified as MDR or XDR. Overall, the highest combined MDR/XDR burden was observed among pus isolates (81.8%), followed by urine isolates (76.9%) (**Figure 3**).

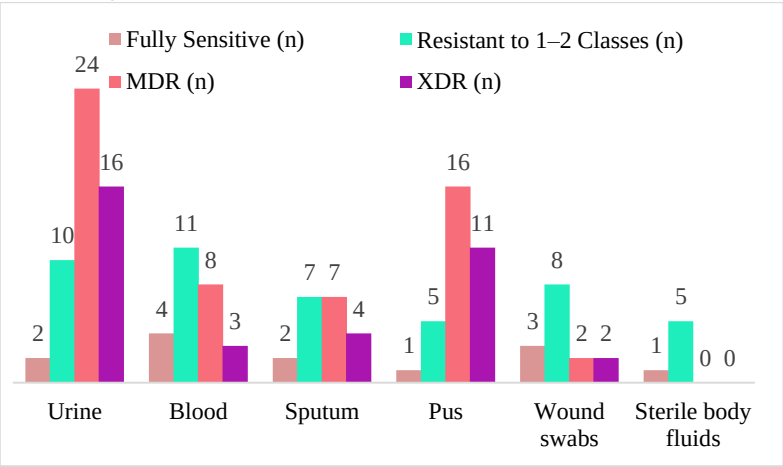


Figure 3: Antimicrobial Susceptibility Categories and Specimen-Wise Distribution of *K. pneumoniae* (n=152).

3.4. Hypermucoviscous Phenotype

The string test revealed a high prevalence of the hypermucoviscous phenotype among the clinical isolates. 114/152

(75.0%) isolates produced a string ≥5 mm and were classified as hypermucoviscous, whereas 38 (25.0%) were string-test negative (**Figure 4**).

Figure 4. The hypermucoviscous phenotype of *Klebsiella pneumoniae*. Formation of a viscous string extending greater than 5 mm from the colony was interpreted as a positive string test result, indicative of a hypermucoviscous *K. pneumoniae* isolate.

3.5. Hemolytic Activity

All *K. pneumoniae* isolates were non-hemolytic, with no detectable zone of hemolysis observed on 5% sheep blood agar after 24–48 h of incubation.

3.6. Biofilm-Forming Capacity

Biofilm formation was also highly heterogeneous across the isolate population. Based on the quantitative microtiter plate assay, 32/152 (21.1%) isolates were strong biofilm producers, while 47 (30.9%) demonstrated moderate biofilm formation. A further 35 (23.0%) isolates were classified as weak biofilm producers, whereas 38 (25.0%) showed no detectable biofilm-forming activity (**Figure 5**).

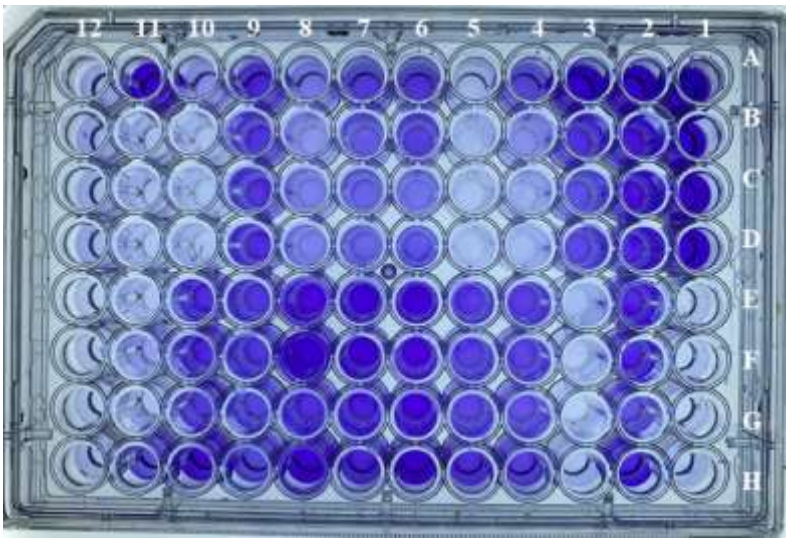


Figure 5. Biofilm formation by *K. pneumoniae* isolates assessed using the microtiter plate assay. (A1-D1 are positive control wells, E1-H1 are negative control wells, darkly stained wells show strong biofilm activity and dull staining shows weak biofilm activity, while absence of stain indicates no biofilm activity).

Discussion

The present study demonstrates a substantial and clinically relevant burden of AMR among *K. pneumoniae* isolates from tertiary-care hospitals in Peshawar, accompanied by a high prevalence of hypermucoviscosity and biofilm formation. More than half of the isolates (61.2%) were classified as MDR or XDR, while 75.0% exhibited a hypermucoviscous phenotype and 75.0% demonstrated some degree of biofilm production. Collectively, these findings indicate that the clinical *K. pneumoniae* population in this setting is characterized not by AMR alone, but by the coexistence of resistance and phenotypic traits that may facilitate persistence and complicate clinical management. This is particularly important in the context of the WHO classification of third-generation-cephalosporin- and carbapenem-resistant Enterobacterales as critical-priority antimicrobial-resistance threats (19) (20).

The marked resistance to β-lactam antibiotics observed in this study is particularly concerning. Resistance exceeded 75% for most third-generation cephalosporins and piperacillin–tazobactam, indicating substantial loss of activity of commonly used therapeutic agents. Fluoroquinolone and aminoglycoside resistance was also prominent, further narrowing conventional treatment options. Although carbapenems retained activity against approximately 80% of isolates, resistance to ertapenem and meropenem in approximately one-fifth of isolates remains clinically important because carbapenems are frequently relied upon for serious infections caused by ESBL-producing and multidrug-resistant Enterobacterales. The comparatively high activity of tigecycline and colistin should be interpreted cautiously, as these agents have important clinical limitations and resistance to both was nevertheless detected. Similar high-level resistance and emergence of carbapenem- and colistin-resistant *K. pneumoniae* have previously been reported from Pakistan, supporting the broader concern that

resistant *K. pneumoniae* is becoming increasingly entrenched in healthcare settings (21).

The high proportion of MDR (37.5%) and XDR (23.7%) isolates represents one of the most important findings of this study. Particularly notable was the concentration of MDR/XDR phenotypes among pus and urinary isolates, suggesting that difficult-to-treat *K. pneumoniae* is not restricted to a single clinical syndrome. This pattern has implications for empirical antimicrobial therapy, particularly where local resistance surveillance is limited. The findings also reinforce the need for routine laboratory-based resistance surveillance and antimicrobial stewardship rather than reliance on conventional empirical regimens. A further important observation was the high prevalence of hypermucoviscosity, with three-quarters of isolates producing a positive string test. This phenotype has traditionally been linked to increased capsular properties and invasive potential; however, hypermucoviscosity should not be interpreted as equivalent to hypervirulence (22). Contemporary evidence emphasizes that the string test has limited specificity for identifying hypervirulent *K. pneumoniae* and molecular characterization is required to establish hypervirulence more reliably (23). The coexistence of hypermucoviscosity with substantial AMR in the present collection is nevertheless noteworthy because the emergence of resistant strains with virulence-associated phenotypes represents an increasingly recognized clinical concern. Recent systematic evidence suggests that hypermucoviscous isolates may, on average, show somewhat lower rates of ESBL and carbapenem resistance than non-hypermucoviscous isolates, but resistant hypermucoviscous strains are clearly emerging and warrant careful surveillance.Biofilm formation was similarly widespread: 75.0% of isolates produced biofilm, including 21.1% strong and 30.9% moderate producers. This finding is clinically relevant because biofilm-associated growth can facilitate persistence on biological surfaces and indwelling medical devices and may reduce antimicrobial penetration and increase tolerance to host defenses (24). The substantial representation of moderate and strong biofilm producers therefore provides an additional explanation for K. pneumoniae persistence in healthcare environments. Importantly, biofilm formation and antimicrobial resistance should not be regarded as interchangeable characteristics; rather, their coexistence may provide complementary advantages, with antimicrobial resistance limiting therapeutic efficacy while biofilm-associated growth promotes persistence (25).

The complete absence of detectable hemolytic activity in all 152 isolates contrasts with the high prevalence of hypermucoviscosity and biofilm formation (10). This finding indicates that the phenotypic characteristics examined in this study were not uniformly expressed as a single virulence profile. Rather, *K. pneumoniae* displayed considerable phenotypic heterogeneity, emphasizing the multifactorial nature of bacterial pathogenicity (26). In particular, the simultaneous presence of hypermucoviscosity and biofilm formation in a substantial proportion of isolates warrants further investigation to determine whether these traits are associated with specific resistance profiles or clinical sources. Overall, this study provides evidence of a convergent phenotypic threat involving AMR, hypermucoviscosity, and biofilm formation among clinical *K. pneumoniae* isolates in tertiary-care hospitals in Peshawar. The findings support strengthening antimicrobial stewardship, routine phenotypic surveillance, infection-prevention measures, and microbiological characterization of MDR/XDR isolates. Future studies incorporating whole-genome sequencing, resistance and virulence determinants, capsular typing, and genomic lineage analysis would be valuable for determining whether the observed phenotypes arise from particular circulating clones or represent independent traits distributed across genetically diverse populations. This distinction is essential for understanding transmission dynamics and designing targeted interventions.

CONCLUSION

This study demonstrates a substantial phenotypic burden among clinical *K. pneumoniae* isolates, characterized by high levels of antimicrobial resistance and a considerable prevalence of MDR and XDR phenotypes. The frequent occurrence of hypermucoviscosity and biofilm-forming capacity further highlights these isolates' ability to exhibit persistence-associated phenotypes alongside resistance. Although all isolates were non-hemolytic, the coexistence of hypermucoviscosity, biofilm formation, and extensive antimicrobial resistance underscores the clinical and infection-control challenges posed by *K. pneumoniae* in tertiary-care settings. These findings support continued phenotypic surveillance, rational antimicrobial stewardship, and strengthened infection-prevention measures to limit the emergence and transmission of difficult-to-treat *K. pneumoniae* in Pakistan.

Ethical Approval

Ethical approval for the study was obtained from the relevant institutional ethics committee before the study commenced. The study was conducted in accordance with applicable institutional ethical guidelines and the principles of the Declaration of Helsinki.

Informed Consent

Since the study encompassed the phenotypic characterization of previously obtained clinical Klebsiella pneumoniae isolates and did not entail direct intervention with patients, individual informed consent was not required.

Conflict of Interest

The authors declare no conflicts of interest related to this study or its publication.

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Data Availability Statement

The data generated and/or analyzed during this study are not publicly available because they are derived from clinical laboratory specimens and may be subject to institutional and ethical restrictions. Relevant data may be available from the corresponding author on reasonable request, subject to institutional approval and applicable confidentiality requirements.

Authors' Contributions (ICMJE)

Aiman Waheed: Conception and design of the study, laboratory investigation, data collection, data analysis and interpretation, manuscript drafting, critical revision, and final approval of the version to be published.

Sofia Bano: Laboratory investigation, data collection, literature review, manuscript drafting and revision, and final approval of the manuscript.

Manahil Zubair: Laboratory investigation, data collection, literature review, manuscript revision, and final approval of the manuscript.

Mudassir Obaid: Laboratory investigation, data collection, literature review, manuscript revision, and final approval of the manuscript.

Sajid Ali: Laboratory investigation, data collection, data interpretation, manuscript revision, and final approval of the manuscript.

Sajjad Ahmad: Conception and design, supervision of the study, laboratory oversight, interpretation of findings, critical revision of the manuscript, correspondence with the journal, and final approval of the version to be published.

All authors agree to be accountable for all aspects of the work and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ICMJE Statement

All authors confirm that they meet the authorship criteria established by the International Committee of Medical Journal Editors (ICMJE). All authors made substantial contributions to the conception or design of the work, acquisition, analysis, or interpretation of data; participated in drafting the manuscript or critically revising it for important intellectual content; approved the final version to be published; and agree to be accountable for all aspects of the work.

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