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Prevalence, Molecular Identification, and Antibiotic Susceptibility Profile of *Klebsiella pneumoniae* in a Tertiary Care Hospital, Bangladesh

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Abstract

Background: *Klebsiella pneumoniae* is a critical contributor to both community- and hospital-acquired infections, with rising multidrug resistance (MDR) posing serious therapeutic challenges. **Objective:** This study assessed the prevalence of *K. pneumoniae* and its antimicrobial susceptibility patterns among clinical specimens collected at Chittagong Medical College, Bangladesh. **Methodology:** This cross-sectional study was conducted from July 2022 to June 2023 in Chittagong Medical College, Chattogram. A total of 320 clinical specimens (urine, wound swabs, sputum, blood) from inpatients and outpatients were subjected to culture and sensitivity. Conventional microbiological identification was confirmed by 16S rRNA PCR. Antimicrobial susceptibility (excluding colistin) was tested via the Modified Kirby–Bauer disk diffusion method (CLSI 2022). Colistin resistance was assessed using the colistin agar test, applying CLSI breakpoints. **Results:** Overall culture positivity was 63.4% (203/320). *Klebsiella pneumoniae* was confirmed in 16.9% (54/320) of samples, most commonly isolated from sputum (51.4%), followed by wound swabs (17.1%), urine (11.8%), and blood (6.5%). Among the 54 *Klebsiella pneumoniae* isolates, resistance rates were highest against amoxiclav (94.4%), with lower resistance observed for aminoglycosides (amikacin, 24.1%) and meropenem (31.5%). Colistin resistance was detected in 12.9% (7/54) of *Klebsiella pneumoniae* isolates. **Conclusion:** The prominence of *Klebsiella pneumoniae* and its variable degree of resistance to multiple antibiotics, underscores an urgent need for enhanced antimicrobial stewardship, accurate diagnostics, and robust surveillance in Bangladesh.

Keywords: antimicrobial resistance surveillance; Colistin agar test; Klebsiella pneumoniae; MDR

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Introduction

Klebsiella species are ubiquitous bacteria commonly found on mucosal surfaces of mammals and in diverse environmental reservoirs such as soil, water, and plants. In humans, *Klebsiella* primarily colonizes the gastrointestinal tract and, less frequently, the nasopharynx[1]. As the second most prevalent aerobic Gram-negative bacterium in the human intestine, the gastrointestinal tract serves as the main reservoir for transmission[2]. The genus *Klebsiella* comprises four recognized species like *Klebsiella pneumoniae, Klebsiella oxytoca*, *Klebsiella terrigena*, and *Klebsiella planticola*. Among these, *Klebsiella pneumoniae*, particularly the subspecies pneumoniae is the most clinically significant[3].

Klebsiella pneumoniae is a leading cause of both community- and hospital-acquired bloodstream

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infections, second only to *Escherichia coli*[4]. It accounts for approximately 3.0% to 7.0% of nosocomial infections, predominantly affecting neonates, the elderly, and immunocompromised patients[5,6]. Transmission in healthcare settings often occurs via direct contact or through contaminated medical devices such as respiratory equipment and catheters[7]. The pathogenicity of *Klebsiella pneumoniae* correlates with its hypermucoviscosity phenotype, an extreme colony stickiness on agar, linked to virulence factors including polysaccharide capsules, adhesins, lipopolysaccharides, and iron-scavenging proteins[8,9]. These virulence factors contribute to a wide spectrum of infections, including urinary tract infections, pneumonia, surgical wound infections, endocarditis, septicemia, necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis[10].

Since the widespread introduction of antimicrobials, the emergence of antimicrobial resistance (AMR) has become a critical global health concern, exacerbated by inappropriate antibiotic use in human and veterinary medicine[11-13]. Multidrug-resistant (MDR) Klebsiella pneumoniae strains, characterized by extended-spectrum β-lactamases (ESBLs) and carbapenemases, have increasingly compromised treatment options. Initially, ESBL-producing strains predominated; however, the extensive use of carbapenems has driven a surge in carbapenem-resistant *Klebsiella pneumoniae* (CRKP), often resistant to multiple antibiotic classes and posing significant therapeutic challenges[10,14].

The rapid global dissemination of MDR and extensively drug-resistant (XDR) Klebsiella pneumoniae, so-called “superbugs” has led to serious clinical and economic impacts, intensified by the horizontal transfer of resistance genes via mobile genetic elements such as plasmids and transposons[15,16]. In Bangladesh, the prevalence of carbapenem-resistant *Klebsiella pneumoniae* is alarmingly high, reported at 37.3% in Dhaka Medical College Hospital, Dhaka, Bangladesh[17]. This trend, combined with the emergence of hypervirulent strains, highlights the urgent need for effective infection control strategies.

This study was aimed to determine the *Klebsiella pneumoniae* prevalence and antimicrobial susceptibility pattern of this clinical isolate which is critical for guiding empirical therapy, curbing resistance development, and improving clinical outcomes.

Methodology

Study Settings and Population: This was a cross-sectional study conducted at the Department of Microbiology, Chittagong Medical College (CMC), Chattogram. The study spanned one year starting from July 2022 to June 2023.

Sample Collection and Eligibility: Clinical specimens including urine, wound swabs, sputum, and blood were collected from both inpatients and outpatients at CMCH who presented with suspected urinary tract infections, respiratory tract infections, wound infections, or bloodstream infections. Patients (or their legal guardians) who did not provide written informed consent were excluded.

Sampling and Sample Size: A non-probability, purposive sampling method was utilized. Sample size was calculated using the standard formula n=Z2pq/d2 , with Z = 1.96 (95% confidence), p = 0.2373 (prevalence of *Klebsiella pneumoniae*) and d = 0.05, resulting in a required sample size of 282.

Data Collection: Following informed consent received, demographic and clinical information including antibiotic history were captured by using a structured questionnaire. Corresponding specimens sent to the microbiology laboratory for culture and sensitivity testing were included.

Culture Procedures and Identification: Specimens were inoculated onto Blood Agar and MacConkey Agar and incubated at 37°C for overnight. Positive blood cultures detected by BD BACTEC FX were subcultured similarly. Organisms were identified based on colony morphology, Gram reaction, and standard biochemical tests (oxidase, catalase, indole, urease, citrate, TSI, MIU media). Confirmation of Klebsiella pneumoniae was performed using 16S rRNA gene PCR[18,19].

Antimicrobial Susceptibility Testing (AST): AST for all isolates (excluding colistin) were performed using the Modified Kirby–Bauer disk diffusion method on Mueller Hinton agar, following CLSI 2022 guidelines. Antibiotic discs {Amikacin (30µg), Amoxiclav (20/10 µg), Ampicillin (10µg), Cefepime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30µg), Ciprofloxacin (30µg), Gentamicin (10µg), Meropenem (10µg), Nitrofurantoin (300µg), Pipperacillin-Tazobactam (100/10µg), Sulphamethoxazole-Trimethoprim (1.25/23.75µg)} were obtained from commercial sources (Oxoid Ltd, UK). E. coli ATCC 25922 served as the quality control strain.

For Colistin, Broth Agar Dilution Method was performed: colistin stock (8 mg/mL) was added to

molten Mueller Hinton agar to obtain plates with concentrations of 1, 2, and 4 µg/mL. A 0.5 McFarland suspension was diluted to approximately 1 × 107 CFU/mL, inoculated onto the plates, and incubated overnight at 37 °C. Interpretation was based on CLSI breakpoints: ≤ 2 µg/mL (intermediate sensitive), ≥ 4 µg/mL (resistant).

Polymerase Chain Reaction (PCR): PCR was performed following standard protocols using gene-specific primers for 16srRNA of *K. pneumoniae*. A forward primer of (5'-3')-ATGTCGCAAGACCAGAGTGG and a reverse primer of (5'-3')- GCACAACCTCCAAATCGACA were used.

DNA Extraction: Genomic DNA was extracted from bacterial cultures using the Monarch Genomic DNA Purification Kit (NEB) per manufacturer instructions. Briefly, bacterial pellets were resuspended in phosphate-buffered saline (PBS), treated with proteinase K and RNase A, followed by lysis buffer incubation at 56°C with agitation. DNA binding, washing, and elution steps were conducted using spin columns, with final elution in 35–100 µL of preheated elution buffer. Extracted DNA was stored at −20°C until further use.

PCR Amplification: Primer stock solutions (100 pmol) were diluted to 20 pmol working concentrations. Each 25 µL PCR reaction contained 12.5 µL master mix (including dNTPs, Taq polymerase, $MgCl_2$, and buffer), 2 µL each of forward and reverse primers, 2 µL DNA template, and 6.5 µL nuclease-free water. Reactions were briefly vortexed and centrifuged before amplification. Thermal cycling was conducted on an Eppendorf Master-cycler Gradient (Hamburg, Germany) with the following conditions for 16S rRNA gene: initial denaturation at 94°C for 3 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 10 minutes.

Agarose Gel Electrophoresis: PCR products were resolved on 2% agarose gels prepared in 1× TBE buffer with SYBR Green staining. Gels were cast, cooled, and electrophoresed at 100 V for 40 minutes. DNA bands were visualized under UV illumination using a gel documentation system. Amplicon sizes were determined by comparison with a 500 bp DNA ladder.

Interpretation: Samples yielding a 657 bp band corresponding to the 16S rRNA gene were considered PCR positive.

Preservation of Isolates: Isolates were preserved by inoculating onto Mueller Hinton agar slants supplemented with 15% glycerol, then stored at -80 °C. Monthly subculturing was performed to maintain viability.

Statistical Analysis: Data were entered into a pre-designed sheet and analyzed using SPSS version 29.0. Results were summarized using tables, charts, and narrative descriptions.

Ethical Consideration: The study protocol received approval from the Committee for Course and Studies (Microbiology) and the Ethical Review Committee of Chittagong Medical College.

Results

Over the one-year study period, 320 clinical specimens were collected from patients at Chittagong Medical College (CMC), including urine (161), wound swabs (76), sputum (37), and blood samples (46). Of these, 203(63.4%) yielded positive cultures, whereas 117(36.6%) showed no microbial growth (Figure I).

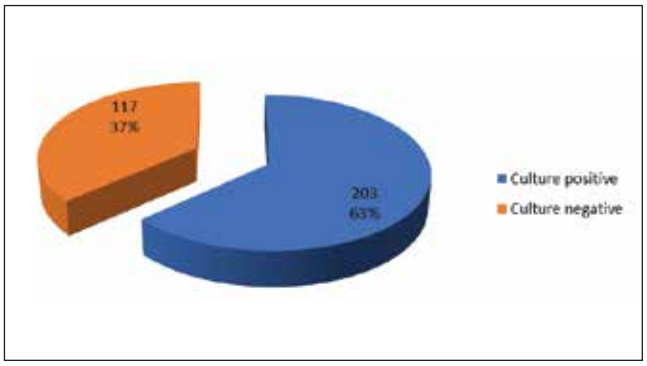

Figure I: Culture Positivity among Study Population (N=320)

Among culture-positive specimens, the vast majority (196; 96.6%) yielded single bacterial isolates, while 7 (3.4%) had polymicrobial growth, resulting in a total of 210 bacterial isolates (Table 1).

Table 1: Number of bacterial isolates from culture

Culture Results	Growth Positive	Bacterial Isolates
Single Growth	196(96.55%)	196(93.54%)
Mixed Growth	7(3.44%)	14((6.45%)
Total	**203**	**210**

Mixed growth=≥ 2 bacteria

Of the 320 total samples, Klebsiella pneumoniae was isolated from 54 (16.9%). The prevalence by specimen type was: sputum (19/37; 51.4%), wound swabs (13/76; 17.1%), urine (19/161; 11.8%), and blood (3/46; 6.5%) (Table 2).

Table 2: Distribution of Klebsiella pneumoniae in Different Specimen (N=320)

Specimens	*Klebsiella pneumoniae*		Total
	Positive	Negative	
Urine	19(11.8%)		161(100.0%)
Wound swab	13(17.1%)		76(100.0%)
Sputum	19(51.4%)		37(100.0%)
Blood	03(6.5%)		46(100.0%)
Total	**54(16.9%)**		**320(100.0%)**

Among the 210 isolates, the most prevalent organisms were *Escherichia coli* (63; 30.0%), *Klebsiella* species (60; 28.6%), *Pseudomonas* species (36; 17.1%), *Staphylococcus aureus* (15; 7.1%), followed by smaller proportions of *Acinetobacter* species(3.8%), *Enterobacter* species (2.9%), *Candida* species (2.4%), *Salmonella* species (1.9%), *Enterococcus* species (1.9%), *Proteus* species (1.4%), *CoNS* (1.4%), and *Citrobacter* species (0.95%) (Figure II).

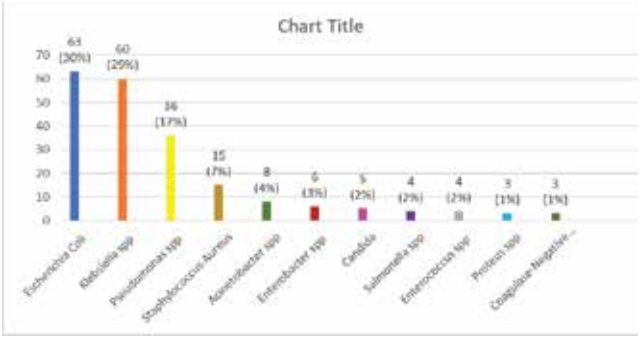

Figure II: Distribution of culture positive fungi identified by PCR (n=80)

Within the 60 phenotypic *Klebsiella isolates*, 3 (5%) were identified as *Klebsiella oxytoca*, and PCR confirmed 3 (5%) as other *Klebsiella* species. The remaining 54(90%) were confirmed by PCR as *Klebsiella pneumoniae*, with a statistically significant difference between phenotypic and genotypic identification ($p < 0.0001$) (Figure 3) (Figure IV) (Table 3).

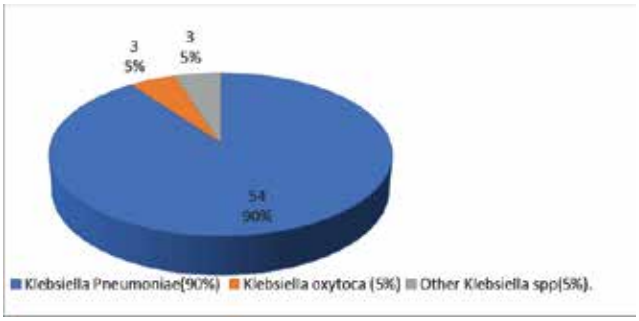

Figure III: Distribution of Klebsiella species (N= 60)

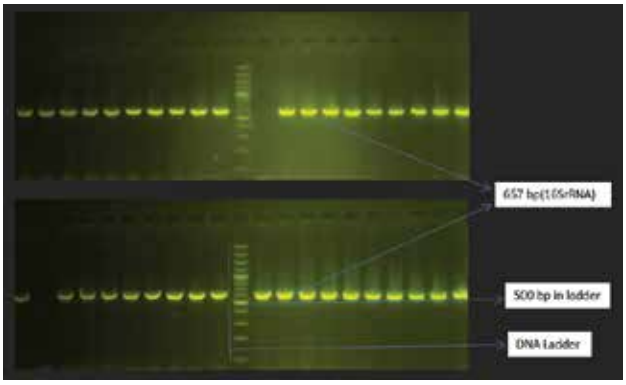

Figure IV: Agarose gel electrophoresis showing the PCR product of 657 bp amplified DNA of 16SrRNA gene of *Klebsiella pneumoniae*

Table 3: Genotypic detection of *Klebsiella pneumoniae* among Phenotypically Suspected Klebsiella species (N=60)

Klebsiella species (Phenotypically identified)	*Klebsiella pneumoniae* Identified		P value
	Phenotypically	PCR	
60	57(95.0%)	54(90.0%)	0.0001

Phenotypically excluding *Klebsiella oxytoca*

Among the 54 *Klebsiella pneumoniae* isolates, 32(59.3%) were recovered from male patients and 22(40.7%) from female patients. The highest frequency of isolates was observed in the 16 to 30 years age group (37.0%), followed by 61 to 75 years (20.4%) and 31 to 45 years (16.7%). The 46 to 60 years age group accounted for 14.8% of isolates, whereas the 0 to 15 year and 76 to 90 years groups each contributed 5.6%. Male patients predominated across most age categories, except for the 16 to 30 years age group, where female isolates (24.1%) exceeded male isolates (13.0%) (Table 4).

Table 4: Distribution of *Klebsiella pneumoniae* cases according to Age and Gender (N= 54)

Age Group	Male	Female	Total
0 to 15 Years	2(3.7%)	1(1.9%)	3(5.6%)
16 to 30 Years	7(13%)	13(24.1%)	20(37%)
31 to 45 Years	6(11.1%)	3(5.6%)	9(16.7%)
46 to 60 Years	5(9.3%)	3(5.6%)	8(14.8%)
61 to 75 Years	10(18.5%)	1(1.9%)	11(20.4%)
76 to 90 Years	2(3.7%)	1(1.9%)	3(5.6%)
Total	**32 (59.3%)**	**22 (40.7%)**	**54(100%)**

Antimicrobial susceptibility testing of these 54 Klebsiella pneumoniae isolates revealed 94.4% resistance to amoxiclav followed by 81.5% to

cefuroxime, 72.2% to ceftriaxone, 70.4% to cefepime, 68.5% to trimethoprim-sulfamethoxazole, and 66.7% to ceftazidime. Resistance rates were lower for ciprofloxacin (59.3%), gentamicin (48.2%), nitrofurantoin (urinary isolates only 36.8%), meropenem (31.5%), piperacillin–tazobactam (27.8%), and amikacin (24.1%) (Figure V).

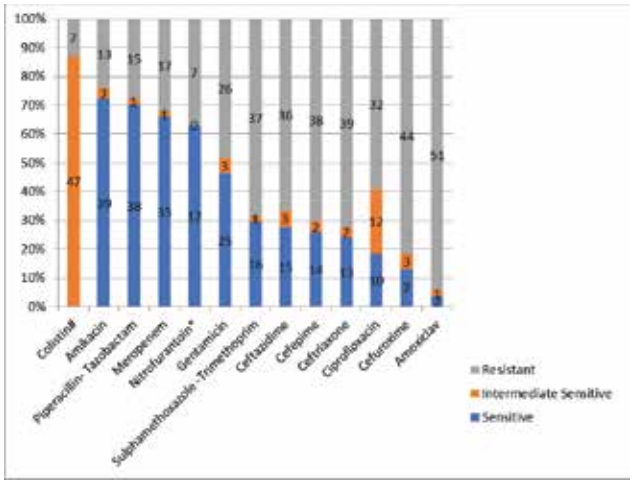

Figure V: Antibiotic Susceptibility Pattern of Isolated *Klebsiella pneumoniae* (N=54) (* Nitrofurantoin was used for 19 uropathogens only; # Colistin susceptibility was performed by colistin agar test)

Discussion

In this one-year cross-sectional study, *Klebsiella pneumoniae* was confirmed in 16.9% of 320 clinical specimens with a culture positivity rate of 63.4%, and notably, 12.9% of *Klebsiella pneumoniae* isolates demonstrated phenotypic colistin resistance. These findings underscore the persistent burden of *Klebsiella* infections in both community and healthcare settings and highlight the emerging challenge of dwindling antimicrobial options.

Our overall culture positivity aligns closely with prior reports from Bangladesh[17,20] and India[21] though outpaced studies[22]. Variation across studies can likely be attributed to differences in regional epidemiology, sample types, and laboratory procedures, as seen in comparisons with Rwandan data[23]

The predominance of single-pathogen isolates (96.6%) is consistent with observations from Nigeria[24] and Rwanda23. E. coli remained the most frequently isolated pathogen (30%), followed by *Klebsiella spp*. (28.6%). These numbers correspond to regional trends[17,20], reinforcing the importance of accurate species identification. Indeed, PCR confirmation showed high consistency fo*r K. pneumoniae* (90%), highlighting gaps in reliance on phenotypic assays.

Antimicrobial susceptibility testing revealed high resistance to amoxiclav (94.4%) and cefuroxime (81.5%), consistent with findings from Iran[25] and Cameroon[36]. Resistance across other agents including cephalosporins, co-trimoxazole, and ciprofloxacin underscores the overuse of these drugs in both clinical and outpatient settings.

Resistance to aminoglycosides (24.1%) and meropenem (31.5%) showed less resistance in this study, but, the percentage was also high enough to consider the loss of reliable treatment options. Alarmingly, 12.9% of *K. pneumoniae* isolates were phenotypically resistant to colistin (MICs up to 32 µg/ml), which is worrisome given its status as a decentralized last-line agent finding that echo recent local and international reports[17,20,27]. Traditional disc diffusion methods fail to detect such resistance accurately, underscoring the value of MIC-based tests in resource-limited settings.

Nonetheless, the study has limitations: data were collected from a single center, limiting generalizability; molecular mechanisms of resistance were not investigated; and the cross-sectional design precludes assessment of temporal trends or causality.

Future research should expand surveillance across diverse healthcare settings, incorporate molecular resistance profiling, and assess environmental reservoirs, particularly in agriculture, which may serve as reservoirs for resistant strains.

Conclusion

In summary, *Klebsiella pneumoniae* remains a prominent and increasingly drug-resistant pathogen in this clinical setting. The detection of multidrug resistance, specially resistance to the cephalosporin group of drugs albeit at high prevalence, is an urgent red flag for clinicians and policymakers. Addressing this threat will require strengthened antimicrobial stewardship, enhanced diagnostic capacity, and coordinated surveillance efforts.

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Conflict of Interest

The authors declare that they have no competing interests.

Financial Disclosure

No external funding was received.

Authors' contributions
The study was conceptualized by Fouzia Ahmed Chowdhury, Abul Kalam and AHM Saiful Karim Chowdhury. Data collection was conducted by Fouzia Ahmed. Formal analysis was done by Milupa Nasrin, Swagata Nandy and methodology were prepared by Fouzia Ahmed Chowdhury, Pompy Dey and Sahed Uddin Ahmed. Laboratory work was performed by Dr. Fouzia Ahmed Chowdhury. Pompy Dey and Ayesha Ahmed Khan drafted the manuscript. Dr. Pompy Dey and Dr. Shaheen Akhter Chowdhury reviewed and edited the final draft. All authors revised the content and approved the manuscript.

Data Availability
Data are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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