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ORIGINAL ARTICLE

Multidrug Resistance in *Klebsiella*: Phenotypic and Genotypic Insights from a Tertiary Care Hospital in India

Sathiya M, Lakshmi Priya N, Ramani C P, Saravana Priya J K

Abstract

Background: Antimicrobial resistance in *Klebsiella* species is a major global health concern, particularly in hospitals where multidrug-resistant strains are prevalent. The emergence of drug resistance has severely limited therapeutic options and increased patient morbidity and mortality. **Aim**: To detect multidrug-resistant *Klebsiella* species from clinical specimens and determine their resistance mechanisms using phenotypic and genotypic methods. **Methods**: A cross-sectional study was conducted at the Institute of Microbiology, Madras Medical College, Chennai, for 12 months . Two hundred clinically significant, consecutive, non-duplicate isolates of *Klebsiella* spp. were identified by standard procedures. Antimicrobial susceptibility was tested by Kirby-Bauer disc diffusion and E-test methods. Phenotypic detection of ESBL, AmpC, MBL, and KPC was performed, and resistant isolates were confirmed by PCR for the blaKPC gene. **Results**: Of 200 isolates, *K. pneumoniae* subsp. *aerogenes* (48%) was most common, followed by *K. oxytoca* (46%) and *K. pneumoniae* subsp. *pneumoniae* (6%). ESBL production was detected in 59.5%, AmpC in 6.5%, MBL in 8%, and KPC in 6%. Enzyme co-production was observed in 12.5%. Mortality among patients with blaKPC-positive isolates was 25%. **Conclusion**: Multidrug resistance in *Klebsiella* spp. is widespread. Early detection and strict antimicrobial stewardship are essential to prevent therapeutic failures and limit hospital transmission.

Key Words

Klebsiella Pneumoniae, Antimicrobial Resistance, ESBL, Carbapenemase, Multidrug Resistance

Introduction

Klebsiella species are opportunistic Gram negative pathogens that have become a major public health concern due to their ability to acquire and disseminate antimicrobial resistance determinants.[1] The widespread use of â lactam antibiotics, especially third generation cephalosporins, has driven the rise of ESBLs, AmpC, MBLs, and KPCs, severely limiting treatment options.[2] Reports from India and Asia show carbapenem resistant Klebsiella prevalence of 20–50% in tertiary hospitals, underscoring the need for surveillance and stewardship.[3,4]

Multiple resistance mechanisms often coexist, complicating detection and therapy; phenotypic methods may miss determinants while molecular techniques, though accurate, are resource intensive.[5] However, comprehensive data from Indian tertiary hospitals linking species distribution, resistance mechanisms, and clinical outcomes is scarce, leaving a critical gap in local

Institute of Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai, Tamil Nadu, India.

Correspondence to: Saravana Priya JK, Post-graduate Microbiology, Madras Medical College, Rajiv Gandhi Government General Hospital (RGGGH), Chennai, Tamil Nadu, India.

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epidemiology. Early identification of resistance patterns is essential to guide empirical therapy and prevent spread.[6]

This study aimed to detect multidrug resistant Klebsiella species from clinical specimens and characterize their resistance mechanisms using phenotypic and genotypic approaches.

Aim

To detect multidrug resistant *Klebsiella* species from clinical specimens and characterize their resistance mechanisms using phenotypic and genotypic methods.

Objectives

Primary:

- Identify clinically significant isolates and determine species distribution.
- Assess antimicrobial susceptibility and detect ESBL, AmpC, MBL, and KPC mechanisms.
- Confirm carbapenemase production by PCR for *blaKPC*.

Secondary:

- Evaluate demographic, clinical, and ward wise distribution.
- Analyse associated risk factors (ICU stay, prior hospitalization, comorbidities).
- Study enzyme co production patterns.
- Correlate phenotypic and genotypic detection of KPC.
- Assess clinical outcomes, including mortality.

Material and Methods

Study design and setting:

This cross sectional study was conducted in the Department of Microbiology, Madras Medical College, Chennai, over 12 months. The hospital is a tertiary care centre serving a large patient population. A total of 200 consecutive, non duplicate, clinically significant *Klebsiella* isolates were collected from blood, urine, respiratory secretions, pus, and wound swabs of patients admitted across multiple wards, including ICU, orthopaedics, medicine, and surgery. Only clinically significant isolates were included; contaminants were excluded. Ethical approval was obtained.

Isolates were identified by standard microbiological methods. Antimicrobial susceptibility was tested by Kirby Bauer disc diffusion and E test, interpreted according to CLSI M100:2025 guidelines. Resistance mechanisms were detected by combined disc method for ESBLs, cefoxitin screening with AmpC disc test, imipenem EDTA combined disc method for MBLs, and Modified Hodge Test with PCR confirmation of *blaKPC*.

Data were analysed using descriptive statistics in Microsoft Excel. Prevalence was expressed as percentages, and associations between resistance mechanisms and clinical outcomes were assessed using chi square test ($p < 0.05$). Confidence intervals (95% CI) and effect sizes were calculated wherever applicable to assess the precision and strength of associations. Sample size was calculated as 196 (based on 50% prevalence, 95% confidence, 7% precision), and 200 isolates were included, fulfilling the requirement.

Results

A total of 200 clinically significant, non duplicate multidrug resistant *Klebsiella* isolates were studied.

Demographics: Male predominance (71%). Most isolates were from patients aged 41–60 years (44.5%), followed by 21–40 years (30.5%). No significant age difference ($\chi^2 = 2.14$, $p = 0.34$).

Specimen distribution: Predominantly pus (38%) and urine (24.5%), followed by sputum (12%) and body fluids (9.5%). Pus and urine predominance was significant ($\chi^2 = 45.6$, $p < 0.001$) (Table 1).

Ward distribution: ICU contributed the highest isolates (29%), followed by orthopaedics (16.5%) and surgery (13.5%). ICU isolates were significantly higher ($\chi^2 = 52.3$, $p < 0.001$) (Table 1).

Risk factors: ICU stay (58 cases) and prior hospitalization (42) were strongly associated with MDR *Klebsiella* ($\chi^2 = 28.7$, $p < 0.001$, OR = 3.2, 95% CI: 1.9–5.4). Diabetes (37) and surgery/trauma (30) were also frequent (Table 2).

Species distribution: *K. pneumoniae* subsp. aerogenes (48%) and *K. oxytoca* (46%) were most common; *K. pneumoniae* subsp. pneumoniae (6%) was less frequent. No significant difference between aerogenes and oxytoca ($\chi^2 = 0.16$, $p = 0.68$).

Clinical profile: Aerogenes and oxytoca were mainly from wound infections; *K. pneumoniae* subsp. pneumoniae was significantly associated with respiratory infections (Fisher's exact test, $p = 0.041$).

Antibiotic susceptibility: Carbapenems and amikacin retained highest activity; cephalosporins showed poor sensitivity. Significant species differences noted for cefotaxime, ciprofloxacin, cotrimoxazole, gentamicin, amikacin, piperacillin tazobactam, and imipenem ($p < 0.05$) (Table 3).

Imipenem resistance: 27.5% resistant by disc diffusion. MIC confirmed high level resistance (33 isolates MIC = 32 µg/ml). Distribution significant ($\chi^2 = 21.9$, $p <$

Table 1: Demographic and clinical specimen distribution of Klebsiella isolates (n=200)

Variable	Category	No. of patients (%)	Statistical analysis
Gender	Male	142 (71)	-
	Female	58 (29)	-
Age (years)	18–20	35 (17.5)	$\chi^2 = 2.14$, $p = 0.34$ (Not significant)
	21–40	61 (30.5)	
	41–60	89 (44.5)	
	>60	15 (7.5)	
Specimens	Pus	76 (38)	$\chi^2 = 45.6$, $p < 0.001$ (Significant)
	Urine	49 (24.5)	
	Sputum	24 (12)	
	Body fluids	19 (9.5)	
	Others (TA, devices, CSF, blood)	32 (16)	
Ward distribution	ICU	58 (29)	$\chi^2 = 52.3$, $p < 0.001$ (Significant)
	Orthopaedics	33 (16.5)	
	Surgery	27 (13.5)	
	Urology/Nephrology	26 (13)	
	Others	56 (28)	

0.001).

Resistance mechanisms:

- ESBL: 59.5% ($\chi^2 = 152.4$, $p < 0.001$).
- AmpC: 6.5% (Fisher's exact test, $p = 0.031$).
- KPC: 6% (Fisher's exact test, $p = 0.004$).

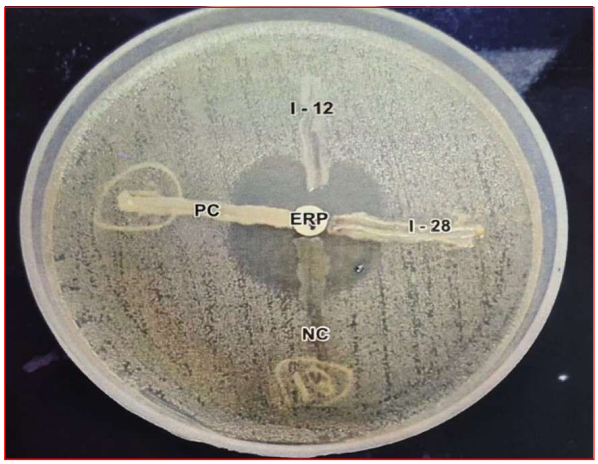

Figure 1: Modified Hodge Test showing Cloverleaf Growth Around Carbapenem Disc, indicating Carbapenemase Production.

- MBL: 8% (Fisher's exact test, $p = 0.045$).
- Co production: 12.5% (ESBL+AmpC most frequent, 40%) (Table 4).

Molecular characterization: Of 40 Modified Hodge Test positive isolates, 16 were phenotypically KPC producers, and 12 were *blaKPC* positive. Correlation

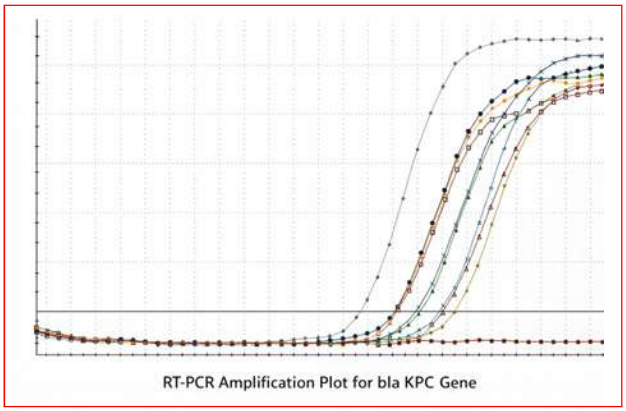

Figure 2: Gel Electrophoresis of bla KPC RT-PCR Products. Amplified Bands Indicate Positive Samples; Lane Intensity Reflects Template Concentration.

Table 2: Risk factors associated with multidrug-resistant Klebsiella infections (n=200)

Risk factor	No. of isolates	Percentage (%)	Statistical analysis
ICU stay	58	29	$\chi^2 = 28.7$, $p < 0.001$ (Significant)
Previous hospitalisation	42	21	$\chi^2 = 18.2$, $p < 0.001$ (Significant)
Diabetes mellitus	37	18.5	$\chi^2 = 12.6$, $p = 0.001$ (Significant)
Surgery/trauma	30	15	$\chi^2 = 9.4$, $p = 0.002$ (Significant)
Chronic illness (TB/COPD)	20	10	Not significant
Alcoholism/chronic liver disease	7	3.5	Not significant
Malignancy	6	3	Not significant

Table 3: Species distribution, clinical infection profile, and antibiotic susceptibility (n=200)

Species	No. (%)	Common infections	Key antibiotic sensitivity (%)	Statistical analysis
K. pneumoniae subsp. aerogenes	96 (48)	Wound, respiratory, urinary	Ciprofloxacin(66), Gentamicin(48), Amikacin(80), Imipenem(67)	χ^2/Fisher's: Significant for CIP (p=0.005), GEN (p=0.006), AK (p=0.011), IPM (p=0.045)
K. oxytoca	92 (46)	Wound, respiratory, urinary	Ciprofloxacin(41), Gentamicin(67), Amikacin(70), Imipenem(79)	χ^2/Fisher's: Significant for CTX (p=0.035), COT (p=0.043), PIT (p=0.012)
K. pneumoniae subsp. pneumoniae	12 (6)	Respiratory	Poor sensitivity across all drugs	Fisher's exact test: respiratory association (significant) p=0.041

Table 4: Resistance mechanisms and molecular characterisation (n=200)

Mechanism	Isolates	Percentage (%)	Statistical analysis
ESBL	119	59.5	$\chi^2 = 152.4$, $p < 0.001$ (Significant)
AmpC	13	6.5	Fisher's exact, $p = 0.031$ (Significant)
MBL	16	8.0	Fisher's exact, $p = 0.045$ (Significant)
KPC	12	6.0	Fisher's exact, $p = 0.004$ (Significant)
Enzyme co-production	25	12.5	$\chi^2 = 0.032$, $p < 0.05$ (Significant)
Other mechanisms	15	7.5	Not significant
Molecular confirmation	12 blaKPC positive	-	$\chi^2 = 18.6$, $p < 0.001$ (Significant)
Clinical outcome	3 deaths among 12 blaKPC cases	25% mortality	$\chi^2 = 4.21$, $p = 0.04$ (Significant)

significant ($\chi^2 = 18.6$, $p < 0.001$) (Figure 1).

Clinical outcomes: Among 12 patients with *blaKPC* positive isolates, 3 died (mortality 25%). Mortality was significantly higher compared to non KPC infections ($\chi^2 = 4.21$, $p = 0.04$, OR = 2.8, 95% CI: 1.1–7.2) (Figure 2). Although based on a small subgroup (n = 12), this represents the entire cohort of genotypically confirmed *blaKPC* infections during the study period. Effect size and confidence intervals were reported to strengthen interpretability, but findings should be interpreted cautiously and validated in larger studies.

Discussion

Klebsiella species are opportunistic Gram negative pathogens that have rapidly emerged as a major public health concern due to their ability to acquire and disseminate resistance determinants.[7–9] In this study of 200 multidrug resistant isolates, males (71%) and middle aged adults (41–60 years, 44.5%) were most affected. Pus (38%) and urine (24.5%) were the predominant specimens, while ICU patients accounted for the highest proportion (29%), consistent with earlier reports linking invasive procedures and prolonged hospital stays to increased risk.[10–13]

Species distribution showed *K. pneumoniae* subsp. aerogenes (48%) and *K. oxytoca* (46%) as predominant, with *K. pneumoniae* subsp. pneumoniae (6%) less frequent. Resistance was highest to third generation cephalosporins (82–89%), while amikacin (80%) and carbapenems (67–78%) retained moderate activity. Ciprofloxacin resistance was lower than regional data, and amikacin consistently outperformed gentamicin, reflecting global trends.[14–20]

ESBL prevalence was 59.5%, AmpC 8.4%, and carbapenem resistance 27.5%. Imipenem resistance was higher in *K. pneumoniae* (33%) than *K. oxytoca* (21%). MHT confirmed carbapenemase in 40 isolates, though 15 were negative, likely due to ESBL/AmpC overproduction with porin loss. MBL was detected in 8%, KPC in 6%, both lower than other reports.[21,22] Importantly, 25 isolates (12.5%) showed co production of enzymes, complicating detection and therapy, consistent with other studies.[23]

The coexistence of diverse mechanisms highlights diagnostic challenges and therapeutic limitations. Mortality among patients with KPC producing infections was 25%, underscoring clinical impact despite the small subgroup size. This analysis, though limited, reflects the entire cohort of genotypically confirmed *blaKPC* infections, with effect size and confidence intervals strengthening interpretability. Current therapy remains restricted, with tigecycline and colistin often used, though monotherapy is less effective than combinations. Novel agents, including polymyxin derivatives and â lactamase inhibitor combinations, may provide future options.

Overall, this study emphasizes the urgent need for early detection of ESBL, AmpC, MBL, and KPC enzymes, strict antimicrobial stewardship, and robust infection control measures to prevent treatment failures and limit the spread of multidrug resistant *Klebsiella* in healthcare settings.

Conclusion

This study highlights the burden of multidrug resistant *Klebsiella* in critical care, with *K. pneumoniae* subsp. aerogenes predominant. High rates of ESBL, AmpC, MBL, and KPC—including 12.5% co production—reflect complex resistance and diagnostic challenges. The link between MBL/KPC and mortality underscores the need for early detection, surveillance, and strict antimicrobial stewardship. Robust infection control and judicious antibiotic use remain vital to curb the spread of these highly resistant strains.

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