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IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Phenotypic detection of β-lactamases producing *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolated from the respiratory tract infections

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Abstract

Introduction: With limited treatment options and emerging antimicrobial resistance amongst bacterial pathogens in hospital settings, the alarming condition needs to be addressed.
Aim and Objective: The study aimed to determine the antibiotic susceptibility (AST) patterns, and extended spectrum β-lactamases (ESBL) and Metallo β-lactamases (MBL) production in *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolated from respiratory tract infections (RTI).
Materials and Methods: Current study, 1624 samples (sputum, tracheal secretions, suction tip) were collected during June 2020 to September 2022, phenotypically characterized by antibiotic sensitivity test (AST), Extended Spectrum Beta lactamases and Metallo Beta lactamases detection.
Results: Of 12.3% (200 isolates), recorded prevalence rate of *K. pneumoniae* was 10.3% while, *A. baumannii* showed 1.9%. Following AST, *K. pneumoniae* displayed highest resistance against Ampicillin (95%), followed by Amoxycillin/clavulanic acid (91%), lowest recorded susceptibility for Tigecycline (39.2%). While, *A. baumannii* expressed susceptibility to Carbapenem group and Ciprofloxacin (84.3%), lowest Tigecycline (50%). Overall study exhibited Colistin retaining its activity with 100% sensitivity. ESBL production in *K. pneumoniae* was 31% and 0% in *A. baumannii.* While, MBL producers was 11 %, *K. pneumoniae* and *A. baumannii* recorded at 9.5% and 25% respectively.
Conclusion: The increasing rates of ESBL producers is concerning, and the current study highlights use of Colistin as the best available antimicrobial for treating such RTI pathogens. Therefore, continuous monitoring of antibiotic-resistant profiles will help to guide effective antimicrobial therapy and management.

Keywords: Antimicrobials, ESBL, MBL, Antibiotic susceptibility test, Colistin.

Received: 05-06-2025; **Accepted:** 20-09-2025; **Available Online**: 19-11-2025

1. Introduction

Respiratory tract infections (RTIs), being the utmost common and frequented infection causes mortality and morbidity in critically ill patients worldwide.[1] RTIs are categorized into upper and lower respiratory tract infections in humans, that are frequently demonstrated by common cold, sore throat, otitis media, sinusitis, and mastoiditis.[2] An estimated 20 % mortality had been reported amongst the infectious diseases in India.[3,4] *K. pneumoniae* is a causative agent of pneumonia, bacteremia, urinary tract infections and myocarditis.[5,6] An encapsulated facultative anaerobe, it possess numerous virulence factors, like endotoxins, cell wall receptors, iron scavenging systems that complicates and restricts treatment options.[7] *A. baumannii*, an opportunistic pathogen in humans with 35 % mortality rate, is a common cause of nosocomial pneumonia in intensive care units (ICU).[8] These pathogens show high prevalence rates of multidrug resistance (MDR) due to intrinsic and acquired factors associated with mortality and morbidity, challenging treatment options.[9,10] The mechanisms involving bacterial antimicrobial resistance

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https://doi.org/10.18231/j.ijmmtd.11625.1758782202

include drug alterations or target sites, low permeability of cell wall, expression of efflux pumps along with resistance genes acquired by horizontal gene transfer (HGT). In recent years, MDR strains have generated with extensive use of antibiotics in hospital environment.[11,12] Though, carbapenems have been typically used for treatment of infectious diseases, emergence of carbapenem-resistant strains has been associated with a higher treatment failure risk.[13,14] Nevertheless, with limited treatment options for MDR causing infectious diseases irrespective of mechanism, there is a limiting ability for developing newer therapeutic agents that has posed a threat to human health.[15] Fewer antibiotics like colistin; a multi-competent polypeptide, is relatively an older polymyxin group, that acts on bacterial cell membrane resulting in increased permeability and cell content leakage leading to cell death.[16] However, use of colistin is limited due to its side effects such as nephrotoxicity and neurotoxicity with antibiotic resistance.[17] Recent years have considered using colistin to treat infectious diseases due to lack of newer ones as resistance to colistin is low.[18]

β-lactam resistance are associated with Extended spectrum β- lactamases (ESBL) that hydrolyze oxyimino β lactams, including cefotaxime, ceftriaxone, ceftazidime, and monobactams without affecting cephamycin, carbapenems, and related compounds.[19] While, *Klebsiella pneumoniae* carbapenem (KPC) β- lactamases mostly found in MDR- *K. pneumoniae* was demonstrated in higher levels and also can exist in *Enterobacter* and *Salmonella* species too.[20,21] Metallo-β-lactamases (MBL) account for 10% of the β-lactamases, which are relevant because they have the capability to hydrolyze the carbapenems. MBLs resistance mechanism in carbapenem-resistant enterobacteriaceae (CRE), is frequented in carbapenems-resistant non-fermentative bacteria such as *A. baumannii* and *P. aeruginosa*, represents challenging chemotherapy against them.[22] The objective of the present study was to determine the incidence of antibiotic profiling and production of β lactamases in *K. pneumoniae* and *A. baumannii* isolated from the RTIs.

2. Materials and Methods

About 1624 clinical samples were collected from patients with signs and symptoms of respiratory tract infections (RTI patients had not started antibiotic therapy) attending Santosh hospitals, Bengaluru. The samples included tracheal aspirates, sputum, and suction tip from both genders. The patient's consent was ensured for the study and the information was maintained confidential. Verbal consents were taken from the patient's relatives, participant's cooperation was solicited, and the ethical approval was obtained from the hospital.

2.1. Isolation and characterization of K. pneumoniae and A. baumannii isolates

The collected samples were processed by inoculating onto the Chocolate agar, Blood agar and MacConkey agar media, and incubated aerobically at 37 ºC for 24-48 h, except for the chocolate agar that was incubated for 24-48 h at 37ºC in an atmosphere of 5-10 % CO_2. Subsequently after incubation, the isolates were purified and their cultural and morphological characteristics were analysed.[23]

2.2. Antimicrobial susceptibility testing (AST)

The AST was detected using the Kirby-Bauer disc diffusion assay.[24] About 100 µl of each standardized McFarland's constant bacterial cultures suspension ($2×10^6$ cfu/ ml) was spread using a sterile glass spreader on Muller Hinton plates (MHA) (Hi-Media, Mumbai) and allowed to set for 5 min. The antibiotics used in the following study included ampicillin (10 mcg), Piperacillin/tazobactam (100/10 mcg), Cefuroxime axetil (30 mcg), Ceftriaxone (30mcg), Cefoperazone/sulbactam (75/10 mcg), Cefepime (30 mcg), Ertapenem (10 mcg), Imipenem (10 mcg), Meropenem (10 mcg), Amikacin (30 mcg), Gentamicin (10 mcg), Ciprofloxacin (05 mcg), Tigecycline (15 mcg), Colistin (10 mcg), and Trimethoprim/sulfamethoxazole (1.25/23.75 mcg). These antibiotic discs were placed aseptically and individually on the surface of the seeded MHA plates, and incubated at 37ºC for 24 h. Following incubation, the results were interpreted using zone scale and percent inhibition was analyzed as per NCCLS guidelines.[25]

2.3. Detection of Extended spectrum β-lactamase producers (ESBL)

The isolates were further screened for ESBL production using a double disc synergy assay as recommended by CLSI guidelines.[26,27] The bacterial colonies were grown in Luria Bertani broth (LB) and growth was adjusted to 0.5 McFarland's standards ($2×10^6$ cfu/ ml). With sterile cotton swab, the inoculum of each isolate was spread individually onto the MHA plates. The discs of cefotaxime (30 mcg) and ceftazidime (30 mcg) individually along with each of these in combination with clavulanic acid (10 mcg) were placed at 20 mm apart and incubated at 37^0C for 18 - 24 h. After incubation, the results were analyzed and an increased zone of inhibition diameter by ≥ 5mm around the combined discs with clavulanic acid as compared to the single antibiotic was considered as ESBL producers. *Klebsiella pneumoniae* ATCC 700603 was used as a standard.[28]

2.4. Detection of Metallo-β-lactamase producers

The imipenem (IMP) resistant isolates were further screened for MBL production by phenotypic detection assay using imipenem-ethylenediaminetetraacetate (IMP-EDTA) combined disc as described by Yong et al.[29] *Pseudomonas aeruginosa* ATCC 27853 was used as a control. The tested inoculum was swabbed on MHA plates as recommended by

CLSI guidelines of 2019[30] and allowed to stay at room temperature for 5 min. Two discs, one with 0.5M EDTA +Imipenem and the other with Imipenem only were placed on the surface of MHA seeded plates at 30 mm apart. The plates were incubated at 37^{0}C for 24 h. The developing inhibition zone of the combined IMP-EDTA disc with a zone diameter of ≥ 7 mm more than the Imipenem disc alone was considered as MBL positive.[29]

2.5. Statistical analysis

The data was further analyzed by using Graphpad Prism software. One-way analysis of variance (ANOVA) was applied to determine any statistically significance of the following data, Wilcoxon Signed-Rank Test and Chi-square Test.

3. Results

3.1. Isolation and characterization of K. pneumoniae and A. baumannii isolates

A total of 1624 clinical samples were collected during June 2020 to September 2022 from the patients with signs and symptoms of RTI. Following screening method on the Chocolate agar, Blood agar and MacConkey agar media, *K. pneumoniae* were mucoid, lactose fermenter, circular, dome shaped, on blood agar pink coloured, non-hemolytic colonies. While, *Acinetobacter baumannii* were very small, pale coloured mucoid colonies on MacConkey agar and non-hemolytic colonies on blood agar (**Figure 1a** and **b**). Both the organisms were Gram negative bacilli and non-motile.

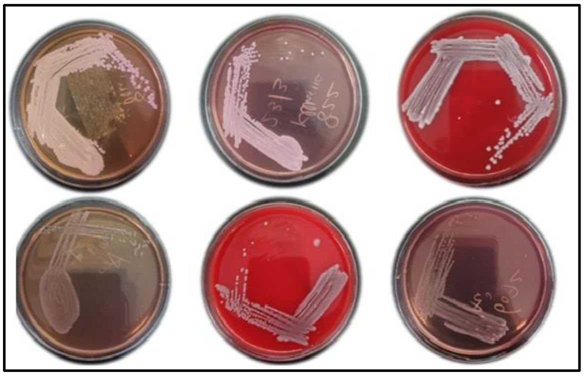

Figure 1: a and **b:** Colony Characteristics of *Klebsiella pneumoniae* and *Acinetobacter baumannii*

A total 12.3 % (n=200) isolates of *K. pneumoniae* and *A. baumannii* were obtained with a prevalence rate of 10.3% and 1.9%, (**Table 1**). The incidence of both the isolates had been shown with respect to gender, revealing incidence of 65% in male as compared to 35% in females.

The incidence with respect to different age groups denoted in the present study is depicted in **Table 3**. The highest prevalence rate of 30% was found in age group of 30-35 years with a carriage rate of 3.69 %, followed by 25-30 years of age group with 19.5% incidence and 2.40% carriage rate. The lowest prevalence was observed with 0-5- and 65-70-years age group with 2.5% as the carriage rate accounted for 0.30% (**Figure 2**). **Table 2**, indicates Chi-square statistics (χ^2) of 0.0062 with p-value being 1.0. Hence, no statistically significant difference was observed with respect to the age group incidence rates that closely coordinated the overall population distribution.

Table 1: Incidence of *K. pneumoniae* and *A. baumannii* observed in clinical samples with respect to gender

S.No.	Gender	No. of positive isolates (n=200)	% incidence (n= 200)	Overall % incidence (n=1624)
1	Male	130	65	8.004
2	Female	70	35	4.31

Table 2: Incidence of *K. pneumoniae* and *A. baumannii* in different age groups

S.No	Age group (years)	No. of positive isolates (n=200)	% incidence (n= 200)	Overall % incidence (n=1624)
1	0-5	5	2.5	0.30
2	15-20	10	5	0.61
3	20-25	20	10	1.23
4	25-30	39	19.5	2.40
5	30-35	60	30	3.69
6	40-45	11	5.5	0.67
7	45-50	8	4	0.49
8	50-55	6	3	0.36
9	55-60	10	5	0.61
10	60-65	20	10	1.23
11	65-70	5	2.5	0.30
12	70-75	6	3	0.36

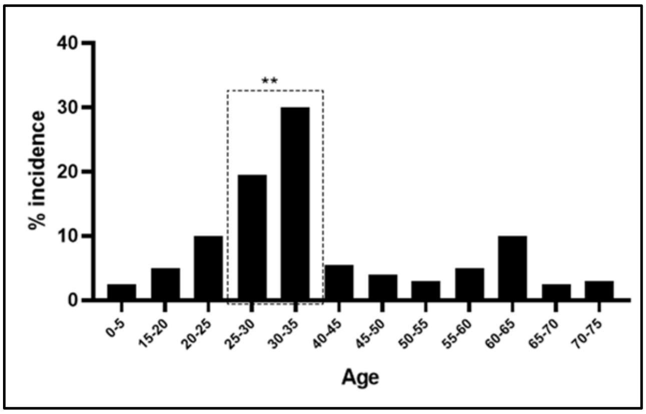

Figure 2: ** P < 0.01 for incidence of *K. pneumoniae* and *A. baumannii* in different age wise distribution associated with RTI pathogens groups, Two-tailed t-test, Prism GraphPad.

This study highlights the prevalence of antibiogram of *K. pneumoniae* and *A. baumannii* causing RTIs as highest prevalent infectious illnesses prevalent diseases. The antimicrobial susceptibility patterns for *K. pneumoniae* are as shown in the **Table 3**. The percent susceptibility patterns were found to highest for Ampicillin recording 95% followed by Amoxycillin/clavulanic acid (91%) and Piperacillin/tazobactam (78.5%). Lowest resistance rate was observed for Tigecycline with 39.2% (**Figure 3**). While, resistance to other antibiotics was found to be more than 50%, signifying an alarming situation with the prescribed antibiotics, including those from the class aminoglycosides, carbapenems, cephalosporin groups, and fluroquinolones. Interestingly, none of the tested bacterial isolates was resistant to Colistin showing 100% sensitivity to this antibiotic.

Table 3: Percent resistance of *K. pneumoniae* (n=168) isolated from the clinical samples

Class of antibiotics	Name	Concentration (mcg/ disc)	(No. of isolates) % R	(No. of isolates) % S
Penicillin & β-lactamase inhibitors	Ampicillin	10	(160) 95.2%	(8) 4.8%
	Piperacillin/tazobactam	100/10	(132) 78.5%	(36) 21.5%
	Amoxycillin/clavulanic acid	20/10	(153) 91.0%	(15) 9%
2nd Generation Cephalosporins	Cefuroxime	30	(120) 71.4%	(48) 28.6%
	Cefuroxime Axetil	30	(120) 71.4%	(48) 28.6%
3rd Generation Cephalosporins	Ceftriaxone	30	(120) 71.4%	(48) 28.6%
	Cefoperazone/Sulbactam	75/10	(112) 66.6%	(56) 33.4%
4th Generation Cephalosporins	Cefepime	30	(117) 69.6%	(51) 30.3%
Carbapenems	Ertapenem	10	(88) 52.3%	(80) 47.7%
	Imipenem	10	(88) 52.3%	(80) 47.7%
	Meropenem	10	(88) 52.3%	(80) 47.7%
Aminoglycoside	Amikacin	30	(97) 57.7%	(71) 42.3%
	Gentamicin	10	(97) 57.7%	(71) 42.3%
Fluoroquinolone	Ciprofloxacin	05	(127) 75.5%	(41) 24.5%
Glycylcycline	Tigecycline	15	(66) 39.2%	(102) 60.8%
Polypeptide	Colistin	10	(0) 0	(168) 100%
Sulfonamides	Trimethoprim/Sulfamethoxazole	1.25/23.75	(104) 61.9%	(64) 38.1%

Table 4: Percent resistance of *Acinetobacter baumannii* (n=32) isolated from the clinical samples

Class of antibiotics	Name	Concentration (µg/ disc)	(No: of isolates) % R	(No. of isolates) % S
Penicillin & β-lactamase inhibitors	Piperacillin/tazobactam	100/10	(18) 56.2%	(14) 43.8%
3rd Gen. Cephalosporins	Cefoperazone/sulbactam	75/10	(20) 62.5%	(12)37.5%
Carbapenems	Ertapenem	10	(27) 84.3%	(5) 15.6%
	Imipenem	10	(27) 84.3%	(5) 15.7%
	Meropenem	10	(27) 84.3%	(5) 15.7%
Fluoroquinolone	Ciprofloxacin	05	(27) 84.3%	(5) 15.7%
Glycylcycline	Tigecycline	15	(16) 50%	(16) 50%
Polypeptide	Colistin	10	(0) 0	(32)100%
Sulfonamides	Trimethoprim/Sulfamethoxazole	1.25/23.75	(20) 62.5%	(12) 37.5%

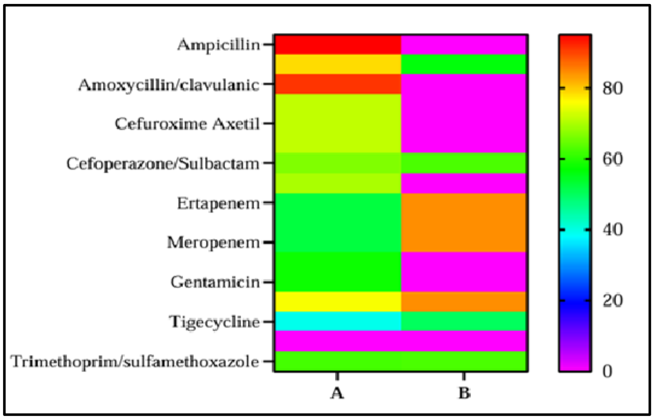

Figure 3: Heat map representing the percent resistance of *K. pneumoniae* (n=168) and *A. baumannii* (n=32) isolated from RTI samples.

The percent resistance of *A. baumannii* isolated from RTIs showed varying results. Highest resistance rate was observed with carbapenem group that included Meropenem, Imipenem, Ertapenem along with Ciprofloxacin recording at 84.3%, followed by 3rd generation Cephalosporins and Trimethoprim/sulfamethoxazole with 62.5%. Lowest resistance rate was observed in Tigecycline with 50% that had been reported but not recommended. All the isolates were found to be 100% sensitive towards Colistin (**Table 4** and **Figure 3**). Following, heat map represents the percentage resistance (%R) of *K. pneumoniae* (n = 168) [Column A] and *Acinetobacter baumannii* (n = 32) [Column B] to various antibiotics used in the study. A higher resistance (darker color) was observed for ampicillin, amoxicillin/clavulanic acid, cefuroxime axetil, and trimethoprim/sulfamethoxazole in both the organisms, indicating poor efficacy. While, carbapenem resistance notably higher in *A. baumannii* than in *K. pneumoniae*, with meropenem and ertapenem showing moderate effectiveness only in *K. pneumoniae*. However, gentamicin retained moderate activity against *A. baumannii* and higher efficacy in *K. pneumoniae*. The study highlights, tigecycline demonstrating lowest resistance rates in the both species, suggesting it may be a potential treatment option against multidrug-resistant strains.

This study also analyzed multidrug resistance (MDR) of total 200 isolates, 107 (53.5%) were observed in *K. pneumoniae* and 9 (4.5%) in *A. baumannii* with a carriage rate of 10% and 0.8%, respectively. The highest percent of MDR was observed in *K. pneumoniae* with a maximum of 9 antibiotics at 25.5% along with a carriage rate of 4.7%, followed by an incidence rate of MDR with 7.5% in three and seven antibiotics and a carriage rate of 1.4%. The lowest rate of 0.18% was observed for eight antibiotics, followed by 0.5% for three antibiotics with a carriage rate of 0.18 and 0.5%, respectively, as indicated in **Table 5** and demonstrated **Figure 4**.

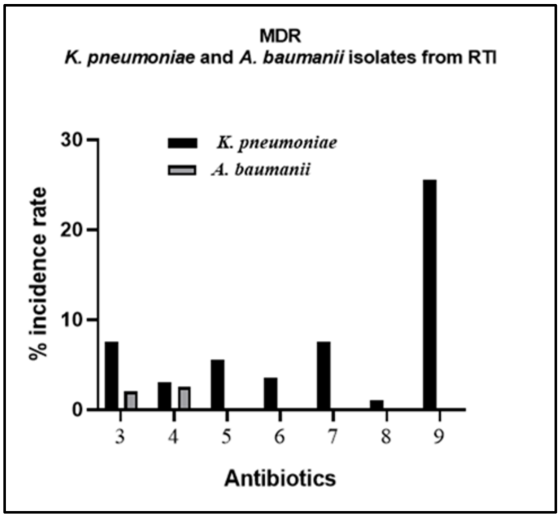

Figure 4: *p<0.05 among *K pneumoniae* and *A baumannii*, MDR strains isolated from RTI. Data analyzed using Wilcoxon signed-rank test using GraphPad Prism.

In *A. baumannii* isolates, MDR was in 3 and 4 antibiotics with prevalence rate of 2 to 2.5%, along with a carriage rate of 0.3 % and 0.4 %, respectively (**Figure 4**). Interestingly, the isolates that showed a maximum of 4 antibiotics and none above are depicted in **Table 5**. The Chi-square statistic (χ^2) rate signifies no statistically significant difference in MDR incidence and carriage rate distributions between *K. pneumoniae* and *A. baumannii* being 0.0 and p-value 1.0.

Table 5: Multidrug resistance in *K. pneumoniae* and *A. baumanii* isolates from RTI

No. of antibiotics	No. of *K. pneumoniae* (n=168)	% Incidence of *K. pneumoniae* (n=200)	% Carriage rate (n=1064)	No. of *A. baumannii* (n= 32)	% Incidence of *A. baumannii* (n=200)	% Carriage rate (n=1064)
3	15	7.5	1.4	4	2	0.3
4	6	3	0.5	5	2.5	0.4
5	11	5.5	1.03	0	0	0
6	7	3.5	0.65	0	0	0
7	15	7.5	1.4	0	0	0
8	2	1	0.18	0	0	0
9	51	25.5	4.7	0	0	0
Total MDR isolates	107	53.5	10.0	9	4.5	0.8

Table 6: Prevalence of ESBL producers in *K. pneumoniae* and *A. baumannii* from different samples of RTI

Samples	*K. pneumoniae* (n=168)	*A. baumannii* (n= 32)	Incidence % (n=200)	Carriage rate % (n=1064)
Sputum	20 (11.9 %)	0 (0)	10	1.8
Suction tip	30 (17.8 %)	0 (0)	15	2.8
Tracheal aspirates	12 (7.14 %)	0 (0)	6	1.1
Total	62 (36.9 %)	0 (0)	62 (31 %)	62 (5.8 %)

Table 7: Incidence of MBL producing *K. pneumoniae* and *A. baumannii* isolated from different RTI samples

Samples	*K. pneumoniae* (n=168)	*A. baumannii* (n= 32)	Incidence % (n=200)	Carriage rate % (n=1064)
Sputum	4 (2.3%)	1 (3.1%)	2.5	0.4
Suction tip	8 (4.7%)	5 (15.6%)	6.5	1.2
Tracheal aspirates	4 (2.3%)	2 (6.2%)	3	0.5
Total	16 (9.5%)	8 (25%)	24 (12%)	24 (2.25%)

The prevalence of extended spectrum β-lactamase producers (ESBL) of *K. pneumoniae* and *A. baumannii* from different clinical samples is denoted in **Table 6**. The analyzed ESBL production in *K. pneumoniae* showed a highest of 15% in suction tip samples, followed by the isolates derived from sputum with 10%, and tracheal aspirates with 6 %. The overall incidence of ESBL production in *K. pneumoniae* was found to be 31% with a carriage rate of 5.8% from all the samples collected. While, *A. baumannii* were non ESBL producers in the present study (**Figure 5**). The Fisher's Exact Test, suggested no statistically significant difference between *K. pneumoniae* and *A. baumannii* incidence across different sample types and limitations could be due to samples numbers. The p-values for sputum (p = 0.218), suction tip (p = 0.080) and tracheal aspirates (p = 0.601).

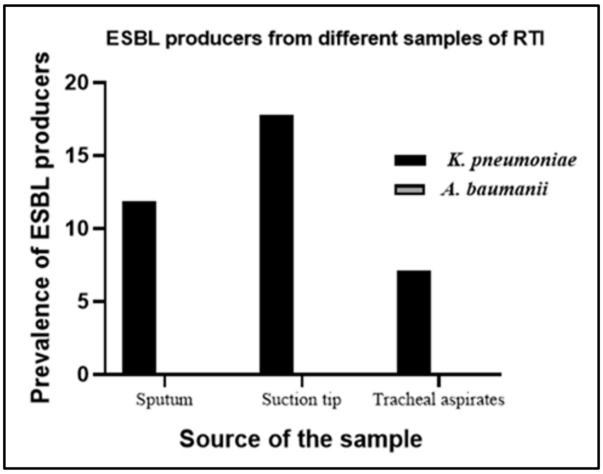

Figure 5: *A. baumannii* isolates were non-ESBL producers as compared to *K. pneumoniae* (No significance in *K. pneumoniae* from different sources). Data analyzed by Two-tailed t-test, Prism GraphPad.

The incidence rate of MBL producing *K. pneumoniae* and *A. baumannii* is shown in **Table 7**. A total of 9.5% *K. pneumoniae* were positive for MBL production, with highest being in suction tip samples with 4.7%, followed by sputum and tracheal aspirates with 2.3%. Subsequently, *A. baumannii* expressed similar results with respect to prevalence rate in the clinical samples, which was highest in suction tip samples with 15.6%, followed by tracheal aspirates and sputum with 6.2 and 2.3% respectively. The overall incidence was found to be 12% for MBL production with a carriage rate of 2.25% (**Figure 6**). The -square statistic showed 0.814 with p-value of 0.666 with no statistically significant difference in MBL production rates between *K. pneumoniae* and *A. baumannii* across different samples.

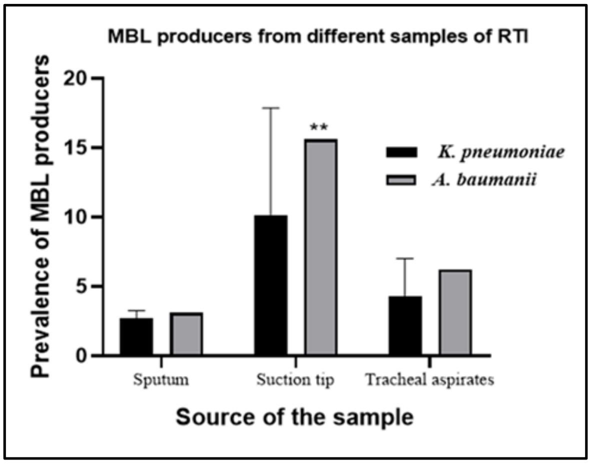

Figure 6: Incidence of Metallo beta lactamases (MBL) producing isolates from different RTI samples. ** P < 0.01 *K. pneumoniae* versus *A. baumannii,* prevalence for producing MBL from suction tip compared to other sources. Data analyzed by Two-tailed t-test, Prism GraphPad.

The overall prevalence of β-lactamases producers of ESBL and MBL has been determined in *K. pneumoniae* and *A. baumannii* (**Figure 7**). Following result analysis revealed 31% of ESBL producers characterized by phenotypic detection, while MBL was 8%, with a carriage rate of 5.8% and 2.8% in *K. pneumoniae*. Meanwhile, *A. baumannii* revealed MBL producers with 12% prevalence rate and 2.25 % carriage rate. The Fisher's Exact Test, p value 1.0 was determined for ESBL incidence and carriage rate while, MBL was p = 1.0 with no statistically significant difference was found between *K. pneumoniae* and *A. baumannii* in terms of ESBL and MBL incidence or carriage rates.

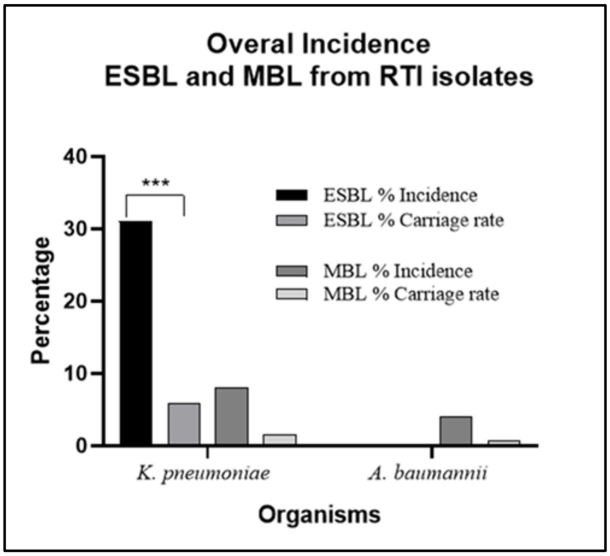

Figure 7: Overall incidence of ESBL and MBL in *K. pneumoniae* and *A. baumannii* isolated from RTIs. *p<0.0001 versus A. baumannii.* Data analyzed using One-way Anova (Brown-Forsythe test), GraphPad Prism.

4. Discussion

Multidrug-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* represents significant threats to the human health, particularly among individuals with compromised immune systems or underlying medication. The treatment of these pathogens often relies on broad-spectrum antibiotics such as carbapenems (*i.e*., Meropenem and Imipenem). However, with the emergence of carbapenemase enzymes (MBLs) from β-lactamase classes D and B has led to rising antibiotic resistance, complicating the treatment strategies globally.[31] Early detection of these resistance genes can help to play a crucial role for managing *such pathogens* in the hospital settings and guiding appropriate antibiotic use.[32] The infectious diseases caused by MDR strains such as *A. baumannii, particularly* carbapenem-resistant strains, pose a major concern in the specialized hospital units and ICUs. These infections primarily affect patients undertaking broad-spectrum antibiotics, those with weakened immune systems, underlying health conditions, and/or undergoing invasive procedures. With limited treatment options and increased resistance antibiotics rates, it often necessitates "last-line" antibiotics, including Tigecycline, Colistin, and Polymyxin B.[33,34] β-lactamases, classified into four molecular classes (A, B, C, and D), contribute significantly to antibiotic resistance. While, β lactamase classification includes the classes A, C, and D is serine-β-lactamases, while class B consists of metallo-β-lactamases (MBLs). Emergence of newer β-lactamases capable of hydrolysing cephamycins, cephalosporins, monobactams, and carbapenems has reduced the treatment options and increased the rates of treatment failure and poor prognosis.[35]

Our study analyzed 1,624 clinical samples, identifying 168 positive isolates of *K. pneumoniae* and 32 positive isolates of *A. baumannii*. We observed a higher incidence in males (8.004%) compared to females (4.31%). The 30–35-year age group had the highest infection prevalence (30%), potentially due to occupational exposure, lifestyle factors, or common underlying conditions. This group also exhibited a 3.69% carriage rate, suggesting a significant number of asymptomatic carriers. The 25–30-year age group had a slightly lower prevalence (19.5%) and a 2.40 % carriage rate, still indicating high infection risk. Conversely, the 0-5- and 65-70-years age groups had lowest prevalence (2.5%) and minimal carriage rate (0.30%), likely attributed to reduced exposure to risk factors.

Our investigation into *K. pneumoniae* from RTIs revealed high susceptibility to Ampicillin (95.2%), Amoxicillin/ clavulanic acid (91%), and Piperacillin/ tazobactam (78.5%). However, resistance to aminoglycosides, carbapenems, cephalosporins, and fluoroquinolones exceeded 50%, thus raising concerns. All isolates were 100% sensitive to Colistin. These findings align with several similar previous studies. Roy et al.,[36] reported that 60% of *Klebsiella* and 75% of *E. coli* isolates from neonates were ESBL producers. A study from India reported high ESBL prevalence in *Klebsiella* (94.87%) and *E. coli* (92%) by Patel et al.,[37] while Vijayakanthi et al.,[38] identified *Klebsiella* as the most common ESBL producer (60%). In this study, carbapenem resistance was observed in 84.3% of *A. baumannii* and 52.3% of *Klebsiella* isolates, in consistence with the emerging reports of increased carbapenem resistance.[39,40]

The resistance profile of *A. baumannii* isolates displayed highest resistance to carbapenems (*i.e*., Meropenem, Imipenem, and Ertapenem) and ciprofloxacin at 84.3%. While, resistance rate towards third-generation Cephalosporins and Trimethoprim/sulfamethoxazole was found to be 62.5%. Tigecycline had lowest resistance at 50%, though not widely recommended. All isolates were 100% sensitive to Colistin in agreement with several previous studies. Smail et al.[41] reported similar findings with MBL-producing *A. baumannii* in various samples. Hans et al.[42] observed resistance to multiple antibiotics such as Erythromycin, Gentamicin, Ceftazidime, and Trimethoprim/ sulfamethoxazole, with sensitivity to Tigecycline and Colistin. While, Farahani et al.[43] also noted high resistance to MDR *Acinetobacter sps* derived Cephalosporinase in the clinical samples derived from the teaching hospital. Peymani et al.[44] found 49% of carbapenem-resistant *A. baumannii* strains produced MBLs. Geographical variations in the resistance rates are significant; resistance to Imipenem and Meropenem ranged from 9.26% in Korea[45] to 49% in Taiwan,[46] 50%-52.4% in China, and 4.5% in Japan,[47] with European rates between 22% and 26%.[48] Our study reported a 56.2% resistance rate to MBLs and 84.3% resistance to third-generation cephalosporins, indicating a more severe resistance profiles compared to the previous reports.

Additionally, this study identified a 9.5% prevalence of MBL-producing *Klebsiella pneumoniae* among RTI samples, with the highest rates in Suction tips (4.7%) and lower rates in Sputum and Tracheal aspirates (2.3% each). For *A. baumannii*, MBL production was more prevalent, with 15.6% in Suction tips, 6.2% in Tracheal aspirates, and 3.1% in Sputum samples, highlighting a higher propensity for MBL production, particularly in the Suction tip samples. This is consistent with Smail et al.[41] who recorded most *A. baumannii* isolates obtained from Sputum samples, with lower rates from Blood and Wound swabs. Studies from Coimbatore and Gurgaon similarly identified high isolation rates from respiratory secretions, reinforcing that the respiratory tract is a common site for these infections.[49,50]

Our study underscores the escalating threat of MDR strains of *A. baumannii* and *K. pneumoniae* in RTIs, highlighting critical resistance patterns that challenge current treatment protocols. *A. baumannii* isolates demonstrated alarmingly high resistance rates to carbapenems; such as Meropenem and Imipenem (84.3%), and third-generation Cephalosporins (62.5%). Furthermore, 56.2% of these isolates were resistant to MBLs, reflecting an advanced level of resistance that complicates the treatment options. In contrast, *K. pneumoniae* showed significant resistance across several antibiotics, however all isolates remained 100% sensitive to Colistin, indicating its continued efficacy despite the increasing rise in drug resistance.

5. Conclusions

Our study reveals significant challenge of MDR- *A. baumannii* and *K. pneumoniae* in RTIs, with *A. baumannii* exhibiting higher resistance rates to Carbapenems and third-generation Cephalosporins with concerning resistance towards MBLs. *K. pneumoniae* showed considerable resistance across various antibiotics, in contrast MBL-producing *A. baumannii* was notably prevalent in suction tip samples. Remarkably, 100 % sensitivity towards colistin with all the tested isolates was observed. However, the resistance profiles reflect alarming situation, emphasizing the necessity for improved surveillance and strict infection control measures. The high prevalence of MBLs and resistance to multiple antibiotic classes underscores the importance of early detection and prudent antibiotic use. The present study suggests colistin effectiveness to treat RTIs, nonetheless to be used as last resort drug, its limited use highlights the ongoing prerequisite for effective treatment approaches. Overall, this study stresses the urgent need for improved infection control practices and continued research to manage these resistant pathogens.

6. Ethical Approval

The study was reviewed by Santosh Hospitals and Diagnostics, by the ethics committee with the approval SHIEC/ECAL/DSU/APR2020.

7. Funding of Source

None.

8. Conflict of Interest

None.

9. Acknowledgement

We would like to acknowledge Santosh Hospitals, Bengaluru, India.

References

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1204–22. https://doi.org/10.1016/S0140-6736(20)30925-9.
2. Roncevic-Babin N, Popadic J, Stojadinovic A. Treatment of Acute Lower Respiratory Tract Infections in Children. *Med Pregl* .2002;55(7–8):299–304. https://doi.org/10.2298/MPNS0208299R.
3. John TJ, Dandona L, Sharma VP, Kakkar M. Continuing Challenge of Infectious Diseases in India. *Lancet.* 2011;377(9761):252–69. https://doi.org/10.1016/S0140-6736(10)61265-2.
4. GBD 2015 LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis*. 2017;17(11):1133–61. https://doi.org/10.1016/S1473-3099(17)30396-1.
5. Chakraborty S, Mohsina K, Sarker PK, Alam MZ, Karim MIA, Sayem SMA. Prevalence, Antibiotic Susceptibility Profiles and ESBL Production in Klebsiella Pneumoniae and Klebsiella Oxytoca among Hospitalized Patients. *Period Biol.* **2**016;118(1):53–8. https://doi.org/10.18054/pb.v118i1.3160.
6. Zhang J, Yang T, Zou M, Wang L, Sai L. The epidemiological features of respiratory tract infection using the multiplex panels detection during COVID-19 pandemic in Shandong province, China. *Sci Rep*. 2023;13(1):6319. https://doi.org/10.1038/s41598-023-33627-9.
7. Nagshetty, K.; Shilpa, B. M.; Patil, S. A.; Shivannavar, C. T.; Manjula, N. G. An Overview of Extended Spectrum Beta Lactamases and Metallo Beta Lactamases. *Adva Microbiol*. 2021;11(01):37–62. https://doi.org/10.4236/aim.2021.111004.
8. Bai R, Guo J. Interactions and Implications of Klebsiella Pneumoniae with Human Immune Responses and Metabolic Pathways: A Comprehensive Review. *Infect Drug Resist*. 2024;17:449–62. https://doi.org/10.2147/IDR.S451013.
9. Gandra S, Tseng KK, Arora A, Bhowmik B, Robinson ML, Panigrahi B, et al. The Mortality Burden of Multidrug-Resistant Pathogens in India: A Retrospective, Observational Study. *Clin Infect Dis*. 2019;69(4):563–70. https://doi.org/10.1093/cid/ciy955.
10. Talebi Bezmin Abadi A, Rizvanov AA, Haertlé T, Blatt NL. World Health Organization Report: Current Crisis of Antibiotic Resistance. *BioNanoSci.* 2019;9(4):778–88. https://doi.org/10.1007/s12668-019-00658-4.
11. Munita JM, Arias CA. Mechanisms of Antibiotic Resistance. *Microbiol Spectr.* 2016;4(2):VMBF-0016-2015. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.
12. Lakshman P, Mayegowda SB, Gadilingappa MN. Extended-Spectrum β-Lactamase (ESBL) Producing Bacterial Pathogens

Associated with Respiratory Tract Infections. *J Pure Appl Microbiol.* 2025;19(1):601–16. https://doi.org/10.22207/JPAM.19.1.50.

13. Perez F, El Chakhtoura NG, Papp-Wallace KM, Wilson BM, Bonomo RA. Treatment Options for Infections Caused by Carbapenem-Resistant *Enterobacteriaceae*: Can We Apply "Precision Medicine" to Antimicrobial Chemotherapy? *Expert Opin Pharmacother.* 2016;17(6):761–81. https://doi.org/10.1517/14656566.2016.1145658.
14. Ghaffarian F, Hedayati M, Sedigh Ebrahim-Saraie H, Atrkar Roushan Z, Mojtahedi A. Molecular Epidemiology of ESBL-Producing Klebsiella Pneumoniae Isolates in Intensive Care Units of a Tertiary Care Hospital, North of Iran. *Cell Mol Biol (Noisy-le-grand).* 2018;64(7):75–9. https://doi.org/10.14715/cmb/2018.64.7.13.
15. Bassetti M, Welte T, Wunderink RG. Treatment of Gram-Negative Pneumonia in the Critical Care Setting: Is the Beta-Lactam Antibiotic Backbone Broken beyond Repair? *Crit Care.* 2016;20(1):19. https://doi.org/10.1186/s13054-016-1197-5.
16. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin Microbiol Rev*. 2017;30(2):557–96. https://doi.org/10.1128/CMR.00064-16.
17. Min KL, Son ES, Kim JS, Kim SH, Jung SM, Chang MJ. Risk factors of colistin safety according to administration routes: Intravenous and aerosolized colistin. *PLoS One*. 2018;13(11):e0207588. https://doi.org/10.1371/journal.pone.0207588.
18. Wu Y, Jiang S, Li D, Wu Y, Li Q, Wang X, et al. Clinical Efficacy and Safety of Colistin Sulfate in the Treatment of Carbapenem-Resistant Organism Infections in Patients with Hematological Diseases. *Infect Dis Ther.* 2024;13(1):141–54. https://doi.org/10.1007/s40121-023-00909-8.
19. Chakraborty A, Chanda DD, Choudhury N, Manjula NG, Shilpa BM. A Retrospective Study on the Pyogenic Pathogens and Their Antibiotic Susceptibility Patterns along with the ESβL Production. *Adv Microbiol*. 2021;11(6):317–26. https://doi.org/10.4236/aim.2021.116024.
20. Sahin K, Tekin A, Ozdas S, Akin D, Yapislar H, Dilek AR, et al. Evaluation of Carbapenem Resistance Using Phenotypic and Genotypic Techniques in Enterobacteriaceae Isolates. *Ann Clin Microbiol Antimicrob.* 2015;14(1):44. https://doi.org/10.1186/s12941-015-0105-1.
21. Baral R, Tuladhar R, Manandhar S, Singh A, Sherchan S. Detection of blaKPC Gene among Carbapenemase Producing Klebsiella Pneumoniae Isolated from Different Clinical Specimens at Tertiary Care Hospital of Nepal. *BMC Microbiol.* 2024;24(1):144. https://doi.org/10.1186/s12866-024-03301-9.
22. Bahr G, González LJ, Vila AJ. Metallo-β-lactamases in the Age of Multidrug Resistance: From Structure and Mechanism to Evolution, Dissemination, and Inhibitor Design. *Chem Rev*. 2021;121(13):7957–8094. https://doi.org/10.1021/acs.chemrev.1c00138.
23. Cheesbrough, M. *District Laboratory Practice in Tropical Countries: Part 1*, Second edition; Cambridge University Press: Cambridge, 2005.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am J Clin Pathol.* 1966;45(4):493–6.
25. Ferraro, M. J.; National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard*, 7th ed.; NCCLS,; NCCLS: Wayne, PA, 2000.
26. Wikler MA. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement*; Clinical and laboratory standards institute,; Clinical and Laboratory Standards Institute: Wayne, PA, 2006.
27. Cockerill F. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement*; Clinical and laboratory standards institute; Clinical and Laboratory Standards Institute: Wayne, PA, 2011.
28. Rasheed JK, Anderson GJ, Yigit H, Queenan AM, Doménech-Sánchez A, Swenson JM, et al. Characterization of the Extended-Spectrum β-Lactamase Reference Strain, *Klebsiella Pneumoniae* K6 (ATCC 700603), Which Produces the Novel Enzyme SHV-18. *Antimicrob Agents Chemother.* 2000**;**44(9):2382–8. https://doi.org/10.1128/AAC.44.9.2382-2388.2000.
29. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA Disk Method for Differentiation of Metallo-β-Lactamase-Producing Clinical Isolates of *Pseudomonas* Spp. and *Acinetobacter* Spp. *J Clin Microbiol.* **2002**;40(10):3798–801. https://doi.org/10.1128/JCM.40.10.3798-3801.2002.
30. Kaye KS, Santerre Henriksen A. Pivmecillinam for Treatment of Uncomplicated Urinary Tract Infections: The Full Picture Combines Extensive Clinical Experience and Emerging Data. *Clin Infect Dis*. 2025;81(3):e70–1. https://doi.org/10.1093/cid/ciaf183.
31. Elshamy AA, Aboshanab KM. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. *Future Sci OA*. 2020;6(3):FSO438. https://doi.org/10.2144/fsoa-2019-0098.
32. Yamin D, Uskoković V, Wakil A, Goni M, Shamsuddin S, Mustafa F, et al. Current and Future Technologies for the Detection of Antibiotic-Resistant Bacteria. *Diagnostics.* 2023**;**13(20):3246. https://doi.org/10.3390/diagnostics13203246.
33. Santajit S, Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *Biomed Res Int*. 2016;2016:2475067. https://doi.org/10.1155/2016/2475067.
34. Alkasaby NM, El Sayed Zaki M. Molecular Study of *Acinetobacter baumannii* Isolates for Metallo-*β*-Lactamases and Extended-Spectrum-*β*-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt. *Int J Microbiol.* 2017;2017:3925868. https://doi.org/10.1155/2017/3925868.
35. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front Microbiol.* 2019;10:539. https://doi.org/10.3389/fmicb.2019.00539.
36. Roy S, Gaind R, Chellani H, Mohanty S, Datta S, Singh AK, et al. Neonatal septicaemia caused by diverse clones of Klebsiella pneumoniae & Escherichia coli harbouring blaCTX-M-15. *Indian J Med Res*. 2013;137(4):791–9.
37. Patel D, Nimbalkar A, Sethi A, Kungwani A, Nimbalkar S. Blood culture isolates in neonatal sepsis and their sensitivity in Anand District of India. *Indian J Pediatr*. 2014;81(8):785-90. https://doi.org/10.1007/s12098-013-1314-2.
38. Vijayakanthi N, Bahl D, Kaur N, Maria A, Dubey NK. Frequency and characteristics of infections caused by extended-spectrum beta-lactamase-producing organisms in neonates: a prospective cohort study. *Biomed Res Int.* 2013;2013:756209. https://doi.org/10.1155/2013/756209.
39. Roy S, Viswanathan R, Singh AK, Das P, Basu S. Sepsis in neonates due to imipenem-resistant Klebsiella pneumoniae producing NDM-1 in India. *J Antimicrob Chemother*. 2011;66(6):1411–3. https://doi.org/10.1093/jac/dkr068.
40. Datta S, Roy S, Chatterjee S, Saha A, Sen B, Pal T, et al. A five-year experience of carbapenem resistance in Enterobacteriaceae causing neonatal septicaemia: predominance of NDM-1. *PLoS One*. 2014;9(11):e112101. https://doi.org/10.1371/journal.pone.0112101.
41. Smail, SB, Al-Otrachi KI. Phenotypic Characterization of Extended-Spectrum Beta-Lactamases and Metallo-Beta-Lactamase of Multi Drug Resistant Acinetobacter Baumannii Causing Nosocomial Infections in Erbil City. *Al-Mustansiriyah J Sci.* 2020:30(4):51–56. https://doi.org/10.23851/mjs.v30i4.671.
42. Hans R, Bisht D, Agarwal R, Irfan M. Phenotypic Detection of MBL, Ampc Beta-Lactamase and Carbapenemases in Multi Drug Resistant Isolates of *Acinetobacter Baumannii*. *Int J Med Res Health Sci.* 2015**;**4(2):311–6. https://doi.org/10.5958/2319-5886.2015.00058.2.

43. Farahani R, Moniri R, Farahani KD, Farahani R. Multi-Drug Resistant Acinetobacter-Derived Cephalosporinase and OXAsetC Genes in Clinical Specimens of Acinetobacter Spp. Isolated From Teaching Hospital. *Jundishapur J Microbiol.* 2013;6(2):181–5. https://doi.org/10.5812/jjm.5059.
44. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing acinetobacter baumannii in a teaching hospital in Tabriz, Iran. *Jpn J Infect Dis*. 2011;64(1):69–71.
45. Jeon BC, Jeong SH, Bae IK, Kwon SB, Lee K, Young D, et al. Investigation of a nosocomial outbreak of imipenem-resistant Acinetobacter baumannii producing the OXA-23 beta-lactamase in Korea. *J Clin Microbiol*. 2005;43(5):2241–5. https://doi.org/10.1128/JCM.43.5.2241-2245.2005.
46. Lin YC, Sheng WH, Chen YC, Chang SC, Hsia KC, Li SY. Differences in carbapenem resistance genes among Acinetobacterbaumannii, Acinetobacter genospecies 3 and Acinetobacter genospecies 13TU in Taiwan. *Int J Antimicrob Agents*. 2010;35(5):439–43. https://doi.org/10.1016/j.ijantimicag.2009.11.020.
47. Xiao YH, Giske CG, Wei ZQ, Shen P, Heddini A, Li LJ. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resist Updat*. 2011;14(4-5):236–50. https://doi.org/10.1016/j.drup.2011.07.001.
48. Turner PJ. MYSTIC Europe 2007: Activity of Meropenem and Other Broad-Spectrum Agents against Nosocomial Isolates. *Diagn Microbiol Infect Dis.* 2009**;**63(2):217–22. https://doi.org/10.1016/j.diagmicrobio.2008.11.004.
49. Muthusamy D, Boppe A, Suresh SP. The Prevalence of Group A Beta Haemolytic Streptococcal Carriers Among School Children in Coimbatore, South India. *J Clin Diagn Res*. 2012;6(7):1181–13. https://doi.org/10.7860/JCDR/2012/.2401.
50. Jaggi N, Sissodia P, Sharma L. Acinetobacter Baumannii Isolates in a Tertiary Care Hospital: Antimicrobial Resistance and Clinical Significance. *J Microbiol Infect Dis.* 1970**;***2*(2):57–63. https://doi.org/10.5799/jmid2012.02.0043.

Cite this article: Lakshman P, Mayegowda SB, Murthy SS, Gadilingappa MN. Phenotypic detection of β-lactamases producing *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolated from the respiratory tract infections. *IP Int J Med Microbiol Trop Dis*. 2025;11(4):483-492.