PHENOTYPIC CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE MECHANISMS AMONG THE CLINICAL ISOLATES OF *ACINETOBACTER* SPP. IN A TERTIARY CARE HOSPITAL, NEPAL: A DESCRIPTIVE CROSS-SECTIONAL STUDY

Ram Prasad Adhikari, Laxmi Kant Khanal, Shusila Khadka, Ritu Pandey, Jyotshna Sapkota, Shiba Kumar Rai

Department of Microbiology, Nepal Medical College Teaching Hospital, Gokarneshwor-8, Kathmandu, Nepal

ABSTRACT

The global emergence and spread of multidrug-resistant *Acinetobacter* spp. have become a significant challenge for managing and treating their infections effectively. The aim of this study was to explore the prevalence of antimicrobial resistance phenotypes (MDR, ESBL, AmpC β-lactamases, MBL, KPC) among *Acinetobacter* spp. and to determine their antimicrobial susceptibility patterns. A descriptive cross-sectional study was conducted from December 2021 to May 2022 at Nepal Medical College Teaching Hospital, Nepal on 55 non-repetitive clinical isolates of *Acinetobacter* spp. Isolation, identification and antimicrobial susceptibility testing was done following standard microbiological techniques. Different β-lactamases (ESBLs, AmpC β-lactamase, MBL and KPC) were detected by standard phenotypic tests. Out of 6,344 clinical specimens processed, 1025 (16.16%) showed bacterial growth. The prevalence of *Acinetobacter* spp. among the grown isolates was of 5.36% (n=55). The highest positivity rate among the processed sample was found in the sputum sample (3.21%) followed by pus (1.21%), body fluids (0.51%), urine (0.47%), and blood (0.31%). The rate of isolation of *Acinetobacter* spp. was higher among the isolates from inpatient (n=378) than the isolates from out-patients (n=647) (10.05% vs 2.63%). The prevalence of MDR, ESBL, AmpC β-lactamases and MBL producing *Acinetobacter* spp. was 67.27 % (n=37), 25.45% (n=14), 14.55% (n=8), and 34.54% (n=19), respectively. Two isolates were detected as KPC phenotypes. Six isolates (10.91%) showed both ESBL and AmpC B-lactamase co-producers. All isolates were susceptible to polymixins and colistin sulphates. Tigecycline resistance was among 29.09 % of isolates. More than 70.00 % of the isolates were resistant to most commonly used first line antibiotics (cefixime - 80.00%, ceftazidime - 78.12%, cefotaxime - 76.36%, ciprofloxacin - 72.72%, ofloxacin - 70.90%, cotrimoxazole - 81.82%). Almost half of the isolates were resistant to carbapenems (imipenem -46.64%, meropenem - 41.82%), piperacillin-tazobactam (49.00%) and amikacin (54.55%). The study shows a high proportion of *Acinetobacter* spp. as MDR with significant presence of ESBL, MBL, AmpC and KPC resistant phenotypes in our set-up. Over 70% resistance to the commonly used antibiotics further highlights the therapeutic challenges posed by these pathogens. The findings from the study emphasize the need for continuous monitoring and implementation of effective control strategies to curb the spread of resistant *Acinetobacter* infections.

KEYWORDS

Acinetobacter spp., MDR, ESBL, AmpC β-lactamase, Nepal

CORRESPONDING AUTHOR

Mr. Ram Prasad Adhikari,
Assistant Professor,
Department of Microbiology,
Nepal Medical College Teaching Hospital, Attarkhel,
Gokarneshwor-8, Kathmandu, Nepal
Email: rampd11@yahoo.com
Orcid No: https://orcid.org/0000-0002-7183-6353
DOI: https://doi.org/10.3126/nmcj.v28i1.92185

***Received on**: November 24, 2025*
***Accepted for publication**: February 18. 2026*

Cite this paper as: Adhikari RP, Khanal LK, Khadka S, Pandey R, Sapkota J, Rai SK. Phenotypic characterization of antimicrobial resistance mechanisms among the clinical isolates of *Acinetobacter* spp. in a tertiary care hospital, Nepal: A descriptive cross-sectional study. *Nepal Med Coll J* 2026; 28: 53-61.

INTRODUCTION

The rising incidence of antimicrobial resistance (AMR) has become a top-priority concern of the 21st century in global health system, with significant impact on clinical outcomes, hospital infection control, and overall public health frameworks.[1] Emergence and spread of multidrug resistance (MDR) bacteria globally with novel mechanisms has taken central stage as a global health issue.[1,2] If this continues, more than 10 million people per year will die by 2050, as projected.[3] Among the MDR gram negative organisms, *Acinetobacter* spp. have emerged as formidable nosocomial pathogens,[4] so WHO has classified these bacteria among the ESKAPE pathogens: a group of highly virulent and MDR bacteria responsible for majority of hospital acquired infections worldwide.[5] Their extraordinary ability to survive under diverse environmental conditions, prolonged persistence on hospital surfaces, colonization as normal flora, acquire resistance genes to multiple drugs etc. have made them the most challenging and highly successful opportunistic pathogens.[6] *Acinetobacter* spp. are non-fermenting, catalase-positive, non-motile, aerobic gram-negative coccobacilli.[6,7] They are widely found in natural water, soil, hospital environment and even in human skin, respiratory tract, digestive tract[6] and can survive in dry conditions for weeks.[6–8] *Acinetobacter* spp. has become increasingly responsible for causing serious healthcare-associated infections including ventilator-associated pneumonia, surgical site infections, urinary tract infections, blood stream infections and meningitis especially in patient admitted to critical care units or undergoing invasive surgical procedures.[9,10] Excellent biofilm producing character of the bacteria, higher colonization rate facilitates their survival and spread in hospital environments.[6–8,11]

Acinetobacter spp. are now showing increasing rate of resistance to nearly all routinely prescribed antimicrobials including aminoglycosides, fluoroquinolones and broad-spectrum β-lactams.[2,3,10] Different resistance mechanisms are involved among these bacteria to make them resistance to these multiple groups of antibiotics.[12] These bacteria naturally produce chromosomally encoded AmpC β -lactamases, specifically *Acinetobacter*-derived cephalosporinase and confer resistance to many penicillins and cephalosporins, but not to extended-spectrum cephalosporins unless overexpressed.[13] Similarly, *Acinetobacter* spp. naturally carry chromosomally encoded, OXA-51 like OXA-type β -lactamases (oxacillinases) that are not highly active against carbapenems but if overexpressed or associated with insertion elements (e.g. ISAba1) make them resistant to carbapenems.[13–15] In addition, these bacteria can acquire plasmid-mediated OXA enzymes like OXA-23, OXA- 24/40, OXA-58 which are the carbapenemases that can hydrolyze carbapenems.[13] Another mechanism of resistance among these bacteria is the production of extended-spectrum β -lactamases (ESBLs) enzymes which are capable of hydrolyzing a wide range of β -lactam antibiotics, including third-generation cephalosporins and monobactams.[16] The most commonly reported ESBLs genes in *Acinetobacter* spp. are PER, VEB, TEM and CTX-M type that may be located on plasmids, integrons, or transposons, facilitating horizontal gene transfer.[16,17] These mobile genetic elements often carry co-resistance determinants for other antibiotic classes such as aminoglycosides, fluoroquinolones and sulphonamides.[18] Additionally, the co-expression of AmpC β -lactamases in ESBLs producing strains further enhances β -lactam resistance.[17,18] Similarly, metallo β -lactamases (MBLs) and *Klebsiella pneumoniae* carbapenemases (KPCs) production are additional resistance mechanisms developing among these bacteria in the recent days making the most commonly prescribed and used carbapenems antibiotics for treating MDR *Acinetobacter* infections, not effective.[18] KPCs are serine β-lactamases enzymes encoded by the bla_{KPC} genes and hydrolyze carbapenems and other β-lactams and are inhibited by β -lactamase inhibitors.[12,19] MBLs are zinc-dependent enzymes (encoded by B-lactamases genes like bla_{IMP} $bla_{VIM,}$ $bla_{NDM,}$ bla_{SIM}) that hydrolyze nearly all β-lactams including carbapenems but not monobactams and not inhibited by β -lactamase inhibitors.[16,20] Horizontal transfer and co-occurrence of these resistance genes among the bacteria often results in resistance to other group of antibiotics like aminoglycosides, fluoroquinolones and sulfonamides.[16–18]

Polymixin, colistins, tigecycline have now become mainstay for treating infections by *Acinetobacter* spp. with these resistance mechanisms as a last-resort antibiotics,[21] however the combined presence of MBLs and/or KPCs with other resistance mechanisms (e.g. efflux pumps, porin mutations, OXA-type carbapenemases) in *Acinetobacter* spp. enhances holistic resistance mechanisms resulting extensive drug resistant (XDR) and pan drug resistant (PDR) isolates to emerge, limiting the efficacy of even these last-resort antibiotics.[16–19] In the recent days, these

resistance mechanisms have been commonly reported from the different parts of the world.[10,12,15–17]

Rapid spread of MDR *Acinetobacter* infections specially in hospital set up with limited and expensive drugs available for treatment in economically underprivileged countries like Nepal is of major concern.[22–25] Some hospital-based studies within the country, have reported increasing rates of MDR and carbapenem resistance, often without the corresponding molecular or phenotypic characterization of the underlying mechanisms.[26,27] Understanding the distribution of these resistance phenotypes: ESBLs, MBsL, AmpC β-lactamases, and KPCs through standardized phenotypic methods is essential in settings like Nepal, where molecular diagnostics are not routinely available.[28,29] Similarly, continuous evaluation and monitoring of these resistance patterns is important in local context to see the changing trends of antimicrobial resistance along with the time.[29]

Therefore, this study aims to phenotypically characterize the antimicrobial resistance patterns of clinical isolates of *Acinetobacter* spp. at Nepal Medical College Teaching Hospital (NMCTH), Kathmandu, Nepal, with particular focus on the detection of ESBL, MBL, AmpC, and KPC producers. The findings are expected to generate locally relevant evidence to guide empirical therapy, strengthen infection control policies, and contribute to national AMR surveillance efforts.

MATERIALS AND METHODS

A descriptive cross-sectional study was conducted from December 2021 to May 2022 in the Clinical Microbiology Laboratory of NMCTH. The study was done in 55 non-repeated bacterial isolates of *Acinetobacter* spp. from clinical specimens (pus, blood, urine, sputum, and body fluids) from patients attending NMCTH.

All the clinical samples received in the Clinical Microbiology Laboratory for culture and sensitivity were processed as a routine diagnostic process according to the guidelines by American Society of Microbiology.[30] In brief, all specimens other than urine and blood were inoculated on blood agar and MacConkey agar (Hi-Media-India) and incubated at 37°C aerobically for 24 hours. The urine specimen was inoculated in a cysteine lactose electrolyte deficient (CLED) medium. Blood samples inoculated in brain heart infusion (BHI) broth and incubated at 37^{0}C aerobically, were sub-cultured each day in blood agar and MacConkey agar until the growth is obtained for a maximum of seven days. Identification of *Acinetobacter* spp. was done based on colony morphology, gram-stain and biochemical tests like catalase test (+), oxidase test (-), motility test (-) and tests on triple sugar iron agar: "No change in color, H_2S (-), no gas production".

Antibiotic susceptibility testing: The antibiotic sensitivity test of the isolates was done by using Mueller Hinton agar (MHA) (Hi-media) by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI).[31] In brief, at least three to five well isolated colonies of the same morphological types were transferred into peptone water and then incubated for 3 hours at 37^{0}C to make the bacteria in their log phase. The obtained turbidity of the bacterial suspension was then be matched with McFarland tube number 0.5 giving standard inoculum size of 1.5x10^8 cells/ml. Lawn culture of bacterial suspension was done on Mueller Hinton agar (MHA) and following antibiotic discs (Hi-media, India) were applied on the surface of the inoculated plate: Cefixime (30 µg), ceftazidime (30 µg), cephotaxime (30 µg), amikacin (30 µg) ciprofloxacin (5 µg), ofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), imipenem (10 µg), meropenem (10 µg), piperacillin–tazobactam (100/10 µg), (10 µg), tigecycline (15 µg).

After overnight incubation at 37^0 C, results were read by measuring diameter of zone of inhibition of each disk applied and was reported as sensitive, resistant and intermediate by comparing with the standard interpretative chart provided by the disc manufacturing company (Hi-Media, India).

For susceptibility testing against polymixin-B and colistin sulphate, broth micro-dilution (BMD) method was used as recommended by both CLSI and the European Committee on antimicrobial susceptibility testing (EUCAST). The minimum inhibitory concentration (MIC) was determined as the lowest antibiotic concentration at which no visible bacterial growth is observed. An MIC of ≤2 µg/mL for colistin was considered as susceptible, while an MIC >2 µg/mL as resistance. Same colistin breakpoints were used for interpretation as suggested by EUCAST.[31,32]

Criteria for MDR: Isolates that are resistant to at least one each from three different classes of antimicrobial agents was regarded as MDR in this study.[33]

Test for ESBL-production: Combination disk method was used for the phenotypic confirmation of ESBL- producing strains in which CTX and CAZ (30 µg), alone and in combination with clavulanic acid (CA) (10 µg) was used (Hi-media, India). An increase ZOI of ≥5 mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirms ESBL. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls, respectively.[31]

Screening and test for AmpC beta-lactamase production: Screening of AmpC beta-lactamase production was done by using cefoxitin disc (30 µg) showing inhibition zone diameter of <18 mm. Isolates with screening test positive were subjected to cefoxitin-cloxacillin (30-200 µg) double disk synergy test. A difference in the cefoxitin-cloxacillin inhibition zones minus the cefoxitin alone zones of 4 mm was considered indicative for AmpC production.[32]

Test for ESBL and AmpC β-lactamase co-producers: Combination disk tests with cefotaxime- clavulanate and cefotaxime-clavulanate with cloxacillin was done for the detection of ESBL and AmpC β-lactamase co-producers. An increase in zone of inhibition size ≥5 mm on cefotaxime-clavulanate with cloxacillin disc than that of cefotaxime-clavulanate disc alone was considered as ESBL and AmpC β-lactamase co-producers.[32]

Test for MBL detection: Combination disk method was used for phenotypic confirmation of MBL producing strains in which two IMP (10 µg) disks, one containing 10 µl of 0.1 M (292 µg) anhydrous EDTA were used. An increase in zone diameter >4 mm around the IMP-EDTA disk compared to that of the IMP disk alone was considered to be positive for MBL.[31]

Test for KPC production: Combination disk method was used for the phenotypic detection of KPC producing strains in which two meropenem (10 µg) disks one alone and other with 10 µl (300 µg /ml) 3-aminophenyl boronic acid (3-APBA) (Hi-media, India) were placed 20 mm apart on the agar plate. An increase in zone diameter >5 mm around the meropenem-APBA disk compared to that of the meropenem disk alone indicated KPC production.[34]

Data processing and analysis: All collected data that are entered into Microsoft Excel (office 365) were analyzed using statistical software (STATA-14). Categorical variables such as antimicrobial resistance patterns, ESBL, MBL, AmpC β-lactamese KPC types are presented as frequencies and percentages. Chi-square (χ^2) test was applied to describe their association. *P*-value of <0.05 was considered statistically significant.

RESULTS

A total of 6,344 clinical specimens (urine: 3380, blood: 1305, sputum: 808, pus: 656 and body fluids: 195) from both inpatients and out-patients of all age groups received for aerobic bacterial culture and antimicrobial

Table 1: Distribution of clinical isolates of *Acinetobacter* spp. according to the age of patients (n=55)

Age of patients (years)	*n* of isolates (%)
<20	5 (9.10)
21-40	10 (18.20)
41-60	18 (32.70)
>60	22 (40.00)

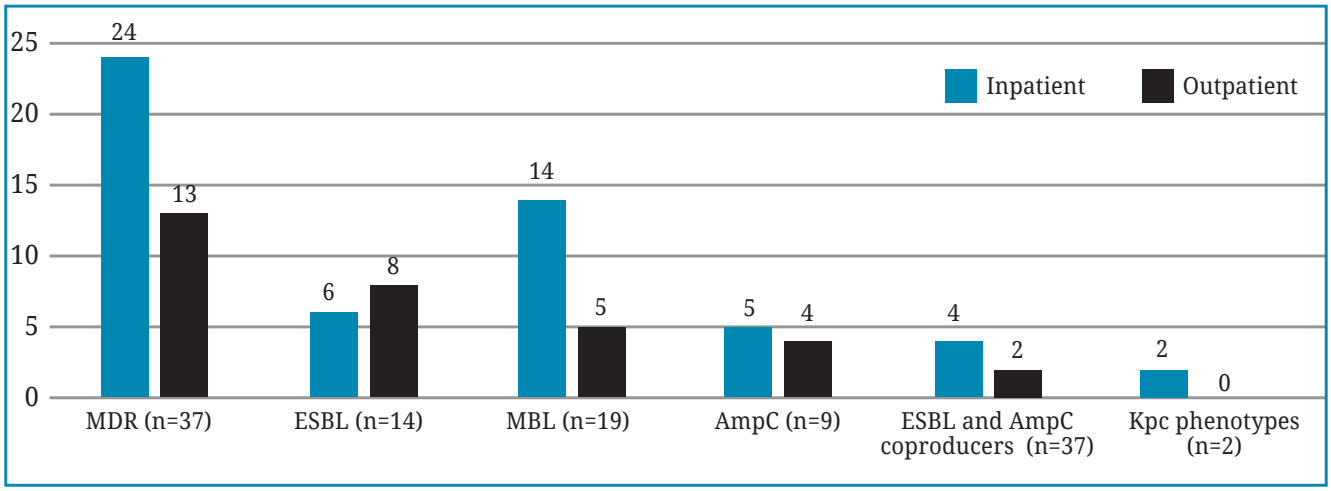

Fig 1: Rate of MDR and different β-lactamases producing *Acinetobacter* spp. in clinical samples from inpatients and out-patients.

Table 2: Resistance pattern of *Acinetobacter* spp. (n=55) to various antibiotics

Antibiotics	*n* of resistance (%)
Cefixime	44 (80.00)
Ceftazidime	43 (78.12)
Cephotaxime	42 (76.36)
cefipime	35 (63.64)
Amikacin	30 (54.55)
Ciprofloxacin	40 (72.72)
Ofloxacin	39 (70.90)
Co-trimoxazole	45 (81.82)
Piperacillin-tazobactum	27 (49.00)
Imepenem	24 (43.64)
Meropenem	23 (41.82)
Polymixin B (MIC >2 µg/mL)	00 (0.00)
Colistin sulphate (MIC >2 µg/mL)	00 (0.00)
Tigecycline	16 (29.09)

susceptibility testing at Clinical Microbiology Laboratory of NMCTH from December 2021 to May 2022 were included in the study. Of the total specimens processed 1025 (378 inpatient's and 647 outpatient's) clinical samples showed bacterial growth with a growth positivity rate of 16.16%.

The prevalence rate of *Acinetobacter* spp. was 5.36% (n=55/1025) among the total bacterial isolates and 0.87 % (n=55/6,344) among the total clinical specimen processed. Of the total 55, (38; 69.09% from inpatients and 17; 30.91% from out-patients) *Acinetobacter* spp. isolates 35 (63.64%) were from males and 20 (36.36%) were from females. The isolates obtained were 26 (47.27%), 16 (29.09%), 8 (14.55%), 4 (7.27%), and 1 (1.82%) from sputum, urine, pus, blood, and body fluids, respectively. The highest positivity rate among the processed sample was found in the sputum sample (3.21%) followed by pus (1.21%), body fluids (0.51%), urine (0.47%), and blood (0.31%). The rate of isolation of *Acinetobacter* spp. was higher among the isolates from inpatient (n=378) than the isolates from out-patients (n=647) (10.05% vs 2.63%). The distribution of the isolates according to the age group of patients is shown in Table 1.

The prevalence of MDR, ESBL, AmpC B-lacatamse and MBLproducing *Acinetobacter* spp. was 67.27 % (n=37), 25.45% (n=14), 14.55% (n=8), and 34.54% (n=19), respectively. Two isolates were detected as KPC phenotypes. Six isolates (10.91%) showed both ESBL and AmpC β-lactamase coproducers. All ESBL, AmpC β-lactamases, MBL, KPC phenotypes as well as ESBL and AmpC β-lactamase coproducing *Acinetobacter* spp. isolates were MDR. The MDR, AmpC β-lactamase and MBL production was higher in isolates among the clinical samples collected from inpatients, however ESBL production was higher from out-patients. (Fig. 1). The antimicrobial susceptibility pattern of *Acinetobacter* spp. is shown in Table 2.

DISCUSSION

In this study, we phenotypically assessed MDR, ESBL, AmpC, MBL, and KPC resistance mechanisms among clinical isolates of *Acinetobacter* spp. and evaluated their antimicrobial susceptibility patterns. This study highlighted the growing clinical and public-health threat posed by MDR *Acinetobacter* spp. in a tertiary care hospital in Nepal. *Acinetobacter* spp. accounted for 5.36% of all bacterial isolates and 0.87% of all specimens processed, which is comparable to several regional and global reports identifying *Acinetobacter* as an emerging nosocomial pathogen in low middle-income countries.[1-3] Previous studies in Nepal have reported prevalence rates ranging from 3-7% of total isolates,[22,24,25] placing our findings within the expected range. The slightly higher proportion compared to some community-based studies may reflect the fact that our samples predominantly originated from a tertiary-care setting, where repeated hospital admissions, device-associated infections, and empirical use of broad-spectrum antibiotics favor the selection of *Acinetobacter* spp.[4,6-9] The isolation rate of bacteria was significantly higher among inpatients (69.1%) than out-patients that aligns with the findings from other Nepalese hospitals and international studies, where *Acinetobacter* is strongly linked with healthcare-associated infections.[7,9,22,24,25] Other studies from Nepal, such as Gurung *et al*[22] and Manandhar *et al*,[24] also reported that the majority of *Acinetobacter* isolates originated from intensive care units and high-dependency wards. This predominance in inpatients can be justified by several factors, including prolonged hospitalization, invasive procedures, mechanical ventilation, and the organism's ability to persist in hospital surfaces through biofilms.[11,12] Age-wise, the highest proportion of isolates was found among patients >60 years (40.0%), which was slightly higher than the 30-35% reported in other Nepalese studies.[25,27] Elderly patients often have weakened immunity, multiple comorbidities, and increase exposure

to antibiotics and medical devices, explaining their enhanced susceptibility.[6,7,9]

Acinetobacter spp. were most frequently isolated from sputum (47.27%), a finding consistent with reports that *Acinetobacter* is strongly associated with lower respiratory tract infections, especially hospital-acquired pneumonia and ventilator-associated pneumonia (VAP).[9-12] The sputum positivity rate of 3.21% in our setting is comparable to the 3.00 to 5.00% reported by Kumari *et al*[25] and Yadav *et al*.[27] Slightly higher sputum positivity in our study could be attributed to the hospital's high inpatient turnover, ventilated patients, and increase prevalence of chronic respiratory diseases in older adults. The lower isolation in urine, pus, blood and body fluids follows the typical trend describe in global literature, where respiratory samples remain the most common source.[6,7,11] The relatively low positivity in blood (7.27%) aligns with findings from Meshram *et al*[7] and Nonyelum *et al*,[9] where bloodstream infections caused by *Acinetobacter* were less frequent but often severe and associated with MDR strains.

The MDR prevalence of 67.27% in this study was substantial but still falls within the ranges documented in Nepal and globally. Studies from Nepal have reported MDR rates ranging from 55.00% to 85.00%.[22,25-27] Our rate is comparable to those found in critical care-oriented studies, for example, Manandhar *et al* 2020 reported 65.00%, but slightly lower than the extremely high MDR rates reported in ICUs (>80.00%).[24]

Similarly, our MDR rate aligned with findings from studies conducted outside Nepal, including meta-analyses from Asia and the Middle East, indicating that MDR *Acinetobacter* has become a pervasive challenge across resource-limited healthcare settings.[10] The high level of MDR observed in our setting can be attributed to several contextual factors such as frequent empirical use of cephalosporins and fluoroquinolones in Nepalese hospitals exerts substantial selective pressure that promotes resistant strains[2,24,28] and the limited availability of rapid diagnostic tools delays the initiation of targeted therapy, allowing inappropriate antimicrobial exposure to further drive resistance.[1,2] Moreover, the inherent environmental persistence of *Acinetobacter* spp. and their strong ability to form biofilms facilitate prolonged survival on hospital surfaces and equipment, contributing to sustained transmission within clinical wards.[11,12,26]

In this study, *Acinetobacter* spp. exhibited a substantial burden of β-lactamase mediated resistance, with ESBL, AmpC, and MBL production observed in 25.45%, 14.55%, and 34.54% of isolates, respectively. The ESBL prevalence of 25.45% is similar with the previous reports from Nepal, which range between 20.00-30.00%,[25,27] but was lower than rates reported from Iran (40.00-60.00%).[17] This discrepancy may reflect variations in local antibiotic prescribing practices, the availability and utilization of β-lactam/β-lactamase inhibitor combinations, and differences in the genetic background of circulating strains.

AmpC production was detected in 14.55% of isolates, which falls within the lower end of global estimates (10.00-25.00%).[18] The relatively lower prevalence of AmpC in our setting may be attributed to restricted use of cephamycins, thereby reducing selective pressure for AmpC-expressing strains.

The presence of ESBLs and AmpC enzymes co-producers (10.91%), indicates the overlapping resistance mechanisms, often mediated by mobile genetic elements carrying additional co-resistance genes.[16-18,25] Co-expression of these enzymes has been associated with broader β-lactam resistance, treatment failure, and rapid dissemination within hospitals.[17,18] Their detection in both inpatient and out-patient isolates, though more frequent among inpatient; suggests community spillover and emphasizes the importance of surveillance beyond hospital settings.

MBL production was observed in 35.54% of isolates, consistent with other Nepalese studies reported 30.00-40.00% MBL-positive *Acinetobacter*.[22,25] The high prevalence of MBLs likely reflects the widespread dissemination of $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-23}}$ like genes in the region, highlighting the ongoing challenge of carbapenem resistance in resource-limited settings.[22,23] The prominence of MBLs is particularly concerning, as these enzymes confer resistance to most β-lactams, including carbapenems, thereby limiting therapeutic options.[3,5,20]

Although rare, the detection of two KPC-producing isolates warrants special concern because KPCs have been traditionally more common in Enterobacteriales than in *Acinetobacter* spp.[12,19] The emergence of KPC in *Acinetobacter* may indicate horizontal acquisition of carbapenemase genes, reflecting evolving genomic adaptability. Reports from Asia and other regions increasingly highlight such acquisitions, underscoring the dynamic nature of carbapenemase epidemiology.[12,15,19,23] Even a low prevalence of KPC, it is clinically

significant due to its ability to spread rapidly via plasmids and its association with high-level carbapenem resistance.

The antimicrobial susceptibility profile of *Acinetobacter* spp. in our study demonstrates high rate of resistance to multiple commonly used antibiotics, reflecting a persistent challenge in both hospital and community settings. Resistance to third and fourth generation cephalosporins (cefixime 80.00%, ceftazidime 78.12%, cefotaxime 76.36%, cefepime 63.64%) and fluoroquinolones (ciprofloxacin 72.72%, ofloxacin 70.90%) is consistent with previous Nepalese reports and global trends, underscoring the widespread dissemination of resistant *Acinetobacter* strains.[16,20,24-27] Co-trimoxazole resistance was notably high (81.82%), aligning with other Asian studies and likely reflecting its frequent empirical use. Aminoglycosides and β-lactam/β-lactamase inhibitor combinations showed moderate activity (amikacin 54.55%, piperacillin-tazobactam 49.00%), whereas carbapenems exhibited resistance in 41-44% of isolates which is moderately high but slightly lower than ICU-based reports from other countries potentially due to more regulated use and stewardship protocols in our set up.[22,24,25]

An encouraging finding of this study was that all isolates still remained susceptible to polymyxin B and colistin (MIC <2 µg /ml), reaffirming their role as last-line therapies for MDR, XDR, and carbapenem-resistant *Acinetobacter* spp.[20,21] However, 29.09% resistance to tigecycline signals an early warning trend, as tigecycline has been one of the relatively accessible options for difficult to treat *Acinetobacter* infections. Tigecycline resistance has been increasingly reported globally and is often linked to efflux pump overexpression and regulatory gene mutations.[16,21] This highlights the importance of preserving tigecycline efficacy through judicious use and stewardship.

The higher prevalence of MDR, MBL, and AmpC phenotypes among inpatient isolates underscores the importance of stringent infection prevention and control practices in hospital wards, particularly intensive care units,[29] environmental decontamination, adherence to hand hygiene, rational antibiotic use, and regular surveillance of resistance mechanisms are critical to break the chain of transmission.[2,3,29] In resource-limited settings like Nepal, where advanced molecular diagnostics are infrequently available, phenotypic screening as used in this study remains an essential and practical approach for early detection of critical resistance mechanisms.[28]

Overall, the findings demonstrated a complex and evolving resistance landscape in *Acinetobacter* spp., driven by coexistence of multiple β-lactamase mechanisms, high MDR burdens, and reduced susceptibility to key therapeutic agents. This scenario threatens the effectiveness of current treatment options and necessitates urgent, coordinated action involving clinicians, microbiologists, hospital administrators, and policymakers.

In conclusion, this study demonstrated a high prevalence of MDR *Acinetobacter* spp. with substantial proportions of ESBL, AmpC, MBL, and KPC producers, highlighting a serious therapeutic and epidemiological challenge in the study setting. The widespread resistance to commonly used antibiotics and increasing carbapenem resistance underscores the growing difficulty in managing *Acinetobacter* infections. The retained susceptibility to polymyxin B and colistin offers some hope for treatment; however, the emergence of tigecycline resistance signals the potential erosion of last-line therapeutic options. These findings emphasize the urgent need for strengthened antimicrobial stewardship, strict infection control measures, and routine surveillance of resistance phenotypes. Expanding diagnostic capacity, promoting rational antibiotic use, and integrating local AMR data into clinical decision-making are critical steps towards mitigating the spread of highly resistant *Acinetobacter* spp. in Nepal.

ETHICAL CONSIDERATION: Ethical approval for this study was taken from the Institutional Review Committee of NMCTH (Ref.: 041-078/079). All data and information were handled with strict confidentiality and used solely for research purposes. Patient identifiers were excluded, and numerical codes were assigned to ensure anonymity and protect privacy throughout the study.

ACKNOWLEDGEMENTS

We thank all the laboratory staffs and post graduate students of the Microbiology Department of NMCTH for their kind support in collection of data and performing the necessary laboratory tests during the study.

Conflict of interest: None

Source of research fund: None

REFERENCES

1. Antimicrobial resistance". Available from: www.who.int (Accessed on: November 2024).
2. Pokharel S, Raut S, Adhikari B. Tackling antimicrobial resistance in low-income and middle-income countries. *Brit Med J Glob Health* 2019; 4: e002104.
3. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. The review on antimicrobial resistance. 2016. Available from: http://amrreview.org/sites/default/files/160525_Final%20paper_with%20cover.pdf (Accessed on: June 2023).
4. Manchanda V, Sanchaita S, Singh N. Multidrug resistant Acinetobacter. J Glob Infect Dis 2010; 2: 291.
5. WHO. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Geneva 2017.
6. Brady MF, Jamal Z, Pervin N. *Acinetobacter*. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430784/?utm_source=chatgpt.com (Accessed on: November 2025).
7. Meshram N and Meshram S. Discernment of Acinetobacter species in world scenario. *J Pure Appl Microbiol* 2023; 17: 1400–9.
8. EL-Hakeem RA, Saleh SE, Aboulwafa MM, Hassouna NA. *Acinetobacter baumannii* virulence factors, resistance mechanisms, and new insights on infection treatment. *Arch Pharm Sci ASU* 2023; 7: 208-30.
9. Nonyelum SC, Marian AA, Chinelo OU. *Acinetobacter baumannii*: Epidemiology, Clinical Manifestations and Associated Infections. In Rangel K, Giovanni De-Simone S, editors. *Acinetobacter baumannii* - The Rise of a Resistant Pathogen. *IntechOpen* 2023. DOI: 10.5772/intechopen.1003618 (Accessed on June 2025).
10. Mohd SLS, Zainal AA, Liew SM, Roberts JA, Sime FB. The global prevalence of multidrug-resistance among *Acinetobacter baumannii* causing hospital-acquired and ventilator-associated pneumonia and its associated mortality: A systematic review and meta-analysis. *J Infect* 2019; 79: 593–600.
11. Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. *J Hosp Infect* 2012; 80: 56-60.
12. Shi J, Cheng J, Liu S, Zhu Y, Zhu M. *Acinetobacter baumannii*: An evolving and cunning opponent. *Front Microbiol* 2024; 15: 1332108.
13. D'Souza R, Pinto NA, Phuong NL *et al*. Phenotypic and genotypic characterization of *Acinetobacter* spp. panel strains: a cornerstone to facilitate antimicrobial development. *Front Microbiol* 2019; 10: 559.
14. Zhang Y, Ding F, Luo Y *et al*. Distribution pattern of carbapenemases and solitary contribution to resistance in clinical strains of *Acinetobacter baumannii*. *Ann Palliat Med* 2021; 10: 9184-91. DOI: https://dx.doi.org/10.21037/ap.
15. Carascal MB, Destura RV, Rivera WL. Molecular genotyping reveals multiple carbapenemase genes and unique blaOXA-51-like (oxaAb) alleles among clinically isolated *Acinetobacter baumannii* from a Philippine tertiary hospital. *Trop Med Health* 2024; 52: 62.
16. Castanheira M, Mendes RE, Gales AC. Global epidemiology and mechanisms of resistance of *Acinetobacter baumannii*-calcoaceticus Complex. *Clin Infect Dis* 2023; 76: 166–78.
17. Abdar MH, Taheri-KM, Taheri K *et al*. Prevalence of extended-spectrum beta-lactamase genes in *Acinetobacter baumannii* strains isolated from nosocomial infections in Tehran, Iran. *GMS Hyg Infect Control* 2019; 14: Doc02.
18. Černiauskienė K, Dambrauskienė A, Vitkauskienė A. Associations between β-lactamase types of *Acinetobacter baumannii* and antimicrobial resistance. *Medicina* 2023; 59:1386.
19. Swathi CH, Chikala R, Ratnakar KS, Sritharan V. A structural, epidemiological & genetic overview of *Klebsiella pneumoniae* carbapenemases (KPCs). *Indian J Med Res* 2016; 144: 21–31.
20. Falagas ME, Kontogiannis DS, Zidrou M, Filippou C, Tansarli GS. Global epidemiology and antimicrobial resistance of metallo-β-lactamase (MBL)-producing *Acinetobacter* clinical isolates: A systematic review. *Pathogens* 2025; 14: 557.
21. Gajic I, Tomic N, Lukovic B *et al*. A comprehensive overview of antibacterial agents for combating multidrug-resistant bacteria: The current landscape, development, future opportunities and challenges. *Antibiotics* 2025; 14: 221.
22. Gurung A, Napit R, Shrestha B, Lekhak B. Carbapenem resistance in *Acinetobacter calcoaceticus-baumannii complex* isolates from Kathmandu Model Hospital, Nepal, is attributed to the presence of $bla_{OXA\text{-}23\text{-}like}$ and $bla_{NDM\text{-}1}$ genes. *BioMed Res Int* 2024; 1: 8842625.
23. Thapa J, Sah AK, Paudel P, Chandra A, Upreti MK. Molecular detection of blaOXA-23, csuE and ompA genes from carbapenem-resistant and biofilm producing *Acinetobacter baumannii* isolated from clinical samples. *J Nepal Biotechnol Assoc* 2025; 6: 48–54.
24. Manandhar S, Zellweger RM, Maharjan N. A high prevalence of multi-drug resistant Gram-negative bacilli in a Nepali tertiary care hospital and associated widespread distribution of extended-spectrum beta-lactamase (ESBL) and carbapenemase-encoding genes. *Ann Clin Microbiol Antimicrob* 2020; 19: 48.
25. Kumari M, Bhattarai NR, Rai K, Pandit TK, Khanal B. Multidrug-resistant *Acinetobacter*: detection of ESBL, MBL, blaNDM-1 genotype, and biofilm formation at a tertiary care hospital in eastern Nepal. *Int J Microbiol* 2022; 10: 1–9.
26. Bhandari K, Chhunju S, Nayaju T *et al*. Biofilm production and antibiotic resistance in clinical isolates of *Acinetobacter* species. *Tribhuvan Univ J Microbiol* 2023; 48–56.
27. Yadav P, Mishra SK, Shrestha S *et al*. Multidrug-resistance and biofilm formation among *Acinetobacter baumannii* isolated from clinical specimens. *J Nepal Health Res Counc* 2025; 22: 662–9.

28. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. *Antimicrob Resist Infect Control* 2017; 6: 67.

29. WHO. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022. Geneva; 2022.

30. Garcia LS. Clinical microbiology procedures handbook: American Society for Microbiology Press; 2010. DOI: 10.1128/9781555817435S.

31. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 34th ed. CLSI supplement M100. CLSI, 2024.

32. EUCAST guideline for the detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance [Internet]. EUCAST; 2017. Available from: http://www.eucast.org/resistance_mechanisms/ (Accessed on: November 2025).

33. Magiorakos AP, Srinivasan A, Carey RB *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268–81.

34. Tsakris A, Poulou A, Pournaras S *et al*. A simple phenotypic method for the differentiation of metallo- -lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. *J Antimicrob Chemother* 2010; 65: 1664–71.