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# Extended-Spectrum β-Lactamase Producing *Klebsiella pneumoniae* Associated with Lower Respiratory Tract Infections from Sputum Specimens

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## Abstract

Lower respiratory tract infection (LRTI) caused by extended-spectrum β-lactamase (ESBL)-producing bacteria is a major health concern worldwide. Prompt diagnosis of ESBL-producing bacteria is critical for guiding appropriate antibiotic therapy and limiting the spread of ESBL pathogens. This study aimed to evaluate the antimicrobial susceptibility profile of Gram-negative isolates from sputum samples and to determine the proportion of multidrug-resistant (MDR) and ESBL Gram-negative isolates. The study was conducted in the microbiology laboratory of B & B Hospital Pvt. Ltd. from January to June 2023. The antimicrobial susceptibility pattern of the Gram-negative isolates was assessed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA), in accordance with Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines, and MDR phenotypes were assigned accordingly. Ceftazidime and cefotaxime discs were used to screen isolates phenotypically for ESBL production, and combination discs with clavulanic acid were used to confirm ESBL production. A total of 148 sputum samples were collected and processed from clinically suspected LRTI patients. Of these, 39 (26.35%) samples showed bacterial growth. Gram-negative bacteria (34, 87.1%) were the most common isolates, most frequently recovered from male patients aged 41-60 years, and the majority of Gram-negative isolates (85.3%) were recovered from inpatients rather than outpatients. Gram-negative isolates were identified by colony morphology, Gram-stain reaction, and standard biochemical tests. Of the total isolates, *Klebsiella pneumoniae* (23, 58.97%) was the most frequently isolated organism. Among the 34 Gram-negative isolates, 20 (59%) were MDR. Of the 34 Gram-negative isolates, 13 (38.23%) were confirmed ESBL producers. Amikacin, gentamicin, and the carbapenems (imipenem and meropenem) were the most effective agents against the ESBL-producing isolates, whereas resistance to third-generation cephalosporins, fluoroquinolones, and cotrimoxazole was high; these agents should therefore be avoided for empirical LRTI therapy pending susceptibility results.

**Keywords:** Gram-negative bacteria, Sputum, *Klebsiella*, MDR, ESBL, antibiotic susceptibility

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## Introduction

Lower respiratory tract infection (LRTI) is inflammation of the lower respiratory tract, i.e. the trachea, bronchi, bronchioles, or lung tissue, and may be bacterial, viral, fungal, or parasitic in origin [1]. Clinically, LRTI presents with a spectrum of symptoms including persistent cough, sputum production, dyspnea, chest pain, and fever [1, 2]. Among Gram-negative bacilli, common causative organisms include *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, which together represent a major cause of severe morbidity and hospitalization [2].

LRTI is a significant cause of morbidity and mortality worldwide [3]. According to the most recent Global Burden of Disease Study (GBD 2021), LRTI accounted for approximately 344 million incident episodes and 2.18 million deaths globally in 2021 [4]. The burden is disproportionately higher in developing countries, where diagnostic delays and limited access to appropriate antimicrobial therapy complicate management [5]. Because the clinical presentation of LRTI—cough, fever, and dyspnea—is non-specific and overlaps with other cardiovascular and upper respiratory conditions, these infections are often under-recognized, misdiagnosed, and consequently mismanaged [6]. This concern is particularly acute in low- and middle-income countries, where LRTI is reported to account for roughly one-fourth to one-half of pediatric hospital visits and remains among the leading causes of mortality in both adults over 70 years and children under 5 years of age [7]. Multidrug-resistant and extensively drug-resistant Gram-negative bacilli (GNB) are also disseminating rapidly across healthcare settings, creating substantial therapeutic challenges and placing additional strain on the healthcare infrastructure of developing nations [8].

Sputum is obtained by a non-invasive technique and is the most widely accepted non-sterile specimen for the diagnosis of LRTI. By the Bartlett/Murray-Washington criteria commonly used to assess specimen quality, an acceptable sputum sample is defined as one containing fewer than 10 squamous epithelial cells per low-power

field (10×) and more than 25 polymorphonuclear cells per high-power (oil-immersion) field (100×); samples meeting this criterion are considered acceptable for culture [9].

β-lactamase production is the principal mechanism of antibiotic resistance in both Gram-positive and Gram-negative bacteria [10]. Extended-spectrum β-lactamases (ESBLs) are enzymes that hydrolyse and confer resistance to oxyimino-cephalosporins (e.g., cefotaxime, ceftriaxone, cefuroxime, and cefepime) and monobactams, but not cephamycins or carbapenems, and are inhibited by clavulanate, sulbactam, and tazobactam. ESBLs belong to Ambler class A β-lactamases [11].

According to the Etiology of Pneumonia in the Community (EPIC) Study, funded by the U.S. Centers for Disease Control and Prevention (CDC) and reported by Jain et al. [12], viral etiologies were identified in more than half of hospitalized patients with community-acquired pneumonia, bacterial pathogens in approximately one-fourth, viral-bacterial co-infection in less than one-tenth, and fungal etiologies in a small minority of cases; the study did not report on mycobacterial infection separately, and this generalization should be interpreted with caution [12]. In a separate study of LRTI, the major bacterial etiologies were Gram-negative rods such as *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., and *Acinetobacter baumannii* [13], while *Streptococcus pneumoniae*, *Staphylococcus aureus*, group A streptococci, and *Enterococcus* spp. were the predominant Gram-positive organisms [14]. In Gram-negative bacteria, plasmids and other mobile genetic elements are major contributors to the dissemination of ESBL genes [15]. Mobile genetic elements also drive multidrug resistance in Gram-positive pathogens, primarily through transposon-mediated resistance or altered penicillin-binding proteins, whereas plasmid-mediated horizontal transfer remains the defining feature of expanding ESBL prevalence among Gram-negative bacilli [15, 16]. Because these plasmids frequently co-harbour resistance determinants alongside ESBL genes, isolates that acquire ESBL genes often concurrently acquire resistance to other antibiotic classes, including fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole, which considerably complicates the empirical treatment of ESBL-associated infections [16, 17].

The prevalence of LRTI varies with the distribution of causative agents [17], season [18], and geographic and socioeconomic conditions [19]. Several phenotypic methods have been developed for the detection of ESBL producers, with variable sensitivity; commonly used techniques include the double-disc synergy test, the combination disc method, and the E-test [20-23]. Molecular assays with high sensitivity and specificity and shorter turnaround time are also available [24].

Antimicrobial resistance (AMR) is a growing challenge to the effective management of infection, and ESBL producers represent a major component of this threat, both in developing countries such as Nepal and worldwide. Despite this concern, data on the antimicrobial resistance profile of ESBL-producing Gram-negative isolates associated with LRTI in Nepal remain limited, complicating the choice of empirical antimicrobial therapy. This study therefore aimed to screen for and confirm ESBL-producing Gram-negative organisms among patients with suspected LRTI and to characterise their antibiotic susceptibility profiles. The findings are intended to inform local antibiotic stewardship and guide empirical treatment choices for patients with suspected ESBL-associated LRTI in this setting.

# Materials and Methods

## Study design

This was a hospital-based cross-sectional study conducted at the Department of Microbiology, B and B Hospital Pvt. Ltd., Gwarko, Lalitpur, from January to June 2023. The study included clinically suspected LRTI patients, both inpatients and outpatients, who attended B and B Hospital Pvt. Ltd. for sputum culture and susceptibility testing. Patients presenting with clinical signs and symptoms consistent with LRTI were included.

## Sample size and inclusion criteria

Sample size (N) = $(Z\alpha)^2$ $p(1-p)/e^2$ (p = expected prevalence; e = allowable error)

Based on a reported prevalence of ESBL-positive Gram-negative bacteria in sputum specimens of 8.74% [25] (used here as p = 0.08 for a conservative estimate),

Sample size (N) = $(1.96)^2 \times 0.08 \times 0.91/0.1^2 = 27.96 \approx 28$

The minimum required sample size was therefore 28 sputum specimens for detection of ESBL producers. Samples collected under sterile conditions in a clean, non-leaking receptacle, properly labelled and accompanied by patient demographic information, were included in the study.

## Culture and bacterial isolation

Sputum samples were inoculated onto blood agar (BA), MacConkey agar (MA), and chocolate agar (CA). Following inoculation, plates were incubated at 37°C overnight; MA and BA plates were incubated aerobically,

and CA plates in a carbon-dioxide-enriched atmosphere using a candle jar. Growth was classified as significant (pathogenic) based on colony morphology, quantity, and correlation with the corresponding Gram-stained smear, in order to distinguish likely pathogens from oropharyngeal commensal (colonizing) flora; specimens without such growth were classified as culture-negative. Isolated colonies showing significant growth were characterised by colony morphology, Gram-stain reaction, and biochemical testing [26] (**Figures 1** and **2**). To ensure valid microbiological results, *Escherichia coli* ATCC 25922 (negative control) and *Klebsiella pneumoniae* ATCC 700603 (positive control for ESBL production) were used for quality control. As this study focused exclusively on the isolation and antimicrobial susceptibility profile of Gram-negative bacilli, these Gram-negative reference strains were selected in accordance with CLSI guidelines for Gram-negative quality control and phenotypic ESBL confirmation.

**Figure 1:** Cultural characteristics of *Klebsiella pneumoniae* on MacConkey agar.

**Figure 2:** Phenotypic biochemical profile for the identification of *Klebsiella pneumoniae* (From left to right: citrate positive, indole negative, triple sugar iron (TSI) acid/acid with no gas production and no hydrogen sulfide; and urease positive).

## Antibiotic susceptibility test

Antibiotic susceptibility testing (AST) of the Gram-negative isolates was performed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA), following CLSI guidelines [27] (**Figure 3**). The antibiotic discs used were amikacin (AK, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), ciprofloxacin (CIP, 5 µg), ofloxacin (OF, 5 µg), gentamicin (GEN, 10 µg), cotrimoxazole (COT, 25 µg), imipenem (IMP, 10 µg), and meropenem (MRP, 10 µg). MDR isolates were defined according to the criteria of Magiorakos et al. [28].


**Figure 3**: Antimicrobial susceptibility testing (AST) of multidrug-resistant *Klebsiella pneumoniae* (Isolate No. 67358) on Mueller-Hinton agar (MHA)

## Confirmation of ESBLs

Suspected ESBL isolates identified on screening were tested against ceftazidime and cefotaxime, each alone and in combination with clavulanic acid (cephalosporin + clavulanate, 30/10 µg) (**Figure 4**). Isolates showing a ≥ 5 mm increase in zone of inhibition with the combination disc compared with the corresponding cephalosporin disc alone, for either combination, were confirmed as ESBL producers [27]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were included as negative and positive controls, respectively, in each test run.

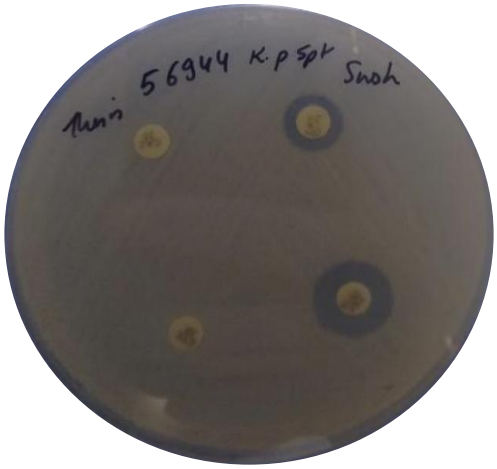


**Figure 4**: Phenotypic double-disc synergy confirmation test for ESBL-producing *Klebsiella pneumoniae*

### Data analysis

Data were analyzed using SPSS. Patient age categories, isolate frequencies, and patient-type distributions were tabulated, and the resistance and sensitivity percentages for each antibiotic, along with the percentage of MDR and ESBL-producing isolates, were calculated. The association between ESBL production and antibiotic resistance pattern was assessed using the chi-square test. Morphology of a representative *Klebsiella pneumoniae* strain (Isolate No. 56944) recovered from a sputum specimen of an LRTI patient, cultured on MacConkey agar and incubated aerobically at 37°C for 24 hours. The image demonstrates characteristic lactose-fermenting, bright pink, large, and highly mucoid colonies, indicative of prominent capsular polysaccharide production.

## Results

### Bacterial identification and prevalence

**Table 1:** Growth pattern of bacteria in sputum samples

| Bacterial isolates | Number | Percent |
|---|---|---|
| *K. pneumoniae* | 23 | 58.97 |
| *P. aeruginosa* | 4 | 10.25 |
| *E. fecalis* | 3 | 7.70 |
| *K. oxytoca* | 3 | 7.70 |
| *C. freundii* | 2 | 5.13 |
| *E. coli* | 2 | 5.13 |
| *S. aureus* | 1 | 2.56 |
| CONS | 1 | 2.56 |
| Total | 39 | 100 |

Of the 148 sputum specimens analysed by culture, 39 (26.35%) showed significant bacterial growth, representing six distinct genera. Gram-negative bacilli (GNB) were the predominant isolates, accounting for 87.1% (34/39) of all pathogens recovered. Among these, *Klebsiella pneumoniae* was the most common species (58.97%, n = 23), followed by *Pseudomonas aeruginosa* (10.25%, n = 4), *Klebsiella oxytoca* (7.7%, n = 3), and *Enterococcus faecalis* (7.7%, n = 3). Other organisms identified included *Escherichia coli* (5.13%), *Citrobacter freundii* (5.13%), and low frequencies of *Staphylococcus aureus* and coagulase-negative staphylococci (2.56% each) (**Table 1**).

### Distribution of Gram negative bacterial isolates among inpatients and outpatients

Among the 34 Gram-negative bacilli (GNB) isolates, the large majority were recovered from inpatients (85.3%, n = 29), while outpatients accounted for 14.7% (n = 5) (**Table 2**). By gender and age, the peak incidence among male patients occurred in the 21-40 year age, whereas among female patients the peak incidence occurred later, in the 61-80 year age (**Table 3**).

**Table 3:** Distribution of Gram negative bacterial isolates among inpatients and outpatients

| Organism | Inpatient | Outpatient |
|---|---|---|
| *K. pneumoniae* | 19 | 4 |
| *P. aeruginosa* | 4 | 0 |
| *K. oxytoca* | 2 | 1 |
| *C. freundii* | 2 | 0 |
| *E. coli* | 2 | 0 |
| Total | 29 (85.30%) | 5 (14.70%) |

### Antibiotic susceptibility pattern of Gram-negative bacteria

Susceptibility testing was performed against nine antibiotics from five classes—amikacin (AK), gentamicin (GEN), cefotaxime (CTX), ceftazidime (CAZ), ciprofloxacin (CIP), ofloxacin (OF), cotrimoxazole (COT), imipenem (IMP), and meropenem (MRP)—for isolates of *E. coli*, *K. pneumoniae*, *K. oxytoca*, *C. freundii*, and *P. aeruginosa*. All *E. coli* isolates (100%) were resistant to ceftazidime, but were 100% sensitive to amikacin, gentamicin, ciprofloxacin, ofloxacin, imipenem, and meropenem.

Among *K. pneumoniae* isolates, the highest resistance was observed against cefotaxime (87%) and ceftazidime (82.6%), followed by cotrimoxazole (78.3%). These isolates also showed 60.9% resistance to gentamicin, ciprofloxacin, and ofloxacin, while resistance to imipenem and meropenem was lower, at 39.1%. *K. oxytoca* isolates showed resistance to all antibiotics tested (100%) except imipenem and meropenem, to which resistance was 33.3%.

**Table 2**: Gender and age distribution of the patients

| Gender<br>Age category | Total number of Male | Infected Male (%) | Total number of Female | Infected Female (%) | Total Number of infected patients |
|---|---|---|---|---|---|
| 0-20 | 35 | 1 (2.84) | 4 | 0 (0) | 1 (2.56) |
| 21-40 | 15 | 8 (52) | 5 | 0 (0) | 8 (40) |
| 41-60 | 17 | 6 (35) | 9 | 0 (0) | 6 (23.08) |
| 61-80 | 17 | 6 (35) | 11 | 9 81.81) | 15(53.57) |
| 81-100 | 27 | 3(11.11) | 8 | 1 (12.5) | 4 (11.4) |
| Total | 111 | 24 | 37 | 10 | 34 |

**Table 4:** Antibiotic susceptibility pattern of Gram-negative bacteria

| Antibiotic class | Antibiotics | Sensitive No (%) | Intermediate No (%) | Resistant No (%) |
|---|---|---|---|---|
| Aminoglycoside | Amikacin | 20 (58.8) | 0 (0) | 14 (41.2) |
| | Gentamycin | 15 (44.1) | 1 (2.9) | 18 (52.9) |
| Beta lactams | Cefotaxime | 4 (11.8) | 0 (0) | 30 (88.2) |
| | Ceftazidime | 5 (14.7) | 0 (0) | 29 (85.3) |
| Fluoroquinolones | Ciprofloxacin | 14 (41.2) | 2 (5.9) | 18 (52.9) |
| | Ofloxacin | 15 (44.1) | 2 (5.9) | 17 (50.0) |
| Sulfonamide Trimethoprim | Cotrimoxazole | 7 (20.6) | 1 (2.9) | 26 (76.5) |
| Carbapenem | Imipenem | 21 (61.8) | 1 (2.9) | 12 (35.3) |
| | Meropenem | 21 (61.8) | 1 (2.9) | 12 (35.3) |

*C. freundii* isolates were susceptible, with 0% resistance to most agents, except for cotrimoxazole (50%) and universal resistance (100%) to cefotaxime and ceftazidime. *P. aeruginosa* isolates showed no resistance (0%) to amikacin and ofloxacin but were fully resistant (100%) to cefotaxime (**Table 4**).

**Distribution of multidrug-resistant Gram-negative bacteria**

**Table 5**: Antibiotic resistance pattern of each Gram-negative bacteria

| Antibiotics / Bacteria | AK No (%) | GEN No (%) | CAZ No (%) | CTX No (%) | CIP No (%) | OF No (%) | COT No (%) | IMP No (%) | MRP No (%) |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* (n=2) | 0 (0%) | 0 (0) | 2 (100) | 1 (50) | 0 (0) | 0 (0) | 1 (50) | 0 (0) | 0 (0) |
| *K. pneumoniae* (n=23) | 11 (47.8) | 14 (60.9) | 19 (82.6) | 20 (87) | 14 (60.9) | 14 (60.9) | 18 (78.3) | 9 (39.1) | 9 (39.1) |
| *K. oxytoca* (n=3) | 3 (100) | 3 (100) | 3 (100) | 3 (100) | 3 (100) | 3 (100) | 3 (100) | 1 (33.3) | 1 (33.3) |
| *C. freundii (n=2)* | 0 (0) | 0 (0) | 2 (100) | 2 (100) | 0 (0) | 0 (0) | 1 (50) | 0 (0) | 0 (0) |
| *P. aeruginosa (n=4)* | 0 (0 ) | 1 (25) | 3 (75) | 4 (100) | 1 (25) | 0 (0) | 3 (75) | 2 (50) | 2 (50) |

MDR was confirmed in 59% of the Gram-negative isolates (**Figure 5**). By species, MDR was most frequent among *K. oxytoca* and *K. pneumoniae* isolates, reflecting the higher resistance rates observed for these species across multiple antibiotic classes above, whereas *C. freundii* isolates, which remained susceptible outside cotrimoxazole and the cephalosporins, contributed the fewest MDR strains.

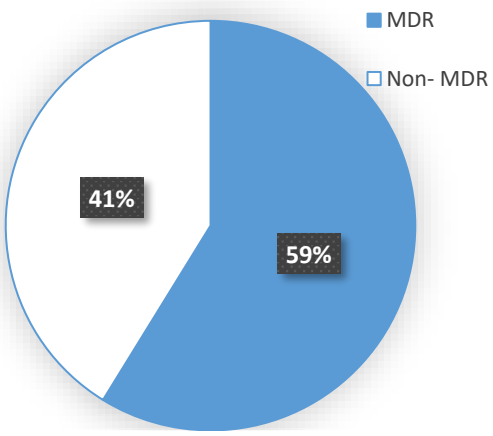


**Figure 5:** Distribution of multidrug resistant Gram-negative bacteria

## Gram-negative ESBL isolates in sputum samples

Phenotypic testing confirmed ESBL production in 38.23% (13/34) of Gram-negative isolates, with *K. pneumoniae* showing the highest proportion of ESBL producers (43.47%) (**Table 6**).

**Table 6:** Gram-negative ESBL isolates in sputum samples

| Bacteria | Total isolates | ESBL screening positive | Confirmed ESBL producers |
|---|---|---|---|
| *E. coli* | 2 | 2 | 1 (50) |
| *C. freundii* | 2 | 2 | 1 (50) |
| *K. pneumoniae* | 23 | 21 | 10 (43.47) |
| *K. oxytoca* | 3 | 3 | 1 (33.34) |
| *P. aeruginosa* | 4 | 4 | 0 (0) |

## Antibiotic resistance in ESBL-producing and non-producing Gram-negative isolates

Among the confirmed ESBL producers, every isolate (13/13, 100%) was resistant to cotrimoxazole, followed by ciprofloxacin and ofloxacin (53%, n = 7). The lowest resistance among ESBL producers was to imipenem and meropenem (30.8%, n = 4). Among ESBL non-producers, the highest resistance was to cefotaxime (81%, n = 17), and the lowest was to amikacin, imipenem, and meropenem (42.9%, n = 9). Statistical analysis showed a significant association between ESBL production and resistance to cotrimoxazole ($P < 0.01$), but no significant association between ESBL production and resistance to amikacin, gentamicin, ofloxacin, ciprofloxacin, imipenem, or meropenem ($P > 0.05$ for all).

# Discussion

In this study, Gram-negative bacteria, and *Klebsiella pneumoniae* in particular, were the predominant etiological agents of LRTI, with MDR and ESBL production observed in 59% and 38.23% of Gram-negative isolates, respectively. Growth was recovered from approximately one-fourth of specimens from suspected LRTI patients, consistent with the observation that not every clinically suspected case yields a culturable pathogen. The predominance of Gram-negative

**Table 7:** Antibiotic resistance pattern of ESBL positive and negative Gram-negative isolates

| Antibiotic class | Antibiotics | ESBL producer No (%) | ESBL non-producer No (%) | p-value |
|---|---|---|---|---|
| Aminoglycosides | Amikacin (AK) | 5 (38.5) | 9 (42.9) | 0.800 |
| | Gentamycin (GEN) | 6 (46.2) | 13 (61.9%) | 0.369 |
| Cephalosporin | Cefotaxime (CTX) | 13 (100) | 17 (81.0) | **0.041** |
| | Ceftazidime (CAZ) | 13 (100) | 16 (76.2) | **0.021** |
| Fluoroquinolone | Ciprofloxacin (CIP) | 7 (53.8) | 13 (61.9) | 0.643 |
| | Ofloxacin (OF) | 7 (53.8) | 12 (57.1) | 0.851 |
| Sulphonamide and trimethoprim | Cotrimoxazole (COT | 13 (100) | 14 (66.7) | **0.005** |
| Carbapenems | Imipenem (IMP) | 4 (30.8) | 9 (42.9) | 0.478 |
| | Meropenem (MRP) | 4 (30.8) | 9 (42.9) | 0.478 |

organisms, and of *K. pneumoniae* specifically, agrees with the findings of Mishra et al. [29] and Pokhrel et al. [30, 31], although Maharjan et al. [25] and Shilpakar et al. [32] reported comparatively lower rates for this pathogen; this variation may reflect differences in patient case-mix, hospital antibiotic policy, or regional colonization patterns between study sites rather than a true difference in underlying epidemiology.

Over three-quarters of GNB-associated LRTI cases in this study occurred in hospitalised patients, consistent with Regmi et al. [33]. This elevated risk among inpatients is plausibly related to hospital exposures such as thoracic or abdominal surgery [34], upper-respiratory colonization with subsequent micro-aspiration [35], or mechanical ventilation and intubation [36, 37]. By contrast, Vijay and Dalela [38] and Emori and Gaynes [39] reported a lower proportion of inpatient cases, which may reflect differences in referral patterns or the relative proportion of ICU/ventilated patients between settings.

The higher prevalence of GNB-associated LRTI observed among males in the 21-40-year age group may plausibly relate to occupational exposures or lifestyle factors such as smoking or alcohol use, consistent with the pattern reported by Bonten et al. [40] and Craven and Steger [41], although this study did not directly assess occupational or behavioural exposures. The higher prevalence among older females (61-80 years) is more consistent with age-related decline in mucociliary clearance and immune function [40] than with a specific gender-linked mechanism.

Approximately 90% of GNB isolates were resistant to the cephalosporins tested (ceftazidime and cefotaxime), indicating that these agents are unsuitable as first-line empirical therapy for LRTI in this setting. Overuse of cephalosporins for minor febrile illness without prior diagnostic testing is a plausible contributor to this high resistance [42], consistent with the higher resistance levels reported by Singla et al. [43] and Tang et al. [44]; lower resistance was reported by Raghubanshi and Karki [45] and Vishwanath et al. [46], a discrepancy that may reflect differences in local antibiotic prescribing practice between settings.

Resistance to the carbapenems (imipenem and meropenem) was observed in approximately 35% of isolates, indicating that these agents remain broadly effective against Gram-negative LRTI in this setting, in agreement with Raghubanshi and Karki [45]; higher resistance was reported by Tang et al. [44]. Because carbapenems are considered agents of last resort [31], they should not be used empirically where alternatives such as amikacin or gentamicin remain effective. Given the resistance pattern observed, cotrimoxazole and third-generation cephalosporins should be avoided for empirical therapy, whereas aminoglycosides and carbapenems currently offer more reliable coverage.

MDR was detected in 59% of Gram-negative isolates, similar to the findings of Bonten et al. [40] and Mishra et al. [29], although Panda et al. [47] reported a substantially higher proportion (over three-quarters) of MDR isolates. Multidrug resistance in Gram-negative bacteria arises through multiple, often co-occurring mechanisms, including chromosomal mutation, horizontal transfer of resistance genes via plasmids, integrons, and transposons, efflux-pump overexpression, and altered outer-membrane permeability [48]. These findings indicate that multidrug resistance is increasing among Gram-negative isolates in this setting and warrants active surveillance and stewardship measures [40].

ESBL production was confirmed in 38.23% of Gram-negative isolates, with roughly half of *E. coli* and *C. freundii* isolates identified as ESBL producers; a similar pattern was reported by Panda et al. [39] and Shilpakar et al. [32] More than two-fifths of *K. pneumoniae* isolates produced ESBL, comparable to the rate reported by Panda et al. [39] Approximately one-third of *K. oxytoca* isolates produced ESBL, a lower proportion than reported by Patel et al. [49] for this species. None of the *P. aeruginosa* isolates were ESBL producers, consistent with Pokhrel et al. [31]. Infection with ESBL-producing organisms is associated with poorer clinical outcomes, including higher mortality [50] and longer hospital stay

[51], underscoring the importance of prompt detection and reporting of ESBL-positive isolates to guide patient management.

All ESBL-positive Gram-negative isolates showed resistance to third-generation cephalosporins (cefotaxime and ceftazidime) and cotrimoxazole, similar to the resistance profiles reported by Kaneria et al. [52], Guzek et al. [53], and Tayh et al. [54] Using these agents empirically for LRTI in this setting therefore carries a substantial risk of treatment failure, as highlighted by Kaneria et al. [52] and Tayh et al. [54] The strong statistical association between ESBL production and cotrimoxazole resistance observed here suggests co-harbouring of ESBL and cotrimoxazole-resistance genes on the same mobile genetic elements [16], though this study did not perform molecular confirmation and this interpretation remains inferential. Approximately half of the ESBL-positive isolates were resistant to the fluoroquinolones (ciprofloxacin and ofloxacin), consistent with an association between ESBL production and fluoroquinolone resistance reported elsewhere [55] and with the findings of Tayh et al. [54]. More than half of ESBL-positive isolates remained susceptible to amikacin and gentamicin, in line with Kaneria et al. [52] and Tayh et al. [8, 54], suggesting these agents remain useful options against ESBL-producing isolates locally. Approximately 30% of ESBL-producing isolates were resistant to the carbapenems (imipenem and meropenem), consistent with Tayh et al. [54], although Guzek et al. reported no carbapenem resistance in their series [53], a discrepancy that may reflect differing local antibiotic exposure and stewardship practices. Overall, these findings support prioritising aminoglycosides and carbapenems over third-generation cephalosporins, cotrimoxazole, and fluoroquinolones when treating suspected ESBL-associated LRTI in this setting.

### Limitations

This study has several limitations. It was conducted at a single centre over a relatively short six-month period, which may limit the generalisability of the findings to other settings or seasons. ESBL production was confirmed phenotypically only, without molecular confirmation.

### Conclusion

Gram-negative bacteria, particularly *Klebsiella pneumoniae*, were the leading cause of LRTI in this cohort, with a high burden of both multidrug resistance and ESBL production. ESBL-producing isolates were most resistant to cotrimoxazole, while ESBL non-producers were most resistant to the cephalosporins; the carbapenems and aminoglycosides remained the most reliable options against both groups. These findings support restricting empirical use of cephalosporins and cotrimoxazole for suspected LRTI in this setting, reserving carbapenems for confirmed or strongly suspected MDR/ESBL infection, and strengthening local antimicrobial stewardship and surveillance. Routine, timely phenotypic screening for MDR and ESBL production in Gram-negative respiratory isolates could help guide more appropriate antibiotic selection and improve patient outcomes in similar hospital settings.

## Author's Contribution

Sushma Shrestha: Conceptualization, Laboratory work, Clinical and Experimental studies, Data collection, Manuscript writing, and Editing; Ms. Roshani Maharjan: Supervision, Methodology validation, Editing, and Review; Mr. Sanjit Shrestha: Technical guidance for laboratory work, Resource management (B and B Hospital) and Data acquisition

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## Competing Interests

There were no disclosures of competing interests.

## Ethical Approval

Ethical approval for this study was granted by the Institutional Review Committee (IRC) of Tribhuvan University, Institute of Science and Technology (TU-IoST), Kirtipur, Kathmandu (Reg. No. IRCIOST-23-0056). Permission to collect samples and access laboratory facilities was also obtained from the hospital administration of B and B Hospital Pvt. Ltd., Lalitpur. All procedures were conducted in accordance with applicable ethical guidelines.

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