# Multi-drug Resistance, β-Lactamases Production, and Coexistence of *bla*$_{NDM-1}$ and *mcr-1* in *Escherichia coli* Clinical Isolates From a Referral Hospital in Kathmandu, Nepal

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**ABSTRACT:** The ability of pathogenic *Escherichia coli* to produce carbapenemase enzymes is a characteristic that allows them to resist various antibiotics, including last-resort antibiotics like colistin and carbapenem. Our objectives were to identify rapidly developing antibiotic resistance (AR), assess β-lactamases production, and detect *mcr-1* and *bla*$_{NDM-1}$ genes in the isolates. A prospective cross-sectional study was carried out in a referral hospital located in Kathmandu from November 2019 to December 2020 using standard laboratory and molecular protocols. Among 77 total *E. coli* isolates, 64 (83.1%) of them were categorized as MDR. Phenotypically 13 (20.3%) colistin-resistant, 30 (46.9%) ESBL and 8 (12.5%) AmpC producers, and 5 (7.8%) ESBL/AmpC co-producers were distributed among MDR-*E. coli*. Minimum inhibitory concentrations (MIC) against the majority of MDR isolates were exhibited at 1 g/L. Of these 77 *E. coli* isolates, 24 (31.2%) were carbapenem-resistant. Among these carbapenem-resistant bacteria, 11 (45.9%) isolates were reported to be colistin-resistant, while 15 (62.5%) and 2 (8.3%) were MBL and KPC producers, respectively. Out of 15 MBL producers, 6 (40%) harbored *bla*$_{NDM-1}$, and 8 (61.5%) out of 13 colistin-resistant pathogens possessed *mcr-1*. The resistance by colistin- and carbapenem were statistically associated ($P < .001$). However, only 2 (18.2%) of the co-resistant bacteria were found to have both genes. Our study revealed the highly prevalent MDR and the carbapenem-resistant *E. coli* and emphasized that the pathogens possess a wide range of capabilities to synthesize β-lactamases. These findings could assist to expand the understanding of AR in terms of enzyme production.

**KEYWORDS:** Multidrug resistance, carbapenem resistance, *mcr-1*, β-lactamases, *bla*$_{NDM-1}$

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## Introduction

Normal habitats for *Escherichia coli* include the intestines of people and other animals, as well as non-host surroundings like foods, soils, and wastes.[1,2] However, some strains of *E. coli* can serve as versatile pathogens and cause illnesses inside the body and outside it.[3] Due to the presence of several efflux pumps, their overexpression, and the development of antibiotic resistance (AR) through horizontal gene acquisition, they may be challenging to treat.[4,5] Antimicrobial resistance (AMR) is primarily caused by the transfer of genes that encode β-lactamases.[6-9] New Delhi metallo-lactamase-1 (NDM-1), a metallo-β-lactamase enzyme encoded by *bla*$_{NDM-1}$ has been described[7,8] and this enzyme may assist in conferring multidrug resistance (MDR).[9-11] Colistin is regarded as a potent antibiotic, even against Enterobacteriaceae that produce NDM-1.[12] Plasmid-mediated encoding genes like *mcr-1*, facilitate the horizontal spread of the drug.[12] The *mcr-1* gene, which confers colistin resistance, was first discovered in China, but later reports of it in other European and Asian nations, including Nepal, were recorded.[14,15] This gene may promote pan-drug resistance as it spreads in carbapenem-resistant *E. coli* (CREco).[13]

Colistin resistance in environmental strains has its origin in poultry and cattle farming, which exploits an inappropriate amount of the antibiotic to stimulate growth.[11,16-18] This reveals why *mcr-1* is found in farms and could be the cause of transmission to humans through the surroundings and food system. Similarly, clinical *E. coli* isolates from Nepal have been reported to contain NDM-1 as well as several NDM variants.[11,19,20] If a plasmid encoding the *bla*$_{NDM-1}$ gene is acquired by the colistin-resistant organism, bacterial infections may become incurable.[20]

This study will help to investigate the prevalent colistin-resistant *E. coli* (ColREco) and CREco and their capacity to produce antagonistic enzymes against antibiotics in Nepal because there hasn't been much research on these topics. Furthermore, research on the coexistence of the *mcr-1* and *bla*$_{NDM-1}$ genes may be crucial for molecular epidemiology. Therefore, our study attempted to explore multidrug resistance, and screen the production of the enzymes involving AmpC β-lactamases (AmpC), Extended-spectrum β-lactamases (ESBL), *Klebsiella pneumoniae* carbapenemase (KPC), and Metallo-β-lactamases (MBL) productions, demonstrate colistin, and carbapenem resistance, determine the

colistin-minimum inhibitory concentration (MIC) and identify the co-existing *mcr-1* and $bla_{NDM-1}$ genes in the pathogen from clinical samples.

## Materials and Methods

### *Sample collection*

The Institutional Review Committee at the Institute of Science and Technology granted ethical approval (IRC/IOST-Regd. No. 26) for conducting this research. Using a hospital-based prospective study design, the research was conducted at Shahid Gangalal National Heart Center and Central Department of Microbiology in Kathmandu. The work duration was from November 2019 to December 2020. Patients provided samples to the hospital laboratory that were allowed for regular culture, including urine, blood, sputum, throat swabs, bodily fluids, endotracheal secretions, stool, cerebrospinal fluid (CSF), wound/pus aspirates, and tissue secretions. As per the accepted microbiological guidelines, the skilled healthcare professionals provided instructions and/or collected the samples as needed under aseptic settings. Within 2 hours of sample collection, all samples were processed for culture.

### *Bacterial culture and identification*

According to the requirements, specimens were inoculated into various culture media (Hi-Media Laboratories Pvt. Ltd., India) and then they were cultured at 37°C. Catheter tips, wound, and pus aspirates were introduced into BA (Blood Agar) and MA (MacConkey Agar) and incubated aerobically overnight. The MA, BA, and Chocolate agar (CA) plates were inoculated with sputum, throat swabs, endotracheal secretions, and body fluids. BA and CA plates were incubated in 5% to 10% carbon dioxide enriched atmosphere. In the same way, in addition to BA and MA, urine was inoculated on CLED (Cysteine Lysine Electrolyte Deficient) Agar and UTI CHROMagar. In CHROMagar, the color of the colony distinct to each pathogen was observed, whereas significant bacteriuria was recognized when there were more than $10^5$ CFU/mL bacteria in CLED agar.[21] Blood specimens were inoculated in Brain-Heart Infusion (BHI) broth in a 1:10 ratio to incubate for up to 7 days. Two times every day for up to 3 days, those culture bottles were visually evaluated for microbial growth. When the growth was suspected, it was further subcultured on BA and MA agar plates. By observing the colony's morphology and color on culture media, isolates were screened.[22] Gram staining and several biochemical assays were also carried out. The presence of *E. coli* on Eosin-methylene blue (EMB) agar was further supported by the presence of their metallic green sheen.[23]

### *Phenotypic antibiotic susceptibility test*

Referencing with Clinical Laboratory Standard Institute's guidelines (CLSI, 2019), the identified *E. coli* were subjected to Kirby-Bauer disk diffusion antibiotic susceptibility testing. Those confirmed isolates were cultured on Mueller Hinton Agar (MHA). Antibiotics (HiMedia Laboratories Pvt Ltd, India) particularly amikacin (30 µg), ampicillin (10 µg), ampicillin/sulbactum (10/10 µg), aztreonam (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), colistin (10 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), norfloxacin (5 µg), piperacillin/tazobactum (100/10 µg), tetracycline (30 µg) and tigecycline (15 µg) were tested for susceptibility.[24]

### *Phenotypic confirmation of ESBL and AmpC producers*

It was anticipated that the organisms showing reduced susceptibility to cefotaxime (30 µg) and ceftazidime (30 µg) with zones of inhibition (ZOI) ⩽27 and ⩽22 mm, respectively, during antibiotic susceptibility tests, could be ESBL producers. According to the CLSI recommendations, the suspected producers were confirmed by combined disk method.[24] This technique allows the use of ceftazidime (30 µg) and cefotaxime (30 µg) disks set both alone as well as in combination arrangement with clavulanic acid (10 µg). The increment in ZOI of ⩾5 mm in combination compared to cefotaxime and ceftazidime disks alone was reported as ESBL producers.

*E. coli* were presumptively examined for AmpC production by measuring their susceptibility in vitro to cefoxitin (30 µg). All the micro-organisms with ZOI ⩽18 mm were suspected to be screening test positive.[25] The screened positive strains were confirmed by inhibition-based methods. The ZOI with the increase in 5 mm around the disk containing boronic acid and cefoxitin in comparison with the diameter around the disk of cefoxitin alone, was regarded as an AmpC producer.[26]

### *Phenotypic identification of CREco and KPC producers*

CREco was determined by using imipenem (10 µg) and meropenem (10 µg). In susceptibility testing by disk diffusion, ZOI ⩽23 mm for both imipenem (10 µg) and meropenem (10 µg) were reported carbapenem-resistant. CREco was further tested for possible carbapenemase production.[24]

All CREco were tested for KPC production by the combined-disk method. These strains, equivalent to 0.5 McFarland in nutrient broth were carpeted on the MHA plates. From the stock solution of Phenylboronic acid [PBA]), 20 µL was dispensed on top of one meropenem disk and allowed to dry. Both meropenem disks with and without PBA were placed onto the MHA plate. An increase of ⩾5 mm ZOI around the meropenem disk combined with PBA compared to meropenem alone was reported as a KPC producer strain.[27]

**Table 1.** Optimization settings of *mcr-1* and $bla_{NDM-1}$ in PCR.

| S.N. | AMPLICON | OPTIMIZED CONDITIONS | | | | REF. |
|---|---|---|---|---|---|---|
| | | CYCLES | DENATURATION | ANNEALING | EXTENSION | |
| 1. | *mcr-1* | 35 | 94°C for 30 s | 57°C for 90 s | 72°C for 90 s | Liu et al[14] |
| 2. | $bla_{NDM-1}$ | 36 | 94°C for 30 s | 52 °C for 40 s | 72°C for 50 s | Nordmann et al[31] |

### *Phenotypic confirmatory test for MBL producers*

MBL production in *E. coli* was determined by the combined-disk method. In this method, the test strains with 0.5 McFarland standard suspension were lawn cultured on MHA plates. Two imipenem disks (10 µg) were placed on an MHA plate, one combined with 0.5 M EDTA (10 µL). After incubating overnight, the ZOI of imipenem and imipenem + EDTA disks were measured. A difference in ZOI by ⩾ 7 mm in combined disk compared to imipenem alone was considered MBL positive.[28,29] All MBL-positive *E. coli* in phenotypic tests were selected for molecular detection of $bla_{NDM-1}$.

### *Determination of MIC using broth dilution test for colistin*

Against MDR-*E. coli*, the in vitro activity of colistin was measured quantitatively by broth dilution method. For evaluating the MIC value of an isolate, a set of tubes that contained an identical volume of Mueller Hinton Broth (MHB) with colistin solution in geometrically increasing concentration (0.5 g /L-32 g/L) following the inoculation with a known number of bacteria ($10^6$ CFU/mL) was prepared. After proper incubation of tubes, MIC was determined by observing the tube with the lowest level of the concentration of antibiotics at which visible growth was absent.[24] MIC results were interpreted based on a joint recommendation by the EUCAST expert system and the CLSI considering the clinical breakpoints ($S \leqslant 2$ g/L and $R > 2$ g/L).

### *Extraction of plasmid DNA*

Plasmids from positive phenotypic tests of MBL producers from total isolates and ColREco conferring multi-drug resistance were extracted for detection of $bla_{NDM-1}$ and *mcr-1* respectively. The inoculated organisms were incubated in Luria-Bertani (LB) broth for overnight with aeration and shaking in a water bath maintaining the temperature of 37°C. Extraction was performed by the phenol-chloroform method.[30] TE buffer (50 µL) was used to preserve the extracted DNA and was stored at −20°C to conduct further investigations.

### *PCR amplification*

Amplification of *mcr-1* (309 bp) from extracted plasmids was performed in a thermocycler (Applied Biosystems, USA) by conventional PCR using primer pairs CLR5-Forward (5′-CGGTCAGTCCGTTTGTTC-3′) and CLR5-Reverse (5′-CTTGGTCGGTCTGTAGGG-3′).[14] NDM-Forward (5′-GGTTTGGCGATCTGGTTTTC-3′) and NDM-Reverse (5′-CGGAATGGCTCATCACGATC-3′) were the primers used to detect $bla_{NDM-1}$, amplifying a 621 bp internal region of the gene.[31] By observing the band in UV-transillumination, the targeted genes were confirmed. The optimized settings for detecting these genes are shown in Table 1.

### *Quality control*

Each batch of prepared media was checked for quality and sterility by incubating one prepared plate/tube for 24 hours at 37°C and performance testing by using standard control strain. To assure that the inoculation utilized for the biochemical assays was pure culture, the purity plate was used. We used control strains of *E. coli* ATCC 25922 for standardization of susceptibility testing and correct interpretation of ZOI of each antibiotic disk. Similarly, the performance of colistin powder in MIC was tested by interpreting the MIC value of colistin for control strain *E. coli* ATCC 25922.[23]

### *Data management and analysis*

GraphPad Prism 8.4.3 and the statistical package for social science (SPSS) software (Version 21.0) were both used to examine all of the experimental data that had been entered into MS Excel 2007. Frequency and percentage were calculated. Nominal data were compared with help of the Chi-square test. When the *P*-value was ⩽.05, the variables were considered statistically significant. MDR-*E. coli* were those that tested positive for 3 or more classes of antibiotics.[32]

## Results

### *Distribution of* E. coli *among clinical specimens*

In the research period, 1252 clinical specimens were processed and only 296 (23.6%) specimens showed bacterial growth. Among 296 growth-positive specimens, 286 (96.6%) had monomicrobial growth, whereas 10 (3.4%) specimens had polymicrobial growth. In total, 311 bacteria were isolated, including 119 (38.3%) Gram-positive and 192 (61.7%) Gram-negative bacteria. The predominant pathogen was *E. coli* (n = 77) from various clinical specimens. The majority of our obtained *E. coli*

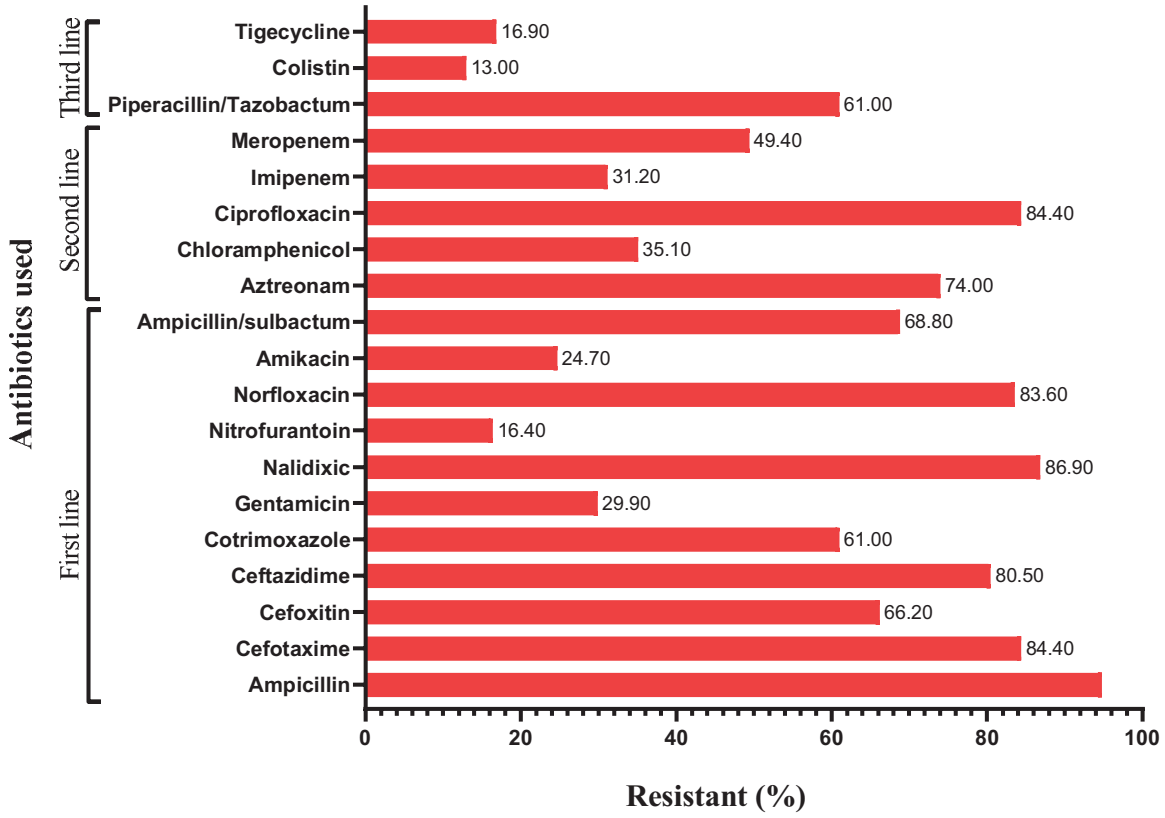


**Figure 1.** Antibiotic resistance profile (%) in *E. coli* (n = 77).

isolates were from urine (79.2%), followed by sputum (7.8%) and blood (5.2%) respectively.

### *Antibiotic susceptibility, MDR, and colistin resistance*

The majority of the isolates exhibited sensitivity toward colistin (87%). Tigecycline was found effective against 83.1% of *E. coli* isolates, and nitrofurantoin for 83.6% of uropathogenic *E. coli* (UPEC). The majority of isolates were resistant to ampicillin (94.8%). Among 77 *E. coli*, 64 (83.1%) were found to be MDR (Figure 1). Out of 64 MDR-*E. coli* isolates, 13 (20.3%) were colistin-resistant.

### *MIC of colistin among MDR*-E. coli

MICs of colistin for those 64 MDR-*E. coli* isolates were found to be ranged from 1 to 16 (g/L). The majority of the isolates had MIC at 1 g/L, then at 2 and 4 g/L (Table 2).

### *Colistin-resistant MDR*-E. coli *harboring* mcr-1

Colistin resistance was determined in 13 out of 64 MDR isolates, and among them, 8 (61.5%) were *mcr-1* positive (Supplemental File Figure 1).

### *ESBL and AmpC producers among MDR*-E. coli

Among 64 MDR-*E. coli* isolates, 30 (46.9%) and 8 (12.5%) were ESBL and AmpC producers respectively and 5 (7.8%) were ESBL/AmpC co-producers. Statistically, there was no significant association between ESBL production and AmpC production in MDR-*E. coli* ($P = .344$) (Table 3).

**Table 2.** MIC value of colistin among MDR *E. coli*.

| S.N. | MIC VALUE (G/L) | NO. OF MDR *E. COLI* |
|---|---|---|
| 1. | 1 | 34 |
| 2. | 2 | 17 |
| 3. | 4 | 8 |
| 4. | 8 | 3 |
| 5. | 16 | 2 |

### *KPC and MBL producers among CREco*

Among the total 77 *E. coli* isolates, 24 (31.2%) were resistant to carbapenem. Among 24 CREco, 62.5% and 8.3% were MBL and KPC producers respectively. Moreover, KPC producers were also found to be co-producer of MBL. Statistically, there was a significant relationship between KPC production and MBL production in *E. coli* ($P = .040$) (Table 4).

### bla$_{NDM-1}$ *in MBL-producing* E. coli

Among 15 MBL producer *E. coli*, 6 (40%) isolates were *bla*$_{NDM-1}$ positive (Supplemental File Figure 2).

**Table 3.** AmpC and ESBL production among MDR *E. coli*. .

| ESBL | AMPC | | TOTAL (%) | *P*-VALUE |
|---|---|---|---|---|
| | PRODUCERS (%) | NON-PRODUCERS (%) | | |
| Producers | 5 (16.7) | 25 (83.3) | 30 (46.9) | .344* |
| Non-producers | 3 (8.8) | 31 (91.2) | 34 (53.1) | |
| Total | 8 (12.5) | 56 (87.5) | 64 (100) | |

*Chi-square test.

**Table 4.** Distribution of KPC and MBL production among carbapenem-resistant *E. coli*.

| MBL | KPC | | TOTAL (%) | *P*-VALUE |
|---|---|---|---|---|
| | PRODUCER (%) | NON-PRODUCER (%) | | |
| Producer | 2 (13.3) | 13 (86.6) | 15 (62.5) | .040* |
| Non- producer | 0 (0.0) | 9 (100) | 9 (37.5) | |
| Total | 2 (8.3) | 22 (91.6) | 24 (100) | |

*Chi-square test.

**Table 5.** Colistin and carbapenem co-resistance in *E. coli*.

| CARBAPENEM | COLISTIN | | TOTAL (%) | *P*-VALUE |
|---|---|---|---|---|
| | RESISTANT (%) | SENSITIVE (%) | | |
| Resistant | 11 (45.8) | 13 (54.2) | 24 (31.2) | <.001* |
| Sensitive | 2 (3.8) | 51 (96.2) | 53 (68.8) | |
| Total | 13 (16.9) | 64 (83.1) | 77 (100) | |

*Chi-square test.

### *Colistin-resistance among CREco*

Out of a total of 24 CREco from total isolates, 11(45.9%) isolates were resistant to colistin. The prevalence of co-resistant (toward colistin and carbapenem) *E. coli* was 14.3% (11/77). Statistically, there was demonstrated a significant link between carbapenem- and colistin resistance ($P < .001$) (Table 5).

### *Co-existing* bla$_{NDM-1}$ *and* mcr-1

Among 11 *E. coli* isolates co-resistant to carbapenem and colistin, only 2(18.2%) isolates were found co-harboring NDM-1 and colistin resistance encoding genes.

### *Co-harboring* bla$_{NDM-1}$ *and* mcr-1 *genes*

Two *E. coli* isolates, EC1 and EC2, isolated from sputum and blood respectively, were found to co-harbor *mcr-1* and *bla*$_{NDM-1}$. Both isolates were sensitive toward colistin, aminoglycosides (Amikacin, and gentamicin), chloramphenicol, and tigecycline.

## Discussion

The emergence of many resistant microorganisms over the last decades has been noticed to create threatening status in human population and could be negatively impact in a higher level if it not controlled in time[33,34] Our study assessed a prevalence of MDR-*E. coli* along with the coproduction of β- lactamases of different classes, the occurrence of AR, and also the coexistence of the resistance-conferring genes in clinical *E. coli*.

Only about one-fourth of the total samples showed bacterial growth positivity. The common isolates were Gram-negative because the infection caused by the group is predominant in hospital settings[35,36] and their persistence in community settings is also reported.[37] The majority of them were *E. coli* (n = 77) since the organism is frequently associated with nosocomial and community-acquired infections.[38] *E. coli* being the principal cause of urinary tract infections is the reason for its higher occurrence in urine compared to other samples in our study.[39,40] High rates of AMR and occurrence of virulence genes are being common in *E. coli* causing urinary tract and blood- associated infections.[41,42]

In the antibiotic susceptibility testing, *E. coli* isolates were commonly resistant to the aminopenicillin antibiotic, ampicillin. Ampicillin resistance has increased extensively due to inappropriate prescription and injudicious use of antibiotics.[43] People can purchase the antibiotics without prescription and some doctors may distribute the antibiotics prior to AST.

There are no such policies to monitor the antibiotics recommended by the doctors. In our finding, the majority of the isolates were sensitive to the polymixin antibiotic, colistin, which indicates the drug is effective in vitro for extensively resistant strains. Tigecycline was found effective antimicrobial for inhibiting *E. coli* isolates to a higher extent, but not for all. Resistance to tigecycline in *E. coli* might have resulted from the occurrence of tigecycline resistance encoding gene, overexpression of efflux pump by chromosomal mutation, or by plasmid-mediated efflux pumps.[44,45]

We observed more than four-fifth of *E. coli* isolates were MDR. Haphazard and imprudent supply of antibiotics in veterinary medicine, and inappropriate prescription in primary health care could be the major reasons for increased antimicrobial resistance.[46] MDR strains were found to produce different β-lactamases. In our study, one-half and one-eighth were ESBL producers and AmpC producers respectively among total MDR-*E. coli*. There are diagnostic and therapeutic problems imposed when there is the coexistence of various types of β-lactamases in a bacterial isolate.[47] Similarly, *E. coli* resistant to both carbapenem antibiotics (Imipenem, and meropenem) was more than 30%. Overproduction of AmpC, ESBL, and MBL together with porin loss, production of carbapenemase, alteration in penicillin-binding protein, and efflux pump overexpression are some mechanisms leading to carbapenem resistance.[48] Among CREco, more than 60% were MBL producers and nearly one-tenth of isolates were KPC producers. High rates of MBL production might be due to the location of MBL encoding genes in mobile genetic elements and the propensity to disseminate by horizontal transfer.[49] It has been indicated in a study that the prevalence of MBL is linked with multi-drug resistance.[50] The lower incidence of KPC in our study was possible because of the low prevalence of $bla_{KPC}$ in *E. coli*.[51]

One-fifth of MDR *E. coli* were found to be ColREco by MIC determination. The increased persistence of ColREco noticed in our investigation might be due to the raising administration of carbapenem and polymixin antibiotics for severely ill patients in Intensive Care Unit (ICU) wards. The incidence of colistin resistance exhibited by *E. coli* from food-producing animals in Nepal is a major concern.[15,35] This indicates a high possibility of zoonotic transmission of this resistance to humans resulting in increased prevalence in clinical isolates.[52,53] In our experiments, the MIC value of colistin was demonstrated up to 16 g/L. Generally, *E. coli* strains with the *mcr-1* are distinguished by resistance with a low MIC range (2 -8 g/L).[54] The presence of multiple plasmids carrying *mcr-1* might be a reason for the increased MIC value of colistin.[55] Overexpression of the efflux pump and other multiple systems of resistance might be the reason for higher MIC in *mcr-1* negative-ColREco.[56] Moreover, two-fifth of MBL producer *E. coli* harbored $bla_{NDM-1}$. MBL production might not be completely influenced by $bla_{NDM-1}$ because there are other different variants of NDM reported in *E. coli*.[11] Besides $bla_{NDM-1}$, the production is also linked with genes such as $bla_{IMP}$, *and* $bla_{VIM}$ which have been reported in *E. coli*.[57]

We reported 2 *E. coli* isolates that harbor both $bla_{NDM-1}$ and *mcr-1*and were susceptible to colistin. Since both $bla_{NDM-1}$ and *mcr-1* are found in self-transferrable plasmids, $bla_{NDM-1}$ can be acquired in *mcr-1*-positive *E. coli* and vice versa.[58] Our results indicate *E. coli* isolates carrying both $bla_{NDM-1}$ and *mcr-1* could produce β-lactamases such as ESBL and KPC. The hospital environment may play important role in incorporating antibiotic-resistant genes.[59] Accumulation of multiple resistant determinants in *E. coli* isolates might have occurred due to the horizontal transfer of plasmids between species and different virulence factors encoding genes that can co-exist in a single host cell.[60-62] Colistin-susceptible isolates with the *mcr-1* gene have already been identified before and are not adequately addressed.[63] In order to prevent infection-related fatalities caused by this pathogen, which is one of the major causes of infection-related deaths globally, further research is needed to address the coexistence of these genes as well as their relationship with other genes.[64]

### *Limitations and strengths*

However, the limitations of the study were the *E. coli* isolates were taken from one hospital, only 2 genes were targeted in this study due to limited time and resources and, the action was determined in vitro. Less focus was given to important variables like the type of patients, infection, age, and gender. Since the mechanisms of MDR-status in the bacteria are still inadequate in Nepal, we couldn't explain the accurate reasons for the scenario. In addition, the exact reason about colistin-susceptible *E. coli* could harbor the *mcr-1* gene is merely addressed.

As far as we know this is the first search regarding the coexistence of *mcr-1* and $bla_{NDM-1}$ in *E. coli*. The findings related to CREco and the detection of AR-encoding genes have not been reported often. These could be the strengths of our study. This study may open up the possibility for authorities to work on creating efficient antimicrobial regulations, efficient treatment and diagnostic procedures, and proper health management.

## Conclusion

The effective antibiotics for inhibiting *E. coli* were shown to be colistin and tigecycline (in vitro). MIC value of colistin against MDR-*E. coli* ranged from 1 to 16 g/L. Production of different classes of β-lactamases could occur in MDR-*E. coli*. MBL and KPC co-production were significantly related among CREco. The co-existence of *mcr-1* and $bla_{NDM-1}$ was considerable in the colistin- and carbapenem-resistant isolates. To combat AMR at the appropriate levels, it is necessary to understand the mechanisms with in-depth molecular analyses.

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## Author Contributions

MRB and BB designed the study. BB performed the laboratory work. MRB, BY, KRR, and PG supervised the laboratory works. BB, PD, and MRB analyzed the obtained data and drafted the manuscript. BY, KRR, and PG reviewed the manuscript. MRB and PD edited and revised the manuscript. All the authors read, finalized, and approved the submitted version.

## Ethical Approval

The Institutional Review Committee of the Institute of Science and Technology at Tribhuvan University in Kirtipur, Kathmandu, granted ethical approval (Regd. No. 26).

## Consent to Participate

The patients' signed consent was obtained before collecting the samples and data.

## Data Availability

Figures showing PCR amplification of $bla_{\text{NDM-1}}$ in MBL-producing *E. coli* and *mcr-1* in ColREco are included in the Supplemental File. Any queries regarding the data concerning the current study would be addressed by the corresponding author upon rational request.

## Supplemental Material

Supplemental material for this article is available online.

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