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Molecular Detection of Colistin Resistant Gene *mcr-1* from Gram Negative Clinical Isolates

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ABSTRACT

Colistin is the last resort antibiotic for treating carbapenem-resistant Gram-negative bacterial infections. The emergence of plasmid-mediated mobile colistin-resistant *(mcr)* genes in Enterobacteriaceae has raised concern due to the possibility of rapid horizontal spread. The global dissemination of mobile colistin-resistant genes in natural and non-natural environments is a major problem in the fight against antimicrobial resistance. This study aimed to determine the prevalence of the mobile colistin-resistant gene *mcr-1* in Gram-negative bacteria obtained from different clinical specimens. 753 clinical samples were collected from patients visiting Annapurna Neurological Institute and Allied Sciences, Maitighar (January 2019 to July 2019). Clinical specimens were cultured and identified using biochemical tests. Antibiotic Susceptibility Testing (AST) was done by the modified Kirby Bauer disc diffusion method using a colistin disc (10µg), colistin resistance was confirmed by using the E-test. In this study, 180 (23.90%) isolates showed growth, among which the identified bacteria were *E. coli, Klebsiella* spp., *Citrobacter* spp., *Acinetobacter* spp. and *Pseudomonas* spp. Colistin resistance was higher in male patients (12.35%) than in female patients (7.69%) and higher in the age group between 21-40 years. AST revealed, 79.44% of isolates were multidrug resistant (MDR) and colistin showed 90% sensitivity towards Gram-negative bacteria. The highest resistance was demonstrated against Cotrimoxazole, 65.26%. Similarly, the highest percentage of colistin resistance was observed in urine samples (14.49%), among isolates from the ICU ward (16.66%) and in *E. coli* (16.07%). Out of the total of 180 isolates, 11.66% were screened as colistin resistant, while E-test confirmed 10% isolates phenotypically. The *mcr-1* genes were detected only in 33.33% of E-test confirmed samples using PCR assays and gel electrophoresis. Early detection of these gene-related infections is of great importance to monitor and control the spread of antibiotic resistance.

Keywords: Gram-negative bacteria, MDR, colistin resistance, *mcr-1* gene

Introduction

Colistin is one of the last lines of defense against the carbapenemase-producing Enterobacteriaceae and other Gram-negative bacteria. The growing threat of colistin-resistant Gram-negative bacteria developing extensive drug resistance (XDR) and/or pan-drug resistance (PDR) is also a major concern (Liu et al., 2016). The effective treatment of various kinds of infections spurred on by Gram-negative bacteria is put at severe threat by the misuse or improper use of antimicrobials, which enhances this process (WHO 2016). Colistin is a bactericidal drug that limits the growth of Gram-negative bacteria with multiple drug resistance, including carbapenemase-producing Enterobacteriaceae and carbapenem-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* as well as other multidrug-resistant Gram-negative bacteria (Wang et al., 2016).

Uses of colistin declined from the early 1970s to the early 2000s as a result of clinical trials that revealed it to be nephrotoxic, and it was superseded by stronger and less harmful antibiotics, including beta-lactams, quinolones, and aminoglycosides. Colistin, however, has been reintroduced into clinical practice as a valuable therapeutic alternative as multidrug-resistant Gram-negative bacteria are more prevalent now (Dalmolin et al., 2018). In addition, there has been a resurgence of interest in colistin as a possible therapeutic option for treating antibiotic resistance. There are two means by which an

individual can become resistant to colistin: plasmid-mediated mutation or chromosomal mutation. The chromosomal mutation occurs in genes encoding the negative regulator MgrB, the PmrA/PmrB, and PhoP/PhoQ, resulting in lipid A molecule changes or even deletion. This mutation has been associated with colistin use (Olaiton et al., 2014). The efficacy of colistin is affected by the prevalent issue of colistin resistance that greatly limits treatment options in critically ill patients and makes it less effective (Irusan et al., 2024). Hence, identifying such resistance genes is crucial for controlling resistance spread, with horizontal gene transfer that has become the primary mechanism among bacteria.

Due to the development of resistance by horizontal gene transmission, plasmid-mediated antibiotic resistance, a crucial topic in the study of antibiotic resistance, demands immediate attention (Bivan et al., 2017). Colistin is frequently used in veterinary medicine, particularly for preventing diarrheal illnesses in pig and poultry production (Ahmed et al., 2020). The fact that the colistin resistance gene *mcr-1* is expressed by plasmids is so ubiquitous demonstrates that an important concern at the convergence of human and animal health has been identified. Initially, colistin resistance was considered to be chromosomally mediated, but in 2015, the plasmid-mediated resistance against colistin was reported. Since then, different *mcr-1* alleles have been described (Hussein et al., 2021). The *mcr-1* gene is mostly located on two common plasmids (IncHI2, IncX4 and IncI2) (Faccone et al., 2020). These plasmids may therefore be vital for the transmission of the *mcr-1* gene. The *mcr-1* gene produces a phosphoethanolamine transferase enzyme that transfers phosphor-ethylamine to Lipid A, causing colistin resistance (Falegas et al., 2005). Because it tends to happen in MDR strains, the development of *mcr-1* in clinical Enterobacteriaceae isolates is particularly concerning because it further reduces the options for treating carbapenem-resistant Enterobacteriaceae infections.

There are ten colistin resistance genes known *(mcr-1 to mcr-10*). In 2008, *mcr-1* to *mcr-5* were identified. Other *mcr* genes (*mcr 6-mcr 10*) have recently been described (Rebelo et al.,2018; Borowaik et al., 2020). High prevalence of plasmid-mediated *mcr-1* gene possessing colistin-resistant *E. coli* strains were observed with co-resistance to both carbapenems and colistin, raising concerns about the potential for pan-drug-resistant bacteria (Dahal et al., 2025). Antibiotic-resistant bacteria are currently being monitored in numerous countries throughout the world. In Nepal, only a few studies on this topic have been conducted. As there is a rise in prevalence of the *mcr-1* gene-mediated resistance to colistin, it has presented a significant threat to the control and treatment of infections. To limit the transmission and cure such highly resistant infections, it is critical to identify both the illnesses and the carriers. The aim of this study was to determine the prevalence of the mobile colistin-resistant gene *mcr-1* in Gram-negative bacteria obtained from different clinical specimens by both phenotypic and genotypic methods. The detection of a colistin resistance gene and its mechanism in the population is necessary for monitoring the emergence and spread of mobile colistin resistance as well as for providing effective treatment options for bacterial infections.

Materials and Methods

Research design, study site and duration

The research was a descriptive, hospital-based cross-sectional study, which was conducted from January 2019 to July 2019. The study site was Annapurna Neurological Hospital and Allied Sciences, and Annapurna Research Center Maitighar, Kathmandu.

Study population and Ethical approval

The study populations were inpatients and OPD patients enrolling at Annapurna Neurological Hospital, Maitighar, Kathmandu. The research protocol was approved by the Ethical Review Board of Nepal Health Research Council (Reg. no: 535/2019).

Sample size of Gram-negative isolates

753 different clinical samples were collected from patients visiting Annapurna Neurological Institute and Allied Sciences, Maitighar. Out of which, l80 Gram-negative isolates were considered as the bacterial samples for determination of colistin resistance, particularly with aim of detecting of *mcr-1 gene*.

Laboratory Methods

Clinical sample selection

Different clinical samples included urine (69), sputum (74), body fluid (15), blood (7) and stool (3). Different Gram-negative isolates (non-duplicate) obtained from various clinical samples processed at the hospital laboratory were included. Duplicate organisms from the same samples were excluded. Similarly, individuals with higher doses of antibiotic therapy and radiation therapy, with contaminated samples and isolates with a zone size of 11mm or more for colistin during AST, were excluded.

Clinical sample collection

All the samples were collected by experienced medical personnel in a clean, leak-proof, sterile container. The specimens were immediately sent to the microbiology laboratory for routine culture and sensitivity testing. All the samples were processed immediately without delay.

Culture and identification of isolates

Samples were cultured on different selective and differential media. Identification of isolates was performed by colony characteristics, Gram staining, followed by various biochemical tests including Catalase, Oxidase, IMViC, TSI, Urease tests, and OF test. Based on the different biochemical reactions, bacterial isolates were identified.

Antibiotic susceptibility test (AST)

Antibiotic susceptibility test of the different clinical isolates towards the various classes of antibiotics was performed by the modified Kirby-Bauer disk diffusion method on Muller Hinton Agar following CLSI guidelines (CLSI 2018).

Screening for colistin-resistant isolates

Isolates that were resistant to any member of the carbapenem were selected for further study using a colistin disk (10µg) on Mueller-Hinton agar (MHA) plates. If the inhibition zone size was less than 11 mm for colistin, then they were considered resistant (CLSI 2018). Colistin resistance was further confirmed by performing the Epsilometer test (E-test) and MIC values were also determined.

Preservation of colistin-resistant isolates

For the preservation of the colistin-resistant isolates, the following methods were used:

Glycerol stock preparation: The isolates were preserved in Tryptic Soya Broth (TSB) containing 20% glycerol. For this organism were inoculated in 1 ml of sterile TSB and incubated overnight, followed by aseptic addition of an equal volume of 40% sterile glycerol. The resulting broth was mixed properly by shaking well and then stored at -20 ºC.

Preservation using Semisolid Media: Brain heart infusion broth was prepared. Then agar-agar was added to make a 0.5% agar concentration of the broth. Then the broth was sterilized and allowed to solidify. Aseptically, the isolates were stabbed as straight lines and incubated overnight and then stored at 4 ºC.

The preserved cultures were later revived on the MacConkey agar.

Antibiotic susceptibility test (AST) of colistin-resistant isolates

All colistin-resistant isolates were subjected to an in vitro antibiotic susceptibility test by the modified Kirby-Bauer disc diffusion method. The antibiotics used were gentamicin, amikacin, ciprofloxacin, polymyxin B, colistin, aztreonam, ceftazidime, ceftriaxone, and piperacillin-tazobactam. The inocula were prepared by transferring 3-4 identical colonies from nutrient agar to sterile normal saline. The turbidity of the inoculum was compared with 0.5 McFarland standard. The lawn culture of the test inoculum was prepared by swabbing Muller-Hinton agar (MHA) with a sterile cotton swab dipped into the inoculum. Antibiotic discs were applied to the inoculated MHA plate and incubated at 37 °C up to 18 hours. After incubation, the zone of inhibition around the discs was noted, and the results were interpreted as susceptible, intermediate or resistant according to CLSI guidelines (CLSI 2018).

Extraction of plasmid DNA

For PCR amplification of the *mcr-1* Gene, 2-3 identified colonies of colistin-resistant isolates from Muller Hinton Agar were transferred into the TE broth in test tubes and incubated at 37 ºC overnight. From broth culture, the plasmid DNA was extracted

using the alkaline hydrolysis method. The alkaline hydrolysis method was followed to extract the plasmid DNA, which involved bacterial cell lysis using sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS), followed by neutralization with potassium acetate. The plasmid DNA was obtained by precipitation with isopropanol after being separated from precipitated chromosomal DNA and proteins (Birnboim & Doly, 1979). Then the extracted plasmids were suspended in TE buffer, labeled well and stored at -20 ºC.

Following PCR mixtures and components were used;
a) PCR mixture volume 25 µl / reaction
b) Master mix composition

Components Composition
FIREPol® DNA polymerase
Components volume (µl) DNA template (2-100 ng) 3
Primer–F (10 picomolar) 1 Primer–R(10 picomolar) 1 Master Mix, ddH_2O
5x Reaction Buffer B 0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20
7.5 mM $MgCl_2$
1x PCR solution – 1.5 mM $MgCl_2$
2 mM dNTPs of each
1x PCR solution – 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM dTTP
Blue dye
Migration equivalent to 3.5-4.5 kb DNA fragment
Yellow dye Migration rate over primers in 1% agarose gel: <35-45 bp

Primer selection, electrophoresis and visualization of PCR product

The primer pair used for the detection of the *mcr-1* gene (309 bp amplicon size) was *mcr-1* Forward (5'-CGGTCAGTCCGTTTGTTC-'3) and *mcr-1* Reverse (5'CTTGGTCGGTCTGTAGGG'3) (Zaki et al.,2018). The thermal cycling process included initial denaturation at 94 ºC for 5 minutes, followed by 30 cycles of 94 ºC for 45 seconds, 60 ºC for 1 minute and 72 ºC for 60 seconds with a final extension at 72 ºC for 7 minutes. This was the PCR optimization condition for the detection of plasmid plasmid-encoded *mcr-1* gene reported by Zaki et al. (2018) using *mcr-1-specific* primers. The *mcr-1* positive plasmid of *E. coli* was used as a positive control. The amplified products were visualized by electrophoresis on 2% agarose gels stained with 0.3 µl ethidium bromide. After gel preparation, 3 µl of 100 bp DNA ladder was added to the first well as a molecular weight marker. 3µl of PCR product was added to the well and 85V of power was supplied for 45 minutes. Finally, the gel was taken for photo documentation in a UV transilluminator.

Quality Control

During sample processing, all the tests were carried out appropriately in aseptic conditions. The reagents used in the study were freshly prepared, while the performance of prepared media was tested using the control strain (*E. coli* ATCC 25922). All the molecular works were performed in a well-isolated molecular laboratory. The working area was equipped with a laminar air flow cabinet with UV light. The cabinet was sterilized using UV light 15 minutes before starting the work, while air flow was on, while preparing the PCR reaction mixture components.

Statistical analysis

Chi-square test was used to determine the association of independent variable [age, sex, specimen types and different wards] with dependent variable [*mcr-1* producing, Gram-negative bacteria]. A value of $p \leq 0.05$ was assumed to be significant for the analyses($\alpha \leq 0.05$).

Results

A total of 753 non-repetitive different clinical samples were processed and 180 (23.90 %) samples exhibited growth for Gram-negative bacteria, among which, the most predominant bacterial isolates found were *E. coli* 56 (31.11%), *Klebsiella* spp. 56 (31.11%), *Citrobacter* spp. 22 (12.22%), *Acinetobacter* spp. 25 (13.88%) and *Pseudomonas* spp. 21 (11.66%) (Figure1). From phenotypically confirmed colistin-resistant isolates, only 6 (33.33%) were *mcr-1* gene positive.

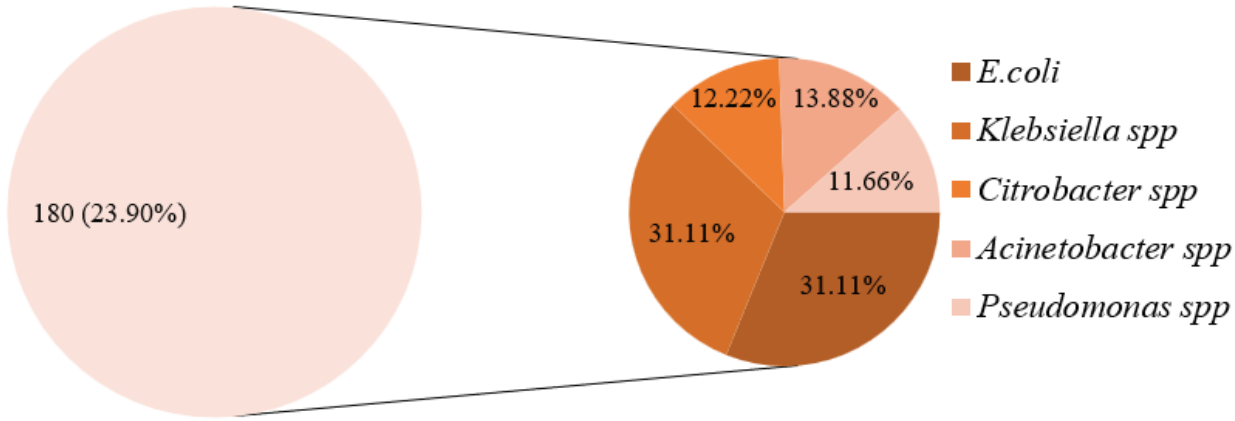

Figure 1: Distribution of bacterial isolates among Gram-negative bacteria

Antibiotic susceptibility and multidrug resistance pattern of Gram-negative isolates

Among the 14 different antibiotics used against Gram-negative isolates, colistin was found to be 90% effective against all isolates, followed by Meropenem, as 75% effective. Gram-negative isolates were highly resistant to Cotrimoxazole (65.26%), followed by Amikacin (64.44%) and Ampicillin (59.44%) (Table 1). Among the total 180 bacterial isolates, 143 (79.44%) bacterial isolates were multidrug resistant, with the highest MDR being *Klebsiella* spp. 51 (35.66%), followed by *E. coli* 45 (31.46%). While 37 (20.56%) isolates were non-MDR. (Table 2)

Table 1: Antibiotic susceptibility pattern of Gram-negative isolates

Antibiotics	**Susceptibility pattern of Gram-negative Bacteria**			
	Sensitive		**Resistant**	
	No	**%**	**No**	**%**
Ampicillin	73	40.55%	107	59.44%
Amikacin	64	35.56%	116	64.44%
Gentamicin	109	60.65%	71	39.35%
Cotrimoxazole	103	57.22%	77	65.26%
Imipenem	112	62.23%	68	37.77%
Colistin	162	90%	18	10%
Ciprofloxacin	125	69.44%	55	30.56%
Levofloxacin	85	47.22%	95	52.78%
Nitrofurantoin	124	70.45%	56	29.55%
Piperacillin Tazobactam	81	45%	99	55%
Meropenem	135	75%	45	25%
Cefotaxime	78	43.33%	102	56.67%
Ceftriaxone	106	58.89%	74	41.11%

Table 2: MDR pattern of Gram-negative isolates

Isolates	**MDR**	**Non -MDR**	**Total**
	No. %	**No. %**	
E. coli	45 (31.46%)	11 (7,69%)	56
Klebsiella spp.	51 (35.66%)	5 (3.4%)	56

Acinetobacter spp.	16 (8.88%)	4 (2.7%)	20
Citrobacter spp.	21 (14.68)	10 (6.99%)	31
Pseudomonas spp.	10 (6.99%)	7 (4.8%)	17
Total	143 (79.44%)	37 (20.56%)	180

Screening of colistin-resistant Gram-negative bacteria

Out of 180 Gram-negative isolates under study, 21 (11.66%) isolates were resistant to colistin. All the colistin-resistant isolates were subjected to further confirmation through the E-test method and Polymerase Chain Reaction. Only 18 (10%) isolates were phenotypically confirmed to produce colistinase by E-test using a colistin strip.

Distribution of colistin-resistant isolates among the types of sample

Out of 180 total samples, including sputum (74), urine (69), pus (8), blood (11), Catheter tips (11) and body fluids (7), where urine isolates (14.49%), sputum sample isolates (6.75%), followed by blood sample and catheter tip sample isolates were found to be colistin resistant (Table 3).

Table 3: Distribution of colistin-resistant isolates among the types of samples

Type of sample		**Colistin resistance**	
	Total	**No.**	**%**
Urine	69	10	14.49%
Sputum	74	5	6.75%
Blood	11	2	18.18%
Catheter tip	11	1	9.09%
Pus	8	_	0.00%
Body fluids	7	_	0.00%

Colistin resistance pattern according to gender and age group

12.35% of colistin resistance was observed in male patients, while in female patients it was found to be 7.69%. The chi-square test revealed no significant association between the total number of colistin-resistant isolates and the different genders of patients, with a p-value found to be >0.05. Isolates obtained from adult patients having the age group 21- 40 years were found to be the highest colistin resistant (Table 4).

Table 4: Distribution of colistin-resistant isolates among age groups

Age group	**Total number of samples**			**P-value**
		Sensitive %	**Resistant %**	
0-20 years	13	11 (84.65%)	2 (15.38%)	
21-40 years	36	30 (83.34)	6 (16.66)	
41-60 years	45	40 (88.89%)	5 (11.11%)	>0.05
61-80 years	48	45 (93.75)	3 (6.25%)	
81-100 years	38	36 (94.74%)	2 (5.2%)	
Total	180	162 (90%)	18 (10%)	

Distribution of colistin resistant isolates among the hospital wards

Among 180 Gram negative isolates, highest resistant isolates were observed in intensive care units 8(16.66%), followed by general ward 6 (10.90%) and furthermore, OPD showed 4 (5.19%).

Distribution of colistin resistance among Gram-negative isolates

The most predominant colistin resistant isolates were in *E. coli* 9 (16.07%) followed by *Klebsiella* spp. 7 (12.5%). Statistically, there was no significant association between type of sample and colistin resistant Gram-negative isolates (Table 5).

Table 5: Distribution of colistin resistance among Gram negative isolates

Isolates	Total number of samples	Colistin		P-Value
		Sensitive %	Resistant %	
E. coli	56	47 (83.92%)	9 (16.07%)	
Acinetobacter spp.	20	19 (95%)	1 (5%)	
Citrobacter spp.	31	30 (96.77%)	1 (3.33%)	>0.05
Klebsiella spp.	56	49 (87.55)	7 (12.5%)	
Pseudomonas spp.	17	17 (100%)	_	
Total	180	162 (90%)	18 (10%)	

Positivity of the *mcr-1* gene among colistin-resistant Gram-negative isolates and distribution of the *mcr-1* gene among types of samples

Out of 18 phenotypic colistin-resistant Gram-negative isolates, only 6 *mcr-1* genes were detected by PCR amplification using *mcr* gene-specific primer. Among the total 6 *mcr* genes detected from different clinical samples, 3 (4.05%) isolates were from sputum samples, followed by 2 (2.89%) from urine samples and 1(33.33%) from catheter tip samples (Table 6).

Table 6: Distribution of the *mcr-1* gene among types of samples

Type of sample		mcr-1 gene		P-Value
	Number of samples	Positive	Negative	
Urine	69	2 (2.89%)	67 (97.10%)	
Sputum	74	3 (4.05%)	71 (95.95%)	
Pus/Swab	12	_	12 (100%)	
Blood	11	_	11 (100%)	>0.05
Body fluids	11	_	11 (100%)	
Catheter tips	3	1 (33.33%)	2 (66.66%)	
Total	180	6	174 (100%)	

Distribution of the *mcr-1* gene among different bacterial species

Among the total 6 *mcr* genes detected from different bacterial species, the most predominant bacterial isolates to harbor the *mcr-1* gene were *Klebsiella* spp. 3(5.35%), followed by *E. coli* 2 (3.57%) and *Acinetobacter* spp. 1(5%). Statistically, there was no significant association between different bacterial species and the detection of the *mcr-1* gene (Table 7).

Table 7: Distribution of the *mcr-1* gene among different bacterial species

Isolates	Total no of sample	mcr-1 gene		P-value
		Positive No. %	Negative No. %	
E. coli	56	2 (3.57%)	54 (96.42%)	
Klebsiella spp.	56	3 (5.35%)	53 (94.64%)	
Pseudomonas spp.	17	_	17 (100%)	>0.05
Acinetobacter spp.	20	1 (5%)	19 (95%)	
Citrobacter spp.	31	_	31 (100%)	
Total	180	6 (3.33%)	174 (96.67%)	

MICs of colistin-resistant isolates

MIC values in colistin-resistant isolates ranged between 2 µg/ml to >256 µg/ml. In our study, all the 18 isolates had an MIC value >2 µg/ml (Figure 2).

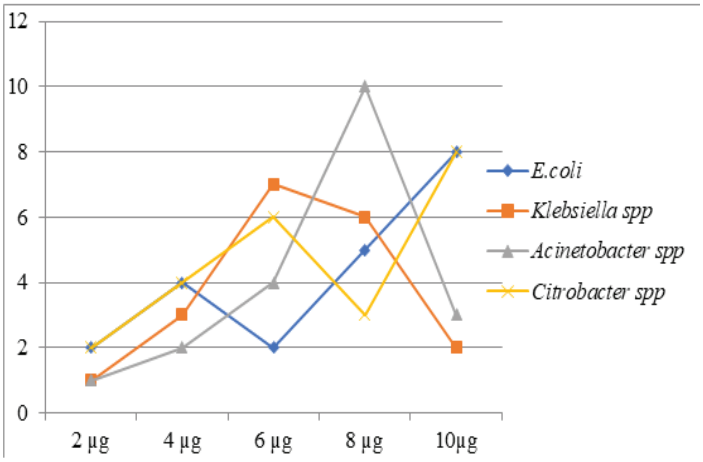

Figure 2: MICs of colistin-resistant isolates

Molecular detection of the *mcr-1* gene

All phenotypically confirmed 18 colistin-resistant isolates were subjected to PCR for molecular detection of the mobile colistin-resistant gene. PCR amplification of the *mcr-1* gene was performed using gene-specific primers specific for the forward and reverse regions of the *mcr-1* gene. Among the colistin-positive isolates, 6 (33.33%) isolates were found to be positive for the *mcr-1* gene (Figure 3).

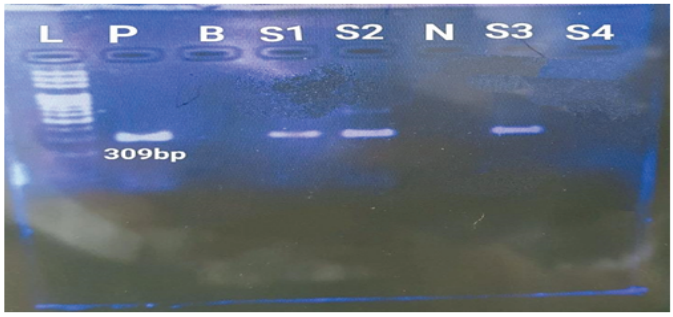

Figure 3: Molecular detection of the *mcr-1* gene

Left to right: Lane L-100bp DNA ladder, Lane P-Positive control(309bp), Lane B-Blank, Lane S1-S2 *mcr-1* positive, Lane N-Negative control lane S3-*mcr-1* positive

Discussion

Gram-negative bacteria have unique structural characteristics, containing an outer membrane that contains lipopolysaccharides, posing huge challenges in the treatment of infections. In this study, 180 (23.90%) samples demonstrated growth of Gram-negative bacteria with altogether 8 different bacterial isolates, most of them being the members of Enterobacteriaceae, indicating the presence of these major human pathogens, which are responsible for infections, particularly in hospital settings. The most predominant bacterial isolates identified were *E. coli* and *Klebsiella* spp., followed by *Citrobacter* spp., *Acinetobacter* spp., and *Pseudomonas* spp., which are consistent with the findings of Wong et al. (2016), who also reported the presence of similar bacterial isolates. The presence of Gram-negative bacteria in clinical samples as major contributors to antibiotic resistance is considered a serious concern. The

AST pattern in this study revealed that the highest number of Gram-negative isolates were resistant to Cotrimoxazole (65.26%) followed by Amikacin (64.44%), Ampicillin (59.44%) and Levofloxacin (52.55%). Most of the Gram-negative isolates were sensitive to colistin (90%) followed by meropenem (75%), Nitrofurantoin (70.45%) and Ciprofloxacin (69.44%). Similarly, a study done by Karki et al. (2020) found colistin, polymyxin B and Tigecycline were the most effective antibiotics. These results indicate that some of these isolates are still treatable by various classes of antibiotics. In this study, 79.44% were multidrug resistant, while 20.56% were non-MDR. Different studies also indicated a similar high proportion of multidrug resistance observed in Gram-negative isolates (Aarthi et al., 2020; Dahal et al., 2025). The emergence of Gram-negative isolates resistant to last-line drugs like colistin and polymyxin B and resistant to various classes of antibiotics is a matter of concern, which may lead to XDR and PDR risk of forming superbugs and limit the treatment options.

In our study, out of 180 Gram-negative isolates, the prevalence of colistin-resistant isolates was 11.66%. The increasing resistance against colistin may be due to increased use of colistin as a treatment option on a daily basis. In this study, the prevalence of colistin-resistant isolates among different types of clinical samples was 14.49% from urine, 6.75% from sputum, 18.18% from blood, and 9.09% from catheter tip samples. This result is commensurate with that reported by Yadav et al. (2024), where out of various specimens received, the highest number of colistin-resistant isolates were obtained from urine samples. This may be attributed to the fact that there is a predominance of uropathogenic bacteria that can be a significant reservoir for the plasmid-mediated colistin resistance.

Slightly higher prevalence was observed for colistin-resistant isolates among the male patients in this study, where 12.35% of colistin-resistant isolates were from male patients and 7.69 % were from female patients. This result is comparable with the finding of Prim et al. (2016), where colistin resistance was higher in urine, sputum and blood samples. Also, it revealed that *E. coli* (0.8%) isolates were from male patients and the remaining isolates (0.7%) from female patients were *mcr-1* positive. This different distribution among males and females according to above mentioned studies showed no significant relationship between colistin production with different genders.

Colistin-resistant isolates were obtained from various hospital wards with different prevalence. High prevalence of colistin resistance was found in the isolates from intensive care units (16.66%) and general wards (10.90%). Similar to this result, in a study conducted, higher rates of colistin resistance were reported from intensive care units (ICUs), which have higher use of antibiotics and invasive procedures (Kang et al., 2017; Paudel et al., 2020). This higher distribution of colistin resistance producing bacteria in the ICU may be related to nosocomial infection, besides being related to excessive use of broad-spectrum antibiotics, invasive procedures, associated septicemia and higher comorbidities among ICU patients. In this study, the most predominant colistin-resistant isolates were *E. coli* (16.07%) and *Klebsiella* spp. (12.5%). Several studies have also reported that *E. coli* is the major colistin-resistant isolate (Wong et al., 2016). This range of prevalence shows that the current research provides valid data regarding the prevalence of colistin-resistant Gram-negative isolates.

Molecular techniques, like PCR, are very critical and promising as these assays target specific regions of the *mcr-1* gene have been successfully used to identify colistin-resistant bacteria harboring this particular gene (Liu et al., 2016). In this study, phenotypic colistin-resistant isolates (10%) were detected by Kirby-Bauer disc diffusion and the E-test method. And, by using PCR assays, the 33.33% *mcr-1* gene was detected. In different studies, the PCR technique was primarily used for the detection of the *mcr-1* gene (Abdalla et al., 2024; Dahal et al., 2025). Thus, it can be concluded that molecular detection of genes is very useful for accurately determining the presence of resistant genes in a particular hospital, while the phenotypic screening can be applied for prophylaxis action.

In our study, out of different colistin-resistant isolates, only *E. coli*, *Klebsiella* spp. and *Acinetobacter* spp. were found to harbor the *mcr-1* gene. Whereas in a study conducted, other organisms (*Pseudomonas* spp. and *Proteus* spp.) were also found to harbor

mcr-1 gene along with *E. coli* and *Klebsiella* spp. (Abdalla et al., 2024). Similar to our result, a study conducted in China also reported the detection of the *mcr-1* gene in *Escherichia coli* isolated from food animals, food products, and human patients (Liu et al., 2016).

During this study, variable colistin MIC values of colistin-resistant isolates were detected that ranged between 2 μg/ml to >256 µg/ml. Similarly, Quan et al. (2016) reported colistin-resistant isolates having MIC values of >256 µg/ml. Study of Walkty et al. (2020) reported the MIC range of 4-16 µg/ml for colistin-resistant *E. coli* isolates, also in another study by Yadav et al. (2024) same MIC value was observed for the resistant isolates. MIC value of 256 µg/ml indicates some isolates were highly colistin resistant. This may be due to the increasing use of colistin antibiotics to treat MDR bacteria in the hospital setting.

In this study, 6 *mcr-1* genes were detected. A result of similar study conducted by Xavier et al. (2016) showed the presence of *the mcr-1* gene in six Gram-negative bacteria isolated from different sources in Portugal, including humans, livestock, and food products. In addition, they found that the *mcr-1* gene was present in plasmids, which are mobile genetic elements that can transfer antibiotic resistance genes between bacteria. Detection of the *mcr-1* gene has been reported in numerous Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Enterobacter* spp. (Liu et al., 2016; Poirel et al., 2017). Another study by Falgenhauer et al. (2016) also reported the spread of *mcr-1* in *K. pneumoniae* isolates from patients in Germany. The rapid spread of the *mcr-1* gene is likely due to its location on a plasmid, which can easily transfer between bacterial species, highlighting the potential for the gene to spread rapidly. Several studies have revealed that the transfer of resistance genes for e.g., the colistin resistance gene, is more effective than chromosomal mutation. This means that bacteria that were previously susceptible to colistin may become resistant through the acquisition of the *mcr-1* gene.

In a study carried out by Zhang et al. (2025), it was revealed that *mcr-1*-positive *E. coli* and *mcr-10*-positive *K. pneumoniae* complex strains were present in wastewater in Yangzhou, China, which highlighted the potential risk of gene spread, even in environments with limited colistin exposure. Additionally, the overuse of colistin in veterinary and human medicine may have contributed to the emergence and spread of the *mcr-1* gene. In another study conducted in the Chitwan district of Nepal, high prevalence of multidrug-resistant *E. coli* isolates, colistin-resistant isolates, and *mcr-1* gene-carrying isolates was found, indicating a serious concern, as this could potentially lead to colistin resistance in human pathogens through horizontal transfer of resistant genes from poultry to humans (Tiwari et al., 2024). Since there is the prevalence of such transferable resistant genes in the environment, molecular detection techniques become especially important that target resistance genes as phenotypic methods cannot accurately identify those genes (Feng et al., 2023).

Regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of resistance patterns, along with specific detection and identification of the sequence that is responsible for acquiring resistance in a particular organism. As there is a rise in the population of bacteria harboring *mcr* genes, the efficacy of colistin is greatly affected. Thus, it is vital for every laboratory to become alert about the early detection of the colistin resistance *mcr* gene, especially in hospital settings, if not diagnosed and left untreated, which may lead to a horrific epidemic condition. Therefore, phenotypic and genotypic identification techniques should be performed for specific diagnosis and continuous surveillance.

Conclusion

Emergence of colistin-resistant Gram-negative bacterial genes, especially *mcr-1,* has led to serious issues globally. Among 180 Gram-negative isolates, 10% showed phenotypic colistin resistance, out of which only 33.33% of isolates were *mcr-1* gene positive. Since most isolates were resistant to most of the routinely used antibiotics, such as cephalosporins, carbapenems, quinolones, amikacin, gentamicin, nitrofurantoin, piperacillin/ tazobactam and colistin, antibiotic resistance of these pathogens should be taken as a serious concern. These data from the hospital environment highlight a comparatively

serious situation, as such settings can serve as a medium for the quick spread of MDR organisms. Therefore, early detection of such types of gene possessing Gram-negative bacteria will be useful for choosing the most appropriate antibiotics and also for controlling the transmission of such bacteria with resistance genes.

Limitations and Recommendations

As this study showed the presence of a comparatively significant proportion of colistin-resistant Gram-negative bacteria, detection of colistin resistance should be done routinely in all clinical laboratories. Phenotypic methods, such as E-test, can be considered confirmatory only in case of immediate antibiotic therapy, while molecular methods (PCR) must be employed as the ultimate choice for the detection of resistant genes. This study highlights usage of colistin should be reviewed, and it should only be applied to infections that are life-threatening.

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Conflict of interest

The authors declare no conflict of interest.

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