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# Detection of AcrAB efflux pump mediated ciprofloxacin resistance in *Escherichia coli* and *Klebsiella pneumoniae* in Nepal

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## Abstract

**Background** Antimicrobial resistance (AMR) has emerged as a serious public health threat with a sharply increasing burden globally. Resource limited countries like Nepal are facing difficulties in the fight against AMR. This has been further worsened due to the lack of adequate molecular data. Ciprofloxacin resistance by AcrAB-TolC efflux pump (EP) genes in Gram-negative bacteria becomes a challenging issue due to its potential role in multidrug resistance (MDR). This hospital-based cross-sectional study investigated these genes in ciprofloxacin-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from patients attending a tertiary care hospital in Kathmandu, Nepal. Different clinical specimens (n = 877) were subjected to bacterial isolation and identification. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion technique and minimum inhibitory concentration (MIC) by agar dilution. AcrAB-TolC genes were detected by polymerase chain reaction (PCR).

**Results** Out of 877 samples, 124 (14.1%) showed bacterial growth, comprising 59 (47.6%) *E. coli*, 39 (31.4%) *K. pneumoniae*, and 26 (20.9%) other bacterial species. MDR was observed in 66.1% of *E. coli* and 89.7% of *K. pneumoniae*. Ciprofloxacin resistance was seen in 57.6% of *E. coli* and 71.8% of *K. pneumoniae* isolates. Majority of *E. coli* (10) and *K. pneumoniae* (8) isolates showed MIC of ciprofloxacin as 256 µg/mL and 512 µg/mL, respectively. There was significant association between ciprofloxacin resistance and MDR ($p < 0.05$). The higher proportion of *AcrAB-TolC* genes was detected in ciprofloxacin-resistant MDR isolates of *E. coli* (87.5%) and *K. pneumoniae* (66.7%) compared to non-MDR isolates.

**Conclusions** Clinical isolates of *E. coli* and *K. pneumoniae* exhibited alarmingly high MDR rates with substantial resistance to commonly prescribed antibiotics in Nepal. We also observed a notable presence of EP-mediated resistance in these isolates. These findings highlight the urgent need for enhanced AMR surveillance (including resistance marker screening and genomic surveillance) and development of evidence-based antibiotic stewardship programs to address the growing burden of MDR Nepal.

**Keywords** Antimicrobial resistance (AMR), Multi-drug resistance (MDR), Minimum inhibitory concentration (MIC), AcrAB-TolC efflux pump gene, *Escherichia coli*, *Klebsiella pneumoniae*, Nepal

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## Introduction

Antimicrobial resistance (AMR) is an urgent and growing global health issue, posing significant threats to health system and clinical outcomes worldwide [1]. An estimated 4.71 million (95% uncertainty interval [UI] 4.23–5.19 million) deaths were associated with bacterial AMR, of which 1.14 million (1.00–1.28 million) were directly attributed to bacterial AMR in 2021 alone [2]. The extensive and inappropriate use of antibiotics in humans and animals, poor health care system as well as inadequate infection prevention and control (IPC) practices in Nepal are major contributors to the increasing burden of AMR [3]. The actions against AMR in Nepal has been impacted Due to other public health priorities, inadequate law enforcement and lack of nation-wide surveillance although a hospital-based AMR surveillance is in existence since 1999 [4]. As a result, AMR was one of the top three causes of mortality in Nepal in 2019 with 6400 casualties directly attributable to it and 23,200 deaths in which AMR was one of the contributing factors [5].

Resistance to fluoroquinolones (such as ciprofloxacin) is caused by variety of mechanisms, such as mutations in the DNA gyrase, efflux pump (EP)-mediated and plasmid-mediated resistance, etc. [6]. EPs are active drug transport that engage in cross-resistance to classes of chemically unrelated compounds with low intrinsic susceptibility thereby enhancing resistance mechanisms [7]. They have substantial impact on the virulence and adaptive responses that lead to the development of AMR during the infection [8]. Additionally, they play a crucial role in the biofilms mediated pathogenesis [9]. They may transport a single substrate or a variety of antibiotics from several classes. MF (major facilitator), MATE (multi-drug and toxic efflux), RND (resistance-nodulation-division), SMR (small multi-drug resistance) and ABC (ATP binding cassette) are the five major families of EPs found in bacteria [10].

Both Gram-positive and Gram-negative bacteria have EPs important for multi-drug resistance (MDR) [11, 12]. AcrAB-TolC EP belongs to RND family and found in Enterobacteriaceae [13]. It is chromosomally encoded pump made up of a tripartite system which may bind to and then expel a wide range of compounds with therapeutic value [14]. Overexpression of this EP is due to genetic mechanism underlying MDR in Gram-negative bacteria [15]. EP inhibitors (EPIs) can be administered to patients with proper antibiotics to prevent from resistance [16]. Therefore, a thorough understanding of EPs and the identification of new EPIs are necessary to tackle the ongoing threat of MDR.

High ciprofloxacin resistance was reported in pathogenic *E. coli* and *K. pneumoniae* from different parts of Nepal, which is a matter of public health concern [17, 18]. There are many driving factors to ciprofloxacin resistance in the Enterobacteriacae family, namely, chromosomal mutations in the *gyrA* and *parC* genes and plasmid-mediated mechanisms like *qnr* genes, EPs, and enzymes [19]. Among these, EP systems are important due to their significant role in actively reducing the intracellular antibiotic concentrations thereby diminishing the treatment efficacy. Therefore, in this study, we explore the antibiotic resistance patterns including minimum inhibitory concentration (MIC) and describe the relationship between ciprofloxacin resistance in these bacteria and the presence of the AcrAB-TolC EP system.

## Material and methods

### Study site, design and sample collection

This was a hospital-based cross-sectional study conducted among patients visiting Sukraraj Tropical and Infectious Disease Hospital (STIDH) in Kathmandu, Nepal from August 2022 to January 2023. Nepal's curative healthcare system includes approximately 122 tertiary-level hospitals, 28 specialized hospitals, and 22 super-specialty hospitals [20]. Among these, STIDH under the Ministry of Health and Population holds a unique position as the nation's only dedicated specialized referral center for tropical and infectious diseases. It plays a crucial role in managing complex or high-risk infectious and tropical disease cases that require specialized care such as diarrheal diseases, dengue, viral hepatitis, HIV/AIDS, and other pandemic potential diseases. STIDH's geographical reach extends nationwide, receiving referrals from all seven provinces. On average, STIDH handles approximately 5 million patients annually [20].

Patients visiting STIDH with possible bacterial infections regardless of age and sex were included in the study. All the samples were collected during the first visit of patients to the hospital. Clinical specimens that included urine, sputum, blood, wound swab, and others (pus, pleural fluid and other body fluids, etc.) were collected from patients following standard procedures (Fig. 1). At the time of sample collection demographic details were recorded using a questionnaire (Supplementary file).

### Sample processing and identification of bacterial isolates

Clinical specimens were collected in sterile, leak-proof pre-labelled containers by qualified healthcare personnel following standard operating procedures [21, 22]. Upon collection, samples were transported to the microbiology laboratory promptly and processed within an hour of sample collection. In the case of some anticipated delay, they were stored in cold condition. Throughout the process, strict adherence to biosafety and quality control measures was maintained to ensure the integrity of the samples and avoid contamination. Blood samples were put into BACTEC blood culture bottles to identify microbial growth using fluorescence-based $CO_2$ detection.

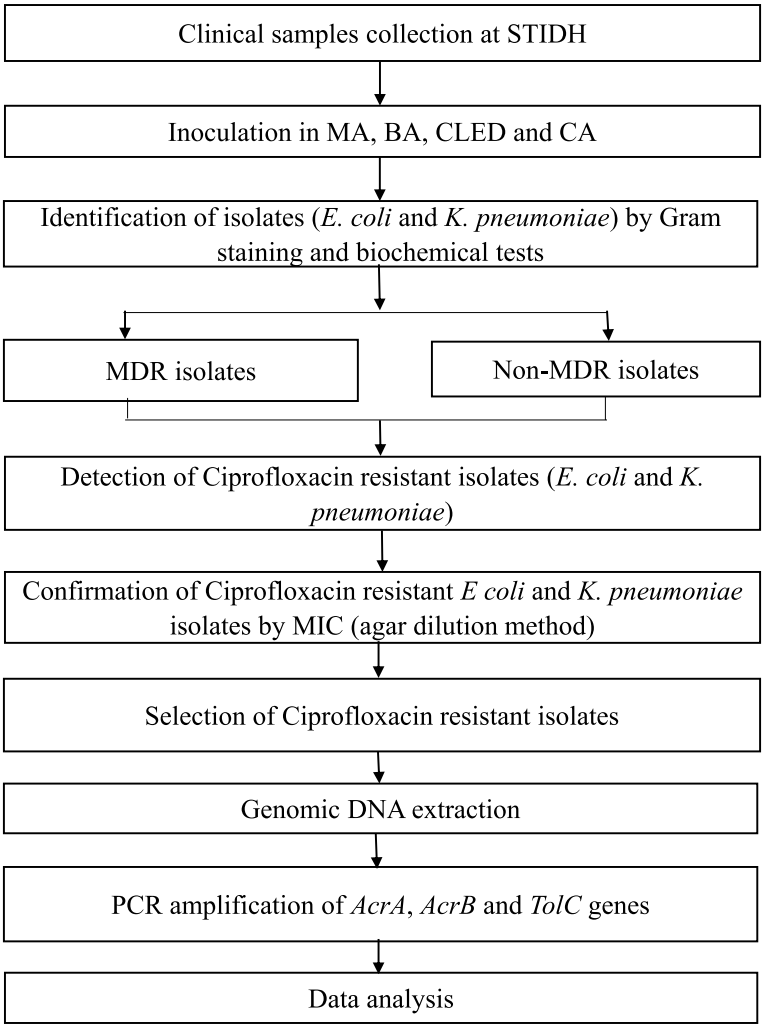


**Fig. 1** Overall flow diagram of study

Blood culture bottles were continuously monitored for up to 7 days at 37˚C in a BACTEC automated blood culture system (company, city, country). From the positive culture bottle, the sample was sub-cultured on Blood agar (BA) and MacConkey agar (MA). Urine specimens were cultured on MA and Cysteine Lactose Electrolyte Deficient (CLED) agar. Sputum and body fluids were cultured in BA, MA and Chocolate agar (CA). While pus and wound swabs were cultured in MA and BA and incubated overnight for 37˚C. The bacterial isolates were identified by exploring their cultural and biochemical characteristics [23]. The bacterial isolates were identified by biochemical tests; catalase test, coagulase test, bacitracin and optochin susceptibility test for Gram-positive bacteria, while sulfide-indole-motility (SIM), Simmons's citrate, triple sugar iron (TSI), urease tests were applied to Gram-negative bacteria. After identification, the isolates were preserved in 30% glycerol stock and kept at −80˚C until further analysis. The isolates were safely cold shipped to the laboratory of Central Department of Microbiology (CDMi), Tribhuvan University where further MIC and molecular works were carried out.

#### Antibiotic susceptibility testing

The screening of ciprofloxacin resistance and MDR among *E. coli* and *K. pneumoniae* isolates were done by Kirby Bauer disc diffusion technique using Mueller–Hinton agar (MHA) (HiMedia Pvt. Ltd., India) according to CLSI guidelines [24, 25]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used for quality control. The following antibiotic discs were used: amoxicillin (10 µg), amikacin (30 µg), ceftazidime (30 µg), cefixime (5 µg), ceftriaxone (30 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), doxycycline (30 µg), 24imipenem (10 µg) meropenem (10 µg), nalidixic acid (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), piperacillin/tazobactam (100/10 µg) and colistin (10 µg).

#### *Definition of MDR*

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes, in accordance with standard criteria [26].

### Confirmation of ciprofloxacin resistance by minimum inhibitory concentration (MIC)

MIC of ciprofloxacin was determined by using agar dilution method following CLSI guidelines [24]. Ciprofloxacin concentration ranged from 0.5 µg/mL to 512 µg/mL for *E. coli* and 0.25 µg/mL to 512 µg/mL for *K. pneumoniae*. Briefly, the inoculum was prepared by adding pure colonies from nutrient agar (NA) plate to sterile distilled water. The inoculum's turbidity was calibrated to 0.5 McFarland standards. Then, the inoculum was applied on MHA surfaces, and plates were incubated at 37°C for 18 h. The antibiotic of lowest concentration that totally prevented the observable growth of bacteria was determined to be the MIC end point**.** The reference strain *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were included in each batch of MIC testing to ensure accuracy and consistency. Any discrepancy greater than one dilution was retested. MIC results were interpreted according to 31 st edition of CLSI M100 (2021) (clinical breakpoints (for ciprofloxacin; ≤ 0.25 as sensitive, 0.5 as intermediate and ≥ 1 as resistant) among Enterobacteriaceae [24, 25].

### Detection of *AcrA, AcrB* and *TolC* genes by polymerase chain reaction (PCR)

#### *DNA extraction and quantification*

From each *E. coli* and *K. pneumoniae* isolate, the genomic DNA was extracted using the alkali lysis method as described previously [27, 28]. Purity assessment and quantitation was done using Nanodrop (Thermo Fisher Scientific Inc., Waltham, MA, USA).

#### *Target gene g amplification*

The final PCR mixture volume was 25 µL which consisted of 13 µL PCR master-mix, 1µL each of forward and reverse primers (Supplementary Table 1), 7 µL nuclease free water and 3 µL template DNA. The PCR cycle for *AcrA* and *AcrB* genes consisted of initial denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min with extension at 72°C for 1 min; final extension at 72°C for 5 min [29]. Similarly, the thermal cycling process for *TolC* gene included initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 secs, annealing at 56°C for 30 secs with extension at 72°C for 1 min; final extension at 72°C for 5 min in GeneAmp Thermocycler (Applied Biosystems, Carlsbad, CA, USA) [30]. *E. coli* K-12 sub-strain MG1655 (ATCC 700926) and *K. pneumoniae* strain BAA-3064 (ATCC BAA-3064) were used as positive controls while ultrapure RNase/DNase free water served as negative controls for every PCR reaction to monitor contamination and reaction efficiency. To further validate the PCR process, duplicate reactions were performed for each sample and target using positive controls and negative controls.

#### *Agarose gel electrophoresis and visualization*

The amplified PCR products were subjected to gel electrophoresis (1.5% agarose containing 0.5 µg/mL ethidium bromide dye) at 70 V for 45 min and visualized under UV-transilluminator (Azure Biosystems, Dublin, CA, USA). The bands of 189 bp, 183 bp and 100 bp were considered positive for the *AcrA, AcrB* and *TolC* genes, respectively (Supplementary Table 1) [30, 31].

#### *Data analysis*

All the data were entered, cleaned and verified in Microsoft office excel spreadsheets (version 16.0). Data was entered by two individual team members and verified to minimize errors. Then, it was imported to statistical package for social science (SPSS) software version 21.0, (IBM Corp., Armonk, NY, USA) and analyzed. Data were presented as proportions. The categorical data was analyzed by chi-square test. Statistical significance was defined as a p-value less than 0.05.

## Results

### Distribution of clinical isolates

Altogether, 877 clinical specimens were collected at STIDH and processed for bacterial analysis. Of these, 124 (14.1%) showed significant bacterial growth. Among which, 59 (47.6%) were *E. coli* followed by 39 (31.5%) *K. pneumoniae* (Table 1) and the rest 26 (20.9%) were others bacterial species (7 *Acinetobacter baumannii*, 6 *Pseudomonas aeruginosa*, 5 *Salmonella enterica* serovar Typhi, 4 each of *Citrobacter spp.* and *Proteus spp.*). Among total *E. coli* isolates, 43 (72.9%) were isolated from female whereas, 16 (41.0%) out of total *K. pneumoniae*, were isolated from female. Majority of *E. coli* isolates were from age group 31–45 (28.8%) and 46–60 (28.8%) years while the age group 31–45 years had the highest number of *K. pneumoniae* isolates (33.3%) (Table 2).

**Table 1** Distribution of *E. coli* and *K. pneumoniae* in different clinical specimens, Kathmandu, Nepal

| Sample type (n) | *E. coli:* n (%) | *K. pneumoniae:* n (%) |
|---|---|---|
| Urine (471) | 53 (11.3) | 14 (3.0) |
| Pus (10) | 3 (30.0) | 3 (30.0) |
| Sputum (263) | 1 (0.4) | 16 (6.0) |
| Wound (17) | 1 (5.9) | 3 (17.7) |
| Body fluid (19) | 1(5.3) | 1 (5.3) |
| Blood (97) | 0 (0.0) | 2 (2.1) |
| Total (877) | **59 (6.7)** | **39 (4.4)** |

**Table 2** Age group-wise distribution of *E. coli* and *K. pneumoniae* isolates, Kathmandu, Nepal

| Age interval (in years) | *E. coli:* n (%) | *K. pneumoniae:* n (%) |
|---|---|---|
| ≤ 16 | 1 (1.7) | 0 (0.0) |
| 17–30 | 9 (15.3) | 12 (30.7) |
| 31–45 | 17 (28.8) | 13 (33.3) |
| 46–60 | 17 (28.8) | 7 (18.0) |
| ≥ 61 | 15 (25.4) | 7 (18.0) |
| Total | 59 (100.0) | 39 (100.0) |

### Antibiotic resistance pattern of *E. coli* isolates

All the *E. coli* isolates were found sensitive towards colistin. Nitrofurantoin was the second most effective drug for *E. coli* (92.5%) whereas, majority of *E. coli* were resistant to amoxicillin (83.1%) (Table 3).

### Antibiotic resistance pattern of the *K. pneumoniae* isolates

All the *K. pneumoniae* isolates were found sensitive to colistin whereas doxycycline (48.7%) and gentamicin (46.1%) were effective to less than half of the isolates. More than half (57.1%) of *K. pneumoniae* isolates were resistant to nitrofurantoin while all of them were resistant to amoxicillin (Table 4).

### High rate of MDR observed in *E. coli* and *K. pneumoniae*

A total of 66.1% (39/59) of *E. coli* and 89.7% (35/39) of *K. pneumoniae* isolates were identified as MDR (Fig. 2). Further, a significant association observed between ciprofloxacin resistance and MDR ($p < 0.05$).

**Table 3** Antimicrobial resistance pattern of the *E. coli* isolates from different clinical specimens, Kathmandu, Nepal

| Antibiotic class | Antibiotics used | Sensitive n (%) | Intermediate n (%) | Resistant n (%) |
|---|---|---|---|---|
| Aminopenicillins | Amoxicillin | 10 (16.9) | 0 (0) | 49 (83.1) |
| Cephalosporins | Ceftriaxone | 22 (37.3) | 0 (0) | 37 (62.7) |
| | Ceftazidime | 23 (39.0) | 0 (0) | 36 (61.0) |
| | Cefixime | 21 (35.6) | 0 (0) | 38 (64.4) |
| Sulphonamides | Cotrimoxazole | 22 (37.3) | 0 (0) | 37 (62.7) |
| Fluoroquinolones | Nalidixic acid | 21 (35.6) | 0 (0) | 38 (64.4) |
| | Ciprofloxacin | 25 (42.4) | 1 (1.7) | 33 (55.9) |
| Nitrofurans | Nitrofurantoin | 49 (92.5) | 3 (5.6) | 1 (1.9) |
| Aminoglycosides | Gentamicin | 42 (71.2) | 0 (0) | 17 (28.8) |
| | Amikacin | 41 (69.5) | 6 (10.2) | 12 (20.3) |
| Carbapenems | Imipenem | 47 (79.7) | 3 (5.0) | 9 (15.3) |
| | Meropenem | 47 (79.7) | 1 (1.7) | 11 (18.6) |
| Tetracyclines | Doxycycline | 38 (64.4) | 7 (11.9) | 14 (23.7) |
| β-lactam with inhibitors | Piperacillin/ tazobactam | 41 (69.5) | 4 (6.8) | 14 (23.7) |
| Lipopeptides | Colistin | 59 (100.0) | 0 (0) | 0 (0) |

A total of 59 *E. coli* isolates were included in the study

**Table 4** Antimicrobial resistance pattern of the *K. pneumoniae* isolates from different clinical specimens, Kathmandu, Nepal

| Antibiotic class | Antibiotics used | Sensitive n (%) | Intermediate n (%) | Resistant n (%) |
|---|---|---|---|---|
| Aminopenicillins | Amoxicilin | 0 (0) | 0 (0) | 39 (100) |
| Cephalosporins | Ceftriaxone | 6 (15.4) | 3 (7.7) | 30 (76.9) |
| | Ceftazidime | 6 (15.4) | 3 (7.7) | 30 (76.9) |
| | Cefixime | 11 (28.2) | 0 (0) | 28 (71.8) |
| Sulphonamides | Cotrimoxazole | 12 (30.7) | 1 (2.6) | 26 (66.7) |
| Fluoroquinolones | Nalidixic acid | 10 (25.7) | 7 (17.9) | 22 (56.4) |
| | Ciprofloxacin | 7 (18.0) | 2 (5.1) | 30 (76.9) |
| Nitrofurans | Nitrofurantoin | 5 (35.7) | 1 (7.2) | 8 (57.1) |
| Aminoglycosides | Amikacin | 14 (35.9) | 8 (20.5) | 17 (43.6) |
| | Gentamicin | 18 (46.1) | 1 (2.6) | 20 (51.3) |
| Carbapenems | Imipenem | 13 (33.3) | 6 (15.4) | 20 (51.3) |
| | Meropenem | 13 (33.3) | 2 (5.1) | 24 (61.6) |
| Tetracyclines | Doxycycline | 19 (48.7) | 8 (20.5) | 12 (30.8) |
| β-lactam with inhibitors | Piperacillin/ tazobactam | 9 (23.1) | 6 (15.4) | 24 (61.5) |
| Lipopeptides | Colistin | 39 (100) | 0 (0) | 0 (0) |

A total of 39 K. *pneumoniae* isolates were included in the study

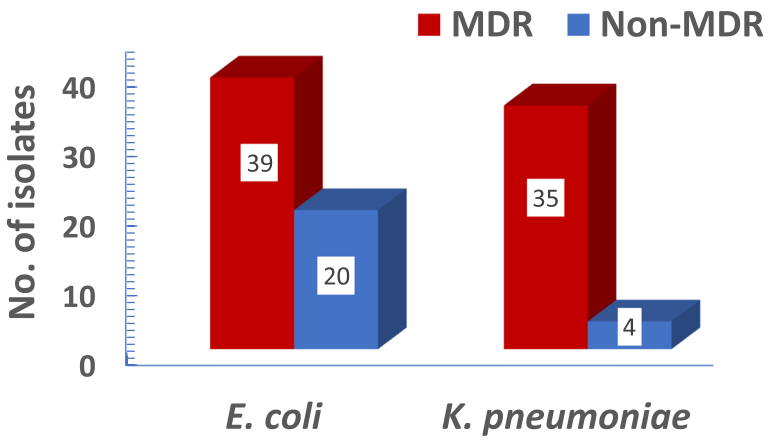


**Fig. 2** Multi-drug resistance in *E. coli* and *K. pneumoniae isolates* from different clinical specimens, Kathmandu, Nepal

**Table 5** MIC of ciprofloxacin among resistant *E. coli* and *K. pneumoniae* isolated from different clinical specimens, Kathmandu, Nepal

| MIC value (µg/mL) | No. of *E. coli* n (%) | No. of *K. pneumoniae* n (%) |
|---|---|---|
| 0.25 | 0 (0) | 4 (12.5) |
| 0.5 | 2 (5.9) | 2 (6.3) |
| 1 | 0 (0) | 1 (3.1) |
| 2 | 1 (2.9) | 0 (0) |
| 4 | 3 (8.8) | 1 (3.1) |
| 8 | 0 (0) | 1 (3.1) |
| 16 | 1 (2.9) | 2 (6.3) |
| 32 | 4 (11.8) | 6 (18.7) |
| 64 | 6 (17.7) | 2 (6.3) |
| 128 | 4 (11.8) | 3 (9.4) |
| 256 | 10 (29.4) | 2 (6.2) |
| 512 | 3 (8.8) | 8 (25) |
| **Total** | **34 (100.0)** | **32 (100.0)** |

### MIC of ciprofloxacin resistant *E. coli* and *K. pneumoniae*

The highest number of *E. coli* isolates showed MIC value of ciprofloxacin as 256 µg/mL [10] followed by 64 µg/mL [6]. Similarly, the highest number of *K. pneumoniae* isolates showed MIC value of ciprofloxacin as 512 µg/mL [8] followed by 32 µg/mL [6] (Table 5; Supplementary Fig. 1). Ciprofloxacin resistance was significantly associated with the bacterial type (higher resistance in *K. pneumoniae* compared *to E. coli* isolates) ($p = 0.012$).

### *AcrA, AcrB and TolC genes among MDR E. coli and K. pneumoniae*

We detected the anticipated amplicon sizes of samples and positive controls in PCR. Negative controls showed no amplification, verifying the specificity of the assay and confirming no contamination. Among MDR *E. coli* isolates, the *AcrA*, *AcrB*, and *TolC* genes were detected in 91.7% (22/24), 81.8% (18/22) and 76.7% (23/30) isolates, respectively. Similarly, among the MDR *K. pneumoniae* isolates, the *AcrA*, *AcrB*, and *TolC* genes were detected in 95% (19/20), 94.7% (18/19) and 90.5% (19/21) isolates, respectively (Supplementary Fig. 2). There was no significant association between the presence of the *AcrAB-TolC* EP genes and MDR in these isolates ($p > 0.05$) (Table 6).

**Table 6** *AcrA*, *AcrB* and *TolC* genes among MDR ciprofloxacin resistant *E. coli* and *K. pneumoniae* isolated from different clinical specimens, Kathmandu, Nepal

| Bacteria | Target gene | MDR isolates, n (%) | Non-MDR isolates, n (%) | Total isolates, n (%) | p-value |
|---|---|---|---|---|---|
| *E. coli* | *AcrA* | 22 (91.7) | 2 (8.3) | 24 (100) | 0.343 |
| | *AcrB* | 18 (81.8) | 4 (18.2) | 22 (100) | |
| | *TolC* | 23 (76.7) | 7(23.3) | 30 (100) | |
| *K. pneumoniae* | *AcrA* | 19 (95) | 1 (5) | 20 (100) | 0.809 |
| | *AcrB* | 18 (94.7) | 1 (5.3) | 19 (100) | |
| | *TolC* | 19 (90.5) | 2 (9.5) | 21 (100) | |

## Discussion

In the hospital setting, high degree of MDR rate was observed in the most frequently encountered pathogens, namely, *K. pneumoniae* (89.7%) and *E. coli* (66.1%) in Nepal. Previous studies conducted in the different parts of Nepal have also reported similar findings [32–34]. Such resistance often poses a burden in the overall health care system. Moreover, the spread of resistant strains into the community may contribute to increased illness burden, mortality, healthcare costs and antibiotic use, although further data are needed to characterize these impacts in the local context [35]. The elevated MDR rates observed in this study may be attributed to increased expression of genes encoding multidrug EPs or the accumulation of multiple resistance genes on resistance (R) plasmids [36]. Apart from this, antibiotic abuse which includes non-empirical usage, inappropriate empirical use, insufficient dosages and easy access to antibiotics (over-the-counter) without a prescription also results in increased MDR [3]. However, empirical treatment may save lives when guided by clinical guidelines and local

resistance patterns. Additionally, the establishment of antibiotic-resistant bacteria has been linked to the widespread use of sub-lethal doses of antibiotics in livestock, including pigs, cattle and poultry for the purpose of treating and preventing diseases and promoting growth [3, 23]. These resistant strains may be transmitted to humans through the food chain [37]. This highlights the need for antimicrobial stewardship, improved training and equitable access to diagnostics and quality-assured drugs to encourage efficient antimicrobial use for patient safety.

A higher proportion (70–95%) of EP genes (*AcrA*, *AcrB*, and *TolC*) were detected in ciprofloxacin resistant and MDR isolates in the PCR analysis. This suggests an EP mediated resistance in our hospital setting. Mutations affecting key amino acid residues in these EP genes could contribute to enhanced MDR by altering their affinity for typical substrates [38]. However, mutation in amino acid residues of EP genes was not examined in this study, which is our limitation.

Nearly 56% of *E. coli* and 77% of *K. pneumoniae* isolates were resistant to ciprofloxacin in the current study. Such ciprofloxacin resistance has been observed in these pathogens in other studies from Nepal too [39–41]. Resistance to fluoroquinolones and β-lactam antibiotics (carbapenems, cephalosporins, and penicillins) contributes to more than two-thirds of deaths attributed to antibiotic resistance [42]. Therefore, the high resistance to fluoroquinolones we found warrants an immediate attention to mitigate a worse future. Three primary mechanisms of ciprofloxacin resistance have been identified: plasmids that protect cells from the drug's lethal effects, mutations that restrict drug accumulation, and mutations in drug targets such as DNA gyrase and DNA topoisomerase IV [19]. Additionally, mutations in EP gene regulators could also contribute to quinolone resistance [41]. All these mechanisms eventually diminishes the effectiveness of antimicrobial therapies and often exacerbates infection severity [43].

Although colistin was found to be sensitive to all *E. coli* and *K. pneumoniae* isolates, carbapenems drugs were resistant to about 57% of *K. pneumoniae* and 17% *E. coli* isolates. Carbapenems and colistin are two common "last-resort" antibiotics which is used to treat infections due to MDR bacteria, especially by extensively drug-resistant (XDR) and pan drug resistant strains [44]. Unfortunately, the efficacy of carbapenems and colistin have decreased recently due to presence of resistant genes, such as $bla_{NDM}$ and *mcr-1* [45]. For instance, a study in China reported the zoonotic transmission of colistin resistance mediated by *mcr-1* from animals to humans [46]. If colistin resistance continues to evolve at this rate, treating MDR bacterial infections may become even more challenging, and a situation called post-antibiotic apocalypse may occur. This can be the most devastating one exacerbating morbidity and mortality [47].

More than 50% of *E. coli* and *K. pneumoniae* isolates were resistant to frequently prescribed antibiotics in Nepal namely, amoxicillin, ceftriaxone, ceftazidime, cefixime and cotrimoxazole. This resistance to commonly used antibiotics is even higher than previous reports from Nepal illustrating an alarming public health threat [48–52]. Observed increase in resistance to third-generation cephalosporins in this study may be due to the production of extended-spectrum β-lactamases as well as bacterial mutations and post-transcriptional modifications [53]. Infections caused by third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* have been associated with increased mortality, prolonged hospital stay, and higher healthcare costs [54]. Thus, it is crucial to monitor spread of resistant strains in the community in Nepal.

Inequitable access to necessary medications and diagnostics is a major contributing factor to AMR in Nepal, especially in rural and resource-constrained areas. Reliance on over-the-counter drug vendors and symptom-based therapies result from a lack of qualified healthcare professionals and testing facilities. High expenses and irregular antibiotic supplies in public health institutions further push the population towards unofficial physicians and traditional healers. These systemic obstacles, which have their roots in availability, affordability and regulatory gaps, encourage improper use of antibiotics and hasten resistance [55]. To prevent the rampant over-the-counter purchasing and use of antibiotics in Nepal, the government of Nepal has very recently issued a circular that some last resort antibiotics (meropenem, polymyxin B, piperacillin + tazobactam, vancomycin, colistin and linezolid) should only be available in the hospital pharmacy and purchased only with prescription. This is a great step towards limiting or tackling the increasing AMR [56]. However, the future will reveal how effectively it is implemented.

About 14% of the clinical specimens showed significant bacterial growth with *E. coli* and *K. pneumoniae* being most common. Several studies during different time frames also state the similar proportions of growth positivity and causative agents [57, 58]. This observation highlights a persistent trend of bacterial infections in Nepal, consistent with patterns observed in the past. However, effective management of patient remains a significant challenge in the region [58]. Most of the *E. coli* were detected in urine samples of female participants. This can be explained by fact that *E. coli* is most common pathogen in urinary tract infection which is common in female [53, 59]. In this study, the majority of *K. pneumoniae* isolates were identified in males similar to reports in the past but exact reason is unknown [60]. The

majority of *K. pneumoniae* was detected from sputum, which is expected, as *K. pneumoniae* is a common pathogen of upper respiratory tract [61]. The infection rates of *K. pneumoniae* and *E. coli* were higher in individuals aged 31–45 (33.3%) and 31–60 (57.6%), respectively. This showed prevalence of infection in highly active populations which certainly affects their daily activity and work potential.

This study serves as a valuable reference for future investigations into the prevalence and causes of ciprofloxacin-resistant isolates in hospital and community settings. While this study utilized conventional methods for confirming ciprofloxacin resistance, including MIC and PCR assays, future research incorporating whole-genome sequencing (WGS) and metagenomics could provide deeper insights into the evolution of resistant strains and their mechanisms. This cross-sectional study was conducted in a single center, which limits its ability to track trends in AMR over time and analysis of temporal variations/seasonal trends. Thus, results may not be generalizable to other settings, limiting the applicability of the findings to a broader population. The most isolates were from the urine and sputum samples which may limit the generalizability of the findings across other infection types or patient populations because the resistance patterns mostly reflect respiratory and urine infection. Another key limitation of this study is that fluoroquinolone resistance was assessed only in relation to the AcrAB efflux pump, without evaluating other common mechanisms like gyrA, gyrB, parC and parE mutations. Thus, the observed association should be interpreted cautiously, and further studies are needed to explore additional resistance determinants. Further, it does not evaluate the long-term clinical or economic outcomes of AMR, such as treatment failures or mortality rates by which the broader impact of AMR on healthcare system and patient outcomes remain unaddressed. Therefore, multi-centric longitudinal studies at molecular level, routine surveillance, and in-depth analyses will provide a more comprehensive understanding of the burden of bacterial infections and AMR in the country. This is important for enforcing AMR policies and interventions like one health approach.

## Conclusions

The findings highlight the dominance of multidrug-resistant *E. coli* and *K. pneumoniae* as major clinical isolates in the tertiary care hospital of Nepal, with alarmingly high resistance to commonly prescribed antibiotics namely amoxicillin, ceftriaxone, cefixime, cotrimoxazole, and ciprofloxacin. The detection of elevated MIC values and the prevalence of EP genes in ciprofloxacin-resistant MDR isolates underscores the presence of EP-mediated resistance. Although our results reflect local trends specific to a tertiary care center, they suggest broader concerns regarding immediate action, including the development of evidence-based antibiotic stewardship programs, implementation of the One Health approach, as well as surveillance using advanced molecular tools to mitigate the public health threat posed by AMR in Nepal.

### Abbreviations

| | |
|---|---|
| AMR | Antimicrobial resistance |
| MDR | Multidrug resistance |
| Ep | Efflux pump |
| MF | Major facilitator |
| MATE | Multidrug and toxic efflux |
| RND | Resistance nodulation division |
| SMR | Small multidrug resistance |
| ABC | ATP binding cassette |
| MA | MacConkey agar |
| BA | Blood agar |
| CA | Chocolate agar |
| CLED | Cysteine lactose electrolyte deficient |
| PCR | Polymerase chain reaction |
| MIC | Minimum inhibitory concentration |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-11555-5.

Supplementary Material 1.

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### Authors' contributions

SPD designed the study. SK, SR and BN did sample collection, and laboratory analysis. SPD, SK and SR performed data analysis. SPD and MR supervised the study. SPD, SK and SR wrote the original draft. SPD, SR, SK, BN, MR, YS, BSC, MMNT, NSB and BKS contibuted to review and editing of manuscript. All authors read and approved the final manuscript.

### Funding

Not applicable.

### Data availability

The datasets generated and/or analyzed during the current study are available in the manuscript.

## Declarations

### Ethics approval and consent to participate

This study received ethical approval from the Institutional Review Committee, Institute of Science and Technology (IRC-IOST), Tribhuvan University, Kathmandu, Nepal (Protocal No. IRCIOST-22–0057, 2022/10/19). The study followed the guidelines outlined in the Declaration of Helsinki and Nepal Health Research Council (NHRC). Each study participant and/or their guardian (if a minor) provided written informed consent.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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