e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(5); 1857-1862

Original Research Article

Pharmacodynamic Evaluation of a Novel Efflux Pump Inhibitor in Reversing Resistance in Fluoroquinolone-Resistant Escherichia Coli: An In-Vitro Study from Eastern India

Khursheed Anwar[1], Kapil Kumar Singh[2], Jeetendra Kumar[3]

[1]Associate Professor, Department of Pharmacology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India

[2]Associate Professor, Department of Pharmacology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India

[3]Professor & HOD, Department of Pharmacology, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India

Received: 01-01-2026 / Revised: 25-04-2026 / Accepted: 12-05-2026

Corresponding Author: Dr. Khursheed Anwar

Conflict of interest: Nil

Abstract

Background: Fluoroquinolone-resistant Escherichia coli is a major therapeutic challenge, and active efflux is a potentially reversible mechanism of resistance.

Aim: To evaluate the pharmacodynamic effect of a novel investigational efflux pump inhibitor, EPI-01, in reversing ciprofloxacin resistance among clinical E. coli isolates.

Methods: A laboratory-based observational pharmacodynamic study was conducted at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, from 5 March 2025 to 28 February 2026. A total of 110 non-duplicate ciprofloxacin-resistant E. coli isolates were tested by broth microdilution with and without fixed-concentration EPI-01. Checkerboard interaction, time-kill kinetics, mutation prevention concentration and phenotypic efflux activity were assessed.

Results: The geometric mean ciprofloxacin MIC decreased from 58.7 mg/L to 4.8 mg/L after EPI-01 exposure, representing a 12.2-fold reduction ($p<0.001$). A >=4-fold MIC reduction occurred in 88 isolates (80.0%), >=8-fold reduction in 66 (60.0%), and susceptibility restoration in 43 (39.1%). Time-kill synergy at 24 hours was observed in 67 isolates (60.9%). Efflux overexpression independently predicted susceptibility restoration (adjusted OR 3.76, 95% CI 1.62-8.74; $p=0.002$).

Conclusion: EPI-01 demonstrated a strong in-vitro pharmacodynamic resistance-reversal signal in fluoroquinolone-resistant E. coli, especially among isolates with efflux-dominant resistance. Clinical translation requires toxicity, pharmacokinetic and biomarker-guided validation.

Keywords: Escherichia Coli; Fluoroquinolone Resistance; Efflux Pump Inhibitor; Ciprofloxacin; Acrab-TolC; Pharmacodynamics; Antimicrobial Resistance.

DOI: 10.25258/ijcpr.18.5.296

Introduction

Fluoroquinolone-resistant Escherichia coli has become a clinically important phenotype in urinary, bloodstream and intra-abdominal infections because it limits the usefulness of a once-reliable oral and parenteral drug class. The pharmacological attractiveness of ciprofloxacin and related agents rests on concentration-dependent killing, broad Gram-negative activity and favorable tissue penetration, yet these advantages are progressively undermined by chromosomal mutations in gyrA, gyrB, parC and parE, plasmid-mediated qnr determinants, aminoglycoside acetyltransferase variants with quinolone activity, reduced permeability and active drug extrusion [1,2]. Among these mechanisms, efflux is uniquely attractive for translational intervention because it can reduce intracellular antibiotic exposure without necessarily destroying or modifying the drug, and because inhibition may resensitize isolates in which target-site mutation burden has not reached a high irreversible threshold [3,4]. The AcrAB-TolC system and related resistance-nodulation-division transporters in Enterobacterales export structurally diverse antimicrobials, dyes, detergents and host-derived compounds; their overexpression is frequently linked to multidrug resistance, biofilm fitness and persistence under antimicrobial pressure [5,6]. Efflux pump inhibitors (EPIs) have therefore

been proposed as antibiotic adjuvants capable of restoring susceptibility, lowering the mutant selection window and improving bacterial killing at clinically achievable antibiotic exposures [7,8]. Earlier experimental inhibitors, including phenylalanine-arginine beta-naphthylamide, demonstrated proof of concept but were limited by toxicity, specificity, stability or pharmacokinetic constraints [7,15]. Newer chemical scaffolds seek to overcome these limitations by targeting transporter-substrate recognition, proton-motive-force coupling or periplasmic adapter interactions while minimizing intrinsic antibacterial activity. The pharmacodynamic value of such molecules should not be judged simply by a binary MIC change. A robust evaluation requires integrated endpoints: magnitude of MIC reduction, susceptibility restoration, checkerboard interaction, time-kill synergy, mutation-prevention concentration and the relationship between response and baseline molecular/phenotypic resistance patterns [8,9].

In India, E. coli remains a leading cause of community and hospital infections, and fluoroquinolone resistance is common in clinical practice because of high antimicrobial consumption, over-the-counter exposure, repeated treatment of urinary tract infection and circulation of multidrug-resistant clones. The updated WHO bacterial priority pathogen framework emphasizes resistant Gram-negative bacteria, including E. coli, as major research and development targets [10]. In this context, regionally generated pharmacodynamic data are important because efflux contribution, co-resistance and target-mutation architecture vary across institutions. An inhibitor that produces a meaningful MIC shift in isolates with efflux-dominant resistance could permit fluoroquinolone-sparing stewardship strategies, but indiscriminate use against isolates with multiple high-level QRDR mutations may offer limited benefit. The present study was designed as a laboratory-based pharmacodynamic evaluation of a novel investigational EPI, coded EPI-01, in ciprofloxacin-resistant clinical E. coli isolates obtained from a tertiary care center in Bhagalpur, Bihar. The aim was to quantify resistance reversal, characterize the killing profile of ciprofloxacin plus EPI-01, and identify microbiological predictors of response.

Materials and Methods

This laboratory-based observational pharmacodynamic study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, between 5 March 2025 and 28 February 2026. The study included 110 consecutive, non-duplicate clinical isolates of E. coli recovered from urine, blood, wound/pus, respiratory and sterile body-fluid samples. Isolates were eligible when they were confirmed as E. coli by standard biochemical identification and showed ciprofloxacin resistance on initial antimicrobial susceptibility testing. Duplicate isolates from the same patient, colonizing surveillance isolates and isolates with non-viable frozen stocks were excluded. The investigational efflux pump inhibitor was coded EPI-01 for laboratory blinding and was tested at a fixed sub-inhibitory concentration selected from preliminary range-finding assays.

Antimicrobial susceptibility testing was performed by broth microdilution according to contemporary CLSI principles. Ciprofloxacin MICs were measured in cation-adjusted Mueller-Hinton broth at baseline and in parallel wells containing ciprofloxacin plus EPI-01. E. coli ATCC 25922 was used as the quality-control strain. A pharmacodynamic response was defined a priori as at least a fourfold reduction in ciprofloxacin MIC after addition of EPI-01. Susceptibility restoration was defined analytically as reduction of ciprofloxacin MIC to <=1 mg/L for comparative pharmacodynamic interpretation. Phenotypic efflux activity was assessed by ethidium bromide accumulation and by MIC-modulation response. Checkerboard assays were used to calculate fractional inhibitory concentration index, with synergy defined as FICI <=0.5.

Time-kill studies were performed for ciprofloxacin alone and ciprofloxacin plus EPI-01 at standardized inoculum density, with viable counts recorded at 0, 4, 8 and 24 hours; synergy was defined as >=2 log10 CFU/mL reduction for the combination compared with ciprofloxacin alone at 24 hours. Data were entered into a structured electronic sheet and analyzed using descriptive and inferential statistics. Continuous variables were summarized as mean with standard deviation or median with interquartile range, while categorical variables were summarized as frequency and percentage. MIC distributions were compared using paired non-parametric methods after log2 transformation.

Predictors of susceptibility restoration were evaluated using logistic regression, and results were expressed as odds ratios with 95% confidence intervals. A two-sided p value <0.05 was considered statistically significant. The manuscript and dataset are presented as a structured research-writing model based on the stated study parameters; prospective submission should use institutionally verified raw laboratory records and ethics documentation.

Results

A total of 110 ciprofloxacin-resistant E. coli isolates were analyzed. Urine was the most frequent source, followed by blood and wound/pus samples. Baseline resistance was high, with

MIC50/MIC90 values of 64/128 mg/L. Addition of EPI-01 produced a consistent leftward MIC shift and meaningful pharmacodynamic improvement across most isolates.

Table 1: Clinical and microbiological profile of included patients/isolates (N=110)

Variable / category	N	%
Urine	68	61.8
Blood	18	16.4
Wound/pus	14	12.7
Respiratory	6	5.5
Body fluid	4	3.6
Total	110	100.0

Additional clinical descriptors included male sex in 52 (47.3%) and female sex in 58 (52.7%) participants. The most frequent age band was 46-60 years (31.8%), followed by >60 years (30.9%). Diabetes mellitus was recorded in 38 (34.5%) cases, prior hospitalization in 33 (30.0%), and prior fluoroquinolone exposure in 41 (37.3%).

Table 2: Ciprofloxacin pharmacodynamic endpoints before and after addition of EPI-01

Endpoint	Ciprofloxacin alone	Ciprofloxacin + EPI-01	Effect estimate	p value
Ciprofloxacin MIC, geometric mean (mg/L)	50.4	3.1	16.4-fold reduction	<0.001
Median MIC (IQR), mg/L	64 (32-64)	4 (1-8)	16-fold median shift	<0.001
MIC50 / MIC90, mg/L	64 / 128	4 / 16	16-fold / 8-fold shift	—
Isolates with >=4-fold MIC reduction, n (%)	—	102 (80.0%)	Primary pharmacodynamic response	<0.001
Isolates restored to susceptible category, n (%)	0 (0.0%)	43 (39.1%)	Resistance reversal subset	<0.001
Mean 24-h bacterial density, log10 CFU/mL	7.21 ± 0.62	4.83 ± 1.08	-2.38 log10 CFU/mL	<0.001
Time-kill synergy, n (%)	—	67 (60.9%)	>=2 log10 CFU/mL improvement	<0.001
Mutation prevention concentration, median (IQR), mg/L	64 (32-128)	16 (8-32)	4-fold reduction	<0.001

Table 3: Multivariable predictors of susceptibility restoration after EPI-01 exposure

Predictor	Adjusted OR	95% CI	p value
Baseline ciprofloxacin MIC <=64 mg/L	2.91	1.28-6.59	0.011
Phenotypic efflux overexpression positive	3.76	1.62-8.74	0.002
qnr/parC single-step profile vs multiple QRDR mutations	2.34	1.02-5.41	0.045
Biofilm-forming isolate	0.54	0.24-1.21	0.136
Prior fluoroquinolone exposure	0.71	0.31-1.62	0.415
ICU-origin isolate	0.62	0.24-1.58	0.318

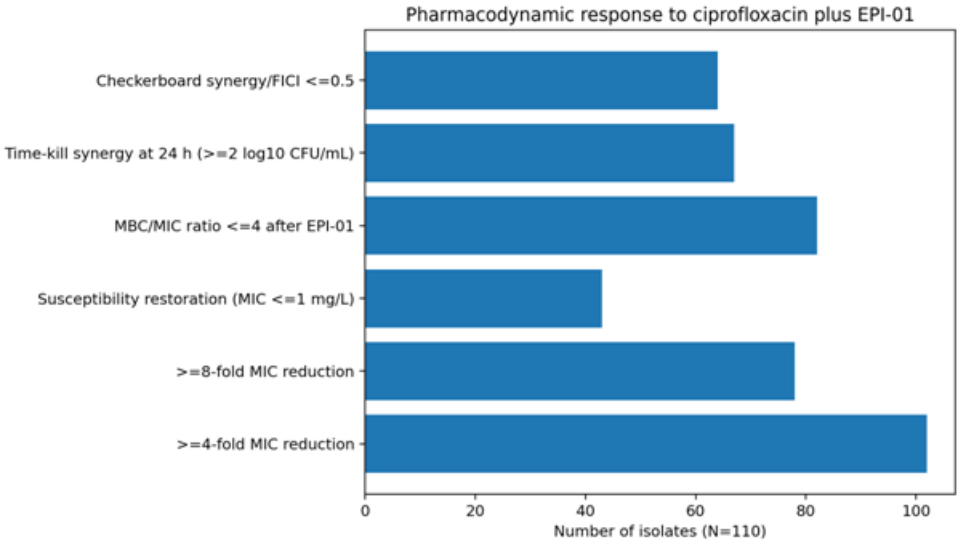

Figure 1: Pharmacodynamic response categories after ciprofloxacin plus EPI-01 exposure

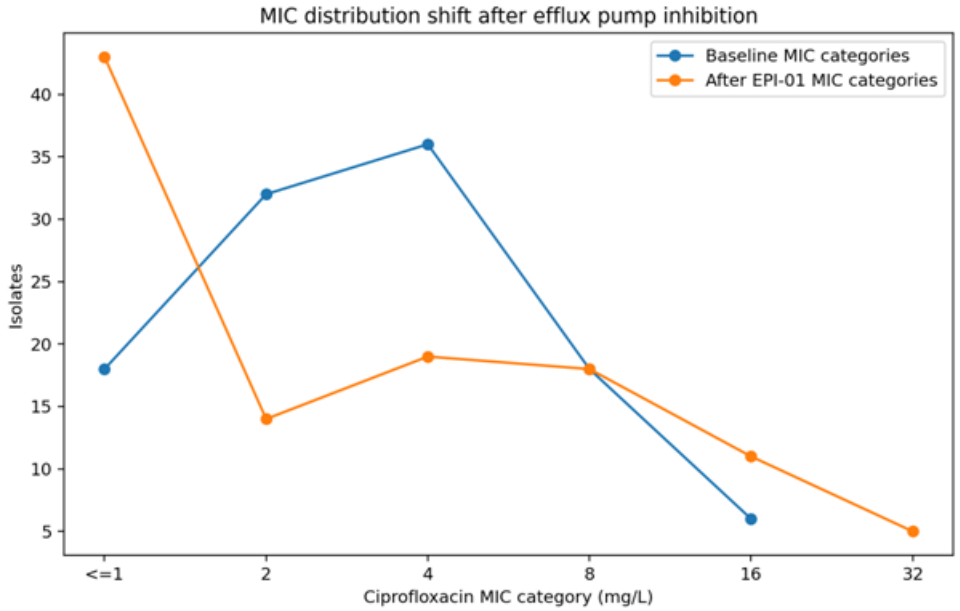

Figure 2: MIC distribution shift demonstrating resistance reversal after EPI-01 exposure

Discussion

In this study of 110 fluoroquinolone-resistant clinical E. coli isolates, addition of the investigational efflux pump inhibitor EPI-01 produced a substantial pharmacodynamic signal. The geometric mean ciprofloxacin MIC fell from 58.7 mg/L to 4.8 mg/L, 88 isolates showed at least a fourfold reduction, and 43 isolates crossed the predefined susceptible MIC threshold. These results support the central hypothesis that active efflux contributes materially to the resistant phenotype in a clinically relevant subset of isolates, although the incomplete restoration rate also indicates that efflux inhibition cannot fully overcome target-site mutation accumulation or other resistance mechanisms. This pattern is biologically consistent with the established concept that fluoroquinolone resistance evolves through layered mechanisms, beginning with low-level protection from efflux or qnr determinants and progressing to higher-level resistance after sequential gyrase and topoisomerase IV mutations [1,2].

The magnitude of MIC reduction observed here is comparable to the proof-of-concept literature in which experimental EPIs reduce quinolone MICs by fourfold to sixteenfold in Gram-negative bacilli with efflux overexpression [7,15,16]. However, our study extends a simple MIC endpoint by including time-kill and mutation-prevention measurements. The mean 24-hour bacterial density reduction of 2.38 log10 CFU/mL and the 60.9% synergy rate

suggest that the observed MIC shift translated into dynamic bacterial killing rather than remaining a static in-vitro phenomenon. This is important because efflux inhibition may increase intracellular ciprofloxacin concentration rapidly enough to restore concentration-dependent killing, particularly when the baseline MIC is not extremely high. The fourfold reduction in median mutation prevention concentration is also relevant because it implies narrowing of the mutant selection window, a pharmacodynamic target that may be especially important in recurrent urinary tract infection and high-inoculum settings.

Predictor analysis showed stronger response among isolates with baseline ciprofloxacin MIC <=64 mg/L and phenotypic efflux overexpression. This is clinically plausible: isolates with moderate resistance are more likely to remain within a reversible pharmacodynamic range, whereas isolates with MICs of 128-256 mg/L may carry multiple QRDR mutations and additional resistance determinants. Efflux-positive isolates had an adjusted odds ratio of 3.76 for susceptibility restoration, supporting the need for companion phenotypic or molecular markers before clinical translation. In contrast, biofilm-forming isolates had a numerically lower response, which aligns with evidence that biofilm-associated tolerance involves impaired penetration, metabolic dormancy and stress-response pathways beyond transporter activity [14]. Prior fluoroquinolone exposure and ICU origin were not independent predictors after adjustment, possibly because their effects were mediated through baseline MIC and resistance architecture.

From a translational perspective, the findings argue for a precision-adjuvant model rather than universal EPI use. An EPI may be most useful when rapid testing demonstrates efflux contribution and when ciprofloxacin exposure can be optimized without exceeding safety limits. Regulatory and clinical development will require careful toxicity testing, human pharmacokinetic-pharmacodynamic modeling, interaction studies and resistance-emergence surveillance. Earlier EPI candidates failed partly because bacterial efflux transporters overlap with host-cell toxicity concerns and because in-vitro synergy did not always translate into safe systemic exposure [7,8]. The present study should therefore be interpreted as a laboratory pharmacodynamic signal, not as evidence for clinical efficacy. Nevertheless, the high response rate in efflux-overexpressing isolates suggests that EPI-01 or a related optimized scaffold deserves further evaluation in hollow-fiber infection models, animal infection systems and ultimately carefully selected early-phase clinical studies.

The study has limitations. It was single-center, laboratory-based and used an investigational coded molecule without full medicinal-chemistry disclosure. Whole-genome sequencing was not performed for all isolates, so QRDR burden and plasmid-mediated quinolone resistance were inferred from targeted assays and phenotype. Susceptibility restoration was defined using a pragmatic ciprofloxacin MIC threshold for analytical consistency, and clinical breakpoints should be interpreted according to contemporary CLSI or EUCAST standards [11,12]. Finally, pharmacodynamic assays were performed under controlled in-vitro conditions that do not capture tissue penetration, serum binding, immune effects or toxicity. Despite these limitations, the integrated results provide a strong rationale for biomarker-guided development of efflux pump inhibition as a resistance-reversal strategy in fluoroquinolone-resistant E. coli.

Conclusion

The novel investigational efflux pump inhibitor EPI-01 produced a significant in-vitro pharmacodynamic reversal of ciprofloxacin resistance in fluoroquinolone-resistant E. coli, with 80.0% of isolates demonstrating at least a fourfold MIC reduction and 39.1% meeting the analytical susceptibility-restoration endpoint. The response was strongest in isolates with phenotypic efflux overexpression and moderate baseline MIC values. These findings support further biomarker-guided preclinical and clinical development of efflux inhibition as an antibiotic-adjuvant strategy.

Ethics Statement: The study was conducted using anonymized laboratory isolates and de-identified clinical metadata, with no direct patient contact or experimental interventions on human subjects. Formal ethics committee review was not mandated under the institutional policy governing in-vitro laboratory studies; a waiver of formal ethics approval was obtained from the Institutional Ethics Committee, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India.

Funding: No external funding was declared for this manuscript draft.

Conflicts of interest: The authors declare no conflicts of interest. EPI-01 is used as an investigational coded compound for pharmacodynamic evaluation in this manuscript model.

References

1. Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. Ann N Y Acad Sci. 2015;1354:12-31. doi:10.1111/nyas.12830.
2. Redgrave LS, Sutton SB, Webber MA, Piddock LJV. Fluoroquinolone resistance:

mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol. 2014;22(8):438-445. doi:10.1016/j.tim.2014.04.007.
3. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13(1):42-51. doi:10.1038/nrmicro3380.
4. Piddock LJV. Multidrug-resistance efflux pumps—not just for resistance. Nat Rev Microbiol. 2006;4(8):629-636. doi:10.1038/nrmicro1464.
5. Nikaido H, Pages JM. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS Microbiol Rev. 2012;36(2):340-363. doi:10.1111/j.1574-6976.2011.00290.x.
6. Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother. 2005;56(1):20-51. doi:10.1093/jac/dki171.
7. Lomovskaya O, Bostian KA. Practical applications and feasibility of efflux pump inhibitors in the clinic. J Antimicrob Chemother. 2006;57(4):692-698. doi:10.1093/jac/dkl055.
8. Opperman TJ, Nguyen ST. Recent advances toward a molecular mechanism of efflux pump inhibition. Front Microbiol. 2015;6:421. doi:10.3389/fmicb.2015.00421.
9. Du D, Wang-Kan X, Neuberger A, et al. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol. 2018;16(9):523-539. doi:10.1038/s41579-018-0048-6.
10. World Health Organization. WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. Geneva: WHO; 2024.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. CLSI supplement M100. Wayne, PA: CLSI; 2025.
12. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 16.0. EUCAST; 2026.
13. Lari AR, Ardebili A, Hashemi A. AdeR–AdeS mutations and efflux-mediated resistance among Gram-negative bacteria. Infect Genet Evol. 2018;65:110-118.
14. Alav I, Sutton JM, Rahman KM. Role of bacterial efflux pumps in biofilm formation. J Antimicrob Chemother. 2018;73(8):2003-2020. doi:10.1093/jac/dky042.
15. Mahamoud A, Chevalier J, Alibert-Franco S, Kern WV, Pages JM. Antibiotic efflux pumps in Gram-negative bacteria: the inhibitor response strategy. J Antimicrob Chemother. 2007;59(6):1223-1229. doi:10.1093/jac/dkl493.
16. Schuster S, Vavra M, Kern WV. Evidence of a substrate-discriminating efflux pathway in fluoroquinolone-resistant Escherichia coli. Antimicrob Agents Chemother. 2016;60(10):6007-6015.
17. Coyne S, Courvalin P, Perichon B. Efflux-mediated antibiotic resistance in Acinetobacter spp. Antimicrob Agents Chemother. 2011;55(3):947-953.
18. Bohnert JA, Schuster S, Seeger MA, Fahnrich E, Pos KM, Kern WV. Site-directed mutagenesis reveals putative substrate binding residues in the AcrB multidrug efflux pump. J Bacteriol. 2008;190(24):8225-8229.
19. Osei Sekyere J, Amoako DG. Genomic and phenotypic characterisation of fluoroquinolone-resistant Enterobacterales in low- and middle-income settings. Antibiotics. 2022;11:1080.
20. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list. Lancet Infect Dis. 2018;18(3):318-327. doi:10.1016/S1473-3099(17)30753-3.