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Research Article

Lateral Flow Immunoassay and the EDTA-Colistin Broth Disk Elution for *mcr-1* gene detection in colistin-resistant *E. coli* and *K. pneumoniae*

Inmunoensayo de flujo lateral y elución de disco en caldo con colistina-EDTA para detectar el gen *mcr-1* en *E. coli* y *K. pneumoniae* resistentes a la colistina

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ABSTRACT

Introduction: The global emergence of colistin-resistant Enterobacterales, especially strains carrying plasmid-mediated *mcr* genes, poses a significant threat to public health. Rapid detection of *mcr*-mediated resistance is critical for timely clinical management and infection control, particularly in resource-limited settings such as Southeast Asia.


2025;54(3):e025076562

Objectives: To evaluate the performance of the lateral flow immunoassay (NG-Test MCR-1) in comparison with the combined reference method of EDTA-Colistin Broth Disk Elution (CBDE/EDTA) and polymerase chain reaction (PCR) for detecting *mcr*-mediated colistin.
Methods: A cross-sectional study was conducted at Cho Ray Hospital, Ho Chi Minh City, Vietnam. A total of 160 clinical isolates of *Klebsiella pneumoniae* (n = 128) and *Escherichia coli* (n = 32), along with 2 additional *E. coli* strains carrying the *mcr-1* gene, were tested for colistin resistance using Broth Microdilution (BMD) combined with the NG-Test MCR-1 in comparison with the CBDE/EDTA combined with PCR detection of *mcr*-1 to *mcr*-10.
Results: Among 32/160 colistin-resistant isolates, only 2 *E. coli* strains were confirmed to carry *mcr*-1 by both NG-Test MCR-1 and PCR, while no *K. pneumoniae* isolates tested positive for *mcr* genes. The NG-Test MCR-1 showed perfect agreement with the CBDE/EDTA method combined with PCR. Furthermore, it achieved 100% PPA and NPA, with wide 95% confidence intervals due to the limited number of mcr-positive isolates.
Conclusions: NG Test MCR-1 provides rapid, specific detection of *mcr-1*, while CBDE/EDTA is a cost-effective screening tool but less definitive. Combining these methods is recommended to improve *mcr*-mediated colistin resistance detection.
Keywords: Edetic Acid; Enterobacteriaceae; Genes; Immunoassay; Plasmids.

RESUMEN

Introducción: La propagación de *Enterobacterales* resistentes a la colistina, especialmente cepas portadoras de genes *mcr* mediados por plásmidos, representa una amenaza para la salud pública y limita las opciones terapéuticas. La detección rápida y precisa de la resistencia mediada por *mcr* es esencial para el manejo clínico y controlar infecciones, sobre todo en entornos con recursos limitados.
Objetivos: Evaluar el rendimiento diagnóstico del inmunoensayo de flujo lateral NG-Test MCR-1 en comparación con el método de referencia EDTA-Colistin Broth Disk Elution (CBDE/EDTA) combinado con reacción en cadena de la polimerasa (PCR) para detectar genes *mcr*.

2025;54(3):e025076562

Métodos: Estudio transversal en el Hospital Cho Ray, Vietnam, con 160 aislamientos clínicos (*Klebsiella pneumoniae* = 128; *Escherichia coli* = 32) y dos cepas adicionales de *E. coli* portadoras de *mcr-1*. La resistencia a colistina se determinó mediante microdilución en caldo (BMD). Las cepas resistentes (CMI ≥ 2 µg/mL) se analizaron con NG-Test MCR-1 y CBDE/EDTA, con confirmación por PCR (*mcr-1* a *mcr-10*).

Resultados: De los 34 aislamientos resistentes, solo 2 de *E. coli* fueron positivos para *mcr-1* por NG-Test MCR-1 y PCR. Ninguna *K. pneumoniae* presentó genes *mcr*. NG-Test MCR-1 mostró concordancia perfecta con CBDE/EDTA + PCR (κ = 1,00; PPA = 100 % [IC95 %: 56–100 %]; NPA= 100 % [IC95 %: 92–100 %]).

Conclusiones: NG-Test MCR-1 permite una detección rápida y específica de *mcr-1*, mientras que CBDE/EDTA es útil como herramienta de cribado. Se recomienda un enfoque combinado para optimizar la detección y apoyar la vigilancia de la resistencia a colistina.

Palabras clave: Ácido edético; Enterobacteriaceae; Genes; Inmunoensayo; Plásmidos.

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INTRODUCTION

The global emergence of carbapenem-resistant Enterobacterales (CRE), particularly *Escherichia coli* (*E. coli)* and *Klebsiella pneumoniae (K. pneumoniae),* poses a significant threat to public health.[1] An even greater concern is the increasing prevalence of multidrug-resistant and extensively drug-resistant strains that exhibit resistance to both carbapenems and colistin, further complicating treatment options.[1,2] In developing regions such as South and Southeast Asia, antimicrobial resistance has escalated significantly, exacerbated by the limited availability of novel antibiotics. In these settings, colistin remains a last-resort therapeutic option for multidrug-resistant


2025;54(3):e025076562

infections, underscoring the critical need to detect colistin-resistant pathogens to guide effective clinical decisions.[3]

Colistin resistance in Enterobacterales is primarily mediated through mechanisms such as chromosomal mutations affecting lipid A and the presence of plasmid-mediated *mcr* genes. Previously, the combination of EDTA-Colistin Broth Disk Elution (CBDE/EDTA) and PCR has been widely supported and utilized for detecting *mcr* genes. As an enzyme-targeted screening tool, CBDE/EDTA identifies the functional presence of MCR enzymes associated with *mcr* genes. In contrast, PCR offers molecular confirmation.[4,5] However, this combined method requires technical expertise and longer turnaround times. In contrast, the NG-Test MCR-1 test by the Lateral Flow Immunoassay method (LFIA) offers a simpler and faster alternative, with high sensitivity and specificity, making it more user-friendly for routine laboratory use.

This study was conducted at Cho Ray Hospital, a major referral center in Vietnam, which serves as a representative site for studying antimicrobial resistance patterns in Southeast Asia. By assessing the performance of this rapid diagnostic tool. It is intended to provide valuable information insights into its potential application in clinical microbiology laboratories across Vietnam and the broader Southeast Asian region.

This study aims to evaluate the performance of the NG-Test MCR-1 in comparison with the combined reference method of EDTA-Colistin Broth Disk Elution (CBDE/EDTA) and polymerase chain reaction (PCR) for detecting *mcr*-mediated colistin resistance.

METHOD

Study design and participants

A prospective, cross-sectional study was conducted at Cho Ray Hospital in Ho Chi Minh City, Vietnam, from October to December 2023.

During this period, 160 non-duplicate clinical isolates were collected (128 *Klebsiella pneumoniae* and 32 *Escherichia coli* strains), along with 2 additional *E. coli* strains carrying the *mcr-1* gene, as confirmed by whole-genome sequencing using the Illumina platform. The isolates were obtained


2025;54(3):e025076562

from inpatients across various clinical departments using a convenience sampling method, based on the availability of eligible specimens during the study period

Study variables

For each bacterial isolate, the following variables were recorded: the bacterial species (*Klebsiella pneumoniae* or *Escherichia coli*), the clinical specimen source (blood, urine, sputum, pus, or body fluids), and the colistin minimum inhibitory concentration (MIC) determined by the broth microdilution (BMD) method by CLSI M100-S34 guidelines. In addition, results from the NG-Test MCR-1 assay (positive or negative) for MCR-1 protein detection, findings from the CBDE and CBDE/EDTA methods (including any MIC changes in the presence of EDTA), and PCR outcomes for *mcr* genes (*mcr-1* to *mcr-10*) were documented. Metadata such as the date of collection and hospital department were also noted, without including any patient-identifiable information.

Data collection

Broth Microdilution (BMD) method, combined with the LFIA: NG Test MCR-1

Colistin's minimum inhibitory concentrations (MICs) were initially determined using the BMD method, following the Clinical and Laboratory Standards Institute (CLSI M100S34) guidelines. In brief, bacterial isolates were cultured in Mueller-Hinton broth and subjected to serial dilutions of colistin. The MIC was defined as the lowest concentration at which no visible bacterial growth was observed after 18–24 hours of incubation at 35°C.[6]

Subsequently, isolates exhibiting colistin resistance (MIC ≥ 2 µg/mL) underwent further testing with the NG Test MCR-1, a lateral flow immunoassay designed to detect MCR-1-producing strains rapidly. Bacterial colonies were re-suspended in an extraction buffer, and the test strip was immersed in the solution. The appearance of a test line indicated a positive result, confirming the presence of the MCR-1 protein.

EDTA-Colistin Broth Disk Elution(CBDE/EDTA) method, combined with the PCR *mcr1-10* test

Initially, colistin resistance was assessed using the CBDE method, following the Clinical and Laboratory Standards Institute (CLSI M100 S34) guidelines. In brief, bacterial isolates were


2025;54(3):e025076562

inoculated into cation-adjusted Mueller-Hinton broth, containing a colistin disk (10 µg) and incubated at 35°C for 16–20 hours. The minimum inhibitory concentration (MIC) was determined based on the turbidity of the bacterial suspension, with an MIC ≥ 2 µg/mL indicating colistin resistance.[6]

To further differentiate *mcr*-mediated colistin resistance from other resistance mechanisms, we performed the CBDE/EDTA test, which involves adding ethylenediaminetetraacetic acid (EDTA) to the CBDE assay.[4] The presence of metalloenzyme activity, which EDTA inhibits, was evaluated by comparing the MIC results between the CBDE and CBDE/EDTA tests. A presumptive positive result for plasmid-mediated colistin resistance (PMCR) by the EDTA-CBDE screening method was defined as any observed reduction in MIC in the presence of EDTA. These screening results were subsequently compared to the corresponding molecular findings for confirmation.

For isolates testing positive in the CBDE/EDTA assay, PCR detection of *mcr* genes (*mcr-1* to *mcr-10*) was conducted at an external reference laboratory using standardized protocols.

Statistical analysis

The agreement between the NG-Test MCR-1 combined with the Broth Microdilution (BMD) method and the CBDE/EDTA method combined with PCR (*mcr* 1–10) was evaluated using Cohen's Kappa coefficient (κ), NPA, and PPA with 95% confidence intervals. Due to the small number of *mcr*-positive isolates, confidence intervals for PPA and NPA were calculated using the Jeffreys method, a Bayesian approach suitable for small sample sizes or when observed proportions are close to 0% or 100%. The association between bacterial species, specimen type, and presence of *mcr* genes using Fisher's exact test. These statistical measures assessed the inter-method concordance for detecting mcr-mediated colistin resistance among Enterobacterales isolates.

Ethical considerations

The study collected frozen bacterial strains that met the selection criteria without patient intervention. The Ethics Committee of the University of Medicine and Pharmacy in Ho Chi Minh City approved this research under contract number 142 /HĐĐĐ-ĐHYD.

2025;54(3):e025076562

RESULTS

Characteristics of the study strains

Among the 160 bacterial isolates, the most common specimen sources were phlegm (30.3%), body fluids (28.0%), and blood (19.3%). The dominant bacterial species identified were *Klebsiella pneumoniae* (80.0%) and *Escherichia coli* (20.0%) (table 1).

Table 1 - Characteristics of the study strains

Characteristics	n	%
Specimens		
Blood	31	19.3
Urine	23	14.3
Phlegm	48	30.3
Pus	13	8.1
Other body fluid	45	28.0
Bacteria		
Klebsiella pneumoniae	128	80.0
Escherichia coli	32	20.0
Total	160	100

Broth Microdilution (BMD) in combination with the NG Test MCR-1 method

Using the broth microdilution (BMD) method, colistin resistance was detected in 2/32 (6.25%) *E. coli* and 30/128 (23.4%) *K. pneumoniae* isolates. Among the 34 colistin-resistant isolates, only two *E. coli* strains were positive for *mcr-1* by NG-Test MCR-1, while no *K. pneumoniae* isolate carried *mcr* genes. All *mcr-1*-positive isolates were confirmed colistin-resistant by BMD, with significant differences observed between species for both colistin resistance ($p = 0.039$) and *mcr-1* positivity ($p = 0.045$).

Using the CBDE/EDTA method, potential *mcr*-mediated resistance was detected in 2/32 *E. coli* and 1/30 *K. pneumoniae* isolates. PCR confirmation identified *mcr-1* only in the two *E. coli* strains,


2025;54(3):e025076562

with no detection in *K. pneumoniae*. Discrepancies between CBDE/EDTA and PCR results highlight limitations of phenotypic screening, particularly in *K. pneumoniae* (p = 0.002) (table 2).

Table 2 - Result of Broth Microdilution (BMD) method in combination with the NG Test MCR-1

Colistin resistance	Colistin resistance by BMD (n/N)	Positive with NGTEST-MCR-1 (n/N)
E.coli	2/32	2/2
K.pneumoniae	30/128	0/30
mcr-1 identified strains	2/2	2/2
Total	34/162	4/34
p-value	0.039	0.045

p-values were calculated using Fisher's exact test.

EDTA-Colistin Broth Disk Elution (CBDE/ EDTA) method in combination with PCR

By the CBDE method, colistin resistance was found in 2/32 *E. coli* isolates and 30/128 *K. pneumoniae* isolates. CBDE/EDTA results were positive in 2/2 colistin-resistant *E. coli* and 1/30 colistin-resistant *K. pneumoniae* isolates. PCR detection of *mcr-1* to *mcr-10* genes confirmed positivity in 2/2 *E. coli* isolates and 0/30 *K. pneumoniae* isolates. Overall, among the 34 colistin-resistant isolates, 5 were positive by CBDE/EDTA and 4 were confirmed by PCR. The p-values for CBDE/EDTA positivity and PCR confirmation were 0.045 and 0.002, respectively (table 3).


2025;54(3):e025076562

Table 3 - Result of EDTA-Colistin Broth Disk Elution(CBDE/ EDTA) method in combination with PCR *mcr1-10* test

Colistin resistance	Colistin resistance by CBDE (n/N)	Positive with CBDE/ EDTA (n/N)	Positive with PCR *mcr*1-10 confirming (n/N)
E.coli	2/32	2/2	2/2
K.pneumoniae	30/128	1/30	0/30
mcr-1 identified strains	2/2	2/2	2/2
Total	34/162	5/34	4/34
p-value	0.045		0.002

p-values were calculated using Fisher's Exact Test.

Interpretation result of BMD with the NG Test MCR-1 to the CBDE/ EDTA

There were 4 isolates confirmed to carry *mcr* genes by PCR were also detected by both the NG Test MCR-1. No false-positive or false-negative results were observed in this small subset, suggesting high concordance between genetic and phenotypic methods for detecting *mcr*-mediated resistance in *E. coli* (table 4).

Table 4 - Confusion matrix between CBDE/EDTA+PCR and BMD+NGTest MCR-1

CBDE/EDTA+PCR	BMD+NGtest MCR-1		Total
	Postive	Negative	
Postive	4	-	4
Negative	-	30	30
Total	4	30	34

The diagnostic agreement between BMD combined with NG-Test MCR-1 and CBDE/EDTA combined with PCR showed a Kappa (κ) value of 1.00 with a 95% confidence interval of [1.00–1.00]. Both Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were 100%, with 95% confidence intervals of [56%–100%] and [92%–100%], respectively. Confidence intervals were calculated using the Jeffreys method (table 5).

2025;54(3):e025076562

Table 5 - Diagnostic agreement indices: Kappa, PPA, and NPA with 95% confidence intervals

Metric	Value	95% Confidence Interval
Kappa (κ)	1.00	[1.00 – 1.00]
PPA	100%	[56% – 100%] (Jeffreys)
NPA	100%	[92% – 100%] (Jeffreys)

PPA: Positive Percent Agreement, NPA: Negative Percent Agreement. Confidence intervals were calculated using the Jeffreys method due to the small sample size.

DISCUSSION

This study provides valuable insights into the characteristics of bacterial isolates and the detection of *mcr*-mediated colistin resistance using different methodologies. Among the 160 clinical isolates analyzed, *K. pneumoniae* accounted for the majority (80%), while *E. coli* represented 20% of cases. The observed distribution of colistin resistance in this study, where 32 out of 160 isolates were resistant to colistin, comprising 30 *Klebsiella pneumoniae* strains without the *mcr* gene and 2 *Escherichia coli* strains both harboring the *mcr-1* gene, aligns with global patterns in colistin resistance mechanisms.[7]

In *K. pneumoniae*, colistin resistance is predominantly associated with chromosomal mutations rather than plasmid-mediated *mcr* genes. Mutations in genes such as *mgrB*, *pmrA*, and *pmrB* lead to modifications in the bacterial outer membrane, reducing colistin binding and efficacy. A study highlighted that colistin resistance in *K. pneumoniae* is often due to the inactivation of the *mgrB* gene, a negative regulator of the PhoPQ signaling system, resulting in lipid A modifications that confer resistance.[8] Conversely, in *E. coli*, colistin resistance is frequently linked to plasmid-mediated *mcr* genes, particularly *mcr-1*. The *mcr-1* gene encodes a phosphoethanolamine transferase that modifies lipid A, diminishing colistin's binding affinity. A comprehensive review reported a global prevalence of *mcr*-mediated colistin-resistant *E. coli* at approximately 6.51%, with significant variations across regions and sources.[9]

In this study, the absence of *mcr* genes in colistin-resistant K. pneumoniae isolates and their presence in all colistin-resistant *E. coli* isolates underscores the distinct resistance mechanisms between these species. This distinction is crucial for developing targeted diagnostic and treatment


2025;54(3):e025076562

strategies, as plasmid-mediated resistance can disseminate more rapidly across bacterial populations than chromosomal mutations.[7]

The NG Test MCR-1 demonstrated high accuracy in detecting the *mcr-1* gene among colistin-resistant *E. coli* isolates, with perfect concordance with PCR results. This LFIA test offers a rapid, specific, and user-friendly approach to identifying *mcr-1* in microbiology laboratories. The speed and ease of NG Test MCR-1 make it a promising tool for early detection, allowing clinicians to adjust antimicrobial therapy promptly. Although *mcr-1* has emerged as the most prevalent variant globally, including in Southeast Asian settings, its major limitation is the potential failure to detect other *mcr* variants (*mcr-2* to *mcr-10*), which may lead to false-negative results in strains carrying alternative resistance mechanisms.[10]

In contrast, the CBDE/EDTA method provides a phenotypic approach to detecting colistin resistance and can identify transferable *mcr*-mediated resistance. This method is considered cost-effective and practical for routine laboratory use. However, its accuracy remains limited, as observed in the study where CBDE/EDTA detected *mcr* genes in one *K. pneumoniae* isolate that later tested negative by PCR. This discrepancy suggests that CBDE/EDTA may not differentiate between *mcr*-mediated resistance and other mechanisms of colistin resistance, such as mutations in the PmrAB and PhoPQ regulatory systems. PCR confirmation is still essential when CBDE/EDTA results are favorable to determine the exact *mcr* variant and avoid misinterpretation.[7]

The comparison between BMD with NG Test MCR-1 and CBDE/EDTA with PCR revealed a perfect agreement ($\kappa = 1.00$), Furthermore, it achieved 100% PPA and NPA, with wide 95% confidence intervals due to the limited number of mcr-positive isolates, reinforcing the reliability of NG Test MCR-1 for detecting *mcr-1* in *E. coli*. However, the absence of *mcr* gene detection in colistin-resistant *K. pneumoniae* suggests that resistance in this species may be attributed to non-*mcr* mechanisms, further supporting the need for a combined approach using phenotypic and molecular tests.

These results are consistent with global studies evaluating the performance of the NG-Test MCR-1. A study published in the *Journal of Clinical Microbiology* assessed the NG-Test MCR-1 LFA


2025;54(3):e025076562

across 238 Gram-negative bacillus isolates, including 126 *Enterobacterales*, 50 *Pseudomonas aeruginosa*, and 50 *Acinetobacter* species. Before repeat testing, the assay demonstrated a positive percent agreement (PPA) of 100% and a negative percent agreement (NPA) of 96.1%. Upon retesting, only one false-positive result remained, attributed to an *MCR-2* producer, indicating the high specificity of the test.[5] As the current study was conducted in Vietnam, where data on NG Test MCR-1 performance remains limited, it provides valuable insights into the effectiveness of this assay for detecting *mcr-1* in Southeast Asia. This research not only expands the application of NG Test MCR-1 but also provides essential data for antimicrobial resistance surveillance and control strategies in the region.

Clinical and Laboratory Implications: The findings of this study highlight the value of incorporating the NG-Test MCR-1 into routine diagnostic workflows for the rapid detection of *mcr-1*, especially in resource-limited settings with restricted access to molecular diagnostics. Its high specificity for *mcr-1* renders it a valuable point-of-care tool, significantly reducing turnaround time and strengthening infection control strategies. A stepwise diagnostic approach, utilizing CBDE/EDTA for initial screening followed by NG-Test MCR-1 or multiplex real-time PCR to identify mcr variants (*mcr-1* to *mcr-10*) comprehensively, could optimize laboratory efficiency and enhance diagnostic accuracy.

Study Limitations and Future Directions: This study has several limitations. First, the number of *mcr*-positive isolates was very small (n = 4), which resulted in wide 95% confidence intervals for both PPA and NPA estimates, despite point estimates being 100%. This statistical limitation reduces the precision of diagnostic performance measurements and warrants cautious interpretation. Second, the research was conducted in a single tertiary-care hospital (Cho Ray Hospital), which may limit the generalizability of the findings to other healthcare settings in Vietnam or Southeast Asia. Third, the study focused exclusively on *mcr-1* detection, and other plasmid-mediated *mcr* variants (*mcr-2* to *mcr-10*) could not be identified, potentially underestimating the overall prevalence of *mcr*-mediated colistin resistance. Finally, only two species (*Klebsiella pneumoniae* and *Escherichia coli*) were examined, which does not capture the full diversity of *Enterobacterales* in clinical practice.


2025;54(3):e025076562

Future research should include larger sample sizes from multiple hospitals across different regions, incorporate a broader range of *Enterobacterales* species, and utilize multiplex PCR or whole genome sequencing to detect all *mcr* variants comprehensively. These improvements would enhance the robustness of prevalence estimates, narrow confidence intervals, and strengthen the evidence base for diagnostic and infection control strategies.

The NG-Test MCR-1 demonstrated perfect agreement with the reference method (κ = 1.00), with 100% PPA and NPA, although wide 95% confidence intervals reflected the limited number of *mcr*-positive isolates. The assay provided rapid and specific detection of *mcr-1*, while CBDE/EDTA served as a cost-effective screening tool. These findings support the combined use of NG-Test MCR-1 and CBDE/EDTA to optimize the detection of *mcr*-mediated colistin resistance, enabling timely clinical decision-making and strengthening antimicrobial stewardship, particularly in resource-limited settings.

NG Test MCR-1 provides rapid, specific detection of mcr-1, while CBDE/EDTA is a cost-effective screening tool but less definitive. Combining these methods is recommended to improve mcr-mediated colistin resistance detection.

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2025;54(3):e025076562

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Conflict of interest

The authors declare that they have no competing interests.

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2025;54(3):e025076562

Data availability

This research data is confidential according to the applicable confidentiality agreements and regulations and, therefore, cannot be publicly displayed or shared. The data are securely stored at the Integrated Planning Department at Cho Ray Hospital. Access to these data requires proper authorization. If you have any questions or need further information, please contact Ngoc Bich Tran at bichngoctran@ump.edu.vn