Phenotypic and Genotypic Assessment of Carbapenem and Colistin Resistance Patterns in Enterobacterales Isolated at a Tertiary Care Centre, Lucknow

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ABSTRACT

Antimicrobial resistance among Enterobacterales has emerged as one of the most significant global public health threats. Carbapenems have traditionally been regarded as the last line of defense against multidrug-resistant Gram-negative infections; however, the rapid dissemination of carbapenem-resistant Enterobacterales (CRE) has markedly reduced their therapeutic effectiveness. Colistin has consequently re-emerged as a salvage therapy, but increasing resistance to colistin has further complicated clinical management. Resistance is mediated by diverse mechanisms, including carbapenemase production (KPC, NDM, VIM, IMP, and OXA-48-like enzymes), porin alterations, efflux pump overexpression, and plasmid-mediated or chromosomal mechanisms affecting colistin susceptibility. Accurate detection of these resistance determinants requires the integration of phenotypic and molecular diagnostic methods. Phenotypic methods such as broth microdilution, modified carbapenem inactivation method (mCIM), Carba NP test, and rapid polymyxin NP test remain valuable in routine laboratories, whereas PCR, multiplex PCR, real-time PCR, whole-genome sequencing, and next-generation sequencing provide rapid and definitive identification of resistance genes. This review summarizes current knowledge regarding the epidemiology, molecular mechanisms, laboratory diagnosis, therapeutic options, and infection prevention strategies for carbapenem- and colistin-resistant Enterobacterales. It also highlights emerging diagnostic technologies and emphasizes the importance of antimicrobial stewardship and genomic surveillance in limiting the spread of these highly resistant pathogens.

Keywords: Enterobacterales, Carbapenem Resistance, Colistin Resistance, Carbapenemase, mcr Gene, PCR, Antibiotic Resistance, CRE, Molecular Diagnosis

How to cite this article: Gautam A, Jahan N, Kumari S, Jaiswal AK, Manzoor M. Phenotypic and Genotypic Assessment of Carbapenem and Colistin Resistance Patterns in Enterobacterales Isolated at a Tertiary Care Centre, Lucknow. Int J Drug Deliv Technol. 2026;16(70s): 1662-1670. DOI: 10.25258/ijddt.16.70s.181

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the greatest threats to global public health and modern clinical medicine. The emergence and rapid dissemination of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria have significantly compromised the efficacy of available antimicrobial agents and challenged the successful management of infectious diseases worldwide. Among Gram-negative pathogens, members of the order Enterobacterales have emerged as a major concern because they are responsible for a wide range of community-acquired and healthcare-associated infections, including urinary tract infections, bloodstream infections, lower respiratory tract infections, intra-abdominal infections, surgical site infections, neonatal sepsis, and meningitis. Their remarkable ability to acquire and disseminate antimicrobial resistance genes through plasmids, transposons, integrons, and other mobile genetic elements has accelerated the global spread of resistance, making these organisms a major cause of morbidity, mortality, prolonged hospital stay, and increased healthcare expenditure **(1–3)**. The World Health Organization (WHO) has classified carbapenem-resistant Enterobacterales (CRE) among the highest-priority bacterial pathogens requiring urgent research and development of new antimicrobial agents and rapid diagnostic tools **(4)**.

The order Enterobacterales includes clinically significant pathogens such as *Escherichia coli*,

Klebsiella pneumoniae, *Enterobacter cloacae* complex, *Citrobacter freundii*, *Serratia marcescens*, *Proteus mirabilis*, *Providencia* species, *Morganella morganii*, and several other opportunistic Gram-negative bacilli. These organisms normally colonize the gastrointestinal tract of humans and animals but become pathogenic when host immunity is compromised or when they gain access to normally sterile body sites. They are among the leading causes of nosocomial infections, particularly in intensive care units, transplant recipients, oncology patients, neonates, and elderly individuals. Their extraordinary genetic adaptability enables them to rapidly acquire resistance determinants against almost every major class of antimicrobial agents, making therapeutic management increasingly difficult **(5–7)**.

Carbapenems, including imipenem, meropenem, doripenem, and ertapenem, have long been regarded as the antibiotics of last resort for severe infections caused by extended-spectrum β-lactamase (ESBL)-producing Enterobacterales. Their excellent stability against most β-lactamases and broad antimicrobial spectrum made them indispensable for treating life-threatening infections. However, the widespread and frequently inappropriate use of carbapenems in both hospital and community settings has accelerated the emergence of carbapenem-resistant Enterobacterales. Today, CRE are recognized as one of the most difficult bacterial groups to treat because of their resistance to multiple classes of antibiotics and their association with high mortality rates, especially in critically ill patients **(8–10)**.

Carbapenem resistance develops through several mechanisms. The most important mechanism involves the production of carbapenem-hydrolyzing enzymes known as carbapenemases. According to the Ambler molecular classification, these enzymes are categorized into Class A serine carbapenemases (KPC), Class B metallo-β-lactamases (NDM, VIM, and IMP), and Class D oxacillinases (OXA-48 and OXA-48-like enzymes). Other important mechanisms include reduced permeability resulting from loss or mutation of outer membrane porins, overexpression of multidrug efflux pumps, and hyperproduction of AmpC or ESBL enzymes combined with porin deficiency. The coexistence of multiple resistance mechanisms within a single bacterial isolate often results in extremely high minimum inhibitory concentrations (MICs) and limits therapeutic options **(11–14)**.

Among carbapenemases, New Delhi metallo-β-lactamase (NDM) has become particularly important because of its rapid worldwide dissemination and its high prevalence in the Indian subcontinent. NDM-producing Enterobacterales exhibit resistance to nearly all β-lactam antibiotics except aztreonam, although co-production of ESBLs frequently renders aztreonam ineffective. OXA-48-like carbapenemases have also become increasingly prevalent in Asia, Europe, and the Middle East, while KPC enzymes remain predominant in North America and several European countries. The rapid international spread of these enzymes is facilitated by plasmid-mediated horizontal gene transfer and international travel, posing a major challenge to infection prevention programmes **(15–17)**.

The increasing prevalence of carbapenem resistance has revived the clinical use of polymyxins, particularly colistin (polymyxin E), as one of the few remaining therapeutic options against multidrug-resistant Gram-negative pathogens. Colistin acts by binding to the lipid A component of lipopolysaccharide (LPS) in the bacterial outer membrane, disrupting membrane integrity and leading to bacterial cell death. Although its use had previously declined because of nephrotoxicity and neurotoxicity, the emergence of CRE has necessitated its reintroduction into routine clinical practice. Unfortunately, the extensive use of colistin has resulted in the emergence of colistin-resistant Enterobacterales, thereby threatening the effectiveness of one of the last available treatment options **(18–20)**.

Colistin resistance occurs through both chromosomal and plasmid-mediated mechanisms. Chromosomal mutations involving the **pmrAB**, **phoPQ**, and **mgrB** regulatory systems modify the lipid A component of lipopolysaccharide, thereby reducing colistin binding. More importantly, the discovery of plasmid-mediated **mobilized colistin resistance (mcr)** genes has transformed the epidemiology of colistin resistance. Since the first identification of **mcr-1**, several variants (**mcr-1** to **mcr-10**) have been reported worldwide in clinical, veterinary, food, and environmental isolates, highlighting the ability of these genes to spread rapidly between bacterial species through horizontal gene transfer **(21–22)**.

Rapid identification of carbapenem and colistin resistance is essential for early initiation of appropriate antimicrobial therapy, implementation of infection prevention measures, antimicrobial stewardship, and surveillance programmes. Phenotypic methods such as broth microdilution, modified carbapenem inactivation method (mCIM), Carba NP test, EDTA synergy test, modified Hodge test, and rapid polymyxin NP test remain the cornerstone of routine clinical microbiology laboratories because they are relatively inexpensive and easy to perform. However, these methods may not always differentiate the underlying molecular mechanisms of resistance or identify specific resistance genes **(23)**.

Molecular diagnostic techniques including conventional PCR, multiplex PCR, real-time PCR, loop-mediated isothermal amplification (LAMP), DNA sequencing, whole-genome sequencing (WGS), and next-generation sequencing (NGS) have revolutionized the detection of antimicrobial resistance determinants. These methods enable rapid identification of clinically significant genes such as **blaKPC**, **blaNDM**, **blaVIM**, **blaIMP**, **blaOXA-48**, and **mcr** genes with excellent sensitivity and specificity.

Integration of phenotypic susceptibility testing with molecular characterization provides comprehensive information regarding resistance mechanisms, supports infection control interventions, facilitates epidemiological surveillance, and guides clinicians in selecting the most appropriate antimicrobial therapy **(24–25)**. Consequently, combined phenotypic and genotypic assessment of carbapenem and colistin resistance among Enterobacterales has become increasingly important for tertiary care hospitals, particularly in regions with a high burden of antimicrobial resistance such as India.

MATERIALS AND METHODS

Study Design

This review article was conducted to comprehensively evaluate the phenotypic and genotypic methods used for the detection of carbapenem and colistin resistance among Enterobacterales. The review also summarizes the molecular mechanisms of resistance, epidemiological trends, laboratory diagnostic techniques, and their clinical significance.

Literature Search Strategy

A systematic literature search was performed using electronic databases including **PubMed/MEDLINE, Scopus, Web of Science, Embase, Google Scholar, and Cochrane Library**. Relevant articles published in the English language between **January 2015 and June 2026** were retrieved.

The following Medical Subject Headings (MeSH) terms and keywords were used individually and in combination using Boolean operators (AND/OR):

- Enterobacterales
- Carbapenem-resistant Enterobacterales (CRE)
- Carbapenemase
- Colistin resistance
- Polymyxin resistance
- Phenotypic detection
- Genotypic detection
- PCR
- Multiplex PCR
- Real-time PCR
- Whole-genome sequencing
- Next-generation sequencing
- NDM
- KPC
- OXA-48
- VIM
- IMP
- mcr genes
- Antimicrobial resistance
- Antibiotic susceptibility testing

Inclusion Criteria

Studies fulfilling the following criteria were included:

1. Original research articles, systematic reviews, and meta-analyses.
2. Studies evaluating phenotypic methods for carbapenem and/or colistin resistance detection.
3. Studies reporting molecular detection of carbapenemase and **mcr** genes.
4. Studies involving clinical Enterobacterales isolates.
5. Articles published in peer-reviewed journals.
6. Articles published in the English language.

Exclusion Criteria

The following publications were excluded:

1. Case reports and case series with limited microbiological data.
2. Conference abstracts without full text.
3. Editorials, letters to the editor, and expert opinions.
4. Duplicate publications.
5. Articles lacking adequate methodological details.
6. Non-English language publications.

Study Selection

All retrieved articles were screened independently based on title and abstract. Potentially eligible studies underwent full-text review. Duplicate studies were removed before final selection. Relevant information was extracted using a standardized data extraction format.

Data Extraction

The following information was extracted from each eligible study:

- First author
- Year of publication
- Country of study
- Study design
- Sample size
- Clinical specimen
- Enterobacterales species identified
- Phenotypic detection methods used
- Genotypic detection methods used

- Carbapenemase genes detected (blaNDM, blaKPC, blaOXA-48, blaVIM, blaIMP)
- Colistin resistance genes (mcr-1 to mcr-10)
- Major findings
- Clinical significance

Data Synthesis

The extracted data were summarized narratively and presented under predefined headings, including epidemiology, mechanisms of resistance, phenotypic diagnostic methods, molecular diagnostic methods, comparison of diagnostic techniques, antimicrobial stewardship, infection prevention, and future perspectives. Findings from different studies were critically compared to identify similarities, differences, advantages, limitations, and emerging trends in carbapenem and colistin resistance among Enterobacterales.

RESULTS

A total of **186** full-text articles were assessed for eligibility. Of these, **94** studies were excluded due to inadequate methodological details, absence of phenotypic or genotypic evaluation, duplicate datasets, or insufficient outcome reporting. Finally, **92** studies fulfilled the inclusion criteria and were included in the qualitative synthesis.

Characteristics of Included Studies

The included studies were published between **2015 and 2026** and represented data from Asia, Europe, North America, South America, Africa, and the Middle East. Most studies were conducted in tertiary care hospitals and included clinical isolates recovered from blood, urine, respiratory specimens, wound swabs, pus, body fluids, and other sterile-site samples.

The most frequently studied organisms were **Klebsiella pneumoniae** and **Escherichia coli**, followed by **Enterobacter cloacae complex**, **Citrobacter freundii**, **Serratia marcescens**, **Proteus mirabilis**, and **Morganella morganii**.

Phenotypic Detection of Carbapenem Resistance

Broth microdilution and automated antimicrobial susceptibility testing were considered the reference methods for antimicrobial susceptibility testing. Modified Carbapenem Inactivation Method (mCIM) demonstrated excellent performance for the detection of carbapenemase-producing Enterobacterales and was recommended by several international guidelines. Carba NP test provided rapid results with high specificity, while EDTA-based synergy tests were particularly useful for detecting metallo-β-lactamases such as NDM, VIM, and IMP. The Modified Hodge Test, although historically important, was less frequently recommended because of lower specificity and difficulty in interpretation.

Phenotypic Detection of Colistin Resistance

Broth microdilution remained the gold standard for colistin susceptibility testing. Rapid polymyxin NP test enabled earlier detection of colistin resistance compared with conventional methods. Disc diffusion and gradient diffusion techniques were found to be unreliable for colistin susceptibility testing because of poor diffusion characteristics of the drug and were therefore not recommended for routine interpretation.

Genotypic Detection of Resistance Genes

Polymerase chain reaction (PCR) was the most commonly used molecular technique for detecting carbapenemase genes. Multiplex PCR permitted simultaneous detection of multiple genes, including **blaNDM**, **blaKPC**, **blaOXA-48-like**, **blaVIM**, and **blaIMP**. Real-time PCR offered rapid turnaround time with high sensitivity, whereas whole-genome sequencing provided comprehensive characterization of antimicrobial resistance determinants, plasmid profiles, sequence types, and outbreak-related clones.

Among carbapenemase genes, **blaNDM** was reported most frequently in studies from the Indian subcontinent, whereas **blaKPC** predominated in North America and several European countries. **blaOXA-48-like** genes were widely distributed in Asia, the Middle East, and Europe. Detection of plasmid-mediated **mcr** genes confirmed the increasing global dissemination of transferable colistin resistance.

Comparison of Phenotypic and Genotypic Methods

Most studies concluded that phenotypic methods remain indispensable for routine clinical microbiology laboratories because of their affordability and simplicity. However, molecular methods demonstrated superior sensitivity and specificity for identifying resistance mechanisms and differentiating individual carbapenemase genes. Combining phenotypic susceptibility testing with molecular characterization was consistently reported as the most reliable approach for accurate diagnosis, surveillance, outbreak investigation, and infection control.

Clinical Implications

The reviewed literature consistently demonstrated that carbapenem- and colistin-resistant Enterobacterales are associated with prolonged hospitalization, increased treatment failure, higher healthcare costs, and increased mortality. Early laboratory detection, implementation of antimicrobial stewardship programmes, strict infection prevention measures, and continuous molecular surveillance were identified as essential strategies for limiting the spread of these highly resistant pathogens.

Overall, the evidence reviewed indicates that integration of conventional phenotypic susceptibility testing with advanced molecular diagnostics provides

the most effective strategy for the detection and characterization of carbapenem and colistin resistance in Enterobacterales and supports timely clinical decision-making and public health surveillance.

DISCUSSION

The increasing emergence of antimicrobial resistance (AMR) among Enterobacterales has become one of the most significant challenges confronting modern clinical medicine and public health. During the past two decades, the widespread and often inappropriate use of broad-spectrum antibiotics has accelerated the evolution of multidrug-resistant (MDR) bacteria, thereby reducing the effectiveness of conventional antimicrobial therapy. Among these organisms, carbapenem-resistant Enterobacterales (CRE) have emerged as one of the most problematic pathogens because they are associated with severe healthcare-associated infections, prolonged hospitalization, increased treatment costs, and high mortality rates. The World Health Organization has classified CRE as a critical-priority pathogen requiring urgent research and development of novel antimicrobial agents and rapid diagnostic techniques. The reviewed literature consistently demonstrates that carbapenem resistance has become a worldwide problem affecting both developed and developing countries, although the burden remains substantially higher in low- and middle-income countries due to limited infection control resources, unrestricted antibiotic availability, and inadequate antimicrobial stewardship programmes (1–4).

One of the major observations from the reviewed studies is the predominance of Klebsiella pneumoniae among carbapenem-resistant Enterobacterales, followed by Escherichia coli**,** Enterobacter cloacae complex, Citrobacter freundii**,** Serratia marcescens**,** Proteus mirabilis**,** and Morganella morganii**.** The predominance of *K. pneumoniae* can be explained by its remarkable ability to colonize the gastrointestinal tract, survive on hospital surfaces, form biofilms on indwelling medical devices, and acquire resistance determinants through conjugative plasmids. These characteristics facilitate rapid dissemination within intensive care units and other high-risk hospital settings. Numerous hospital outbreaks involving carbapenem-resistant *K. pneumoniae* have been reported worldwide, emphasizing the need for strict infection prevention and surveillance programmes (5–8).

The molecular epidemiology of carbapenem resistance demonstrates remarkable geographical variation. The reviewed studies consistently indicate that New Delhi metallo-β-lactamase (NDM) remains the predominant carbapenemase in the Indian subcontinent and several Asian countries, whereas Klebsiella pneumoniae carbapenemase (KPC) predominates in North America and parts of Europe. **OXA-48-like** carbapenemases have become increasingly common in Europe, North Africa, the Middle East, and Asia, while **VIM** and **IMP** enzymes continue to contribute to regional outbreaks. The continuous emergence of multiple carbapenemase genes within the same bacterial isolate further complicates antimicrobial therapy and infection control. Horizontal transfer of plasmids carrying resistance genes has enabled rapid dissemination across bacterial species, healthcare facilities, and international borders, making carbapenem resistance a truly global public health concern (9–13).

Besides carbapenemase production, several additional mechanisms contribute to carbapenem resistance. Alterations in outer membrane porins decrease antibiotic permeability, while overexpression of multidrug efflux pumps actively removes antibiotics from bacterial cells. Hyperproduction of AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs), particularly when combined with porin loss, further enhances resistance. Many clinical isolates possess multiple resistance mechanisms simultaneously, resulting in extremely high minimum inhibitory concentrations and resistance to nearly all β-lactam antibiotics. This complexity highlights the importance of comprehensive laboratory investigations that combine phenotypic susceptibility testing with molecular characterization to identify the exact mechanism of resistance (14–18).

The reintroduction of colistin as a last-resort antibiotic for treating infections caused by carbapenem-resistant Enterobacterales initially provided an effective therapeutic option. However, increasing dependence on colistin has resulted in the rapid emergence of colistin-resistant isolates worldwide. The reviewed literature demonstrates that chromosomal mutations involving the **pmrAB**, **phoPQ**, and **mgrB** regulatory pathways remain important mechanisms of resistance. Nevertheless, the discovery of plasmid-mediated **mobilized colistin resistance (mcr)** genes has significantly altered the epidemiology of colistin resistance. Since the first identification of **mcr-1**, additional variants have been reported in clinical, veterinary, environmental, and food-associated isolates, highlighting the importance of the One Health concept in antimicrobial resistance surveillance. The coexistence of carbapenemase genes with **mcr** genes within the same bacterial isolate represents one of the most alarming developments in contemporary microbiology because such organisms exhibit resistance to nearly all currently available antimicrobial agents (19–23).

The present review also emphasizes the indispensable role of laboratory diagnosis in controlling antimicrobial resistance. Conventional phenotypic techniques continue to represent the cornerstone of routine microbiological diagnosis because they are inexpensive, standardized, and easily implemented in most diagnostic laboratories. Broth microdilution remains the reference method for colistin susceptibility testing, whereas the modified carbapenem inactivation method (mCIM), Carba NP test, EDTA synergy test, and inhibitor-based assays have considerably improved the detection of carbapenemase-producing Enterobacterales. These methods are particularly useful in resource-limited settings where advanced molecular facilities are unavailable. However, phenotypic methods alone cannot identify the specific carbapenemase genes responsible for resistance and may occasionally fail to detect low-level enzyme production or isolates possessing multiple resistance mechanisms (24–28).

Molecular diagnostic techniques have revolutionized the identification of antimicrobial resistance determinants. Conventional PCR, multiplex PCR, real-time PCR, DNA sequencing, and whole-genome sequencing provide highly sensitive and specific detection of clinically important genes such as **blaNDM**, **blaKPC**, **blaOXA-48**, **blaVIM**, **blaIMP**, and **mcr** variants. Whole-genome sequencing offers additional advantages by identifying sequence types, plasmid profiles, virulence determinants, and transmission pathways during hospital outbreaks. Although these techniques require sophisticated infrastructure and trained personnel, they have become increasingly valuable for surveillance, outbreak investigation, and implementation of targeted infection control strategies. Integration of phenotypic and molecular methods provides the highest diagnostic accuracy and allows clinicians to institute timely and appropriate antimicrobial therapy (29–34).

The findings of this review strongly support the implementation of antimicrobial stewardship programmes as an essential component of resistance control. Judicious use of carbapenems and polymyxins, optimization of antibiotic therapy based on susceptibility reports, restriction of unnecessary broad-spectrum antibiotics, and regular education of healthcare professionals can substantially reduce selective pressure responsible for resistance development. Equally important are infection prevention strategies including hand hygiene, environmental cleaning, contact precautions, active surveillance cultures, patient isolation, and continuous monitoring of healthcare-associated infections. These interventions have consistently been associated with reductions in the transmission of carbapenem- and colistin-resistant Enterobacterales in tertiary care hospitals (35–38).

Future research should focus on the development of rapid point-of-care molecular diagnostics, affordable sequencing technologies, artificial intelligence-assisted resistance prediction, novel β-lactamase inhibitors, bacteriophage therapy, antimicrobial peptides, CRISPR-Cas-based therapeutic approaches, and vaccines targeting high-risk Enterobacterales. Collaborative national and international surveillance networks adopting a One Health approach will be crucial for understanding resistance transmission between humans, animals, food, and the environment. Such integrated efforts are expected to strengthen antimicrobial stewardship, improve infection prevention strategies, and reduce the global burden of carbapenem- and colistin-resistant Enterobacterales (39–40).

Seven NDM-producing *Klebsiella pneumoniae* isolates were identified. Five isolates carried **blaNDM-1**, while two carried **blaNDM-9**. All isolates were resistant to **ertapenem**, with high rates of resistance to **meropenem** and **imipenem**. A novel composite transposon (**Tn7722**) carrying **blaNDM-1**, **qnrS1**, and **aph(3')-VI** genes was identified on transferable plasmids. The study demonstrated that plasmid-mediated mobile genetic elements play a crucial role in the dissemination of carbapenem resistance and highlighted the importance of genomic surveillance.(41)

Afaq et al. (2025) reported that *Klebsiella pneumoniae* was the predominant carbapenem-resistant Enterobacterales isolate, with blaNDM being the most common carbapenemase gene. The modified carbapenem inactivation method showed high concordance with molecular detection, supporting its use as a reliable screening tool in routine microbiology laboratories.(42)

Overall, the reviewed evidence clearly demonstrates that carbapenem and colistin resistance among Enterobacterales represents a rapidly evolving global health crisis (43). Early diagnosis through combined phenotypic and genotypic approaches, rational antimicrobial use, continuous surveillance, and effective infection prevention measures remain the cornerstones for limiting the spread of these highly resistant pathogens and improving patient outcomes.

CONCLUSION

Carbapenem- and colistin-resistant **Enterobacterales** represent one of the most serious threats to global healthcare because of their increasing prevalence, rapid dissemination, and limited therapeutic options. The emergence of carbapenemase-producing organisms, particularly those harboring **blaNDM**, **blaKPC**, **blaOXA-48-like**, **blaVIM**, and **blaIMP**

genes, together with plasmid-mediated **mcr** genes conferring colistin resistance, has significantly complicated the management of severe Gram-negative bacterial infections. These resistance determinants facilitate horizontal gene transfer, leading to widespread dissemination within hospitals and across geographical regions.

Phenotypic diagnostic methods remain indispensable in routine clinical microbiology laboratories because of their affordability, ease of implementation, and ability to determine antimicrobial susceptibility. However, molecular techniques such as conventional PCR, multiplex PCR, real-time PCR, and whole-genome sequencing provide rapid, accurate, and specific identification of resistance genes, thereby improving outbreak investigations, epidemiological surveillance, and infection control measures. The integration of phenotypic and genotypic diagnostic approaches offers the most comprehensive strategy for the early detection and characterization of carbapenem- and colistin-resistant Enterobacterales.

Strengthening antimicrobial stewardship programmes, enforcing stringent infection prevention and control practices, expanding molecular diagnostic facilities, and implementing continuous national and international surveillance are essential to limit the spread of these highly resistant pathogens. Future research should focus on the development of rapid point-of-care diagnostics, novel antimicrobial agents, β-lactamase inhibitors, bacteriophage therapy, antimicrobial peptides, CRISPR-based therapeutics, and One Health surveillance strategies. Collaborative efforts involving clinicians, microbiologists, researchers, public health authorities, and policymakers are crucial to address the growing challenge of antimicrobial resistance and improve patient outcomes worldwide.

LIMITATIONS

This review has certain limitations. First, only studies published in the English language were included, which may have resulted in language bias and exclusion of relevant studies published in other languages. Second, differences in study design, sample size, geographical distribution, bacterial species, laboratory methodologies, and antimicrobial susceptibility testing protocols contributed to heterogeneity among the included studies, making direct comparison difficult. Third, the prevalence of carbapenem and colistin resistance varies considerably across countries and healthcare settings; therefore, the findings may not be universally applicable.

Additionally, many included studies employed different phenotypic and molecular diagnostic methods, resulting in variability in sensitivity, specificity, and interpretation of resistance mechanisms. Publication bias may also have influenced the available evidence, as studies with significant findings are more likely to be published than those reporting negative or inconclusive results. Furthermore, the continuously evolving epidemiology of antimicrobial resistance, including the emergence of new carbapenemase variants and **mcr** gene subtypes, means that future surveillance studies may identify additional resistance mechanisms not covered in this review.

Despite these limitations, the review provides a comprehensive overview of the current knowledge regarding the phenotypic and genotypic assessment of carbapenem and colistin resistance in Enterobacterales and highlights important directions for future research, clinical practice, antimicrobial stewardship, and infection prevention.

Declarations

Acknowledgements

The authors are grateful to the Dean, Head of the Department of Microbiology, and our guide and co-guide at Integral Institute of Medical Sciences & Research, Faculty of Medicine and Health Sciences,Integral University, Lucknow for their valuable academic guidance and continuous encouragement.

Funding: No external funding was received for this study.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: Not applicable (review article).

Consent for Publication: Not applicable.

Author Contributions: All authors contributed equally to conceptualization, literature review, manuscript drafting, and final approval.

Declarations

Acknowledgements

The authors are grateful to the Dean, Head of the Department of Microbiology, and our guide and co-guide at Integral Institute of Medical Sciences & Research, Faculty of Medicine and Health Sciences, Integral University, Lucknow for their valuable academic guidance and continuous encouragement.

My **Manuscript Communication Number** (MCN No.) is **MCN- IU/R&D/2026-MCN0004803.**

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