

# Phenotypic and Genotypic Characterisation of *Citrobacter*: Implications in Clinical Microbiology and Antimicrobial Resistance - A Narrative Review

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## Abstract

*Citrobacter* species are increasingly recognised as significant opportunistic pathogens within the *Enterobacteriaceae* family, capable of causing a variety of infections, including urinary tract infections, sepsis, and neonatal meningitis. Their clinical relevance has been magnified by the rapid emergence of multidrug-resistance, particularly due to the acquisition of extended-spectrum  $\beta$ -lactamases and carbapenemases. Across regions, *Citrobacter* spp. including *C. koseri* implicated in severe neonatal central nervous system infection are increasingly recognised in healthcare-associated infections, while rising multidrug-resistance and carbapenemase production add to the global AMR burden. Traditional phenotypic methods often fall short in distinguishing closely related *Citrobacter* species, necessitating the integration of advanced genotypic techniques such as PCR, MLST, and whole-genome sequencing. This narrative review explores the phenotypic and genotypic characteristics of *Citrobacter* species, their role in human disease, and the mechanisms underlying their antimicrobial resistance. The review emphasises the importance of precise identification and molecular characterisation in guiding clinical management and informing public health interventions.

**Keywords:** *Citrobacter*, Phenotypic Identification, Genotypic Characterisation, Antimicrobial Resistance, ESBL, Clinical Microbiology

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## INTRODUCTION

The genus *Citrobacter* belongs to the family *Enterobacteriaceae*. It comprises facultative anaerobic, Gram-negative bacilli that are commonly found in soil, water, food, and the intestinal tracts of animals and humans. Initially grouped under the *Salmonella* genus due to phenotypic similarities, *Citrobacter* was later recognised as a distinct genus based on biochemical and molecular differences. It includes several species, of which *Citrobacter freundii*, *Citrobacter koseri*, and *Citrobacter braakii* are most frequently associated with human infections.<sup>1</sup> These organisms are opportunistic pathogens, often colonising the gastrointestinal tract without causing harm, but are capable of causing serious infections, particularly in immunocompromised individuals.<sup>2</sup>

Clinically, *Citrobacter* species have been implicated in a wide range of infections, including urinary tract infections, sepsis, pneumonia, wound infections, and, most notably, neonatal meningitis. *C. koseri* is particularly notorious for its ability to invade the central nervous system in neonates, often resulting in brain abscesses and high morbidity.<sup>3</sup> The ability of these organisms to cause diverse infections across various patient populations underscores their growing clinical importance.<sup>4</sup>

A major concern in recent years has been the emergence and spread of antimicrobial resistance (AMR) among *Citrobacter* species. These bacteria have demonstrated an alarming capacity to acquire and disseminate resistance genes, including those encoding extended-spectrum  $\beta$ -lactamases (ESBLs) and carbapenemases, making treatment options increasingly limited. The presence of mobile genetic elements such as plasmids and integrons facilitates rapid horizontal transfer of resistance determinants within hospital and community settings.<sup>5</sup>

Although *Enterobacterales* such as *Escherichia coli* and *Klebsiella pneumoniae* remain the most frequently emphasised pathogens, *Citrobacter* spp. warrant renewed attention because they combine severe invasive disease potential with an increasing antimicrobial resistance burden. In particular, *C. koseri* is associated with invasive neonatal central nervous

system infection and can be complicated by brain abscess formation, contributing to significant morbidity.<sup>3</sup> In parallel, *Citrobacter* spp. have shown a marked capacity to acquire and disseminate resistance determinants, including ESBLs and carbapenemases, supported by mobile genetic elements that facilitate spread in healthcare and community settings.<sup>5</sup>

Given the rising clinical burden and therapeutic challenges posed by *Citrobacter*, accurate identification and characterisation of these organisms are crucial. Phenotypic methods, though widely used, are often insufficient for precise species-level identification due to overlapping biochemical profiles with other *Enterobacterales*.<sup>6</sup> In contrast, genotypic tools such as polymerase chain reaction (PCR), multilocus sequence typing (MLST), and whole-genome sequencing (WGS) offer greater resolution. They are essential for understanding the molecular epidemiology, virulence factors, and resistance mechanisms of these pathogens.<sup>7</sup> This narrative review aims to provide a comprehensive overview of the phenotypic and genotypic characteristics of *Citrobacter* species, with a focus on their implications in clinical microbiology and antimicrobial resistance. It highlights the current diagnostic challenges, resistance trends, and the significance of integrated characterisation approaches in improving infection management and guiding therapeutic decisions.

### Taxonomy and species diversity

The taxonomy of the genus *Citrobacter* has undergone substantial revision since early descriptions in the late 19<sup>th</sup> and early 20<sup>th</sup> centuries, with the genus formally proposed by Werkman and Gillen in 1932.<sup>3</sup> Early classifications were frequently inconsistent, and *Citrobacter* species were at times grouped with other genera such as *Escherichia* and *Salmonella*; for example, *Citrobacter freundii* was historically referred to as *Bacterium freundii* and *Escherichia freundii*.<sup>8</sup> This ambiguity largely reflected phenotypic overlap and the limited availability of molecular tools, with clearer recognition of the genus emerging by the mid-20<sup>th</sup> century.<sup>9</sup> Subsequently, DNA hybridisation, expanded biochemical schemes, and more recently whole-genome sequencing have refined species boundaries, revealing a complex

genus with at least 19 genomospecies and several clinically important members.<sup>10</sup>

Among the various species of *Citrobacter*, three are particularly significant in clinical microbiology: *Citrobacter freundii*, *Citrobacter koseri* (formerly known as *C. diversus*), and *Citrobacter braakii*.<sup>3</sup> *C. freundii* is the most commonly encountered species in clinical settings and is associated with a range of opportunistic infections, including urinary tract infections (UTIs), sepsis, and wound infections. *C. koseri* is especially notable for its propensity to cause severe infections in neonates, such as meningitis and brain abscesses, conditions that can have high morbidity and mortality.<sup>11</sup> *C. braakii* is less frequently isolated but is increasingly recognised as a cause of nosocomial infections. Other species, such as *C. amalonaticus* and *C. youngae*, are occasionally implicated in human disease but are less common in clinical practice.<sup>3</sup>

*Citrobacter* is a member of the *Enterobacteriaceae* family, which includes other well-known genera such as *Escherichia*, *Klebsiella*, and *Salmonella*. These bacteria share several characteristics: they are Gram-negative, facultatively anaerobic rods, commonly found in the intestinal tract of humans and animals, and are capable of fermenting glucose and reducing nitrate.<sup>12</sup> However, *Citrobacter* is distinguished from its relatives by certain biochemical traits, most notably its ability to utilise citrate as a sole carbon source. Additionally, some *Citrobacter* species, such as *C. koseri*, are indole positive, which helps differentiate them from other closely related genera.<sup>3</sup> Despite these differences, there is considerable phenotypic overlap among *Enterobacterales*, which can complicate identification based solely on traditional biochemical tests.<sup>13</sup> Modern molecular techniques, including whole-genome sequencing and multilocus sequence typing, have greatly improved the accuracy of species identification and have revealed the substantial genetic diversity within the genus *Citrobacter*.<sup>13</sup>

### Phenotypic characteristics of *Citrobacter*

The phenotypic characteristics of *Citrobacter* species are central to their identification in clinical microbiology, although several challenges persist due to their close

resemblance to other *Enterobacterales*.<sup>14</sup> Morphologically, *Citrobacter* species are Gram-negative rods, typically measuring between 1 and 3 micrometres in length. They are non-spore-forming and facultatively anaerobic.<sup>15</sup> Most species are motile, possessing peritrichous flagella that allow movement in liquid media. When subjected to Gram staining, these bacteria appear as pink to red rods under the microscope, confirming their Gram-negative nature.<sup>15</sup>

Culturally, *Citrobacter* species grow well on standard laboratory media.<sup>16</sup> On nutrient agar, colonies are usually smooth, convex, and translucent to opaque with a shiny surface, while on blood agar they appear circular and flat and may occasionally show red pigmentation.<sup>16</sup> On MacConkey agar, *Citrobacter* typically forms pink colonies due to lactose fermentation, although this reaction may take up to 48 hours to fully develop. On selective media such as Salmonella–Shigella (SS) agar, colonies may appear colourless with grey or black centres, reflecting hydrogen sulfide (H<sub>2</sub>S) production by some species.<sup>17</sup> Growth on XLD and EMB agar may be inhibited or may present as yellow to brown colonies, sometimes lacking the characteristic metallic sheen seen with *Escherichia coli*.<sup>18</sup>

Biochemically, *Citrobacter* species are notable for their ability to utilise citrate as a sole carbon source, which remains a key differentiating feature within the *Enterobacterales*. Indole production is species-dependent; for example, *C. koseri* is typically indole positive, whereas *C. freundii* is often indole negative.<sup>19</sup> Urease activity is generally weak or negative but can be variable, and H<sub>2</sub>S production is common, particularly in *C. freundii*, appearing as blackening on iron-containing media.<sup>19</sup> Automated systems such as API 20E and VITEK 2 are widely used and interpret panels of reactions (including citrate, indole, urease, and H<sub>2</sub>S) to support species-level identification.<sup>20</sup> However, overlapping biochemical profiles with other *Enterobacterales*, including *Enterobacter* and *Escherichia*, can sometimes lead to misidentification.<sup>21</sup>

This overlap in phenotypic characteristics presents a significant challenge in accurately identifying *Citrobacter* species using traditional biochemical methods. Misidentification can have clinical consequences, as different species may

vary in pathogenicity and antimicrobial resistance profiles.<sup>22</sup> To overcome these limitations, modern clinical laboratories increasingly rely on Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS).<sup>23</sup> This technology analyses the unique protein spectra of bacterial isolates, allowing for rapid and highly accurate identification. MALDI-TOF MS has proven particularly valuable in distinguishing *Citrobacter* from other closely related genera and even differentiating between species and subspecies within the genus.<sup>23</sup> Its accuracy and speed have made it a gold standard for phenotypic identification in many clinical settings, complementing and sometimes replacing traditional biochemical approaches.<sup>23</sup>

### Genotypic characterisation

Genotypic characterisation has become essential for the accurate identification and understanding of *Citrobacter* species, especially given the limitations of phenotypic methods.<sup>24</sup> Among the most widely used molecular techniques are PCR-based assays that target conserved genetic sequences. The 16S rRNA gene is commonly amplified for genus-level identification, but due to its high conservation, it sometimes lacks the discriminatory power needed for precise species identification.<sup>25</sup> To overcome this, other housekeeping genes such as *recN* and additional loci are targeted, providing greater specificity for distinguishing closely related *Citrobacter* species. These PCR-based methods are rapid and sensitive, making them valuable for routine diagnostics and outbreak investigations.<sup>26</sup>

The advent of whole-genome sequencing (WGS) has dramatically enhanced our understanding of *Citrobacter* at both the species and strain levels. WGS provides a comprehensive view of the entire genetic makeup of an isolate, revealing not only species identity but also insights into population structure, gene content, and evolutionary dynamics.<sup>27</sup> Through WGS, researchers can construct core genome phylogenies and analyse the pan-genome, which includes all genes found within the genus. This high-resolution approach has clarified previously ambiguous species boundaries and uncovered extensive genetic diversity within and between *Citrobacter* species.<sup>28</sup> Multilocus Sequence Typing (MLST),

which involves sequencing several housekeeping genes to assign isolates to specific sequence types, is another genotyping tool that has proven invaluable for epidemiological surveillance. MLST data can be extracted directly from WGS, enabling standardised comparison across laboratories and facilitating the tracking of outbreak strains.<sup>29</sup> WGS-based detection of resistance determinants and assessment of clonal relatedness can be translated into practice by supporting timely selection of active therapy and guiding targeted infection-prevention actions during suspected transmission events or outbreaks.

Phylogenetic analyses based on genomic data have provided deep insights into the genetic relationships among *Citrobacter* strains. Studies using core genome alignments and advanced computational methods have identified at least 11 distinct phylogenetic groups within the genus.<sup>30</sup> Some of these groups correspond to well-known species such as *C. koseri* and *C. rodentium*. In contrast, others reveal the remarkable genetic heterogeneity of species like *C. freundii*, which is distributed across multiple phylogenetic clusters.<sup>27</sup> These analyses have also shed light on evolutionary divergence within the genus, identifying pathogenic clades and highlighting the genetic basis for differences in virulence and ecological adaptation. The use of metrics such as average nucleotide identity (ANI) and pan-genome analysis further supports the delineation of species and the identification of unique gene sets associated with pathogenic potential.<sup>31</sup>

The genotypic diversity of *Citrobacter* is closely linked to its repertoire of virulence factors and the presence of mobile genetic elements. Genomic studies have identified numerous virulence genes, including those encoding fimbriae that facilitate adhesion to host tissues, siderophores for iron acquisition, and a variety of toxins such as Shiga-like and heat-stable toxins.<sup>32</sup> Some *Citrobacter* strains possess pathogenicity islands and prophage-associated genes that may enhance their ability to form biofilms, evade the immune system, or resist antimicrobial agents. Mobile genetic elements, such as plasmids and integrons, play a pivotal role in the dissemination of both virulence and resistance genes.<sup>33</sup> These elements enable horizontal gene transfer, allowing *Citrobacter* to rapidly acquire new traits, including

resistance to critical antibiotics like carbapenems and extended-spectrum beta-lactams. The dynamic nature of these genetic elements contributes to the adaptability and clinical significance of *Citrobacter* species, underscoring the importance of ongoing genomic surveillance.<sup>34</sup>

**Clinical implications of *Citrobacter* infections**

*Citrobacter* species cause a wide range of infections, with urinary tract infections being the most common. They are also responsible for bloodstream infections, wound and soft tissue infections, and respiratory and intra-abdominal infections.<sup>35</sup> Notably, *C. koseri* is a significant cause of neonatal meningitis and brain abscesses, which can result in serious complications in infants. Patients at greatest risk for *Citrobacter* infections include immunocompromised individuals, such as those with diabetes, cancer, or chronic illnesses.<sup>36</sup> Hospitalised patients, especially those in intensive care or with prolonged stays, are more vulnerable. Device-associated infections, particularly catheter-related UTIs, are frequent, emphasising the role of invasive procedures in infection risk. Infants and the elderly also represent high-risk groups due to their weaker immune defences.<sup>37</sup> Clinical implications of *Citrobacter* infections are shown in Table 1.

**Antimicrobial resistance patterns**

Antimicrobial resistance in *Citrobacter* species is a growing concern in clinical microbiology, as these organisms frequently display multidrug resistance that complicates treatment.<sup>3</sup> Phenotypically, *Citrobacter* isolates often exhibit resistance to several major antibiotic classes. Resistance to β-lactam antibiotics is particularly common, with many strains showing reduced susceptibility or complete resistance to penicillins, cephalosporins, and monobactams.<sup>45</sup> In recent years, resistance to carbapenems- considered last-resort antibiotics- has also been reported, especially among hospital-acquired isolates. Additionally, resistance to fluoroquinolones is increasingly observed, often due to mutations in genes encoding DNA gyrase and topoisomerase IV.<sup>46</sup> Aminoglycoside resistance is also notable, frequently mediated by enzymes that modify and inactivate these drugs. The cumulative effect is that more than half of clinical *Citrobacter* isolates

**Table 1.** Clinical Implications of *Citrobacter* Infections

Type of Infection	Common Species Involved	At-Risk Patient Groups	Associated Complications
Urinary Tract Infection (UTI) <sup>38</sup>	<i>C. freundii</i> , <i>C. koseri</i>	Elderly, catheterised patients, diabetics	Pyelonephritis, bacteremia
Bloodstream Infections (BSI) <sup>39</sup>	<i>C. freundii</i> , <i>C. braakii</i>	Immunocompromised, ICU patients	Septic shock, multiorgan dysfunction
Neonatal Meningitis <sup>40</sup>	<i>C. koseri</i>	Preterm infants, neonates	Brain abscess, hydrocephalus, and high mortality
Wound and Surgical Site <sup>41</sup>	<i>C. freundii</i> , <i>C. braakii</i>	Postoperative patients, trauma cases	Delayed healing, localised sepsis
Respiratory Tract Infections <sup>42</sup>	<i>C. freundii</i>	Ventilated patients, COPD patients	Hospital-acquired pneumonia
Intra-abdominal Infections <sup>43</sup>	<i>C. freundii</i> , <i>C. koseri</i>	Post-abdominal surgery, peritonitis cases	Abscess formation, peritonitis
Osteomyelitis and Septic Arthritis <sup>44</sup>	<i>C. freundii</i>	Diabetics, immunosuppressed patients	Chronic infection, joint destruction

may be classified as multidrug-resistant (MDR), limiting therapeutic options and increasing the risk of treatment failure.<sup>47</sup>

### **Clinical consequences of MDR *Citrobacter***

The high frequency of multidrug-resistant phenotypes in *Citrobacter* spp. constrains empiric treatment choices and increases the likelihood of delayed effective therapy and clinical failure, particularly in hospital-acquired infections.<sup>47</sup> In addition, the potential for dissemination of resistant strains within healthcare environments heightens outbreak risk and reinforces the need for prompt susceptibility-guided optimisation of therapy alongside stringent infection-prevention measures.<sup>45</sup>

The mechanisms underlying this resistance are diverse and often work in combination. One of the most significant contributors is the production of extended-spectrum beta-lactamases (ESBLs). These enzymes hydrolyse a broad range of  $\beta$ -lactam antibiotics, including third-generation cephalosporins and monobactams.<sup>48</sup> The emergence of carbapenemase-producing *Citrobacter* strains has further complicated management. Reported enzymes include KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo- $\beta$ -lactamase), and VIM (Verona integron-encoded metallo- $\beta$ -lactamase), which confer resistance to carbapenems. In addition to these enzymatic mechanisms, structural changes in the bacterial cell can also drive resistance.<sup>49</sup> Overexpression of efflux pumps can actively expel antibiotics from the cell. Porin loss or mutations reduce antibiotic entry; together these changes contribute to high-level resistance, particularly when combined with beta-lactamase production.<sup>50</sup>

Horizontal gene transfer plays a pivotal role in the spread of antimicrobial resistance among *Citrobacter* and other *Enterobacterales*. Plasmids, which are mobile genetic elements, frequently carry genes encoding ESBLs, carbapenemases, and aminoglycoside-modifying enzymes.<sup>51</sup> These plasmids can move between bacteria via conjugation, enabling rapid dissemination of resistance traits within the hospital environment and the broader community. Transposons and integrons further facilitate the integration and movement of resistance genes across different genomic locations.<sup>51</sup> Environmental reservoirs,

such as contaminated water sources and hospital surfaces, can serve as persistent sources for resistant *Citrobacter* strains, leading to outbreaks and inter-facility transmission. The ability of these bacteria to acquire and share resistance genes with other members of the *Enterobacterales* order highlights the importance of vigilant infection control, robust antimicrobial stewardship, and ongoing surveillance in healthcare settings.<sup>51</sup>

### **Diagnostic approaches: phenotypic vs. genotypic methods**

Phenotypic methods like culture and biochemical tests are commonly used for *Citrobacter* identification. They are cost-effective and provide important information on antibiotic susceptibility, but can be slow and sometimes inaccurate due to overlapping traits with related bacteria.<sup>52</sup> Genotypic methods such as PCR and DNA sequencing offer faster and more precise identification by detecting specific genetic markers. These methods work well even when bacteria are difficult to culture, but require specialised equipment and expertise. Combining phenotypic and genotypic approaches in clinical labs improves diagnostic accuracy and speed.<sup>53</sup> Integrated workflows help clinicians make better treatment decisions and enhance infection control. New technologies like CRISPR-based diagnostics allow rapid, sensitive detection of pathogens and resistance genes at low cost. Nanopore sequencing provides real-time, detailed analysis of bacterial genomes, aiding comprehensive diagnosis.<sup>54</sup> Comparison of phenotypic and genotypic diagnostic methods for *Citrobacter* identification are shown in Table 2.

### **Infection control and therapeutic strategies**

Accurate species-level identification of *Citrobacter* is fundamental for effective infection control and therapeutic decision-making. Different species within the genus, such as *C. freundii* and *C. koseri*, exhibit varying degrees of pathogenicity and antimicrobial resistance profiles.<sup>61</sup> This variability means that misidentification can lead to inappropriate antibiotic use, delayed patient recovery, and increased risk of the spread of resistant strains within healthcare settings.<sup>62</sup> Precise laboratory identification enables clinicians to tailor antimicrobial therapy more effectively. It

**Table 2.** Comparison of Phenotypic and Genotypic Diagnostic Methods for *Citrobacter* Identification

Parameter	Phenotypic Methods	Genotypic Methods
Basis of Identification <sup>55</sup>	Morphology, biochemical reactions, and culture characteristics	DNA/RNA sequence detection and analysis
Common Techniques <sup>56</sup>	Gram staining, API 20E, VITEK 2, MALDI-TOF MS	PCR, MLST, 16S rRNA sequencing, WGS
Turnaround Time <sup>57</sup>	Rapid (within 24-48 hours)	Variable; may take 1-5 days depending on the method
Species-Level Accuracy <sup>58</sup>	Moderate; often misidentifies closely related species	High; allows accurate species and strain-level differentiation
Detection of Resistance Genes <sup>59</sup>	Indirect, inferred through susceptibility profiles	Direct; identifies specific resistance genes (e.g., <i>bla</i> genes)
Cost and Infrastructure <sup>60</sup>	Lower cost; basic microbiology lab required	Higher cost; requires molecular lab setup
Expertise Required <sup>60</sup>	Basic microbiological skills	Specialised molecular biology expertise
Application in Outbreak Analysis <sup>60</sup>	Limited utility	Crucial for phylogenetics and source tracking
Detection of Novel Strains <sup>60</sup>	Rarely possible	Enables the discovery of new strains and variants
Limitations <sup>60</sup>	Low discriminatory power; influenced by environmental factors	High cost, infrastructure-dependent, sometimes time-consuming

supports infection control teams in implementing targeted measures to prevent outbreaks, especially in environments where multidrug-resistant (MDR) organisms are prevalent.<sup>62</sup>

Antibiotic stewardship plays a critical role in managing *Citrobacter* infections, particularly given the rising incidence of MDR and extended-spectrum beta-lactamase (ESBL) producing strains. Stewardship programs promote the judicious use of antibiotics by encouraging treatment based on susceptibility testing and local resistance patterns rather than empirical broad-spectrum therapy.<sup>63</sup> Carbapenems such as imipenem and meropenem, along with agents like tigecycline and colistin, have demonstrated efficacy against many resistant *Citrobacter* isolates. Nonetheless, empirical therapy should be promptly adjusted once laboratory results are available to avoid unnecessary exposure to broad-spectrum agents and reduce selective pressure.<sup>64</sup> Stewardship interventions such as prospective audit and feedback, preauthorisation of selected antibiotics, and adherence to institutional guidelines reduce inappropriate prescribing. These measures improve clinical outcomes and help limit the emergence and transmission of resistant *Citrobacter* strains within hospitals.<sup>65</sup>

In addition to conventional antibiotics, novel therapeutic approaches are being explored to combat resistant *Citrobacter* infections. Bacteriophage therapy, which uses viruses that specifically infect and kill bacteria, has shown promise in experimental models and some clinical cases.<sup>66</sup> Phage therapy can be particularly effective when used in combination with antibiotics, as synergistic effects may enhance bacterial clearance even at lower antibiotic doses. Furthermore, antimicrobial peptides and nanomaterials such as silver nanoparticles have demonstrated *in vitro* activity against *Citrobacter*, offering potential alternative or adjunctive therapies.<sup>67</sup> While these innovative treatments are still under investigation and require further clinical validation, they represent hopeful avenues for addressing infections caused by highly resistant *Citrobacter* strains.<sup>67</sup>

### Future perspectives

Significant advances in genomics and molecular biology are shaping the future of

*Citrobacter* research. With the advent of whole-genome sequencing (WGS), researchers are uncovering the extensive genetic diversity within the genus, identifying new genomospecies, and tracking the emergence of dominant clones in clinical settings.<sup>68</sup> Comparative genomics has highlighted the plasticity of the *Citrobacter* genome, revealing thousands of gene families and substantial differences in core metabolic and virulence-related genes. Together, these findings improve our understanding of the evolutionary adaptability of *Citrobacter*. They also suggest mechanisms that may underpin persistence in both environmental and healthcare settings.<sup>69</sup> Notably, the identification of specific virulence clusters, such as the high-pathogenicity island in *C. koseri*, points to previously unrecognised factors that may contribute to severe infections, especially in vulnerable populations like neonates.<sup>69</sup>

Another promising trend is the development of rapid diagnostic tests that can detect *Citrobacter* species and resistance determinants directly from clinical specimens. Traditional culture-based methods, while reliable, are time-consuming and may delay appropriate therapy. In contrast, molecular techniques such as multiplex nucleic acid assays and real-time PCR now enable the identification of *Citrobacter* and key resistance genes within hours.<sup>70</sup> These rapid diagnostics are being increasingly integrated into clinical workflows, allowing for more timely and targeted treatment decisions. Additionally, advances in serotyping and *in silico* analysis based on O-antigen gene clusters are improving our ability to track outbreaks and monitor the epidemiology of *Citrobacter* in both hospital and community settings.<sup>70</sup>

Despite these technological advancements, several important research gaps remain. The full spectrum of *Citrobacter* virulence mechanisms is still not completely understood. While some genes associated with serum resistance, cytotoxicity, and cell surface properties have been identified, their precise roles in pathogenesis and their regulation during infection require further exploration. Environmental persistence is another area that warrants more investigation. Although

*Citrobacter's* genetic flexibility suggests a strong capacity for survival in diverse habitats, the specific factors that facilitate its long-term persistence and transmission, particularly in healthcare environments, are not yet fully mapped.

## CONCLUSION

*Citrobacter* species, once considered relatively innocuous gut commensals, have now emerged as important opportunistic pathogens with a growing role in serious healthcare-associated infections. Their increasing involvement in a wide range of clinical presentations, coupled with their alarming potential to develop and disseminate antimicrobial resistance, poses significant challenges to clinicians and microbiologists alike. Accurate identification through a combination of phenotypic and genotypic methods is critical not only for appropriate clinical management but also for epidemiological surveillance and infection control. Advancements in molecular diagnostics and whole-genome-based analyses offer promising tools for a deeper understanding of *Citrobacter's* pathogenicity and resistance dynamics. Continued research, integrated diagnostic strategies, and stringent antimicrobial stewardship are essential to combat the rising threat posed by these organisms in clinical settings. Strengthening laboratory capacity and antimicrobial resistance surveillance for *Citrobacter* spp., particularly in low- and middle-income countries and high-risk settings such as neonatal and intensive care units, will be crucial for early detection of emerging resistant lineages and for guiding locally appropriate stewardship and infection-control policies.

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The authors declare that there is no conflict of interest.

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