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SHORT REPORT

Characterization of NDM-4-Producing *Citrobacter amalonaticus* Isolates from China

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Objective: The aim of this study was to investigate the genomic features of carbapenem-resistant *Citrobacter* spp. carrying bla_{NDM-4} on a novel IncFII-116 plasmid.

Methods: Carbapenem-resistant *Citrobacter* spp. isolates were collected from diarrheal inpatients. Antibiotic susceptibility testing was routinely performed. Whole-genome sequencing and bioinformatic analyses were conducted on the isolates with bla_{NDM-4} gene.

Results: Whole- genome sequencing was performed on the two *C. amalonaticus* isolates carrying bla_{NDM-4}. Whole-genome analysis revealed that pL5091_bla_{NDM-4} and pL5094_bla_{NDM-4}belong to a new type of plasmid (IncFII-116) with lengths of 87124 bp and 87952 bp in two *C. amalonaticus*, respectively. Moreover, bla_{NDM-4} was encoded in the *trpF-ble-*bla_{NDM-4}-IS15 cassette array.

Conclusion: We identified a novel IncFII-116 plasmid carrying bla_{NDM-4} in *C. amalonaticus* for the first time, raising concerns about the emergence of carbapenem-resistant *Citrobacter* spp.

Keywords: carbapenem-resistant, *C. amalonaticus*, IncFII-116, NDM-4, whole-genome sequencing

Introduction

Citrobacter spp. is a common zoonotic pathogen comprising 11 species.[1] Various species of *Citrobacter* spp. may cause infections, including diarrhea, sepsis, meningitis, or respiratory and urinary system infections in neonates, the elderly, and immunocompromised hosts.[2] Although the majority of cases are sporadic, mortality associated with invasive *Citrobacter* spp. infections is high.[3,4]

A recent increase in multidrug resistance among *Citrobacter* spp. has been reported.[4] Previous studies have demonstrated that *Citrobacter* spp., especially *C. freundii*, harbor carbapenemase genes and contribute to a high proportion of carbapenem-resistant Enterobacterales, representing an emerging infection control and public health challenge.[5–7] Different carbapenemase genes, such as bla_{NDM-1}, bla_{KPC-2}, bla_{IMP-4}, bla_{VIM-2} and bla_{OXA-48}, have been identified in *Citrobacter* spp.[5] In addition, several specific plasmids and other genetic mobile elements for carbapenemase gene carriage have been identified, such as IncN[pMLST15] for KPC-2 and Tn*AS3 for* NDM-1.[5,8] Among plasmids harboring bla_{NDM}, in Enterobacteriaceae, IncX3 was predominant, followed by the IncFII type.[7] Several studies have reported different carbapenemase genes and transmission mechanisms in *C. freundii, C. koseri*, and *C. braakii*.[5,8] However, genomic analyses of NDM-producing *C. amalonaticus* have been limited. In this study, the genomic characterization of two carbapenem-resistant *C. amalonaticus* isolates harboring bla_{NDM-4} were analyzed.

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Methods

Collection and Identification of Bacterial Strains

Fecal samples (1.0 g) from inpatients with diarrhea were cultured on MacConkey agar supplemented with 2 mg/L meropenem for 18–24 h at 37°C from 2016 to 2023 at The First Affiliated Hospital, Zhejiang University School of Medicine. Isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker, Bremen, Germany). *Citrobacter* spp. isolates were collected for further analysis. Carbapenemase-encoding genes (bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{OXA-48}) were tested using PCR.[9]

Antibiotic Susceptibility Testing

The MICs of 18 antibiotics were determined according to Clinical and Laboratory Standards Institute (CLSI) recommendations. The results were interpreted in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) guidelines.[10]

Whole Genome Sequencing (WGS) and Bioinformatics Analysis

Genomic DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, United States) and sequenced using Illumina HiSeq 2000 (Novogene Bioinformatics Technology Co., Ltd., Beijing, China) and Oxford Nanopore Technologies platform (Oxford Nanopore Technologies, Oxford, UK), Complete genome sequences were generated by performing a hybrid assembly of short-read and long-read sequences using Unicycler v0.4.8.

The Whole Genome Shotgun BioProject for the included isolates was deposited under accession number PRJNA1118925. The plasmid sequence was hosted in the corresponding BIGSdb database under accession number IncFII-116.

The antimicrobial resistance genes were identified using ResFinder version 2.1 (http://cge.cbs.dtu.dk/services/resfinder). Plasmid Finder v. 1.3 was used to identify the plasmid incompatibility type.[11] All sequenced genomes were annotated using Prokka.[12] The Pyani and Python3 modules calculate the average nucleotide identity (ANI) and related measures for species comparisons (https://github.com/HuttonICS/pyani). Plasmid alignment was performed and visualized using BLAST ring image generator (BRIG) software.

Results

Characteristics of the Carbapenem-Resistant Citrobacter Spp

Forty-two carbapenem-resistant *Citrobacter* spp. were identified, including 32 *C. freundii*, three *C. koseri*, three *C. braakii*, two *C. amalonaticus*, and two *C. youngae*.

Among *C. freundii* isolates, PCR revealed that 19 isolates carried bla_{NDM}, four isolates carried bla_{KPC}, one isolate carried bla_{IMP}, one isolate carried bla_{VIM} and one isolate carried bla_{OXA-48} (Supplementary Table S1). In addition, bla_{KPC} coexisted with bla_{NDM} in three *C. freundii* isolates. All *C. koseri* isolates harbored bla_{KPC} and *C. amalonaticus* harbored bla_{NDM-4}.

Antimicrobial Susceptibility

Two NDM-4-producing *C. amalonaticus* isolates (L5091 and L5094) were resistant to multiple antibiotics (Table 1).

Genetic Characteristics of NDM-4-Producing C. amalonaticus

Strains L5091hy and L5094hy had the highest ANI values compared to the type strain of *C. amalonaticus* (GCA_001558935.2) (Figure S1). A circular chromosome and several plasmids were confirmed in two *C. amalonaticus* isolates (L5091hy and L5094hy) by whole-genome sequencing. Plasmids pL5091_bla_{NDM-4} and pL5094_bla_{NDM-4} are novel IncFII-116 plasmids. The lengths of the two plasmids were 87124 bp and 87952 bp, respectively. The average GC content of the two plasmids was 53.6% and 53.6%, respectively.

The genetic structures of pL5091_bla_{NDM-4} and pL5094_bla_{NDM-4} are similar (Figure 1). The genetic environment for bla_{NDM-4} in the two *C. amalonaticus* isolates was *trpF-ble-*$bla_{\mathrm{NDM-4}}$-IS15. However, it is of note that a large fragment of recombination in IS15 were identified downstream of bla_{NDM-4} in pL5094_bla_{NDM-4}.

Table 1 Antimicrobial Drug Susceptibility Profiles

Antibiotic	MIC (mg/l)/Antimicrobial Susceptibility		
	L5091	L5094	ATCC25922
Amoxicillin/Clavulanic acid	>128/R	128/R	8/S
Piperacillin/Tazobactam	>128/R	>128/R	4/S
Ceftazidime	>128/R	>128/R	4/S
Ceftriaxone	>128/R	>128/R	≤0.03/S
Cefepime	128/R	64/R	0.06/S
Cefotaxime	>128/R	>128/R	0.125/S
Ciprofloxacin	64/R	64/R	≤0.004/S
Levofloxacin	16/R	16/R	0.03/S
Imipenem	32/R	32/R	0.25/S
Meropenem	32/R	16/R	≤0.008/S
Ceftazidime/Avibactam	>128/R	>128/R	0.125/S
Trimethoprim/Sulfamethoxazole	≤0.125/S	0.25/S	≤0.125/S
Amikacin	>128/R	>128/R	4/S
Gentamicin	>128/R	>128/R	1/S
Aztreonam	4/S	2/S	0.125/S
Fosfomycin	>512/R	>512/R	0.5/S
Tigecycline	0.5/S	0.25/S	0.125/S
Polymixin B	0.25/I	0.5/I	0.5/I

Abbreviations: S, susceptible; R, resistant; I, intermediate.

Discussion

The continued increase in the prevalence of multidrug-resistant *Citrobacter* spp. poses a global health security threat.[3,5] The present and previous studies have demonstrated *C. freundii* is the predominant epidemic isolate.[5,6] The bla_{NDM} gene is commonly detected in carbapenem-resistant *Citrobacter* spp. In addition, $bla_{NDM\text{-}4}$ in two *C. amalonaticus* isolates were first identified in this study.

A previous study reported *C. freundii* accounted for 83% of *Citrobacter* spp., which was higher than our result (76.2%).[5,13] This difference may be because the strains in our study were isolated only from fecal samples. As previously described for *C. freundii*, the transmission mechanisms of $bla_{\text{NDM-1}}$-, $bla_{\text{VIM-1}}$-, and $bla_{\text{OXA-48}}$-carrying plasmids were IncF, IncN, and IncM1, respectively.[14] Notably, bla_{NDM} was the most frequent carbapenemase detected in *C. freundii*, which differs from other strains in France.[13] Both local and global carbapenem-resistant *Citrobacter* spp. are genetically diverse.[6] In addition, several earlier studies have reported the co-production of two or three carbapenemases, such as KPC-2 plus NDM-1, KPC-2 plus VIM-2, VIM-1 plus OXA-48, or KPC and NDM plus SHV, in *Citrobacter* spp. isolates.[5,8,15,16]

C. amalonaticus is seldom isolated from fecal or urine samples. A study described the coexistence of $bla_{NDM\text{-}1}$ and *mcr-1-like* genes in *C. amalonaticus*.[17] To the best of our knowledge, this is the first report of *C. amalonaticus* harboring $bla_{NDM\text{-}4}$, significantly extends our understanding of the structural diversification of plasmids. These results highlight the necessity of continuously monitoring the dissemination of carbapenem-resistant *Citrobacter* spp. in the clinical setting.

The most prevalent plasmids carrying bla_{NDM} are IncX3, IncFIB, IncFII, and IncC types.[18] Here, we report two carbapenem-resistant *C. amalonaticus* isolates in China, carrying a novel IncFII-116 plasmid containing $bla_{NDM\text{-}4}$, which is relatively close to IncFII. Therefore, the comprehensive resistance surveillance of *Citrobacter* spp. should be further strengthened.

Conclusion

Here, we characterized the first IncFII-116 plasmid carrying $bla_{NDM\text{-}4}$ in two *C. amalonaticus* isolates from China. The genetic environment of NDM-4 has been elucidated, highlighting the need to monitor the dissemination of carbapenemase-encoding genes and plasmids.

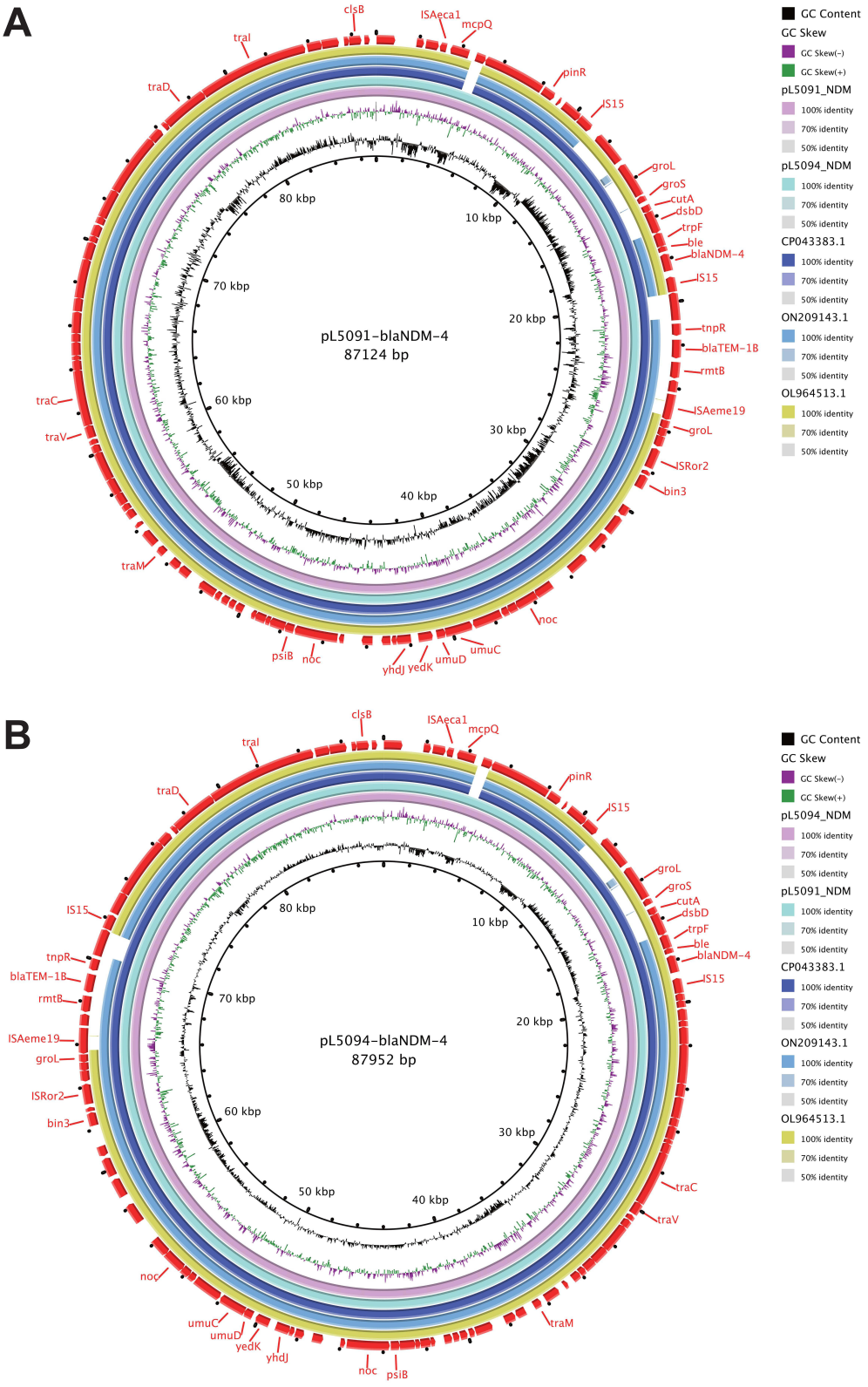

Figure 1 Major structural features and comparison of NDM-encoding plasmids. Genomic map of the *bla*$_{NDM-4}$ producing pL5091_*bla*$_{NDM-4}$/pL5094_*bla*$_{NDM-4}$ plasmids with three closely related plasmids (CP043383.1, ON209143.1, ON964513.1). (**A**) pL5091_*bla*$_{NDM-4}$; (**B**) pL5094_*bla*$_{NDM-4}$. ORFs are portrayed by arrows and colored according to their putative functions. The alignment of the plasmids was performed and visualized by BLAST ring image generator (BRIG) software.

Data Sharing Statement

This Whole Genome Shotgun BioProject for the two *Citrobacter amalonaticus* isolates has been deposited in GenBank under the accession number PRJNA1118925. The plasmid sequence was hosted in the corresponding BIGSdb database under accession number IncFII-116.

Ethics Approval and Consent to Participate

This study was retrospective in nature, so informed consent was waived by the Clinical Research Ethics Committee of The First Affiliated Hospital, Zhejiang University School of Medicine. Patient treatment information was de-identified and complied with the Declaration of Helsinki. All experiments strictly followed relevant guidelines and regulations, and the ethical protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (no. 2018-752).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Brenner DJ, Grimont PA, Steigerwalt AG, Fanning GR, Ageron E, Riddle CF. Classification of citrobacteria by DNA hybridization: designation of *Citrobacter farmeri* sp. nov. *Citrobacter youngae* sp. nov. *Citrobacter braakii* sp. nov. *Citrobacter werkmanii* sp. nov. *Citrobacter sedlakii* sp. nov. and three unnamed *Citrobacter* genomospecies. *Int J Syst Bacteriol*. 1993;43(4):645–658. doi:10.1099/00207713-43-4-645
2. Doran TI. The role of Citrobacter in clinical disease of children: review. *Clin Infect Dis*. 1999;28(2):384–394. doi:10.1086/515106
3. Shih CC, Chen YC, Chang SC, Luh KT, Hsieh WC. Bacteremia due to Citrobacter species: significance of primary intraabdominal infection. *Clin Infect Dis*. 1996;23(3):543–549. doi:10.1093/clinids/23.3.543
4. Lee R, Choi SM, Jo SJ, et al. Clinical characteristics and antimicrobial susceptibility trends in Citrobacter Bacteremia: an 11-year single-center experience. *Infect Chemother*. 2019;51(1):1–9. doi:10.3947/ic.2019.51.1.1
5. Yao Y, Falgenhauer L, Falgenhauer J, et al. Carbapenem-resistant Citrobacter spp. as an emerging concern in the hospital-setting: results from a genome-based regional surveillance study. *Front Cell Infect Microbiol*. 2021;11:744431. doi:10.3389/fcimb.2021.744431
6. Babiker A, Evans DR, Griffith MP, et al. Clinical and genomic epidemiology of Carbapenem-Nonsusceptible Citrobacter spp. at a tertiary health care center over 2 decades. *J Clin Microbiol*. 2020;58(9):e00275–20. doi:10.1128/JCM.00275-20
7. Yoon EJ, Kang DY, Yang JW, et al. New Delhi Metallo-Beta-Lactamase-producing Enterobacteriaceae in South Korea between 2010 and 2015. *Front Microbiol*. 2018;9:571. doi:10.3389/fmicb.2018.00571
8. Li Y, Fang C, Qiu Y, Dai X, Zhang L. Genomic characterization of a carbapenem-resistant Citrobacter freundii cocarrying bla(KPC-2) and bla (NDM-1). *J Glob Antimicrob Resist*. 2022;29:289–292. doi:10.1016/j.jgar.2022.04.014
9. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70 (1):119–123. doi:10.1016/j.diagmicrobio.2010.12.002
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 34th informational supplement 2024. Available from: http://www.clsi.org/. Accessed February 2024.
11. Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. 2014;58(7):3895–3903. doi:10.1128/AAC.02412-14
12. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068–2069. doi:10.1093/bioinformatics/btu153
13. Arana DM, Ortega A, González-Barberá E, et al; Spanish Antibiotic Resistance Surveillance Programme Collaborating Group. Carbapenem-resistant Citrobacter spp. isolated in Spain from 2013 to 2015 produced a variety of carbapenemases including VIM-1, OXA-48, KPC-2, NDM-1 and VIM-2. *J Antimicrob Chemother*. 2017;72(12):3283–3287. doi:10.1093/jac/dkx325
14. Biez L, Bonnin RA, Naas T, Dortet L. Characterization of VIM-1-, NDM-1- and OXA-48-producing Citrobacter freundii in France. *J Antimicrob Chemother*. 2022;77(4):1200–1202. doi:10.1093/jac/dkac005

15. Ma J, Xu R, Li W, Liu M, Ding X. Whole-genome sequencing of clinical isolates of Citrobacter Europaeus in China carrying bla(OXA-48) and bla (NDM-1). *Ann Clin Microbiol Antimicrob*. 2024;23(1):38. doi:10.1186/s12941-024-00699-y
16. Luo X, Yu L, Feng J, et al. Emergence of extensively drug-resistant ST170 Citrobacter portucalensis with Plasmids pK218-KPC, pK218-NDM, and pK218-SHV from a Tertiary Hospital, China. *Microbiol Spectr*. 2022;10(5):e0251022. doi:10.1128/spectrum.02510-22
17. Faccone D, Albornoz E, Tijet N, et al. Characterization of a multidrug resistant Citrobacter amalonaticus clinical isolate harboring bla(NDM-1) and mcr-1.5 genes. *Infect Genet Evol*. 2019;67:51–54. doi:10.1016/j.meegid.2018.10.020
18. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM Metallo-β-Lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev*. 2019;32(2):e00115–18. doi:10.1128/CMR.00115-18

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