AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

LETTER TO THE EDITOR

Coexistence of bla_{OXA-58} and bla_{NDM-1} on a Novel Plasmid of GR59 from an *Acinetobacter towneri* Isolate

Ying Li,[a,b] Yichuan Qiu,[a] Chengju Fang,[a] Xiaoyi Dai,[a] Luhua Zhang[a]

[a]The School of Basic Medical Science and Public Center of Experimental Technology, Southwest Medical University, Luzhou, Sichuan, China

[b]Immune Mechanism and Therapy of Major Diseases of Luzhou Key Laboratory, Public Center of Experimental Technology, School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan, China

KEYWORDS *Acinetobacter*, GR59, bla_{NDM-1}, bla_{OXA-58}, carbapenemase

Carbapenem-resistant *Acinetobacter* spp. are commonly reported worldwide, which becomes a serious public health issue (1). The genetic plasticity of *Acinetobacter* allowed this bacterium to rapidly acquire and accumulate carbapenemase genes, such as bla_{OXA-23} and bla_{NDM-1}, making it a problematic nosocomial persister as well as an important reservoir of resistance genes (2). Plasmid-mediated horizontal gene transfer is relevant to the dissemination of carbapenemase genes (2, 3). To date, 58 plasmid groups (GR1 to GR58) have been proposed in *Acinetobacter* based on the nucleotide identity of the *rep* genes (4, 5). Here, we report a multidrug-resistant (MDR) plasmid carrying bla_{OXA-58} and bla_{NDM-1}, which represents a new plasmid type, GR59.

Strain SCLZS30 was recovered from the sewage outlet of Luzhou People's Hospital, Sichuan Province, China, in August 2019. It was resistant to meropenem, cefotaxime, cefoxitin, tetracycline, and gentamicin, intermediate to ciprofloxacin and sulfamethoxazole/trimethoprim, and susceptible to tigecycline and colistin. The whole-genome sequence of SCLZS30 was determined using the PacBio RS II and Illumina HiSeq 2000 platforms. The genome of SCLZS30 consists of a 2,916,803-bp circular chromosome (41.1% GC content) and two circular plasmids, pNDM_SCLZS30 (47,845 bp) and p1_SCLZS30 (64,918 bp). An additional 23,275-bp contig could not be circularized but has two putative plasmid replication genes. Average nucleotide identity (ANI) analysis showed that SCLZS30 belongs to *Acinetobacter towneri*, as it has 95.27% identity (77.31% query coverage) to the *A. towneri* reference strain, GX3 (CP071766) (6). SCLZS30 contains 13 antibiotic resistance genes (ARGs), conferring resistance to aminoglycosides, β-lactams, macrolides, tetracyclines, sulfonamides, and amphenicols. Among them, *aadB*-$bla_{OXA-392}$ (bla_{OXA-1} family)-*catB3*-*qacEΔ1*-*sul1* and *aadA2b* are located on the chromosome, and *aacC2d*, *aphA6*, bla_{NDM-1}, bla_{OXA-58}, *mph*(E), *msr*(E), and *tet*(39) are carried by pNDM_SCLZS30.

pNDM_SCLZS30 has a replication gene, *repB*, encoding a replicase protein of the Rep_3 family (Pfam:01051). As the *repB* gene has <74% nucleotide identity to the *rep* genes of all 58 existing *Acinetobacter* plasmid groups (4, 5), pNDM_SCLZS30 defines a novel group designated GR59. The backbone of pNDM_SCLZS30, with an average GC content of 36.59%, has only 88.6% identity (45% coverage) with its closest match, p80-1-2-tetX3 (CP041297, *Acinetobacter indicus*, animal origin, China). Determinants for conjugation were not identified on pNDM_SCLZS30, while a *mobA* gene for plasmid mobilization is present (Fig. 1). Transconjugants of *Escherichia coli* J53/EC600 were not obtained for pNDM_SCLZS30 after repeated attempts.

All seven resistance genes in pNDM_SCLZS30 are clustered in a 21,469-bp MDR region, which has a higher GC content (43.46%) than the backbone, suggesting different origins. Also, the MDR region is mosaic with areas of high and low GC contents, indicating that it might be shaped by multiple genetic events. The bla_{NDM-1} gene is found in a 4,149-bp Tn*125* remnant (56.35% GC), with the genetic structure *dsbC-trpF-ble-bla*$_{NDM-1}$-IS*Aba125*, downstream

Address correspondence to Xiaoyi Dai, daixiaoyi@swmu.edu.cn, or Luhua Zhang, zhluhua@swmu.edu.cn.

The authors declare no conflict of interest.

Published 12 May 2022

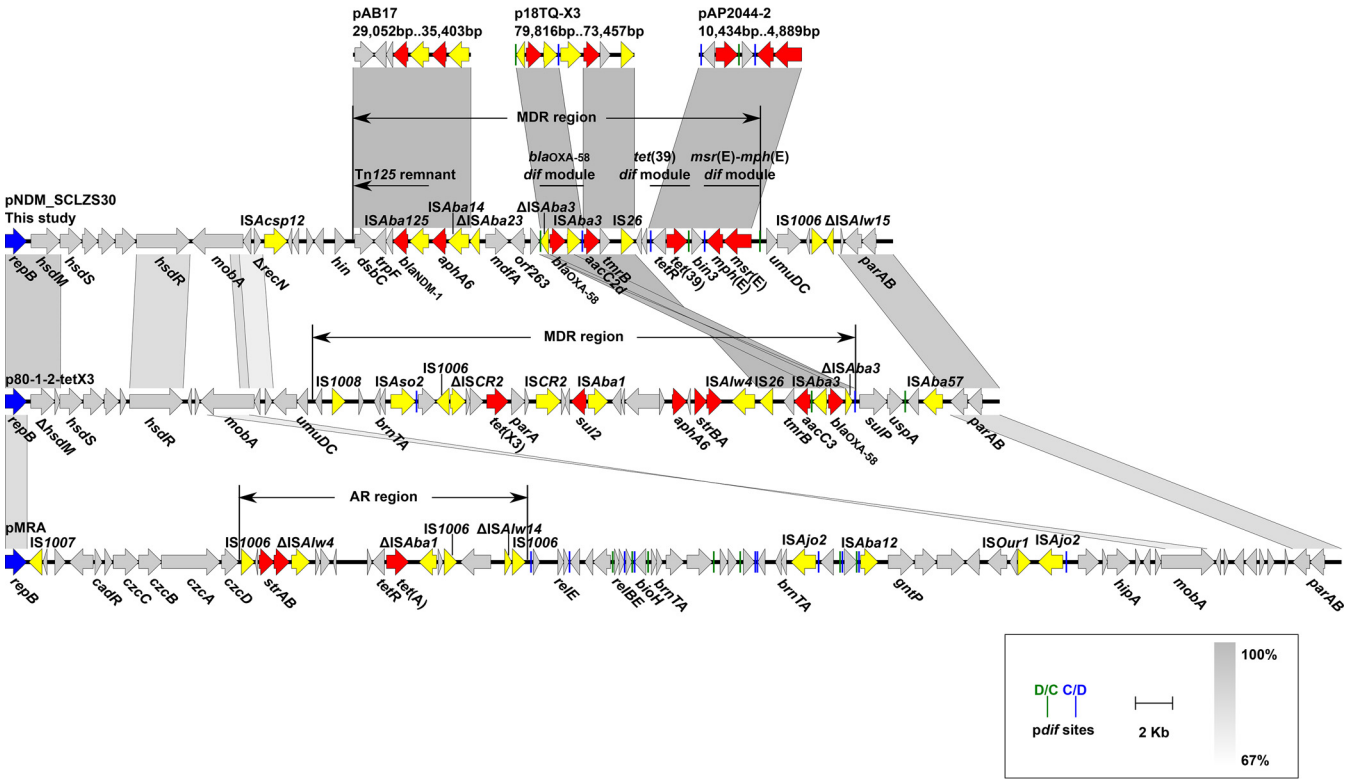

FIG 1 Genetic characteristics of pNDM_SCLZS30. The MDR region of pNDM_SCLZS30 is compared with segments from pAB17 (MT002974, *Acinetobacter baumannii*, patient origin, Brazil), p18TQ-X3 (CP045132, *Acinetobacter indicus*, animal origin, China), and pAP2044-2 (CP087718, *Acinetobacter pittii*, patient origin, China). The complete nucleotide sequence of pNDM_SCLZS30 is compared with GR59 plasmids p80-1-2-tetX3 (CP041297, *A. indicus*, animal origin, China) and pMRA (CP079749, *Acinetobacter johnsonii*, environmental origin, China). Genes are indicated by arrows. *repB*, resistance genes, and mobile genetic elements are highlighted in blue, red and yellow, respectively. Short vertical lines represent p*dif* sites, D/C orientation in green and C/D in blue. Regions of >67% nucleotide sequence identity are indicated by gray shading. MDR, multidrug-resistant; AR, antibiotic resistance.

of the IS*Aba14-aphA6* segment. As previously found in many *Acinetobacter* plasmids, bla_{OXA-58} and its flanking inverted IS*Aba3*, one of which is truncated, are harbored in a 2,313-bp *dif* module (35.15% GC) (7), which is located within an *mdfA-orf263-orf159*-p*dif*-ΔIS*Aba3*-bla_{OXA-58}-IS*Aba3*-p*dif-aacC2d-tmrB*-IS*26* region, revealing a novel genetic context. In addition, a *tet*(39) *dif* module (39.15% GC content) and an *msr*(E)-*mph*(E) *dif* module (36.67% GC content) (8), separated by a resolvase gene, are successively located 3.7-kb downstream of the bla_{OXA-58} *dif* module. The three *dif* modules were previously commonly found in *Acinetobacter* (8, 9), but their coexistence on a single plasmid is rare. BLASTn analysis showed that the three *dif* modules are also simultaneously present on pDETAB2 (CP047975) and pNDM_SCLZS86 (CP090865), but the genetic context of each *dif* module is different (10, 11). These results suggest that site-specific recombination via the p*dif* sites may account for the acquisition of ARGs and the rearrangement of multidrug resistance regions (8–10).

Three complete GR59 plasmids, p80-1-2-tetX3 (CP041297), pMRA (CP079749), and pXMC5X702-195k (CP084301), which contain a *repB* gene >79.64% identical (>98% coverage) to that of pNDM_SCLZS30 (4), were identified in GenBank. A 3.8-kb region, covering *repB* and *parAB* genes, is conserved in these GR59 plasmids (see Fig. S1 in the supplemental material). Six tandem 22-bp repeats were found 535 to 562 bp upstream of *repB*, likely iterons (Fig. S2). A potential origin of plasmid replication, characterized by an array of AT-rich repeat sequences, was identified close to these tandem repeats in each GR59 plasmid (Fig. S2). In addition to pNDM_SCLZS30, p80-1-2-tetX3 and pMRA also contain multiple clinically important ARGs (Fig. 1), and p80-1-2-tetX3 harbors similar bla_{OXA-58} and *aacC* modules as pNDM_SCLZS30, suggesting this plasmid group as a potential vehicle in mediating the dissemination of resistance determinants.

In summary, this study identifies a novel plasmid type, designated GR59, from an environmental *A. towneri* isolate, which carries both bla_{NDM-1} and bla_{OXA-58}. This plasmid group may

serve as an important platform to allow evolution and rearrangement of ARGs to adapt to different antimicrobial selection pressures. More studies are needed to monitor the prevalence of GR59 plasmids and their role in the spread of ARGs in *Acinetobacter*.

Data availability. Complete sequences of the chromosome, pcontig1, pNDM_SCLZS30, and p1_SCLZS30 of SCLZS30 were deposited in GenBank under accession numbers CP090382 to CP090385.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (31900125), the Scientific and Technological Project in Sichuan Province (2022JDRC0144), and the Joint Funds of the Luzhou and Southwest Medical University Natural Science Foundation (2019LZXNYDJ47). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

We declare no competing interests.

REFERENCES

1. Hamidian M, Nigro SJ. 2019. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. Microb Genom 5: e000306. https://doi.org/10.1099/mgen.0.000306.
2. Ramirez MS, Bonomo RA, Tolmasky ME. 2020. Carbapenemases: transforming *Acinetobacter baumannii* into a yet more dangerous menace. Biomolecules 10:720. https://doi.org/10.3390/biom10050720.
3. Salto IP, Torres Tejerizo G, Wibberg D, Puhler A, Schluter A, Pistorio M. 2018. Comparative genomic analysis of *Acinetobacter* spp. plasmids originating from clinical settings and environmental habitats. Sci Rep 8:7783. https://doi.org/10.1038/s41598-018-26180-3.
4. Salgado-Camargo AD, Castro-Jaimes S, Gutierrez-Rios RM, Lozano LF, Altamirano-Pacheco L, Silva-Sanchez J, Perez-Oseguera A, Volkow P, Castillo-Ramirez S, Cevallos MA. 2020. Structure and evolution of *Acinetobacter baumannii* plasmids. Front Microbiol 11:1283. https://doi.org/10.3389/fmicb.2020.01283.
5. Castro-Jaimes S, Guerrero G, Bello-Lopez E, Cevallos MA. 2022. Replication initiator proteins of *Acinetobacter baumannii* plasmids: an update note. Plasmid 119–120:102616. https://doi.org/10.1016/j.plasmid.2021.102616.
6. Ma J, Wang J, Feng J, Liu Y, Yang B, Li R, Bai L, He T, Wang X, Yang Z. 2020. Characterization of three porcine *Acinetobacter towneri* strains co-harboring *tet(X3)* and bla_{OXA-58}. Front Cell Infect Microbiol 10:586507. https://doi.org/10.3389/fcimb.2020.586507.
7. Wang J, Wang Y, Wu H, Wang ZY, Shen PC, Tian YQ, Sun F, Pan ZM, Jiao X. 2020. Coexistence of bla_{OXA-58} and *tet(X)* on a novel plasmid in *Acinetobacter sp.* from pig in Shanghai, China. Front Microbiol 11:578020. https://doi.org/10.3389/fmicb.2020.578020.
8. Blackwell GA, Hall RM. 2017. The *tet39* determinant and the *msrE-mphE* genes in *Acinetobacter* plasmids are each part of discrete modules flanked by inversely oriented p*dif* (XerC-XerD) sites. Antimicrob Agents Chemother 61:e00780-17. https://doi.org/10.1128/AAC.00780-17.
9. Cameranesi MM, Moran-Barrio J, Limansky AS, Repizo GD, Viale AM. 2018. Site-specific recombination at XerC/D sites mediates the formation and resolution of plasmid co-integrates carrying a bla_{OXA-58}- and TnaphA6-resistance module in *Acinetobacter baumannii*. Front Microbiol 9:66. https://doi.org/10.3389/fmicb.2018.00066.
10. Liu H, Moran RA, Chen Y, Doughty EL, Hua X, Jiang Y, Xu Q, Zhang L, Blair JMA, McNally A, van Schaik W, Yu Y. 2021. Transferable *Acinetobacter baumannii* plasmid pDETAB2 encodes OXA-58 and NDM-1 and represents a new class of antibiotic resistance plasmids. J Antimicrob Chemother 76:1130–1134. https://doi.org/10.1093/jac/dkab005.
11. Li Y, Qiu Y, Fang C, Tang M, Dai X, Zhang L. 2022. Characterisation of a novel GR31 plasmid co-harbouring *blaNDM-1* and *blaOXA-58* in an Acinetobacter sp. isolate. J Glob Antimicrob Resist 29:212–214. https://doi.org/10.1016/j.jgar.2022.03.015.