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NDM-5 and OXA-181 Beta-Lactamases, a Significant Threat Continues To Spread in the Americas

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ABSTRACT Among Gram-negative bacteria, carbapenem-resistant infections pose a serious and life-threatening challenge. Here, the CRACKLE network reports a sentinel detection and characterization of a carbapenem-resistant *Klebsiella pneumoniae* ST147 isolate harboring bla_{NDM-5} and $bla_{OXA-181}$ from a young man who underwent abdominal surgery in India. bla_{NDM-5} was located on an IncFII plasmid of ≈90 kb, whereas $bla_{OXA-181}$ was chromosomally encoded. Resistome and genome analysis demonstrated multiple copies of the transposable element IS*26* and a "hot-spot region" in the IncFII plasmid.

KEYWORDS NDM-5, OXA-181, *Klebsiella pneumoniae*

Carbapenem-resistant *Klebsiella pneumoniae* (CR *Kp*) infections are among the most problematic clinical challenges worldwide, since few antimicrobials retain activity against them, and they are associated with high morbidity and mortality (1, 2). The New Delhi metallo-beta-lactamase (NDM-1) was first identified in 2008 (3). bla_{NDM} has emerged as a major global health challenge not only because of its ability to confer resistance to nearly all beta-lactam antibiotics but also because of its rapid spread worldwide. At present, 16 bla_{NDM-5} variants are reported in >40 countries, and it is endemic in several areas (Southeast Asia, the Balkans, and the Middle East) (4). The NDM-5 variant differs from NDM-1 at amino acid positions Val88Leu and Met154Leu, and this metallo-beta-lactamase was first identified in a multidrug-resistant *Escherichia coli* ST648 isolate from a patient in the United Kingdom who had a recent history of hospitalization in India (5). NDM-5 has since been detected in Algeria Spain, Japan, Australia, China, and Egypt (6–10).

The OXA-48 beta-lactamase was initially identified in a *K. pneumoniae* isolate from a patient residing in Istanbul, Turkey, in 2001 (11). Soon after, the gene encoding this carbapenemase (the "phantom menace") rapidly disseminated through Turkish hospitals, with outbreaks in the main cities of the country reaching endemic levels (12, 13). Since then, 11 variants of $bla_{OXA-48-like}$ have been designated and have spread to the Middle East, North Africa, and Europe. In addition, several nosocomial outbreaks were reported in Mediterranean countries, including France, Spain, Lebanon, Israel, Tunisia, Morocco, and Malta, and have reached an endemic situation (14). OXA-181, a variant of OXA-48 that differs by four amino acid substitutions (Thr104Ala, Asn110Asp, Glu175Gln, and Ser179Ala), was initially identified in *Enterobacter cloacae* and *K. pneumoniae*

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TABLE 1 MICs for CR *Kp*-1 and CR *Kp*-2, evaluated by Etest

Antibiotic	MIC (mg/liter) for:	
	CR *Kp*-1	CR *Kp*-2
Tigecycline	0.5	0.5
Amikacin	>256	2
Gentamicin	>256	2
Ampicillin-sulbactam	>256	>256
Piperacillin/tazobactam	256	>256
Ceftazidime/avibactam	>256	0.5
Ceftazidime	>256	48
Cefepime	>256	96
Ceftriaxone	>32	>32
Cefotaxime	>32	>32
Ertapenem	>32	>32
Imipenem	>32	4
Meropenem	>32	24
Doripenem	>32	8
Polymyxin B[a]	≤0.5	1

[a]Polymyxin B susceptibility was evaluated by broth microdilution.

isolates that were recovered in 2007 in India (15). Since then, OXA-181-producing *Enterobacteriaceae* were reported in several other countries on the Indian subcontinent, including Bangladesh, Sri Lanka, and Nepal (16, 17), followed by Canada, France, the Netherlands, New Zealand (in a *K. pneumoniae* isolate from a patient from Nepal), Norway (in a patient from Romania), Oman, Romania, Singapore, South Africa, and the United Kingdom (in a patient from India) (18).

Herein, we report a *K. pneumoniae* isolate harboring $bla_{\text{NDM-5}}$ and $bla_{\text{OXA-181}}$ and a paired isolate from the same patient exhibiting highly plastic genomic regions associated with the IS*26* insertion element involved in the loss of $bla_{\text{NDM-5}}$ and several other resistance determinants. These isolates were detected as part of a surveillance network dedicated to characterizing the *K. pneumoniae* carbapenemase (KPC) epidemic in the United States and elsewhere.

A young man underwent sleeve gastrectomy for management of obesity in India in 2014. The surgery was complicated by a postoperative anastomotic leak, and the patient was transferred to a U.S. hospital a month after surgery. An abdominal washout was required, and the patient was placed on bowel rest and started on total parenteral nutrition. However, he developed an enterocutaneous fistula and was transferred to one of the hospitals participating in CRACKLE (The Consortium on Resistance against Carbapenems in *Klebsiella pneumoniae*), a prospective, multicenter, observational study in the Great Lakes Region of the United States (2), for a Roux-en-Y gastric bypass procedure and resection of the fistula. This surgery was performed 4 months after his initial operation. Cultures sent from abdominal fluid revealed a carbapenem-resistant *K. pneumoniae* strain (CR *Kp*-1) (Table 1) and *Candida albicans*. The patient was initially treated with ciprofloxacin, metronidazole, and micafungin. After CR *Kp*-1 was confirmed, ciprofloxacin was switched to tigecycline. He remained febrile, and a repeat abdominal computed tomography scan revealed persistent fluid collections, which were drained percutaneously. Cultures from this procedure again revealed a carbapenem-resistant *K. pneumoniae* strain (CR *Kp*-2) (Table 1). He was treated for a total of 16 days with tigecycline. His symptoms resolved, and he was discharged in stable condition and off of antibiotics after a 28-day admission.

The Check MDR CT103 XL assay (Check-Points, Wageningen, Netherlands) was used to detect beta-lactamase genes, including extended-spectrum beta-lactamases (bla_{SHV} and bla_{TEM} ESBLs), plasmid-mediated AmpCs, and carbapenemases, including bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, $bla_{\text{OXA-48-like}}$, bla_{GES}, bla_{GIM}, bla_{SPM}, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24/40-like}}$, $bla_{\text{OXA-48-like}}$. CR *Kp*-1 contained bla_{NDM}, $bla_{\text{OXA-48-like}}$, $bla_{\text{CTX-M-15-like}}$, $bla_{\text{SHV-WT}}$, and $bla_{\text{TEM-WT}}$. CR *Kp*-2 contained $bla_{\text{OXA-48-like}}$, $bla_{\text{CTX-M-15-like}}$, and $bla_{\text{SHV-WT}}$. PCR amplification and sequencing performed to identify bla_{NDM} and $bla_{\text{OXA-48-like}}$ variants con-

TABLE 2 Resistome of CR *Kp*-1 and CR *Kp*-2

Strain	Genome size (bp)	No. of contigs	Resistance determinants
CR *Kp*-1	5,608,317	87	*oqxA*, *oqxB*, bla_{SHV-11}, *fosA*, *rmtF*, *aacA4*, *aac(6′)Ib-cr*, *qnrB12*, *dfrA14*, $bla_{OXA-181}$, *ARR-2*, $bla_{CTX-M-15}$, *mph*(A), *sul1*, *erm*(B), *dfrA12*, *aadA2*, *rmtB*, bla_{TEM-1}, bla_{NDM-5}
CR *Kp*-2	5,587,222	79	*oqxA*, *oqxB*, bla_{SHV-11}, *fosA*, *rmtF*, *aacA4*, *aac(6′)Ib-cr*, *qnrB12*, *dfrA14*, $bla_{OXA-181}$, *ARR-2*, $bla_{CTX-M-15}$

firmed that CR *Kp*-1 carried bla_{NDM-5} and $bla_{OXA-181}$, whereas CR *Kp*-2 carried only $bla_{OXA-181}$.

Conjugation experiments were performed using *K. pneumoniae* clinical strains as donors and the azide-resistant *E. coli* J53 as a recipient; however, transconjugants were not obtained. Therefore, plasmid DNA was extracted and electroporated into *E. coli* DH10B (19). Transformants were selected on ampicillin-containing lysogeny broth agar, and the presence of bla_{NDM-5} and $bla_{OXA-181}$ was confirmed by PCR amplification. Plasmid characterization on CR *Kp*-1 and CR *Kp*-2 isolates, as well as on transformants, was performed by PCR-based replicon typing using the PCR-based replicon typing kit (Diatheva, Fano, PU, Italy) following the manufacturer's instructions. Only an IncFII amplicon was obtained for both strains and transformants. S1 nuclease pulsed-field gel electrophoresis (PFGE) followed by Southern hybridization with bla_{NDM-5}, $bla_{OXA-181}$, and IncFII replicon probes for clinical strains and transformants (see Fig. S1 in the supplemental material) and I-CeuI PFGE followed by Southern hybridization with $bla_{OXA-181}$ and 16S probes for clinical strains (see Fig. S2 in the supplemental material) suggested that bla_{NDM-5} was located on an IncFII plasmid of ≈90 kb, while $bla_{OXA-181}$ was encoded chromosomally (20, 21).

Draft whole-genome sequences were obtained from Illumina paired-end reads. Use of ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) with assemblies as input confirmed that CR *Kp*-2 possesses a resistome smaller than that of CR *Kp*-1 (Table 2) (22). Likewise, BLAST revealed that for CR *Kp*-1, bla_{NDM-5} was localized on an IncFII plasmid of ≈90 kb (99% similarity with pCC1409, a bla_{NDM-5}-harboring IncFII plasmid from a *K. pneumoniae* ST147 isolated in Poland; KT725789.1) (Fig. 1A). Interestingly, this same plasmid was present in CR *Kp*-2; however, it was slightly smaller due to the loss of an ≈25-kb region, including not only bla_{NDM-5} but also several other resistance determinants (e.g., bla_{TEM-1}, *mphA*, *ermB*, *dfrA12*, *aadA2*, and *rmtB*) (Fig. 1A). ISFinder (https://www-is.biotoul.fr) showed the presence of multiple transposable elements, including IS*26*, distributed along the above-mentioned region (Fig. 1B) (23). Of note, the finding of *rmtB* is manifested by the difference in susceptibility to aminoglycosides (256 versus 2 mg/liter) (Table 1). In addition, the presence of numerous resistance determinants may indicate that this is a "hot-spot region." Given the replicative transposition mechanism of IS*26* and its previously shown critical role in the mobilization and reorganization of antibiotic resistance genes in Gram-negative bacteria, we hypothesize that the excision of this region might have been aided by IS*26* (24, 25).

IS*Ecp1* was identified upstream of $bla_{OXA-181}$, as previously described (18); I-CeuI PFGE and probe hybridization suggested that this carbapenemase was chromosomally located in both clinical strains. Interestingly, transformation experiments revealed that $bla_{OXA-181}$ was also carried by a plasmid on the CR *Kp*-2 *E. coli* transformant, which, based on size and IncFII probe hybridization, is the same plasmid that lost the above-mentioned ≈25-kb bla_{NDM-5}-harboring region. This might indicate a transposition event aided by the flanking IS*Ecp1* accompanying this gene, highlighting the plasticity of this plasmid to incorporate or lose genes (Fig. S1 and S2 in the supplemental material).

In the United States, bla_{NDM} has been circulating since 2010 (26), whereas bla_{OXA-48} was imported in 2012 by patients who were initially hospitalized in Saudi Arabia and India (27). The rapid and widespread dissemination of $bla_{OXA-181}$ throughout Southeast Asia, the recent finding of $bla_{OXA-181}$ (associated with the IS*Ecp1* mobile genetic element) in the chromosome of a wastewater *Shewanella xiamenensis* isolate (28), and

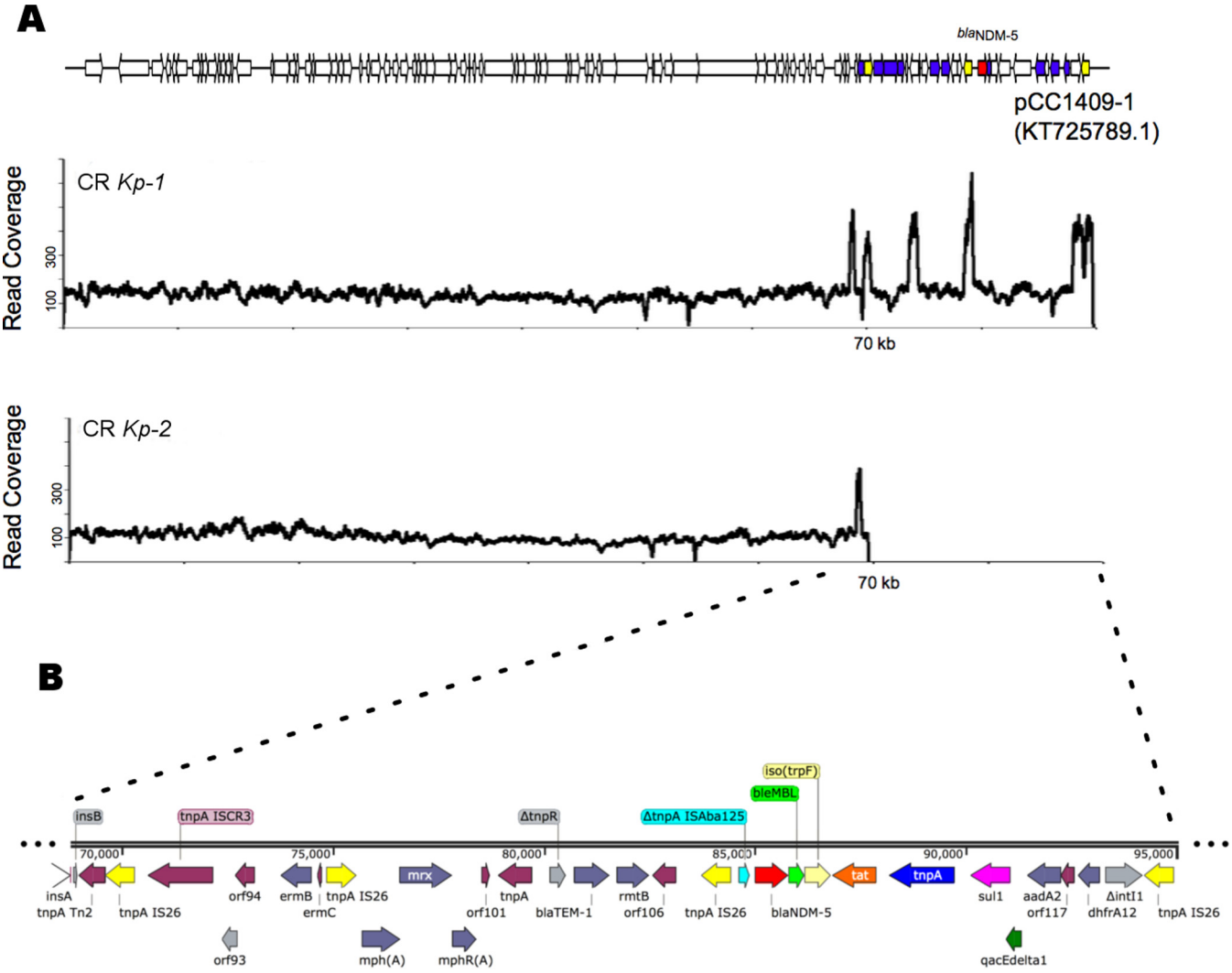

FIG 1 (A) Coverage of Illumina reads from *K. pneumoniae* isolates CR *Kp*-1 and CR *Kp*-2 mapped using Bowtie2 (33) across the reference IncFII plasmid pCC1409 (KT725789.1). (B) Organization of the 25-kb bla_{NDM-5}-containing region of the IncFII plasmid present only in CR *Kp*-1. This entire region was excised out of the IncFII plasmid present in CR *Kp*-2.

the recognition that most cases outside of this area of the world are from patients that had a recent travel history to Southeast Asia and the Asian Pacific region suggest that this is the likely place of origin for $bla_{OXA-181}$ and an important reservoir of this carbapenemase gene. Multilocus sequence typing (MLST) (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) revealed that CR *Kp*-1 and CR *Kp*-2 both belong to ST147. A *K. pneumoniae* isolate belonging to the same ST type (ST147) and carrying an IncFII plasmid with $bla_{OXA-181}$ and bla_{NDM-5} was previously reported in South Korea from a patient transferred from a tertiary care hospital in Abu Dhabi, United Arab Emirates (29). However, the first report of a *K. pneumoniae* isolate harboring $bla_{OXA-181}$ and bla_{NDM-5} occurred in Singapore in 2013 and was isolated from the urine of a patient that was transferred from Bangladesh (16). Another isolate harboring both carbapenemase genes was described in Egypt; however, it was an *E. coli* that carried each gene on a different plasmid (30). In the United States, a *K. pneumoniae* isolate carrying bla_{NDM-1} and $bla_{OXA-232}$ was reported in another patient transferred from India (31). However, bla_{NDM-5} and $bla_{OXA-181}$ have been reported only once before in the same patient but in different *E. coli* isolates (32).

In summary we describe the occurrence of two clinically important carbapenemases (the "evil twins"), $bla_{OXA-181}$ (chromosomally encoded) and bla_{NDM-5} (plasmid encoded),

in a carbapenem-resistant *K. pneumoniae* clinical isolate in the United States. Our results also underscore the ability of ceftazidime/avibactam to indicate the presence of metallo-beta-lactamases when carbapenem-resistant isolates show resistance to this combination. These findings underscore the importance of molecular surveillance programs such as CRACKLE that characterize resistant strains and highlight the emergence of novel genotypes in the United States.

Accession number(s). The nucleotide sequence of the draft genomes for CR *Kp-1* (1699_2676) and CR *Kp-2* (1699_2677) were deposited in GenBank under accession numbers NZ_MPYK00000000.1 and NZ_MPYL00000000.1, respectively.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00454-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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REFERENCES

1. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP. 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis 13:785–796. https://doi.org/10.1016/S1473-3099(13)70190-7.
2. van Duin D, Perez F, Rudin SD, Cober E, Hanrahan J, Ziegler J, Webber R, Fox J, Mason P, Richter SS. 2014. Surveillance of carbapenem-resistant *Klebsiella pneumoniae*: tracking molecular epidemiology and outcomes through a regional network. Antimicrob Agents Chemother 58:4035–4041. https://doi.org/10.1128/AAC.02636-14.
3. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. 2009. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother 53:5046–5054. https://doi.org/10.1128/AAC.00774-09.
4. Mojica MF, Bonomo RA, Fast W. 2016. B1-Metallo-β-lactamases: where do we stand? Curr Drug Targets 17:1029–1050. https://doi.org/10.2174/1389450116666151001105622.
5. Hornsey M, Phee L, Wareham DW. 2011. A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother 55:5952–5954. https://doi.org/10.1128/AAC.05108-11.
6. Pitart C, Solé M, Roca I, Román A, Moreno A, Vila J, Marco F. 2015. Molecular characterization of bla_{NDM-5} carried on an IncFII plasmid in an *Escherichia coli* isolate from a nontraveler patient in Spain. Antimicrob Agents Chemother 59:659–662. https://doi.org/10.1128/AAC.04040-14.
7. Sassi A, Loucif L, Gupta SK, Dekhil M, Chettibi H, Rolain J-M. 2014. NDM-5 carbapenemase-encoding gene in multidrug-resistant clinical isolates of *Escherichia coli* from Algeria. Antimicrob Agents Chemother 58:5606–5608. https://doi.org/10.1128/AAC.02818-13.
8. Soliman AM, Khalifa HO, Ahmed AM, Shimamoto T, Shimamoto T. 2016. Emergence of an NDM-5-producing clinical *Escherichia coli* isolate in Egypt. Int J Infect Dis 48:46–48. https://doi.org/10.1016/j.ijid.2016.05.003.
9. Wailan AM, Paterson DL, Caffery M, Sowden D, Sidjabat HE. 2015. Draft genome sequence of NDM-5-producing *Escherichia coli* sequence type 648 and genetic context of bla_{NDM-5} in Australia. Genome Announc 3:e00194-15. https://doi.org/10.1128/genomeA.00194-15.
10. Zhu YQ, Zhao JY, Xu C, Zhao H, Jia N, Li YN. 2016. Identification of an NDM-5-producing *Escherichia coli* sequence type 167 in a neonatal patient in China. Sci Rep 6:29934. https://doi.org/10.1038/srep29934.
11. Poirel L, Héritier C, Tolün V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:15–22. https://doi.org/10.1128/AAC.48.1.15-22.2004.
12. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL. 2015. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 20(45). http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21300.
13. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. 2008. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. Antimicrob Agents Chemother 52:2950–2954. https://doi.org/10.1128/AAC.01672-07.
14. Girmenia C, Serrao A, Canichella M. 2016. Epidemiology of carbapenem resistant *Klebsiella pneumoniae* infections in Mediterranean countries. Mediterr J Hematol Infect Dis 8:e2016032. https://doi.org/10.4084/MJHID.2016.032.
15. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. 2011. Early dissemination of NDM-1-and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob Agents Chemother 55:1274–1278. https://doi.org/10.1128/AAC.01497-10.
16. Balm MND, La MV, Krishnan P, Jureen R, Lin RTP, Teo JWP. 2013. Emergence of co-producing NDM-type and OXA-181 carbapenemases. Clin Microbiol Infect 19:E421–E423. https://doi.org/10.1111/1469-0691.12247.
17. Hall JM, Corea E, Sanjeewani HDA, Inglis TJJ. 2014. Molecular mechanisms of β-lactam resistance in carbapenemase-producing *Klebsiella*

pneumoniae from Sri Lanka. J Med Microbiol 63:1087–1092. https://doi.org/10.1099/jmm.0.076760-0.
18. Poirel L, Potron A, Nordmann P. 2012. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 67:1597–1606. https://doi.org/10.1093/jac/dks121.
19. Heringa SD, Monroe JD, Herrick JB. 24 October 2007. A simple, rapid method for extracting large plasmid DNA from bacteria. Nature Precedings. http://precedings.nature.com/documents/1249/version/1.
20. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220.
21. Liu S-L, Hessel A, Sanderson KE. 1993. Genomic mapping with *I-Ceu I*, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella spp.*, *Escherichia coli*, and other bacteria. Proc Natl Acad Sci U S A 90:6874–6878. https://doi.org/10.1073/pnas.90.14.6874.
22. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.
23. Siguier P, Pérochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32–D36. https://doi.org/10.1093/nar/gkj014.
24. Harmer CJ, Moran RA, Hall RM. 2014. Movement of IS*26*-associated antibiotic resistance genes occurs via a translocatable unit that includes a single IS*26* and preferentially inserts adjacent to another IS*26*. mBio 5:e01801-14. https://doi.org/10.1128/mBio.01801-14.
25. He S, Hickman AB, Varani AM, Siguier P, Chandler M, Dekker JP, Dyda F. 2015. Insertion sequence IS*26* reorganizes plasmids in clinically isolated multidrug-resistant bacteria by replicative transposition. mBio 6:e00762-15. https://doi.org/10.1128/mBio.00762-15.
26. Centers for Disease Control and Prevention. 2010. Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase—United States, 2010. MMWR Morbid Mortal Weekly Rep 59:750.
27. Mathers AJ, Hazen KC, Carroll J, Yeh AJ, Cox HL, Bonomo RA, Sifri CD. 2013. First clinical cases of OXA-48-producing carbapenem-resistant *Klebsiella pneumoniae* in the United States: the "menace" arrives in the New World. J Clin Microbiol 51:680–683. https://doi.org/10.1128/JCM.02580-12.
28. Potron A, Poirel L, Nordmann P. 2011. Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in *Shewanella xiamenensis*. Antimicrob Agents Chemother 55:4405–4407. https://doi.org/10.1128/AAC.00681-11.
29. Cho SY, Huh HJ, Baek JY, Chung NY, Ryu JG, Ki C-S, Chung DR, Lee NY, Song J-H. 2015. *Klebsiella pneumoniae* co-producing NDM-5 and OXA-181 carbapenemases, South Korea. Emerg Infect Dis 21:1088–1089. https://doi.org/10.3201/eid2106.150048.
30. Gamal D, Fernandez-Martinez M, El-Defrawy I, Ocampo-Sosa AA, Martinez-Martinez L. 2016. First identification of NDM-5 associated with OXA-181 in *Escherichia coli* from Egypt. Emerg Microb Infect 5:e30. https://doi.org/10.1038/emi.2016.24.
31. Doi Y. 2014. Co-production of NDM-1 and OXA-232 by *Klebsiella pneumoniae*. Emerg Infect Dis 20:163–165. https://doi.org/10.3201/eid2001.130904.
32. Hasassri ME, Boyce TG, Norgan A, Cunningham SA, Jeraldo PR, Weissman S, Patel R, Banerjee R, Pogue JM, Kaye KS. 2016. An immunocompromised child with bloodstream infection caused by two *Escherichia coli* strains, one harboring NDM-5 and the other harboring OXA-48-like carbapenemase. Antimicrob Agents Chemother 60:3270–3275. https://doi.org/10.1128/AAC.03118-15.
33. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.