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Detection of NDM-19, a novel variant of the New Delhi metallo-β-lactamase with increased carbapenemase activity under zinc-limited conditions, in Switzerland

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ABSTRACT

A novel New Delhi metallo-β-lactamase (NDM) variant, NDM-19, was identified in a carbapenem-resistant *E. coli* strain isolated from a subcutaneous infection of a laparotomy scar from an Egyptian patient in a Swiss hospital. NDM-19 is a derivative of NDM-7, from which it differs by a single amino acid substitution (Ala233Val). Under zinc-limiting growth conditions, *E. coli* DH5α transformants producing NDM-19 displayed reduced susceptibility towards expanded-spectrum cephalosporins and carbapenems as compared to transformants producing NDM-1 or NDM-7.

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In clinically relevant Gram-negative bacteria, resistance to β-lactams can be mediated by numerous β-lactamases. Among them, Ambler B metallo-β-lactamases (MBL) are the most worrisome ones, as they virtually hydrolyze all β-lactam antibiotics (penicillins, cephalosporins and carbapenems), with the monobactam aztreonam being the only exception (Nordmann, 2014). Since the discovery of NDM-1 in 2008, NDM-β-lactamases have become one of the most widespread carbapenemases worldwide (van Duin and Doi, 2017). Rapid evolution of bla_{NDM} genes and their dissemination in a broad range of Gram-negative bacteria through multidrug resistance plasmids have established NDM as a global major public health threat. To date, 24 different NDM variants have been reported (Naas et al., 2017), with single or multiple amino acid substitutions in 18 positions, some of which are associated with a significant increase in carbapenemase activity as compared to that of NDM-1 (*e.g.* NDM-5, NDM-7, NDM-12, NDM-13, NDM-15, NDM-16) (Cheng et al., 2018). In this report, we describe the first detection of the NDM-19 variant in an *Escherichia coli* clinical isolate (hereafter designated EC1918) in Switzerland.

EC1918 was isolated from a subcutaneous infection of a laparotomy scar from an Egyptian patient admitted to the University Hospital Zurich, Switzerland, in February 2018. The clinical isolate was identified as *E. coli* by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MALDI, biotyper, Brucker Daltonics, France). Antimicrobial Susceptibility testing was performed using the E-test (*BD*, Franklin Lakes, NJ) or the broth microdilution method following interpretation guidelines set by EUCAST (http://www.eucast.org/clinical_breakpoints/). EC1918 was resistant to penicillins, penicillin/inhibitor combinations, cephalosporins and carbapenems, ceftazidime/avibactam and ceftolozane-tazobactam combinations, gentamicin and fluoroquinolones (Table 1). In contrast, EC1918 remained susceptible to sulfamethoxazole-trimethoprim, aztreonam, tigecycline, fosfomycin and colistin. Antimicrobial susceptibility testing (AST) data initially suggested the presence of a class Ambler B metallo-β-lactamase (Maurer et al., 2015), which was confirmed by PCR targeting NDM-1 (Poirel et al., 2011). Sequencing revealed a novel bla_{NDM} variant, namely bla_{NDM-19} (Genbank accession number MF370080).

NDM-19 differs from NDM-1 (Genbank accession number AHM26723) by amino acids substitutions at positions 130 (Asp→Asn), 154 (Met→Leu) and 233 (Ala→Val), and from NDM-7 (Genbank accession number AKN35289) by one substitution at position 233 (Ala→Val) (Figure S1). To evaluate the impact of the Ala233Val substitution on the hydrolytic activity towards β-lactam antibiotics, bla_{NDM-1}, bla_{NDM-7} and bla_{NDM-19} together with their native (identical) promoters were amplified by PCR from clinical isolates harboring the corresponding genes using the previously described forward primer pre-NDM-for (5′-CACCTCATGTTTGAATTCGCC-3′) and reverse primer pre-NDM-rev (5′-CTCTGTCACATCGAAATCGC-3′) (Nordmann et al., 2012). The resulting PCR products of 983 bp were cloned in the ZeroBlunt TOPO vector (Invitrogen, France) and then transformed in *E. coli* DH5α. Expression of each bla_{NDM} gene conferred high-level resistance towards penicillins, penicillin-inhibitors combinations, 2nd and 3rd generation cephalosporins and cephalosporin-inhibitor combinations (Table 1). All expression clones remained susceptible to the monobactam aztreonam. Transformants harboring bla_{NDM-7} or bla_{NDM-19} exhibited a significantly lower susceptibility to cefepime, ertapenem, imipenem and meropenem, as compared to the strain carrying the NDM-1-encoding plasmid. However, *E. coli* DH5α harboring bla_{NDM-7} and bla_{NDM-19}

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Table 1
Antimicrobial susceptibility testing of the *E. coli* clinical isolate and derivatives. Shown are the MICs (mg/L) for β-lactams of the *E. coli* EC1918 clinical isolate, the *E. coli* JE53 transconjugant and the *E. coli* DH5α harboring bla_{NDM1}, bla_{NDM-7} and bla_{NDM-19}, as determined by E-test.

	Strain						
	E. coli EC1918	*E. coli* JE53	*E. coli* JE53 pNDM-19	*E. coli* DH5α	DH5α pTOPO-NDM-1	DH5α pTOPO-NDM-7	DH5α pTOPO-NDM-19
Ampicillin	>256	4	>256	1	>256	>256	>256
Amoxicillin/clavulanic acid	12	3	24	2	48	48	48
Piperacillin/tazobactam	>256	1.5	>256	0.25	>256	>256	>256
Cefuroxime	>256	2	>256	0.38	>256	>256	>256
Cefoxitin	>256	2	>256	1	>256	>256	>256
Cefpodoxime	>256	0.5	>256	0.25	>256	>256	>256
Ceftazidime	>256	0.64	>256	0.16	>256	>256	>256
Ceftriaxone	>256	0.32	>256	<0.16	>256	>256	>256
Cefepime	>256	0.64	3	0.47	3	24	12
Ertapenem	>256	0.003	0.25	0.002	0.125	0.75	1
Imipenem	4	0.125	0.75	0.125	0.38	12	8
Meropenem	8	0.16	0.38	0.12	0.25	2	1.5
Aztreonam	0.23	0.23	0.23	0.16	0.23	0.23	0.23
Ceftazidime/avibactam	>256	0.64	>256	0.32	>256	>256	>256
Ceftolozane/tazobactam	>256	0.19	>256	0.19	>256	>256	>256

displayed comparable MICs for all tested β-lactams. Previous works have shown that M154L, the most common point mutation found among clinical NDM variants, enhances β-lactamase activity when zinc is scarce, a condition that is likely to occur in the infection sites (Palmer and Skaar, 2016). This prompted us to investigate whether the additional mutation A233V, which differentiates NDM-19 from NDM-7 and appears to be distantly located from the active site (Figure S2), further enhances this effect. In accordance with previous report (Cheng et al., 2018; Stewart et al., 2017), MICs for *E. coli* DH5α harboring bla_{NDM-7} were significantly higher for ampicillin, 3rd generation cephalosporins and carbapenems as compared to the *E. coli* strain carrying bla_{NDM-1} (Table 2). More importantly, under the same condition MICs for the same drugs were significantly higher for the NDM-19-producing strain than for *E. coli* DH5α bla_{NDM-7}, with an 8-fold increase for ceftazidime and cefotaxime MICs and a 4-fold increase for meropenem and ertapenem.

Transferability of bla_{NDM-19} was analyzed by a mating-out assay, using the azide-resistant *E. coli* JE53 as the recipient and EC1918 as the donor strain. Transconjugants were selected on LB agar plates containing sodium-azide (100 mg/L) and ceftazidime (10 mg/L). The bla_{NDM-19} gene was successfully transferred to *E. coli* JE53, confirming its location on a plasmid. *E. coli* JE53 transconjugants were resistant to all penicillins, penicillin-inhibitor combinations, cephalosporins and ertapenem, while remaining susceptible to imipenem, meropenem, aztreonam (Table 1). Moreover, transconjugants were susceptible to gentamicin and fluoroquinolones, indicating that no additional resistance determinants were located on the same plasmid. Analysis of the plasmidic DNA of EC1918 and the *E. coli* JE53 transconjugants by agarose gel electrophoresis revealed the presence of a plasmid of approximately 50 kb in size in both strains. In addition, a low-molecular-weight plasmid of approximately 3.6 kb was detected in EC1918. To gain further insight into the genetic context of the bla_{NDM-19} gene, whole genome sequencing (WGS) was performed with the plasmid content of an *E. coli* JE53 transconjugant, which was extracted using the Kieser method (Kieser, 1984). Whole genomic sequencing (WGS) of EC1918 plasmidic DNA was performed on an Illumina MiSeq platform (Illumina®, San Diego, CA). Identification of antibiotic resistance genes from sequencing reads (fastq) was performed using the ARIBA pipeline (Hunt et al., 2017), querying the ARG-ANNOT and CARD databases (Gupta et al., 2014; McArthur et al., 2013). WGS confirmed that the bla_{NDM-19} gene was located on a 48-kb plasmid, hereafter referred to as pCH18-NDM-19 (Figure S3). Sequence analysis of pCH18-NDM-19 (GenBank accession number MK091521) revealed that the plasmid belongs to IncX3 incompatibility type, which is the predominant type associated with bla_{NDM} genes (Espinal et al., 2018). Moreover, pCH18-NDM-19 was highly similar to the 45'122 bp plasmid pOM26-1-NDM-7 (Genbank accession number KP776609), sharing an almost identical region of 42'583 bp (Figure S4). The schematic representation, including sequence alignment of pCH18-NDM-19 with the closely related pOM26-1-NDM-7 plasmid, was performed with EasyFig 3.3.1 (Sullivan et al., 2011). Analysis of the genetic context revealed the presence of two insertion sequences upstream of bla_{NDM-19}, namely IS*Aba125* and IS5 (Figure S4). Of note, the IS*Aba125* insertion element, which was previously shown to be responsible for the mobilization of the bla_{NDM-1} gene in *A. baumannii* (*Bontron* et al., *2016*), was disrupted by a copy of an IS5 insertion element. Downstream of bla_{NDM-19}, genes coding for bleomycin resistance, a putative phosphoribosylanthranilate isomerase, the oxidoreductase DsbC superfamily protein and a copy of an IS26 insertion element were identified. This genetic context was identical to that of previously described bla_{NDM-7} genes (Espinal et al., 2018; Gottig et al., 2013; Pal et al., 2017). Multilocus sequence typing showed that *E. coli* EC1918 belongs to sequence type (ST) 167.

Table 2
MICs for the NDM variants under zinc-limiting conditions (50 μM EDTA). Shown are the MICs (mg/L) for β-lactams of the *E. coli* DH5α harboring bla_{NDM1}, bla_{NDM-7} and bla_{NDM-19}, as determined by the broth dilution method.

Condition	No EDTA			+ 50 μM EDTA		
Variant	NDM-1	NDM-7	NDM-19	NDM-1	NDM-7	NDM-19
Ampicillin	1280	5120	10240	2	80	1280
Cefoxitin	128	512	1024	<1	8	32
Cefotaxime	32	128	128	<1	4	32
Ceftazidime	1024	>1024	>1024	4	128	1024
Ceftriaxone	32	256	256	<1	8	16
Cefuroxime	1024	2048	>2048	8	256	516
Cefepime	4	>16	>16	<0.0156	2	2
Aztreonam	<0.0156	<0.0156	<0.0156	<0.0156	<0.0156	<0.0156
Imipenem	2	32	64	0.125	0.5	1
Meropenem	1	32	32	<0.0156	0.125	0.5
Ertapenem	1	32	32	<0.0156	0.125	0.5

In summary, in this study we identified a novel NDM variant, NDM-19, in a ST167 *E. coli* clinical isolate. Under zinc-limiting conditions, expression of NDM-19 confers lower susceptibility to large-spectrum cephalosporins and carbapenems as compared to NDM-7, from which it differs by a single amino acid substitution. These findings indicate an ongoing evolution of the bla_{NDM} genes driven by the combined selective pressure of β-lactam antibiotics and zinc deprivation. This observation must be taken into account for analysis of novel NDM-variants.

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Transparency Declarations

There are no conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2019.06.003.

References

Bontron S, Nordmann P, Poirel L. Transposition of *Tn125* Encoding the NDM-1 Carbapenemase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2016;60(12):7245–51. https://doi.org/10.1128/AAC.01755-16. Epub 2016/09/28. PubMed PMID: 27671058; PubMed Central PMCID: PMCPMC5119004.

Cheng Z, Thomas PW, Ju L, Bergstrom A, Mason K, Clayton D, et al. Evolution of New Delhi metallo-beta-lactamase (NDM) in the clinic: Effects of NDM mutations on stability, zinc affinity, and mono-zinc activity. J Biol Chem 2018;293(32):12606–18. https://doi.org/10.1074/jbc.RA118.003835. Epub 2018/06/18. PubMed PMID: 29909397.

Espinal P, Miro E, Segura C, Gomez L, Plasencia V, Coll P, et al. First Description of bla_{NDM-7} Carried on an IncX4 Plasmid in *Escherichia coli* ST679 Isolated in Spain. Microb Drug Resist 2018;24(2):113–9. https://doi.org/10.1089/mdr.2017.0039. Epub 2017/06/27. PubMed PMID: 28650275.

Gottig S, Hamprecht AG, Christ S, Kempf VA, Wichelhaus TA. Detection of NDM-7 in Germany, a new variant of the New Delhi metallo-beta-lactamase with increased carbapenemase activity. J Antimicrob Chemother 2013;68(8):1737–40. https://doi.org/10.1093/jac/dkt088. Epub 2013/04/06. PubMed PMID: 23557929.

Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, et al. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 2014;58(1):212–20. https://doi.org/10.1128/AAC.01310-13. PubMed PMID: 24145532; PubMed Central PMCID: PMCPMC3910750.

Hunt M, Mather AE, Sanchez-Buso L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom 2017;3(10), e000131. https://doi.org/10.1099/mgen.0.000131. PubMed PMID: 29177089; PubMed Central PMCID: PMCPMC5695208.

Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid 1984;12(1):19–36. Epub 1984/07/01. PubMed PMID: 6387733.

Maurer FP, Castelberg C, Quiblier C, Bloemberg GV, Hombach M. Evaluation of carbapenemase screening and confirmation tests with *Enterobacteriaceae* and development of a practical diagnostic algorithm. J Clin Microbiol 2015;53(1):95–104. https://doi.org/10.1128/JCM.01692-14. PubMed PMID: 25355766; PubMed Central PMCID: PMCPMC4290934.

McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, et al. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 2013;57(7):3348–57. https://doi.org/10.1128/AAC.00419-13. PubMed PMID: 23650175; PubMed Central PMCID: PMCPMC3697360.

Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, et al. Beta-lactamase database (BLDB) - structure and function. J Enzyme Inhib Med Chem 2017;32(1):917–9. https://doi.org/10.1080/14756366.2017.1344235. Epub 2017/07/20. PubMed PMID: 28719998.

Nordmann P. Carbapenemase-producing *Enterobacteriaceae*: overview of a major public health challenge. Med Mal Infect 2014;44(2):51–6. https://doi.org/10.1016/j.medmal.2013.11.007. Epub 2013/12/24. PubMed PMID: 24360201.

Nordmann P, Boulanger AE, Poirel L. NDM-4 metallo-beta-lactamase with increased carbapenemase activity from *Escherichia coli*. Antimicrob Agents Chemother 2012;56(4):2184–6. https://doi.org/10.1128/AAC.05961-11. Epub 2012/01/19. PubMed PMID: 22252797; PubMed Central PMCID: PMCPMC3318389.

Pal T, Ghazawi A, Darwish D, Villa L, Carattoli A, Hashmey R, et al. Characterization of NDM-7 Carbapenemase-Producing *Escherichia coli* Isolates in the Arabian Peninsula. Microb Drug Resist 2017;23(7):871–8. https://doi.org/10.1089/mdr.2016.0216. Epub 2017/02/06. PubMed PMID: 28156193.

Palmer LD, Skaar EP. Transition Metals and Virulence in Bacteria. Annu Rev Genet 2016;50:67–91. https://doi.org/10.1146/annurev-genet-120215-035146. PubMed PMID: 27617971; PubMed Central PMCID: PMCPMC5125913.

Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70(1):119–23. https://doi.org/10.1016/j.diagmicrobio.2010.12.002. Epub 2011/03/15. PubMed PMID: 21398074.

Stewart AC, Bethel CR, VanPelt J, Bergstrom A, Cheng Z, Miller CG, et al. Clinical Variants of New Delhi Metallo-beta-Lactamase Are Evolving To Overcome Zinc Scarcity. ACS Infect Dis 2017;3(12):927–40. https://doi.org/10.1021/acsinfecdis.7b00128. Epub 2017/10/03. PubMed PMID: 28965402; PubMed Central PMCID: PMCPMC5726261.

Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. Bioinformatics 2011;27(7):1009–10. https://doi.org/10.1093/bioinformatics/btr039. Epub 2011/02/01. PubMed PMID: 21278367; PubMed Central PMCID: PMCPMC3065679.

van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence 2017;8(4):460–9. https://doi.org/10.1080/21505594.2016.1222343. Epub 2016/09/07. PubMed PMID: 27593176; PubMed Central PMCID: PMCPMC5477705.