AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

NDM-35-Producing ST167 *Escherichia coli* Highly Resistant to β-Lactams Including Cefiderocol

Laurent Poirel,[a,b,c] José Manuel Ortiz de la Rosa,[a,b] Zeynep Sakaoglu,[a] Ayda Kusaksizoglu,[a] Mustafa Sadek,[a,b] Patrice Nordmann[a,b,c,d]

[a]Emerging Antibiotic Resistance, Medical and Molecular Microbiology, Department of Medicine, University of Fribourg, Fribourg, Switzerland
[b]Swiss National Reference Center for Emerging Antibiotic Resistance, Fribourg, Switzerland
[c]INSERM European Unit (LEA), (IAME, Paris), University of Fribourg, Fribourg, Switzerland
[d]Institute for Microbiology, Lausanne University hospital and University of Lausanne, Lausanne, Switzerland

ABSTRACT A multidrug-resistant (carbapenems, aztreonam + avibactam, and cefiderocol) ST167 *Escherichia coli* clinical isolate recovered from a patient hospitalized in Switzerland produced NDM-35 showing ca. 10-fold increased hydrolytic activity toward cefiderocol compared to NDM-1. The isolate co-produced a CMY-type β-lactamase, exhibited a four amino-acid insertion in PBP3, and possessed a truncated iron transporter CirA protein. Our study identified an association of unrelated resistance mechanisms leading to resistance to virtually all β-lactams in a high-risk *E. coli* clone.

KEYWORDS metallo-β-lactamase, NDM, cefiderocol, aztreonam-avibactam

The trend of increasing carbapenem resistance observed in Gram-negative bacteria is mainly related to the dissemination of the carbapenemase-encoding genes (1). Among these genes, those encoding metallo-β-lactamases (MBLs) are the most problematic, since their production leads to very limited treatment options (2, 3). MBLs of the NDM group are the most frequently identified acquired carbapenemases worldwide, hydrolyzing all β-lactams except monobactams, and they are not inactivated by currently commercialized β-lactamase inhibitors (4). Even novel β-lactam/β-lactamase inhibitor combinations, such as ceftazidime-avibactam and ceftolozane-tazobactam, are ineffective against MBL-producers. Nevertheless, very recent therapeutic options are promising, such as cefiderocol (FDC) and aztreonam/avibactam (ATM-AVI) (5, 6). ATM-AVI is a drug combination which is now being tested in clinical trials for its efficacy in treating Gram-negative bacterial infections, particularly those that produce MBLs, since MBLs do not hydrolyze aztreonam (7). FDC is a novel siderophore cephalosporin with broad-spectrum activity against a large variety of Gram-negative bacteria (8, 9), penetrating into Gram-negative bacterial cells by binding to iron molecules and using the bacterial iron transport system to enter the bacterial periplasmic space. Once across the outer membrane, FDC binds to penicillin-binding proteins (PBPs), mainly PBP3, to disrupt cell wall synthesis, eventually leading to cell death (5). Interestingly, it was shown that MBLs have overall a poor catalytic activity toward FDC, making this drug effective against most MBL-producers (10). However, we recently reported that NDM-like β-lactamases significantly contributed to reduced susceptibility to FDC in *Acinetobacter baumannii* (11). More recently, we also demonstrated that some NDM variants may confer reduced susceptibility to FDC in *E. coli* and *Pseudomonas aeruginosa*, along with the extended-spectrum β-lactamases of the PER type (12).

This study was initiated by the isolation of a carbapenem- and FDC-resistant *E. coli* isolate (N1949) producing a novel NDM-1 variant (NDM-35), recovered from the rectal swab of a patient hospitalized in Switzerland. Susceptibility testing was performed and interpreted according to EUCAST guidelines (https://eucast.org/clinical_breakpoints/). Noteworthy, iron depleted-cation adjusted Mueller-Hinton broth (ID-CAMHB) was prepared and used for

Address correspondence to Laurent Poirel, laurent.poirel@unifr.ch.

The authors declare no conflict of interest.

Received 28 February 2022
Returned for modification 8 May 2022
Accepted 13 June 2022
Published 11 July 2022

TABLE 1 MICs of β-lactams for *E. coli* N1949 and NDM-producing *E. coli* recombinant strains determined by broth microdilution

	MICs (ug/mL)					
	E. coli isolate					
β-Lactam(s)	N1949	TOP10 (NDM-35)	TOP10 (NDM-1)	TOP10 (NDM-5)	TOP10 (NDM-9)	TOP10
Amoxicillin	>512	>512	>512	>512	>512	4
Ticarcillin	>512	>512	>512	>512	>512	4
Piperacillin	>512	256	128	128	256	2
Cefoxitin	>512	512	256	256	>512	2
Ceftazidime	>512	>512	>512	>512	>512	0.5
Cefotaxime	>512	128	256	256	256	0.125
Cefepime	256	8	16	16	16	0.5
Aztreonam	64	0.125	0.125	0.125	0.25	≤0.125
Aztreonam + avibactam[a]	8	0.125	0.125	0.125	0.125	≤0.125
Imipenem	64	32	16	>32	16	0.125
Meropenem	128	8	4	>32	4	0.03
Ertapenem	>512	32	32	32	32	0.015
Temocillin	>512	32	32	32	256	8
Cefiderocol	256	2	1	1	2	≤0.125

[a]Avibactam supplemented at a concentration of 4 μg/mL.

determination of FDC MICs following the latest EUCAST recommendation (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Addenda/Cefiderocol_addendum_20200501.pdf). Interpretation was also performed following the EUCAST breakpoints, with resistance determined as >2 μg/mL for FDC and > 4 μg/mL for ATM, the latter being also applied here for ATM-AVI. *E. coli* N1949 was resistant to fluoroquinolones, sulfonamides, and tetracycline, and highly resistant to all β-lactams, including FDC and ATM-AVI (Table 1). In contrast, it remained susceptible to aminoglycosides, nitrofurantoin, tigecycline, and fosfomycin.

Whole-genome sequencing (WGS) was performed using an Illumina platform (Illumina, San Diego, CA) as described previously (13). Briefly, the total genomic DNA (gDNA) was extracted using a QIAamp DNA minikit and QIAcube (Qiagen) according to the manufacturer's instructions, and WGS was performed on an Illumina MiSeq instrument using the Nextera sample preparation method with 2 × 150 bp paired-end reads. *De novo* genome assembly was performed using the CLC Genomic Workbench (version 20.0.4; CLC Bio, Aarhus, Denmark), and contigs with a minimum length of 800 nucleotides (nt) were generated. The resulting assembled sequences were analyzed using ResFinder 4.1 (for antimicrobial resistance genes) and MLST 2.0 for multilocus sequence typing (MLST) analysis on the Center for Genomic Epidemiology server (http://www.genomicepidemiology.org/). The raw sequence data project had been deposited at GenBank under accession no PRJNA836848.

WGS analysis revealed that *E. coli* N1949 belonged to sequence type ST167. In addition to the $bla_{\text{NDM-35}}$ carbapenemase gene, *E. coli* N1949 possessed a series of antibiotic resistance genes, including *sul1* (sulfonamides), *dfrA12* (trimethoprim), *aadA2* (aminoglycosides), *mdf(A)*, *mph(A)* (macrolides), *tet(B)* (tetracycline), *catA1* (chloramphenicol), and $bla_{\text{CMY-145}}$, a derivative of $bla_{\text{CMY-2}}$ (broad-spectrum cephalosporins).

Mating-out assays, performed as described previously (14), gave an *E. coli* J53 transconjugant producing the NDM-35 carbapenemase with resistance or reduced susceptibility to β-lactams, as expected. Antibiotic susceptibility testing showed that this transconjugant was co-resistant to tetracycline, chloramphenicol, sulfonamides, and trimethoprim-sulfamethoxazole (data not shown). Plasmid extraction from the *E. coli* transconjugant followed by gel electrophoresis showed a plasmid size of ca. 250 kb. Plasmid-based replicon typing (15) identified this plasmid as belonging to the IncFIA/IncFIB incompatibility group.

Analysis of the PBP3 sequence showed a YRIN amino-acid insertion in comparison with the PBP3 sequence of the wild-type *E. coli* MG1655 (GenBank no. NC_000913.3) as reference. This 4-amino-acid insertion was previously shown to be associated with increased resistance to ATM-AVI (13). However, it remains to be determined whether this amino acid insertion plays a role in decreased susceptibility to FDC.

TABLE 2 Kinetic parameters of purified NDM enzymes[a]

	NDM-1			NDM-5			NDM-9			NDM-35		
β-Lactam(s)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
Penicillin G	220	110	2	320	100	3.2	18	60	0.3	230	150	1.5
Amoxicillin	220	170	1.3	220	1,000	0.22	12	75	0.16	140	300	0.45
Ticarcillin	40	100	0.4	35	150	0.23	45	170	0.26	30	75	0.4
Cephalothin	290	20	14.5	60	10	6	1.2	5	0.24	55	11	5
Cefotaxime	200	20	10	160	25	6.4	15	20	0.75	65	65	1
Ceftazidime	810	>1,000	0.8	450	>1,000	0.45	105	>1,000	0.10	115	>1,000	0.1
Imipenem	50	50	1	430	110	4	35	90	0.4	50	50	1
Ertapenem	45	20	2.2	65	65	1	20	12	1.7	70	60	1.2
Meropenem	35	40	0.85	550	80	6.8	27	27	1	90	45	2
Cefiderocol	0.9	280	0.003	0.7	230	0.003	4.6	200	0.02	7	270	0.03

[a]Standard deviations were below 15%. k_{cat}, catalytic efficiency; K_m, Michaelis constant; k_{cat}/K_m, specificity constant.

Analysis of the *cirA* gene encoding an iron transporter showed that it was truncated in isolate N1949, leading to a CirA-deficient strain, and therefore was likely a source of reduced susceptibility to FDC, as previously evidenced by complementation assays in *K. pneumoniae* (14). Finally, analysis of the iron-catecholate transporter FiuA encoding gene previously shown to be involved in reduced susceptibility to FDC (16) showed a wild-type sequence, and therefore it was not involved in FDC resistance here.

The high-level resistance to FDC observed in *E. coli* N1949 was therefore considered to be multifactorial, including at least the truncation of the CirA iron transporter, the production of a CMY-type AmpC β-lactamase, and possibly the production of a specific NDM β-lactamase.

To address the question of whether NDM-35 possesses specific hydrolytic properties in comparison with other NDM variants that could further enhance resistance to FDC, and whether NDM variants possess similar or distinct hydrolytic activities against FDC, we carried out a comparative study on the substrate selectivity of four NDM variants (NDM-1, NDM-5, NDM-9, NDM-35) by using isogenic strain backgrounds. The corresponding genes were cloned into the high-copy pUCp24 vector and expressed in the same *E. coli* TOP10 background, giving rise to recombinant strains producing NDM-1, NDM-5, NDM-9, and NDM-35, respectively (17). High-level resistance to amoxicillin, ticarcillin, piperacillin, cefoxitin, ceftazidime, and cefotaxime (MICs from 128 to >512 μg/mL) was observed for all of these recombinant strains (Table 1). However, significant differences were observed in MIC values for temocillin, carbapenems, and to a lesser extent, FDC. In particular, the NDM-5-producing *E. coli* recombinant strain showed ca. 4- to 16-fold higher MIC values of imipenem and meropenem compared to the NDM-1- and NDM-9-producing strains. Surprisingly, the NDM-35-producing *E. coli* recombinant strain showed significantly lower MIC values for these two carbapenems compared with the NDM-5-producing strain (Table 1). Notably, the MICs of FDC were 2 μg/mL for the NDM-35- and NDM-9-producing *E. coli* strains, 2-fold higher than those for the NDM-1- and NDM-5-producing strains (Table 1).

To further assess the variable hydrolytic properties of the different NDM enzymes suggested by the different MIC values observed, steady-state kinetic parameters were determined for the NDM-1, NDM-5, NDM-9, and NDM-35 enzymes. Purification of the NDM β-lactamases was performed using a two-step purification process in an AKTA Prime chromatography machine as described (18). A HiTrap Q column (GE Healthcare) was used in boths step after pre-equilibration with 20 mM piperazine buffer (pH 5.84) in the first step and 20 mM Tris buffer (pH 7.62) in the second step. The purified β-lactamase extracts were immediately used for enzymatic determinations (18).

Although all of the NDM variants hydrolysed penicillins at high levels in a similar manner, significant differences were observed for cephalosporins. NDM-1 and NDM-5 variants showed higher catalytic efficiencies for cefotaxime and ceftazidime compared to NDM-9 and NDM-35. The hydrolytic activity of NDM-35 toward imipenem and meropenem was weaker compared to that of NDM-5, although similar to those of NDM-1 and NDM-9. With respect to FDC, the hydrolytic activity of all variants remained relatively low (Table 2). Nevertheless, ca. 10-fold

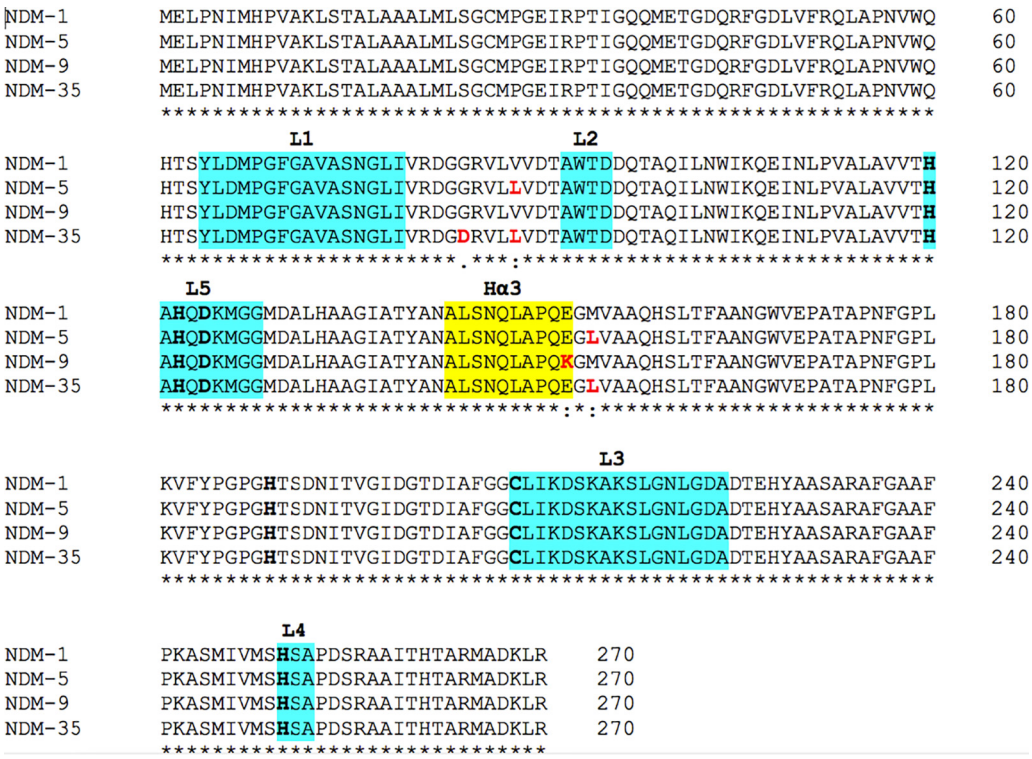

FIG 1 Alignment of NDM-1, NDM-5, NDM-9, and NDM-35 amino acid sequences. Active site loops and helix are highlighted in blue and yellow, respectively. Asterisks indicate the identical residues in the β-lactamases. Red residues are the amino acid substitutions of the different NDM variants in comparison with NDM-1.

higher k_{cat}/K_m values were observed for NDM-35 and NDM-9 compared to those of NDM-1 and NDM-5 (Table 2).

Alignment of the NDM variants showed that NDM-5, NDM-9, and NDM-35 proteins contained one, two, and three amino acid substitutions, respectively, compared to NDM-1 (Fig. 1). NDM-5 and NDM-35 share two amino acid substitutions (Val88Leu and Met154Leu) in comparison with NDM-1, which are not located in any active site loops (19, 20). NDM-35 differs from NDM-5 by a Gly-to-Asp amino acid substitution at position 84 (Gly84Asp), located between the L1 and L2 loops (Fig. 1). This substitution slightly impacted the ability of NDM-35 to compromise the efficacy of carbapenems, as observed through MIC and kinetic data determinations, but conversely increased its hydrolytic activity toward FDC.

The impact of NDM-35 on susceptibility to FDC was therefore proven here, and the high-level resistance to that antibiotic observed in *E. coli* N1949 could be explained by a combination of different mechanisms, including enzymatic degradation (NDM-35 production) and lower penetration into the bacterial cell (siderophore defect). As observed in this study for *E. coli*, we recently showed that susceptibility to FDC was impacted by the production of an NDM-type enzyme, as in *A. baumannii* (11). Interestingly, it was also recently shown that NDM enzymes facilitated the emergence of FDC resistance in *Enterobacter cloacae* through mutations in the CirA catecholate siderophore (21).

Finally, the FDC-resistant *E. coli* isolate was shown to belong to ST167, which is currently widely disseminating worldwide. Noteworthy, the wide spread of NDM-5 (only one single substitution with respect to NDM-35)-producing carbapenem-resistant ST167 *E. coli* has been recently evidenced in different European countries, including Switzerland, Germany, and Italy (22–28). It is therefore classified as a high-risk clone, being community-occurring but also a source of nosocomial outbreaks (27). This clonal strain accumulated diverse resistance mechanisms that made it resistant to almost all β-lactams, including carbapenems, the newly developed ceftazidime-avibactam and ceftolozane-tazobactam combinations, and aztreonam-avibactam. Here, we demonstrated that high-level resistance to FDC might be the ultimate step toward pan-β-lactam resistance. Interestingly, Simner et al. (29) recently

reported increased MICs of FDC upon selective pressure in a series of consecutive ST167 *E. coli* isolates recovered from a same patient from the United Arab Emirates and showed that the decreased susceptibility to FDC was related to increased bla_{NDM-5} expression through multiplication of bla_{NDM-5} copies. This suggests that close monitoring of ST167 *E. coli* strains should be carried out with respect to their susceptibility to FDC.

ACKNOWLEDGMENTS

This work was financed by the University of Fribourg, Switzerland, the NARA, and the Swiss National Science Foundation (grant no. FNS 310030_1888801).

REFERENCES

1. Nordmann P, Poirel L. 2019. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin Infect Dis 69:S521–S528. https://doi.org/10.1093/cid/ciz824.
2. Iovleva A, Doi Y. 2017. Carbapenem-resistant *Enterobacteriaceae*. Clin Lab Med 37:303–315. https://doi.org/10.1016/j.cll.2017.01.005.
3. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF. 2018. Carbapenemase-producing organisms: a global scourge. Clin Infect Dis 66:1290–1297. https://doi.org/10.1093/cid/cix893.
4. Nordmann P, Poirel L, Walsh TR, Livermore DM. 2011. The emerging NDM carbapenemases. Trends Microbiol 19:588–595. https://doi.org/10.1016/j.tim.2011.09.005.
5. Zhanel GG, Golden AR, Zelenitsky S, Wiebe K, Lawrence CK, Adam HJ, Idowu T, Domalaon R, Schweizer F, Zhanel MA, Lagacé-Wiens PRS, Walkty AJ, Noreddin A, Lynch Iii JP, Karlowsky JA. 2019. Cefiderocol: a siderophore cephalosporin with activity against carbapenem-resistant and multidrug-resistant Gram-negative bacilli. Drugs 79:271–289. https://doi.org/10.1007/s40265-019-1055-2.
6. Yahav D, Giske CG, Grāmatniece A, Abodakpi H, Tam VH, Leibovici L. 2020. New β-lactam-β-lactamase inhibitor combinations. Clin Microbiol Rev 34:e00115-20. https://doi.org/10.1128/CMR.00115-20.
7. Bush K. 2018. Past and present perspectives on β-lactamases. Antimicrob Agents Chemother 62:e01076-18. https://doi.org/10.1128/AAC.01076-18.
8. Kohira N, West J, Ito A, Ito-Horiyama T, Nakamura R, Sato T, Rittenhouse S, Tsuji M, Yamano Y. 2015. *In vitro* antimicrobial activity of a siderophore cephalosporin, S-649266, against *Enterobacteriaceae* clinical isolates, including carbapenem-resistant strains. Antimicrob Agents Chemother 16:729–734. https://doi.org/10.1128/AAC.01695-15.
9. Sato T, Yamawaki K. 2019. Cefiderocol: discovery, chemistry, and in vivo profiles of a novel siderophore cephalosporin. Clin Infect Dis 69:S538–S543. https://doi.org/10.1093/cid/ciz826.
10. Doi Y. 2019. Treatment options for carbapenem-resistant Gram-negative bacterial infections. Clin Infect Dis 69:S565–S575. https://doi.org/10.1093/cid/ciz830.
11. Poirel L, Sadek M, Nordmann P. 2021. Contribution of PER-type and NDM-type β-lactamases to cefiderocol resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 65:e0087721. https://doi.org/10.1128/AAC.00877-21.
12. Poirel L, Ortiz de la Rosa JM, Sadek M, Nordmann P. 2022. Impact of acquired broad-spectrum β-lactamases on susceptibility to cefiderocol and newly developed β-lactam/β-lactamase inhibitor combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 66:e0003922. https://doi.org/10.1128/aac.00039-22.
13. Sadek M, Juhas M, Poirel L, Nordmann P. 2020. Genetic features leading to reduced susceptibility to aztreonam-avibactam among metallo-β-lactamase-producing *Escherichia coli* isolates. Antimicrob Agents Chemother 64:e01659-20. https://doi.org/10.1128/AAC.01659-20.
14. Poirel L, Aires-de-Sousa M, Kudyba P, Kieffer N, Nordmann P. 2018. Screening and characterization of multidrug-resistant Gram-negative bacteria from a remote African area, São Tomé and Príncipe. Antimicrob Agents Chemother 62:e01021-18. https://doi.org/10.1128/AAC.01021-18.
15. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018.
16. Ito A, Sato T, Ota M, Takemura M, Nishikawa T, Toba S, Kohira N, Miyagawa S, Ishibashi N, Matsumoto S, Nakamura R, Tsuji M, Yamano Y. 2018. *In vitro* antibacterial properties of cefiderocol, a novel siderophore cephalosporin, against Gram-negative bacteria. Antimicrob Agents Chemother 62:e01454-17. https://doi.org/10.1128/AAC.01454-17.
17. Ortiz de la Rosa JM, Nordmann P, Poirel L. 2019. ESBLs and resistance to ceftazidime/avibactam and ceftolozane/tazobactam combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. J Antimicrob Chemother 74:1934–1939. https://doi.org/10.1093/jac/dkz149.
18. Poirel L, Ortiz De La Rosa JM, Kieffer N, Dubois V, Jayol A, Nordmann P. 2019. Acquisition of extended-spectrum β-lactamase GES-6 leading to resistance to ceftolozane-tazobactam combination in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 63:e01809-18. https://doi.org/10.1128/AAC.01809-18.
19. Khan S, Ali A, Khan AU. 2018. Structural and functional insight of New Delhi metallo β-lactamase-1 variants. Future Med Chem 10:221–229. https://doi.org/10.4155/fmc-2017-0143.
20. 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.
21. Nurjadi D, Kocer K, Chanthalangsy Q, Klein S, Heeg K, Boutin S. 2022. New Delhi metallo-β-lactamase facilitates the emergence of cefiderocol resistance in *Enterobacter cloacae*. Antimicrob Agents Chemother 66:e0201121. https://doi.org/10.1128/AAC.02011-21.
22. Findlay J, Poirel L, Kessler J, Kronenberg A, Nordmann P. 2021. New Delhi Metallo-β-lactamase-producing *Enterobacterales* bacteria, Switzerland, 2019–2020. Emerg Infect Dis 27:2628–2637. https://doi.org/10.3201/eid2710.211265.
23. McElheny CL, Fowler EL, Iovleva A, Shields RK, Doi Y. 2021. *In vitro* evolution of cefiderocol resistance in an NDM-producing *Klebsiella pneumoniae* due to functional loss of CirA. Microbiol Spectr 9:e0177921. https://doi.org/10.1128/Spectrum.01779-21.
24. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. 2019. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clin Microbiol Rev 32:e00115-18. https://doi.org/10.1128/CMR.00115-18.
25. Chakraborty T, Sadek M, Yao Y, Imirzalioglu C, Stephan R, Poirel L, Nordmann P. 2021. Cross-border emergence of *Escherichia coli* producing the carbapenemase NDM-5 in Switzerland and Germany. J Clin Microbiol 59:e02238-20. https://doi.org/10.1128/JCM.02238-20.
26. Garcia-Fernandez A, Villa L, Bibbolino G, Bressan A, Trancassini M, Pietropaolo V, Venditti M, Antonelli G, Carattoli A. 2020. Novel insights and features of the NDM-5-producing *Escherichia coli* Sequence Type 167 high-risk clone. mSphere 5:e00269-20. https://doi.org/10.1128/mSphere.00269-20.
27. Giufrè M, Errico G, Accogli M, Monaco M, Villa L, Distasi MA, Del Gaudio T, Pantosti A, Carattoli A, Cerquetti M. 2018. Emergence of NDM-5-producing *Escherichia coli* sequence type 167 clone in Italy. Int J Antimicrob Agents 52:76–81. https://doi.org/10.1016/j.ijantimicag.2018.02.020.
28. Bibbolino G, Di Lella FM, Oliva A, Lichtner M, Del Borgo C, Raponi G, Trancassini M, Mengoni F, Arcari G, Antonelli G, Carattoli A. 2021. Molecular epidemiology of NDM-5-producing *Escherichia coli* high-risk clones identified in two Italian hospitals in 2017–2019. Diagn Microbiol Infect Dis 100:115399. https://doi.org/10.1016/j.diagmicrobio.2021.115399.
29. Simner PJ, Mostafa HH, Bergman Y, Ante M, Tekle T, Adebayo A, Beisken S, Dzintars K, Tamma PD. 2021. Progressive development of cefiderocol resistance in *Escherichia coli* during therapy is associated with increased bla_{NDM-5} copy number and gene expression. Clin Infect Dis ciab888. https://doi.org/10.1093/cid/ciab888.