AMERICAN SOCIETY FOR MICROBIOLOGY | mSphere®

OBSERVATION
Clinical Science and Epidemiology

IMP-68, a Novel IMP-Type Metallo-β-Lactamase in Imipenem-Susceptible *Klebsiella pneumoniae*

Hiroaki Kubota,[a] Yasunori Suzuki,[a]* Rumi Okuno,[a] Yumi Uchitani,[a] Tsukasa Ariyoshi,[a] Nobuyuki Takemura,[b] Fuminori Mihara,[b] Kazuhisa Mezaki,[c] Norio Ohmagari,[d] Mari Matsui,[e] Satowa Suzuki,[e] Tsuyoshi Sekizuka,[f] Makoto Kuroda,[f] Keiko Yokoyama,[a] Kenji Sadamasu[a]

[a]Department of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan
[b]Department of Hepato-Biliary Pancreatic Surgery, National Center for Global Health and Medicine, Tokyo, Japan
[c]Microbiology Laboratory, National Center for Global Health and Medicine, Tokyo, Japan
[d]Disease Control and Prevention Center, National Center for Global Health and Medicine, Tokyo, Japan
[e]Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, Japan
[f]Pathogen Genomics Center, National Institute of Infectious Diseases, Tokyo, Japan

ABSTRACT We recently detected a novel variant of an IMP-type metallo-β-lactamase gene (bla_{IMP-68}) from meropenem-resistant but imipenem-susceptible *Klebsiella pneumoniae* TA6363 isolated in Tokyo, Japan. bla_{IMP-68} encodes a Ser262Gly point mutant of IMP-11, and transformation experiments showed that bla_{IMP-68} increased the MIC of carbapenems in recipient strains, whereas the MIC of imipenem was not greatly increased relative to that of other carbapenems, including meropenem. Kinetics experiments showed that IMP-68 imipenem-hydrolyzing activity was lower than that for other carbapenems, suggesting that the antimicrobial susceptibility profile of TA6363 originated from IMP-68 substrate specificity. Whole-genome sequencing showed that bla_{IMP-68} is harbored by the class 1 integron located on the IncL/M plasmid pTMTA63632 (88,953 bp), which was transferable via conjugation. The presence of plasmid-borne bla_{IMP-68} is notable, because it conferred antimicrobial resistance to carbapenems, except for imipenem, on *Enterobacteriaceae* and will likely affect treatment plans using antibacterial agents in clinical settings.

IMPORTANCE IMP-type metallo-β-lactamases comprise one group of the "Big 5" carbapenemases. Here, a novel bla_{IMP-68} gene encoding IMP-68 (harboring a Ser262Gly point mutant of IMP-11) was discovered from meropenem-resistant but imipenem-susceptible *Klebsiella pneumoniae* TA6363. The Ser262Gly substitution was previously identified as important for substrate specificity according to a study of other IMP variants, including IMP-6. We confirmed that IMP-68 exhibited weaker imipenem-hydrolyzing activity than that for other carbapenems, demonstrating that the antimicrobial susceptibility profile of TA6363 originated from IMP-68 substrate specificity, with this likely to affect treatment strategies using antibacterial agents in clinical settings. Notably, the carbapenem resistance conferred by IMP-68 was undetectable based on the MIC of imipenem as a carbapenem representative, which demonstrates a comparable antimicrobial susceptibility profile to IMP-6-producing *Enterobacteriaceae* that previously spread in Japan due to lack of awareness of its existence.

KEYWORDS *Enterobacteriaceae*, *Klebsiella*, antibiotic resistance, carbapenems, enzyme kinetics, genome analysis, plasmid-mediated resistance

IMP-type metallo-β-lactamases are among the most common families of acquired carbapenemases detected from *Enterobacteriaceae* and have been reported mainly in East Asia, including Japan (1). Although IMP-type metallo-β-lactamases generally hydrolyze carbapenems, this activity on imipenem by several IMP variants, including

Citation Kubota H, Suzuki Y, Okuno R, Uchitani Y, Ariyoshi T, Takemura N, Mihara F, Mezaki K, Ohmagari N, Matsui M, Suzuki S, Sekizuka T, Kuroda M, Yokoyama K, Sadamasu K. 2019. IMP-68, a novel IMP-type metallo-β-lactamase in imipenem-susceptible *Klebsiella pneumoniae*. mSphere 4:e00736-19. https://doi.org/10.1128/mSphere.00736-19.

Editor Mariana Castanheira, JMI Laboratories

Address correspondence to Hiroaki Kubota, Hiroaki_Kubota@member.metro.tokyo.jp, or Yasunori Suzuki, ysuzuki@vmas.kitasato-u.ac.jp.

* Present address: Yasunori Suzuki, Laboratory of Animal Hygiene, Kitasato University School of Veterinary Medicine, Aomori, Japan.

Hiroaki Kubota and Yasunori Suzuki contributed equally to this work.

Received 11 October 2019
Accepted 16 October 2019
Published 30 October 2019

TABLE 1 MICs of selected antimicrobial agents for the *K. pneumoniae* TA6363 strain and for the transformants and transconjugants carrying bla_{IMP-68} or bla_{IMP-11}

Antimicrobial agent	MIC (μg/ml) for strain:					
	K. pneumoniae TA6363	*E. coli* DH5α (pHSG398-bla_{IMP-68})	*E. coli* DH5α (pHSG398-bla_{IMP-11})	*E. coli* DH5α	*E. coli* J53 (pTMTA63632)	*E. coli* J53
Ampicillin	>256	16	64	2	>256	4
Piperacillin	>256	1	2	1	>256	2
Ceftazidime	8	64	>256	≤0.06	4	0.125
Cefotaxime	>32	>32	>32	≤0.06	>32	≤0.06
Cefepime	6	4	16	≤0.06	2	≤0.06
Aztreonam	8	<0.06	<0.06	<0.06	0.125	≤0.06
Meropenem	16	32	8	≤0.06	2	≤0.06
Imipenem	0.5	0.5	8	0.125	0.25	0.25
Doripenem	16	4	4	≤0.06	1	≤0.06
Ertapenem	>32	8	1	≤0.06	0.5	≤0.06
Gentamicin	>256	0.125	0.125	0.125	0.25	0.25
Amikacin	8	0.5	0.5	0.5	4	1
Ciprofloxacin	16	≤0.06	≤0.06	≤0.06	≤0.06	≤0.06
Tigecycline	2	0.125	0.125	0.125	0.125	0.125
Polymyxin B	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125	≤0.125

IMP-6, is weak (2–4). IMP-1 Ser262 is replaced with glycine in these variants, and *Enterobacteriaceae* encoding IMP-6 frequently show susceptibility to imipenem. This feature caused the spread of bla_{IMP-6}-harboring plasmids in Japan as determined after screening the antibiotic susceptibility of infectious bacteria to imipenem as a representative carbapenem (5–7). Here, we report a novel variant, IMP-68, which is a point mutant of IMP-11 corresponding to the Ser262Gly substitution.

Klebsiella pneumoniae TA6363 was isolated from ascites obtained from a hospitalized male patient suffering from peritonitis at the National Center of Global Health and Medicine in 2016. The patient was suspected to be domestically infected by this pathogen, because he had not been abroad from Japan within at least 90 days. This study was approved by the ethics committee of the Tokyo Metropolitan Institute of Public Health. TA6363 was initially determined to be resistant to meropenem by MicroScan walkaway 96 SI (Beckman Coulter, Brea, CA, USA) using the MicroScan Neg Combo EN 1T test card (Beckman Coulter), which employs meropenem as a carbapenem representative. However, this strain was subsequently found to be susceptible to imipenem using the dry strip method using Etest (bioMérieux, La Balme-Les-Grottes, France) (Table 1). TA6363 was positive for a modified carbapenem-inactivation method (8) and found to produce metallo-β-lactamase using the double-disk synergy test involving meropenem and sodium mercaptoacetate (Eiken Chemical, Tokyo, Japan) disks. Pulsed-field gel electrophoresis using an S1-nuclease-digested DNA plug (S1-PFGE) showed that TA6363 carried three plasmids (Fig. 1), with the second larger one (pTMTA63632; ~90 kbp) harboring the novel bla_{IMP} gene according to sequencing plasmids extracted from the S1-PFGE gel (MiSeq; Illumina, San Diego, CA, USA) (9, 10). We found that this bla_{IMP} gene encoded a Ser262Gly point mutant of IMP-11 metallo-β-lactamase, and we named this novel variant "bla_{IMP-68}" (see Fig. S1 in the supplemental material).

We tested the influence of IMP-68 on antibiotic resistance to β-lactams by transformation experiments. We cloned the open reading frame of bla_{IMP-68} into a chloramphenicol-resistant pHSG398 vector (Takara Bio, Shiga, Japan) at the EcoRI-KpnI site and used it to transform *Escherichia coli* DH5α cells (Takara Bio). Additionally, we tested bla_{IMP-11} for comparison, and conjugation transfer was tested using *E. coli* J53, as previously described (9). The MICs of β-lactams were determined by the dry strip method using Etest. To determine whether IMP-68 production was detectable by several phenotypic tests other than the modified carbapenem-inactivation method, TA6363 and the *E. coli* J53 transconjugant were applied to the modified Hodge (11), Carba NP (12), and Blue-Carba (13) tests.

To purify IMP-11 and IMP-68, the nucleotide sequence for the restriction site of the

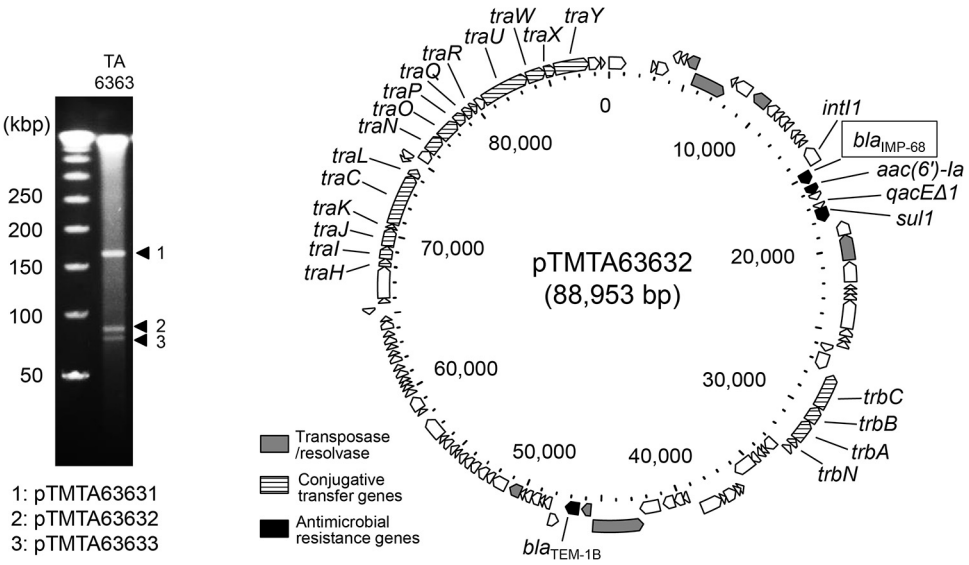

FIG 1 The bla_{IMP-68}-carrying plasmid pTMTA63632. S1-PFGE pattern of TA6363 showing that three plasmids were carried by TA6363 (left). The circular map of pTMTA63632 (right), which harbored bla_{IMP-68}, was generated by GView server (https://server.gview.ca/).

PreScission protease (GE Healthcare, Chicago, IL, USA) was added to the N terminus of bla_{IMP-11} and bla_{IMP-68} after the signal peptide region (first 19 amino acids) by PCR, followed by cloning into the pETBK vector (BioDynamics, Tokyo, Japan) for expression in *E. coli* Rosetta II cells (Merck-Millipore, Darmstadt, Germany). His-tagged proteins were purified using a nickel-nitrilotriacetic acid (Ni-NTA) resin (Qiagen, Hilden, Germany) and digested by PreScission protease to remove the His tag.

Kinetics experiments were performed by spectrophotometry (14–16), with initial hydrolysis rates for each β-lactam measured using a Biomate 3 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 298 nm for imipenem and meropenem, 260 nm for ceftazidime and cefotaxime, and 235 nm for ampicillin at 24 $\pm$ 1°C in 50 mM sodium phosphate buffer (pH 7.0) supplemented with 50 μM $ZnCl_2$. K_m and k_{cat} values were determined by the Michaelis-Menten equation. Experiments were performed in triplicate.

Table 1 shows the MICs for TA6363 and *E. coli* transformants. Although TA6363 was resistant to meropenem, doripenem, and ertapenem, the MIC of imipenem for TA6363 was classified as susceptible (S) according to Clinical and Laboratory Standards Institute criteria (12). *E. coli* transformation by bla_{IMP-68} alone significantly increased the MICs of β-lactams, and the substrate specificity of IMP-68 and IMP-11 differed. The lower MIC of imipenem for IMP-68 than that for IMP-11 was similar to the relationship between IMP-6 and IMP-1, where the MIC of imipenem for IMP-6 is lower than that for IMP-1 (2). Furthermore, the bla_{IMP-68}-carrying native plasmid pTMTA63632 was transferred by conjugation from TA6363 to *E. coli* J53, resulting in enhanced antibiotic resistance via pTMTA63632. TA6363 and the transconjugant were positive for all the above-mentioned tested phenotypic methods. Despite the use of imipenem, the Carba NP and Blue-Carba tests were sensitive enough to detect IMP-68 production.

Several differences in substrate specificity between IMP-11 and IMP-68 identified during antibiotic susceptibility testing were consistent with the results of kinetics experiments (Table 2). The k_{cat}/K_m values of IMP-68 against imipenem were lower than those of IMP-11, whereas IMP-68 exhibited higher meropenem-hydrolyzing activity. Additionally, the k_{cat}/K_m values of IMP-68 against ceftazidime were lower than those of IMP-11. These differences in substrate specificity between IMP-68 and IMP-11 correlated with those between IMP-6 and IMP-1 (2).

Additionally, genomic DNA extracted from TA6363 was sequenced on MiSeq and

TABLE 2 Kinetic parameters of IMP-11 and IMP-68

Antimicrobial agent	IMP-68			IMP-11		
	K_m (μM)[a]	k_{cat} (s^{-1})[a]	k_{cat}/K_m (μM^{-1} s^{-1})	K_m (μM)[a]	k_{cat} (s^{-1})[a]	k_{cat}/K_m (μM^{-1} s^{-1})
Ampicillin	465 ± 200	1.7 ± 0.44	0.0037	830 ± 248	15.0 ± 1.9	0.018
Ceftazidime	326 ± 131	3.9 ± 2.1	0.012	232 ± 18.9	8.8 ± 3.7	0.038
Cefotaxime	10.3 ± 2.3	25.7 ± 1.3	2.5	11.8 ± 2.2	9.0 ± 1.6	0.84
Meropenem	10.1 ± 0.94	9.7 ± 1.7	0.89	14.8 ± 2.8	5.5 ± 0.50	0.37
Imipenem	347 ± 52.2	36.7 ± 7.8	0.11	41 ± 18.4	21.9 ± 4.7	0.54

[a]Presented as the mean ± standard deviation.

MinION platforms (Oxford Nanopore, Oxford, United Kingdom), and the obtained reads were assembled by Unicycler (v.0.4.7) (17). Genes were predicted and annotated using Prokka (v.1.11) (18), NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and ResFinder (v.3.1) (19). The Inc types of the plasmids were determined by PlasmidFinder (v.2.0) (20), with the threshold of nucleotide coverage and identity used for Inc-type identification established at 96% and 98%, respectively (21).

We obtained four circular sequences (Table S1) corresponding to a chromosome and three plasmids (Fig. 1 and Fig. S2). TA6363 was classified as ST268, and pTMTA63632 was determined to be an IncL/M plasmid. Chromosomal mutations corresponding to antimicrobial resistance to carbapenems (*ompK36*, *ompK37*, and *acrR*) and fluoroquinolones (*gyrA* and *parC*) were not found (Table S1). Class 1-integron-harboring bla_{IMP-68} in pTMTA63632 (Fig. 1), which was assigned to In1702 in the INTEGRALL database (22), was similar to previously reported IMP genes harboring class 1 integrons, as the gene cassettes were located between *intI1* and *qacEΔ1-sul1* (10, 23, 24). Specifically, In1702 was compared with the class 1 integrons harbored by pNUH14_ECL028_1 and pIMP-A2015-49 plasmids previously found in Japan (24), which carried IMP-1 and IMP-11, respectively (Fig. S3). The inclusion of conjugation transfer genes by pTMTA63632 was consistent with the conjugation experiment performed using *E. coli* J53 (Table 1). The higher MICs of ampicillin and piperacillin for the transconjugant than for bla_{IMP-68} alone (Table 1) likely originated from the effect of bla_{TEM-1} in pTMTA63632. TA6363 was resistant to both amikacin and gentamicin, whereas the transconjugant was resistant only to amikacin. This difference was attributable to the carriage of aminoglycoside resistance genes by pTMTA63632 [*aac(6′)-Ia*] and pTMTA63633 [*aac(3)-IId*, *aph(3′)-Ib*, and *aph(6)-Id*] (Table S1) (25); namely, pTMTA63632 conferred antimicrobial resistance to amikacin, whereas pTMTA63633 was additionally necessary for the gentamicin resistance.

In conclusion, IMP-68 should be noted as a novel carbapenemase that does not sufficiently confer resistance to imipenem on *Enterobacteriaceae* due to the Ser262Gly substitution from IMP-11. IMP-68 production would have been missed if the MIC of imipenem had been used to investigate the carbapenemase-producing *Enterobacteriaceae*. The diversity of substrate specificity among IMP-type enzymes might affect treatment plans using antibacterial agents in clinical settings.

Data availability. We deposited the bla_{IMP-68} sequence in GenBank (MF669572) and the whole-genome sequence of TA6363 containing chromosome (AP019665), pTMTA63631 (AP019666), pTMTA63632 (AP019667), and pTMTA63633 (AP019668) in the DNA Data Bank of Japan (DDBJ).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00736-19.

FIG S1, TIF file, 1.5 MB.

FIG S2, TIF file, 1.7 MB.

FIG S3, TIF file, 1 MB.

TABLE S1, DOCX file, 0.02 MB.

ACKNOWLEDGMENTS

This work was supported by the Research Program on Emerging and Re-emerging Infectious Diseases from the Japan Agency for Medical Research and Development, AMED (grants 17fk0108121j and 18fk0108048j) and by a grant for Research on Emerging and Re-emerging Infectious Diseases and Immunization from the Japanese Ministry of Health, Labour and Welfare (grant H28-Shinko Gyosei-Ippan-006).

REFERENCES

1. Nordmann P, Poirel L. 2014. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 20:821–830. https://doi.org/10.1111/1469-0691.12719.
2. Yano H, Kuga A, Okamoto R, Kitasato H, Kobayashi T, Inoue M. 2001. Plasmid-encoded metallo-beta-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. Antimicrob Agents Chemother 45:1343–1348. https://doi.org/10.1128/AAC.45.5.1343-1348.2001.
3. Iyobe S, Kusadokoro H, Ozaki J, Matsumura N, Minami S, Haruta S, Sawai T, O'Hara K. 2000. Amino acid substitutions in a variant of IMP-1 metallo-beta-lactamase. Antimicrob Agents Chemother 44:2023–2027. https://doi.org/10.1128/aac.44.8.2023-2027.2000.
4. Tada T, Nhung PH, Miyoshi-Akiyama T, Shimada K, Phuong DM, Anh NQ, Ohmagari N, Kirikae T. 2015. IMP-51, a novel IMP-type metallo-β-lactamase with increased doripenem- and meropenem-hydrolyzing activities, in a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother 59:7090–7093. https://doi.org/10.1128/AAC.01611-15.
5. Yano H, Ogawa M, Endo S, Kakuta R, Kanamori H, Inomata S, Ishibashi N, Aoyagi T, Hatta M, Gu Y, Yamada M, Tokuda K, Kunishima H, Kitagawa M, Hirakata Y, Kaku M. 2012. High frequency of IMP-6 among clinical isolates of metallo-β-lactamase-producing *Escherichia coli* in Japan. Antimicrob Agents Chemother 56:4554–4555. https://doi.org/10.1128/AAC.00617-12.
6. Shigemoto N, Kuwahara R, Kayama S, Shimizu W, Onodera M, Yokozaki M, Hisatsune J, Kato F, Ohge H, Sugai M. 2012. Emergence in Japan of an imipenem-susceptible, meropenem-resistant *Klebsiella pneumoniae* carrying blaIMP-6. Diagn Microbiol Infect Dis 72:109–112. https://doi.org/10.1016/j.diagmicrobio.2011.09.019.
7. Kanazawa S, Sato T, Kohira N, Ito-Horiyama T, Tsuji M, Yamano Y. 2017. Susceptibility of imipenem-susceptible but meropenem-resistant bla_{IMP-6}-carrying *Enterobacteriaceae* to various antibacterials, including the siderophore cephalosporin cefiderocol. Antimicrob Agents Chemother 61:e00576-17. https://doi.org/10.1128/AAC.00576-17.
8. Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, Bobenchik AM, Lockett ZC, Charnot-Katsikas A, Ferraro MJ, Thomson RB, Jr, Jenkins SG, Limbago BM, Das S. 2017. Modified carbapenem inactivation method (mCIM) for phenotypic detection of carbapenemase production among Enterobacteriaceae. J Clin Microbiol 55:2321–2333. https://doi.org/10.1128/JCM.00193-17.
9. Kubota H, Uwamino Y, Matsui M, Sekizuka T, Suzuki Y, Okuno R, Uchitani Y, Ariyoshi T, Aoki W, Suzuki S, Kuroda M, Shinkai T, Yokoyama K, Sadamasu K, Funakoshi T, Murata M, Hasegawa N, Iwata S. 2018. FRI-4 carbapenemase-producing *Enterobacter cloacae* complex isolated in Tokyo, Japan. J Antimicrob Chemother 73:2969–2972. https://doi.org/10.1093/jac/dky291.
10. Sekizuka T, Matsui M, Takahashi T, Hayashi M, Suzuki S, Tokaji A, Kuroda M. 2018. Complete genome sequence of bla_{IMP-6}-positive *Metakosakonia* sp. MRY16-398 isolate from the ascites of a diverticulitis patient. Front Microbiol 9:2853. https://doi.org/10.3389/fmicb.2018.02853.
11. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing; 27th informational supplement. CLSI Document M100. CLSI, Wayne, PA.
12. Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing; 29th informational supplement. CLSI Document M100. CLSI, Wayne, PA.
13. Pires J, Novais A, Peixe L. 2013. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol 51:4281–4283. https://doi.org/10.1128/JCM.01634-13.
14. Poirel L, Le Thomas I, Naas T, Karim A, Nordmann P. 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum beta-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob Agents Chemother 44:622–632. https://doi.org/10.1128/aac.44.3.622-632.2000.
15. Boschi L, Mercuri PS, Riccio ML, Amicosante G, Galleni M, Frère JM, Rossolini GM. 2000. The Legionella (Fluoribacter) gormanii metallo-beta-lactamase: a new member of the highly divergent lineage of molecular-subclass B3 beta-lactamases. Antimicrob Agents Chemother 44:1538–1543. https://doi.org/10.1128/aac.44.6.1538-1543.2000.
16. Queenan AM, Shang W, Flamm R, Bush K. 2010. Hydrolysis and inhibition profiles of beta-lactamases from molecular classes A to D with doripenem, imipenem, and meropenem. Antimicrob Agents Chemother 54:565–569. https://doi.org/10.1128/AAC.01004-09.
17. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
18. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
19. 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.
20. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
21. 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.
22. Moura A, Soares M, Pereira C, Leitão N, Henriques I, Correia A. 2009. INTEGRALL: a database and search engine for integrons, integrases and gene cassettes. Bioinformatics 25:1096–1098. https://doi.org/10.1093/bioinformatics/btp105.
23. Zhao WH, Chen G, Ito R, Kimura S, Hu ZQ. 2012. Identification of a plasmid-borne blaIMP-11 gene in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. J Med Microbiol 61:246–251. https://doi.org/10.1099/jmm.0.035626-0.
24. Tetsuka N, Hirabayashi A, Matsumoto A, Oka K, Hara Y, Morioka H, Iguchi M, Tomita Y, Suzuki M, Shibayama K, Yagi T. 2019. Molecular epidemiological analysis and risk factors for acquisition of carbapenemase-producing Enterobacter cloacae complex in a Japanese university hospital. Antimicrob Resist Infect Control 8:126. https://doi.org/10.1186/s13756-019-0578-3.
25. Shaw KJ, Rather PN, Hare RS, Miller GH. 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 57:138–163.