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Description of a novel IncP plasmid harboring bla_{KPC-2} recovered from a SPM-1-producing *Pseudomonas aeruginosa* from ST277

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ABSTRACT

The high rates of carbapenem resistance among Brazilian *Pseudomonas aeruginosa* isolates are mainly associated with the clone ST277 producing the carbapenemase SPM-1. Here, the complete genetic composition of a IncP plasmid harboring bla_{KPC-2} in isolates of this endemic clone carrying chromosomal bla_{SPM-1} was described using whole genome sequencing. These results confirm the association of these two carbapenemases in ST277 and also describe the genetic composition of a novel bla_{KPC-2}-plasmid. Considering the fact that this association occurs in a high-risk clone, monitoring the dissemination of this plasmid should be a public health concern.

Dears,

The carbapenemase encoding gene bla_{KPC} has disseminated to various species (Brandt et al., 2019; Villegas et al., 2007; Yigit et al., 2001). In Brazil, despite KPC being disseminated in *Klebsiella pneumoniae* and other *Enterobacterales* since 2006, its first report in *P. aeruginosa* was in 2012 (De Araújo Jácome et al., 2012). By 2014, Brazilian isolates from different pulsotypes carrying both KPC and SPM-1 were observed (Rizek et al., 2014). Since then, different Brazilian *P. aeruginosa* clones carrying chromosomal and plasmid bla_{KPC-2} have been reported (Carrara-Marroni et al., 2015; de Oliveira Santos et al., 2018; de Paula-Petroli et al., 2018; Galetti et al., 2019).

Incompatibility group for a Brazilian *P. aeruginosa* KPC-plasmid was described once: a IncQ1 plasmid in a single ST2584 isolate (de Oliveira Santos et al., 2018). IncP plasmids carrying bla_{KPC} in *P. aeruginosa* have been described in China and Colombia (Dai et al., 2016; Naas et al., 2013; Wang et al., 2021).

The high rates of carbapenem resistance among Brazilian *P. aeruginosa* isolates are associated with ST277, an endemic clone producing metallo-β-lactamase (MβL) SPM-1 (de Oliveira Santos et al., 2019; Labarca et al., 2014), which acquired other specific genes assuming a multidrug resistant profile (Silveira et al., 2020). Here we describe the genetic composition of a bla_{KPC-2} harboring IncP plasmid in ST277 isolates carrying chromosomal bla_{SPM-1}.

From April to July 2020, three carbapenem-resistant *P. aeruginosa* isolates (CCBH28525, CCBH28189 and CCBH28529) were sent to the LAPIH-IOC/Fiocruz, where bla_{KPC} and bla_{SPM} genes were detected by PCR. They were isolated from tracheal secretion of different patients admitted to a hospital in Bahia state (Brazilian Northeast).

Isolates were sequenced with Illumina technology and CCBH28525 was also submitted to nanopore long-read sequencing . A hybrid assembly for CCBH28525 was performed with Unicycler (Wick et al., 2017) and for the others only Illumina reads were used. Draft genomes were submitted to GenBank and annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) with accession numbers CP086064-CP086065 (CCBH28525), JAHFYU000000000.1 (CCBH28189), and JAHFYV000000000.1 (CCBH28529) (Supplementary Table 1).

Two circularized contigs were obtained for CCBH28525, one

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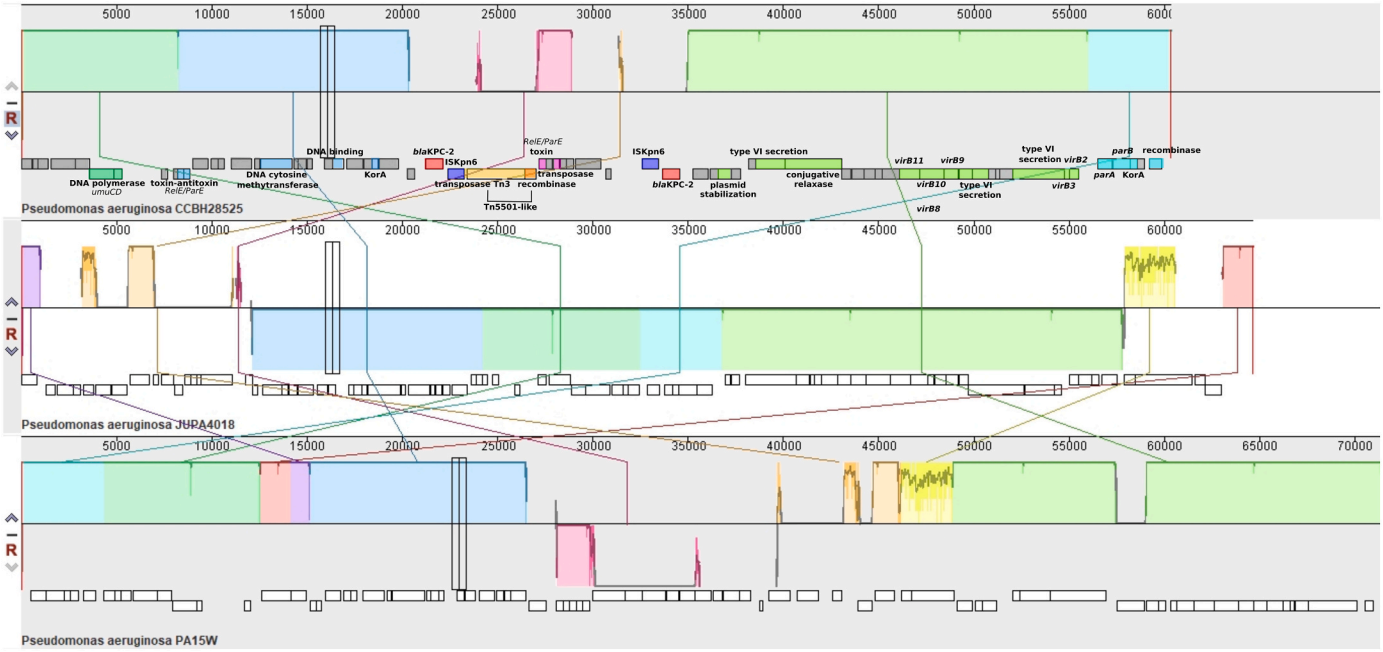

Fig. 1. Comparison of plasmid pCCBH28525 from *P. aeruginosa* CCBH28525 against pJUPA4018 from *P. aeruginosa* JUPA4018 and pPA15W from *P. aeruginosa* PA15W. The CDS for proteins shared by the three plasmids are highlighted according to Localized Co-linear Blocks (LCB) colors. CDS for hypothetical proteins or only domain annotations are highlighted in gray.

chromosome and a plasmid of 60,312 bp. S1-nuclease PFGE confirmed a single plasmid in all isolates with the same molecular weight (data not shown).

Using ABRicate (T. Seemann, https://github.com/tseemann/abricate) against ResFinder (Zankari et al., 2012), the antimicrobial resistance genes bla_{PAO}, *fosA*, *sul1*, *aph(3')-IIb*, *rmtD*, *aadA7*, $bla_{OXA-396}$, bla_{OXA-56}, *catB7*, *cmx* and bla_{SPM-1} were found in CCBH28525's chromosome . Duplicated copies of bla_{KPC-2} were identified in the CCBH28525 plasmid (pCCBH28525). The same genes were detected in CCBH28189 and CCBH28529.

Co-production of KPC-2 and SPM-1 can influence therapeutic options. New agents against carbapenem-resistant gram-negative pathogens must be carefully considered. For example, ceftazidime-avibactam (CZA), meropenem-vaborbactam, and imipenem-cilastatin-relebactam have activity against class A carbapenemases but not against MβL. Besides, most β-lactamase inhibitors are unable to inhibit MβL activity (Doi, 2019).

Susceptibilities were determined by disk diffusion and interpreted according to CLSI breakpoints (2020). All isolates were resistant to CZA, amikacin, piperacillin/tazobactam, ceftazidime, cefepime, meropenem, gentamicin and ciprofloxacin. The underlying resistance mechanisms to CZA in *P. aeruginosa* are variable but for our isolates the MβL SPM-1 could explain it. Mutations in AmpC (T105A, R79Q) and NalC (S209R, G71E) could also be related to this phenotype (López-Causapé et al., 2018; Wang et al., 2020) (Supplementary Table 1).

All isolates analyzed were assigned to ST277. Only now we can clearly associate bla_{SPM-1} and bla_{KPC-2} to the high-risk endemic clone ST277, as in previously report MLST was not performed (Rizek et al., 2014).

Most characteristic ST277 genomic islands were present or partially present in CCBH28525 (Supplementary Fig. 1). Genes conferring antibiotic resistance such as bla_{SPM-1}, bla_{OXA-56}, *rmtD, cmx,* and *sul1* were in PAGI-15 and -25, which are nearly complete (Silveira et al., 2016). CRISPR-Cas systems were present in all isolates, carrying the characteristic 39 spacers of ST277 (Silveira et al., 2020).

pCCBH28525 was taken as a representative plasmid. PlasmidFinder (Carattoli et al., 2014) analysis could not determine the plasmid Inc. group, while Mob-typer tool (Robertson and Nash, 2018) results classified it as a conjugative IncP plasmid. pCCBH28525 backbone can be divided into distinct modules: replication (polymerase *umuC/umuD*), stability (*parA*, *parB*, toxin-antitoxin system RelE/ParE family), propagation (protein complex of the type IV secretion system -VirB homologous-, conjugative relaxase) and adaptation (bla_{KPC-2}). The mechanisms of active segregation and post segregational cell killing promoted by *parA-parB* operon and type II toxin−/antitoxin system respectively, can avoid plasmid loss and contribute to the maintenance of the KPC carrying plasmid (Yano et al., 2019).

The best matches from pCCBH28525 BLASTn alignments against NCBI nr database were eight plasmids carrying MβL genes bla_{VIM} or bla_{IMP}. pBH6 from *P. aeruginosa* strain BH9 (NZ_CP029714), which was the only fully sequenced and assembled bla_{KPC-2}-plasmid from this specie isolated in Brazil, covers only 9% of pCCBH28525.

Alignments between pCCBH28525 and representative bla_{VIM} (pJUPA4018) and bla_{IMP} (pPA15W) plasmids showed shared replication, stability, and propagation modules, but not the bla_{KPC-2} and its related ISs (Darling et al., 2004) (Fig. 1). All encode the KorA, a plasmid-encoded global transcription regulator characteristic of IncP plasmids (Rajasekar et al., 2016). Mob-typer tool results confirmed pJUPA4018 and pPA15W as conjugative IncP plasmids. Other IncP plasmids carrying the bla_{KPC-2} have been described in *P. aeruginosa*, although the overall genetic structure is different (Dai et al., 2016; Naas et al., 2013; Wang et al., 2021). So, it seems more likely that genetic recombination occurred between IncP/MβL plasmid and KPC transposons than a genetic transfer from *P. aeruginosa* strains.

Tn4401 is the most prominent transposon type for bla_{KPC-2}, also including *P. aeruginosa* KPC-plasmids (Brandt et al., 2019). We could not detect *ISKpn7* (CP004367 region: 32468..34423) or *ISKpn8* (KF826292 region: 9739..10822), which are ISs described in *Tn4401*-like transposons (Knapp et al., 2018), upstream of bla_{KPC-2}. However, this same absence has been previously observed in Brazilian *P. aeruginosa* (Carrara-Marroni et al., 2015; de Paula-Petroli et al., 2018). Upstream both copies of the bla_{KPC-2} gene only a 73 bp segment is identical to *Tn4401* (EU176011), which is interrupted by non-coding DNA sequences. Similar structure has been described in a KPC-2 positive Colombian

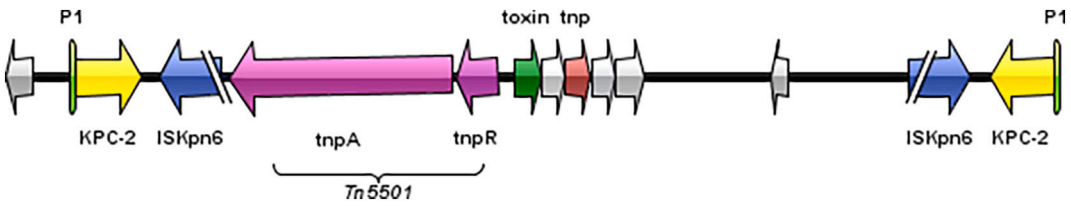

Fig. 2. Genetic context of *bla*$_{KPC2}$in plasmid pCCBH28525. The CDS for hypothetical proteins are highlighted in gray. ISKpn6 truncation is represented by // symbol.

P. aeruginosa isolate, in a plasmid lacking genes involved in mobilization, partitioning and conjugation. The 73 bp segment contains the P1 promoter, and in this situation the *bla*$_{KPC-2}$ gene is likely to still be expressed (Naas et al., 2013) (Fig. 2).

A truncated *ISKpn6* was detected downstream the first *bla*$_{KPC-2}$ gene, with a conserved IRR and disrupted by a Tn3 family transposon in the other end, but in the same sense (https://www-is.biotoul.fr/). Mobile Element Finder (Johansson et al., 2021) annotated this as *Tn5501* from *Pseudomonas putida*. The complete *Tn5501* was present, including recombinase (*tnpR*) and transposase (*tnpA*). After about 5000 bp, another truncated *ISKpn6*, also conserving the IRR, was detected downstream the second *bla*$_{KPC-2}$ gene (Fig. 2).

Presumably, truncation of *Tn4401* prevents further transposition (Sheppard et al., 2018). However, *Tn5501-like* could provide alternative routes for *bla*$_{KPC-2}$ mobilization. Furthermore, the chimera of transposon-associated elements indicated a novel genetic environment for the *bla*$_{KPC-2}$ gene in Brazilian ST277 *P. aeruginosa* isolates.

Although the co-presence of *bla*$_{SPM}$ and *bla*$_{KPC}$ in *P. aeruginosa* isolates has been reported, neither the sequence type nor the genetic context was characterized. Since *bla*$_{KPC}$ is located in a novel IncP plasmid, this information could impact on treatment and may expand epidemiological surveillance regarding nosocomial Brazilian *P.aeruginosa* isolates. As ST277 is widespread in Brazil, these strains could act as a novel reservoir.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105302.

Author contributions

AC-A conceived the research. RL, TO, CT-T, IS were responsible for the execution of phenotypic and molecular tests to identify and detect resistance of Gram-negative bacteria. IS executed the PFGE analyses. AC-A and CR-S participated in the selection of sequenced strains. MS, CR-S, RL, EM and RMA participated in method design. MS, RL, RMA and GK were responsible for data handling. CR-S, RL, RP and GK executed the whole genome sequencing. MS performed the data analysis. MS and RMA made the figures. AC-A, MS, CR-S, RMA, RP and GK wrote parts and edited the complete manuscript. All authors have read and approved the manuscript.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Brandt, C., Viehweger, A., Singh, A., Pletz, M.W., Wibberg, D., Kalinowski, J., Lerch, S., Müller, B., Makarewicz, O., 2019. Assessing genetic diversity and similarity of 435 KPC-carrying plasmids. Sci. Rep. 9, 11223. https://doi.org/10.1038/s41598-019-47758-5.

Carattoli, A., Zankari, E., Garciá-Fernández, A., Larsen, M.V., Lund, O., Villa, L., Aarestrup, F.M., 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.

Carrara-Marroni, F.E., Cayô, R., Streling, A.P., Da Silva, A.C.R., Palermo, R.L., Romanin, P., Venâncio, E., Perugini, M.R.E., Pelisson, M., Gales, A.C., 2015. Emergence and spread of KPC-2-producing P*seudomonas aeruginosa* isolates in a Brazilian teaching hospital. J. Glob. Antimicrob. Resist. 3, 304–306. https://doi.org/10.1016/j.jgar.2015.07.002.

Dai, X., Zhou, D., Xiong, W., Feng, J., Luo, W., Luo, G., Wang, H., Sun, F., Zhou, X., 2016. The IncP-6 plasmid p10265-KPC from *Pseudomonas aeruginosa* carries a novel ΔISEc33-associated *bla*$_{KPC-2}$ gene cluster. Front. Microbiol. 0, 310. https://doi.org/10.3389/FMICB.2016.00310.

Darling, A.C.E., Mau, B., Blattner, F.R., Perna, N.T., 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res. 14, 1394–1403. https://doi.org/10.1101/gr.2289704.tion.

De Araújo Jácome, P.R.L., Rodrigues Alves, Í., Borges Cabral, A., Lopes, A.C.S., Vieira Maciel, M.A., 2012. First report of KPC-producing *Pseudomonas aeruginosa* in Brazil. Antimicrob. Agents Chemother. 56, 4990. https://doi.org/10.1128/AAC.00699-12.

de Oliveira Santos, I.C., Albano, R.M., Asensi, M.D., D'Alincourt Carvalho-Assef, A.P., 2018. Draft genome sequence of KPC-2-producing *Pseudomonas aeruginosa* recovered from a bloodstream infection sample in Brazil. J. Glob. Antimicrob. Resist. 15, 99–100. https://doi.org/10.1016/j.jgar.2018.08.021.

de Oliveira Santos, I.C., Pereira de Andrade, N.F., da Conceição Neto, O.C., da Costa, B. S., de Andrade Marques, E., Rocha-de-Souza, C.M., Asensi, M.D., D'Alincourt Carvalho-Assef, A.P., 2019. Epidemiology and antibiotic resistance trends in clinical isolates of *Pseudomonas aeruginosa* from Rio de janeiro - Brazil: importance of mutational mechanisms over the years (1995–2015). Infect. Genet. Evol. 73, 411–415. https://doi.org/10.1016/J.MEEGID.2019.05.015.

de Paula-Petroli, S.B., Campana, E.H., Bocchi, M., Bordinhão, T., Picão, R.C., Yamada-Ogatta, S.F., Carrara-Marroni, F.E., 2018. Early detection of a hypervirulent KPC-2-producing *Pseudomonas aeruginosa* ST235 in Brazil. J. Glob. Antimicrob. Resist. 12, 153–154. https://doi.org/10.1016/j.jgar.2018.01.014.

Doi, Y., 2019. Clinical infectious diseases treatment options for Carbapenem-resistant gram-negative bacterial infections. Clin. Infect. Dis. ® 69, 565–575. https://doi.org/10.1093/cid/ciz830.

Galetti, R., Andrade, L.N., Varani, A.M., Darini, A.L.C., 2019. A phage-like plasmid carrying *bla*$_{KPC-2}$ gene in carbapenem-resistant *Pseudomonas aeruginosa*. Front. Microbiol. 10, 572. https://doi.org/10.3389/fmicb.2019.00572.

Johansson, M.H.K., Bortolaia, V., Tansirichaiya, S., Aarestrup, F.M., Roberts, A.P., Petersen, T.N., 2021. Detection of mobile genetic elements associated with antibiotic resistance in *salmonella enterica* using a newly developed web tool: MobileElementFinder. J. Antimicrob. Chemother. 76, 101–109. https://doi.org/10.1093/JAC/DKAA390.

Knapp, C.W., Novais, A., Zhao, Z., Huang, J., Ding, H., Shi, Y., Zhao, Y., Hu, X., Ren, J., Huang, G., Wu, R., 2018. Further spread of a *bla*$_{KPC}$-harboring untypeable plasmid in *Enterobacteriaceae* in China. Front. Microbiol. https://doi.org/10.3389/fmicb.2018.01938 www.frontiersin.org 1.

Labarca, J.A., Salles, M.J.C., Seas, C., Guzmán-Blanco, M., 2014. Carbapenem Resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the Nosocomial Setting in Latin America. doi:10.3109/1040841X.2014.940494 42, 276–292. https://doi.org/10.3109/1040841X.2014.940494.

López-Causapé, C., Cabot, G., del Barrio-Tofiño, E., Oliver, A., 2018. The versatile mutational resistome of *Pseudomonas aeruginosa*. Front. Microbiol. 9, 1–9. https://doi.org/10.3389/fmicb.2018.00685.

Naas, T., Bonnin, R.A., Cuzon, G., Villegas, M.-V., Nordmann, P., 2013. Complete sequence of two KPC-harbouring plasmids fro*m Pseudomonas aeruginosa*. J. Antimicrob. Chemother. 68, 1757–1762. https://doi.org/10.1093/JAC/DKT094.

Rajasekar, K.V., Lovering, A.L., Dancea, F., Scott, D.J., Harris, S.A., Bingle, L.E.H., Roessle, M., Thomas, C.M., Hyde, E.I., White, S.A., 2016. Flexibility of KorA, a plasmid-encoded, global transcription regulator, in the presence and the absence of its operator. Nucleic Acids Res. 44, 4947. https://doi.org/10.1093/NAR/GKW191.

Rizek, C., Fu, L., Cavalcanti dos Santos, L., Leite, G., Ramos, J., Rossi, F., Guimaraes, T., Levin, A.S., Figueiredo Costa, S., 2014. Characterization of carbapenem-resistant

Pseudomonas aeruginosa clinical isolates, carrying multiple genes coding for this antibiotic resistance. https://doi.org/10.1186/s12941-014-0043-3.

Robertson, J., Nash, J.H.E., 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb. Genom. 4 https://doi.org/10.1099/mgen.0.000206.

Sheppard, A.E., Stoesser, N., German-Mesner, I., Vegesana, K., Walker, A.S., Crook, D.W., Mathers, A.J., 2018. Tetyper: a bioinformatic pipeline for classifying variation and genetic contexts of transposable elements from short-read whole-genome sequencing data. Microb. Genom. 4 https://doi.org/10.1099/mgen.0.000232.

Silveira, M.C., Albano, R.M., Asensi, M.D., Carvalho-Assef, A.P.D.A., 2016. Description of genomic islands associated to the multidrug-resistant *Pseudomonas aeruginosa* clone ST277. Infect. Genet. Evol. 42, 60–65. https://doi.org/10.1016/j.meegid.2016.04.024.

Silveira, M.C., Rocha-De-Souza, C.M., Albano, R.M., De Oliveira Santos, I.C., Carvalho-Assef, A.P.D.A., 2020. Exploring the success of Brazilian endemic clone *Pseudomonas aeruginosa* ST277 and its association with the CRISPR-Cas system type I-C. BMC Genomics 21. https://doi.org/10.1186/s12864-020-6650-9.

Villegas, M.V., Lolans, K., Correa, A., Kattan, J.N., Lopez, J.A., Quinn, J.P., 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing-lactamase. Antimicrob. Agents Chemother. 51, 1553–1555. https://doi.org/10.1128/AAC.01405-06.

Wang, Y., Wang, J., Wang, R., Cai, Y., 2020. Resistance to ceftazidime–avibactam and underlying mechanisms. J. Glob. Antimicrob. Resist. https://doi.org/10.1016/j.jgar.2019.12.009.

Wang, L., Chen, E., Yang, L., Feng, D., Xu, Z., Chen, D., 2021. Emergence of clinical *Pseudomonas aeruginosa* isolate Guangzhou-PaeC79 carrying *crpP*, bla_{GES-5}, and bla_{KPC-2} in Guangzhou of China. Microb. Drug Resist. 27, 965–970. https://doi.org/10.1089/MDR.2020.0420.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. https://doi.org/10.1371/journal.pcbi.1005595.

Yano, H., Shintani, M., Tomita, M., Suzuki, H., Oshima, T., 2019. Reconsidering plasmid maintenance factors for computational plasmid design. Comput. Struct. Biotechnol. J. https://doi.org/10.1016/j.csbj.2018.12.001.

Yigit, H., Queenan, A.M., Anderson, G.J., Domenech-Sanchez, A., Biddle, J.W., Steward, C.D., Alberti, S., Bush, K., Tenover, F.C., 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. 45, 1151–1161. https://doi.org/10.1128/AAC.45.4.1151-1161.2001.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644. https://doi.org/10.1093/jac/dks261.