A Conjugative IncI1 Plasmid Carrying \textit{erm}(B) and \textit{bla}_{CTX-M-104} That Mediates Resistance to Azithromycin and Cephalosporins

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ABSTRACT In this study, an IncI1 plasmid encoding resistance to both cefotaxime and azithromycin was recovered from a clinical \textit{Klebsiella pneumoniae} strain. The azithromycin resistance was confirmed to be mediated by the \textit{erm}(B) gene. This plasmid could be readily conjugated to strains of \textit{Escherichia coli} and \textit{Salmonella}, promoting rapid dissemination of azithromycin- and ceftriaxone-resistance-encoding elements among Gram-negative bacterial pathogens. Transmission of this plasmid in \textit{Salmonella} is of particular concern, since it could mediate expression of phenotypic resistance to azithromycin and ceftriaxone, which are the current choices for treatment of \textit{Salmonella} infections. Our findings suggest a need to monitor the efficiency and pattern of transmission of this plasmid among key Gram-negative bacterial pathogens.

IMPORTANCE Since the approval by the FDA of azithromycin for treatment of \textit{Salmonella} infections, efforts have been made to monitor the development of resistance to azithromycin in these organisms. In this study, we report an IncI1 plasmid from a clinical \textit{K. pneumoniae} strain that encodes resistance to both cefotaxime and azithromycin. This plasmid could be readily conjugated to strains of \textit{Escherichia coli} and \textit{Salmonella}, promoting rapid dissemination of azithromycin- and ceftriaxone-resistance-encoding elements among Gram-negative bacterial pathogens. Furthermore, data from this study confirmed for the first time the role of the \textit{erm}(B) gene in mediating resistance to azithromycin in various bacterial species, particularly \textit{Salmonella}.

KEYWORDS \textit{Klebsiella pneumoniae}, azithromycin resistance, colistin resistance, \textit{erm}(B), mcr-8

Azithromycin, a derivative of erythromycin, is a semisynthetic macrolide antibiotic (1, 2). It was generated by incorporating a nitrogen atom in the macrocyclic lactone ring of erythromycin, resulting in a stable 15-membered azalide (3). Since azithromycin exhibits reduced toxicity, improved pharmacokinetics, metabolic stability, and high tolerability, it has been used worldwide after being approved for clinical use in 1988 (3–5). Classic macrolides exhibit low levels of activity against \textit{Enterobacteriaceae}, due to their poor membrane penetration potential (6). In contrast, azithromycin is membrane permeable and thus exhibits excellent potential for clinical treatment of infections caused by members of \textit{Enterobacteriaceae}, such as \textit{Escherichia coli}, \textit{Shigella} spp., or \textit{Salmonella} spp. (7, 8). Like other macrolides, azithromycin binds to the bacterial ribosome and inhibits mRNA translation, thus interfering with protein synthesis and preventing bacterial growth (6). Several mechanisms of resistance to macrolides have been described; one involves reduction in binding affinity of the drug via modification of either the bacterial ribosome or the drug molecule (9). A second mechanism is efflux of the drug from the bacterial cell via enhancement of efflux pump expression (10).
**Klebsiella pneumoniae**, which belongs to the *Enterobacteriaceae* family, is regarded as a reservoir of drug resistance genes due to its widespread presence among humans and in the environment (11). Although macrolides are not recommended for treatment of infections caused by *K. pneumoniae*, a variety of macrolide resistance genes in this species have been described (12). However, whether these genes confer cross-resistance to azithromycin remains unclear (10). In this report, we describe the identification of a conjugative plasmid harboring the erythromycin ribosome methylase gene, *erm* (B). The plasmid was recovered from a clinical *K. pneumoniae* strain that was confirmed to exhibit resistance to azithromycin. The *erm* (B) gene was demonstrated to mediate expression of phenotypic resistance to azithromycin in strains of various members of the *Enterobacteriaceae* family, particularly *Salmonella*, since azithromycin is an FDA-approved drug for treatment of clinical *Salmonella* infections. Widespread transmission of this kind of plasmid among clinical *Enterobacteriaceae* strains, or dissemination of clinical strains harboring such a plasmid, would result in significant reduction in the clinical value of azithromycin, thereby severely limiting the choices of effective antimicrobial agents for the treatment of life-threatening infections.

**RESULTS AND DISCUSSION**

A strain suspected to be *K. pneumoniae*, namely, HK31, was recovered from a hospitalized patient in Hong Kong SAR in 2016 (Table 1). Antimicrobial susceptibility tests performed on strain HK31 showed that it was resistant to azithromycin, the β-lactam antibiotics ampicillin, aztreonam, cefotaxime, and ceftazidime, and the aminoglycoside antibiotics gentamicin and amikacin, as well as ciprofloxacin, chloramphenicol, and colistin, but remained susceptible to meropenem and tigecycline (Table 2).

Strain HK31 was then subjected to whole-genome sequencing using both the Illumina NextSeq 500 platform and the long-read Oxford Nanopore Technologies MinION platform. The genome size of strain HK31 was found to be 5,798,010 bp.

**TABLE 1** Strains and plasmids used in this study

| Strain or plasmid | Relevant genotype | Source |
|-------------------|------------------|--------|
| **Strains** | | |
| *E. coli* | | |
| DH5α | F−φ80lacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rK− mK−) supE44 thi-1 gyrA96 relA1 λ− | Invitrogen |
| JS3 | Derivative of *E. coli* K-12; azide | Laboratory stock |
| 25922 | Quality control strain | ATCC |
| **K. pneumoniae** | | |
| HK31 | Clinical strain; AZIr, erm(B) | This study |
| **S. Typhimurium** | | |
| PY1 (14028s) | Derivative of CDC6516-60 | ATCC |
| **Plasmids** | | |
| pCR2.1 TOPO | Amp′, Kan′; pUC ori TA cloning vector, topoisomerase | Invitrogen |
| pCR2.1/erm(B) | erm(B) in pCR2.1 | This study |

*a* azide′, sodium azide resistance; AZIr, azithromycin resistance; Amp′, ampicillin resistance; Kan′, Kan resistance.

**TABLE 2** Phenotypic and genotypic characteristics of *K. pneumoniae* strain HK31 and its transconjugants

| Strain | Species | MIC (μg/ml) for: | Conjugation efficiency |
|--------|---------|------------------|-----------------------|
|        | AZI | CTX | CAZ | CIP | CHL | ATM | AMP | AMK | GEN | MEM | CLS | TGC | erm(B) |        |
| HK31   | *K. pneumoniae* | 128 | >128 | >128 | 128 | 64 | >128 | >128 | >128 | >128 | 0.25 | 32 | 0.5 | + | NA |
| JS3    | *E. coli* | 2 | <0.25 | 0.5 | <0.25 | 2 | <0.25 | 8 | 2 | 2 | <0.25 | <0.25 | <0.25 | − | NA |
| JS3TC  | *E. coli* | 64 | >128 | 2 | <0.25 | 2 | 64 | >128 | 2 | 2 | <0.25 | <0.25 | <0.25 | + | 1.33E−06 |
| PY1    | *S. Typhimurium* | 4 | <0.25 | 8 | <0.25 | 4 | <0.25 | 8 | 2 | 2 | <0.25 | 1 | 0.5 | − | NA |
| PY1TC  | *S. Typhimurium* | 64 | >128 | 32 | <0.25 | 4 | 128 | >128 | 2 | 2 | <0.25 | 1 | 0.5 | + | 3.5E−07 |
| 25922  | *E. coli* | 2 | <0.25 | 0.5 | <0.25 | 4 | <0.25 | 8 | 2 | 2 | <0.25 | 0.5 | 0.5 | NA | |

*a* All tests were performed in duplicate, and each test included three biological replicates. AZI, azithromycin; CTX, cefotaxime; CAZ, ceftazidime; CIP, ciprofloxacin; CHL, chloramphenicol; ATM, aztreonam; AMP, ampicillin; AMK, amikacin; GEN, gentamicin; MEM, meropenem; CLS, colistin; TGC, tigecycline.

*b* NA, not available.
including a 5.13-Mb chromosome and three plasmids with sizes of 204,580 bp, 115,662 bp, and 98,579 bp. Strain HK31 was found to belong to sequence type 273-1 based on multilocus sequence typing (MLST) and the KL14 serotype based on capsular typing. BLAST searches against the reference sequences showed that this strain harbored multiple resistance genes, including the quinolone resistance genes qnrB4 and qnrA8, the β-lactam resistance genes blaCTX-M-15, blaCTX-M-104, and blaSHV-12, and the aminoglycoside resistance genes armA, ant(2')-Ia, and aph(3')-Ia, the macrolide resistance genes msh(E), msr(E), and erm(B), the fosfomycin resistance gene fosA, the sulfonamide resistance gene sul1, and the colistin resistance gene mcr-8. The blaCTX-M-15 and blaCTX-M-104 genes were found to be located in the 204,580-bp plasmid, which was designated pHK31_1 (Fig. 1a). This plasmid was an IncFIBK plasmid, possessing 239 coding sequences.
128 μg/ml (Table 3). The results indicated that *erm*(B) also encoded resistance to azithromycin.

Because pHK31_3 was shown to carry the *tra* gene cluster of the IncI plasmid, which encodes plasmid conjugation functions, a conjugation experiment was then performed.

**FIG 1** Alignment of plasmids pHK31_1 and pHK31_2 with structurally similar plasmids by BRIG and Easyfig. (a) Plasmid pHK31_1 exhibits the highest degree of similarity (77% coverage and 99.88% identity) to plasmid p10057-catA (GenBank accession number MN423364.1). (b) Plasmid pHK31_2 exhibits the highest degree of similarity to pKP91 (GenBank accession number MG736312.1) (72% coverage and 99.72% identity) and plasmid pHNAH8I-1 (GenBank accession number MK347425.1) (84% coverage and 99.12% identity). (c) Alignment of plasmid pHK31_1 with plasmids p10057-catA and pHKU57_1 using Easyfig. (d) Alignment of plasmid pHK31_2 with plasmids pKP91 and pHNAH8I-1 using Easyfig.
FIG 2 Alignment of plasmid pHK31_3 with structurally similar plasmids by BRIG. (a) Plasmid pHK31_3 exhibits the highest level of similarity (100% coverage and 99.75% identity) to plasmid pQD23-1 (GenBank accession number MN548042.1). Plasmid p12-6919.1 (GenBank accession number CP039604.1) from *Salmonella enterica* subsp. *enterica* serovar strain PNCS014868 and plasmid pRHB20-C04_2 (GenBank accession number CP057639.1) from *E. coli* strain RHB20-C04 harbored similar plasmid backbones but lacked the mosaic resistance region. (b) Alignment of plasmid pHK31_3 with plasmid pQD23-1 using Easyfig. (c) The *erm*(B) gene in plasmid pHK31_3 is located in the truncated Tn6295 element within the plasmid p2246-CTXM.
to test its potential to be transferred to strains of *E. coli* and *Salmonella* species, which are common pathogens that belong to the *Enterobacteriaceae* family. The results showed that pHK31_3 could be directly conjugated from strain HK31 to *E. coli* strain J53, with an efficiency of $1.33 \times 10^6$ (Table 2 and Fig. 3). The transconjugant obtained was found to exhibit XbaI pulsed-field gel electrophoresis (PFGE) profiles that were identical to those of the recipient strain, but there was evidence of acquisition of the 100-kb plasmid according to the S1 nuclease PFGE profiles (Fig. 3). A transconjugant strain of J53 was then treated as the donor to conjugate this plasmid to the *Salmonella enterica* subsp. *enterica* serovar Typhimurium PY1 recipient strain. The results showed that plasmid pHK31_3 could be directly conjugated from strain J53 to strain PY1 with an efficiency of $3.5 \times 10^7$ (Table 2 and Fig. 3). These transconjugants were also confirmed to have acquired the ability to express phenotypic resistance to azithromycin and ceftriaxone (Table 2). The ability to conjugate such a plasmid to *Salmonella* spp. highlighted the clinical significance of this conjugative plasmid encoding both azithromycin and cephalosporin resistance, since these two types of antibiotics are current choices for treatment of clinical *Salmonella* infections. Since the approval by the FDA of azithromycin for treatment of *Salmonella* infections, efforts have been made to monitor the development of resistance to azithromycin in these organisms. Azithromycin resistance has been increasingly reported in *Salmonella* strains, while the exact mechanisms remain unclear. The *mphA* gene has been shown to be associated with azithromycin resistance in *Salmonella* strains, while a large proportion of *mphA*-positive *Salmonella* strains in our collection are susceptible to azithromycin (unpublished data, Table 3).

**TABLE 3** MICs of strains that had acquired the *erm* (B) gene via transformation

| Strain          | Azithromycin MIC (µg/ml) |
|-----------------|---------------------------|
| DH5α            | 2                         |
| DH5α/erm(B)     | >128                      |
| PY1             | 4                         |
| PY1/erm(B)      | >128                      |

**FIG 3** XbaI PFGE and S1 PFGE analyses of strain HK31, recipient strain *E. coli* J53, *S. Typhimurium* PY1, and their corresponding transconjugants J53TC and PY1TC. Asterisks denote the conjugative plasmid pHK31_3. XbaI PFGE and S1 PFGE were repeated twice for all strains to confirm the consistency of the XbaI PFGE and S1 PFGE profiles.
Chen KC and Chen S), which contradicts the role of mphA as an azithromycin resistance gene in Salmonella. Data from this study confirmed for the first time the role of the erm(B) gene in mediating resistance to azithromycin in various bacterial species, particularly Salmonella.

CONCLUSION

In this study, we characterized a conjugative plasmid that harbored the erm(B) gene from a clinical K. pneumoniae strain and confirmed the role of this gene in mediating azithromycin resistance in various bacterial species. This plasmid was also able to conjugate to strains of E. coli and Salmonella and encode phenotypic azithromycin and ceftriaxone resistance. Surveillance and close monitoring of the transmission of this plasmid in Gram-negative bacteria, particularly Salmonella, should be implemented.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Strains and plasmids used in this work are listed in Table 1. K. pneumoniae strain HK31 was isolated from a clinical patient in a hospital located in Hong Kong SAR and was identified by the Vitek 2 system (bioMérieux, France), with confirmation by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker, Germany). E. coli strains 25922 and J53 and S. Typhimurium strain PY1 were from our laboratory stocks. Strains were grown in Luria-Bertani (LB) broth at 37°C. Kanamycin (Kan) was added at a concentration of 50 μg/ml where appropriate.

Cloning of the erm(B) gene. The erm(B) gene was amplified using the primers erm-B-F (AGAAGGAGGGATTCGTCATG) and erm-B-R (TCTTGCTAGTCTAGGGACCT). Briefly, fragments of 200 bp upstream and downstream of the erm(B) gene were amplified, ligated into the pCR2.1 TOPO vector (Invitrogen), and introduced into E. coli DH5α by heat shock at 42°C. The resulting plasmid recoverable from the transformants was verified by sequencing and electroporated into S. Typhimurium strain PY1.

Conjugation assay. To evaluate the transferability of the azithromycin-resistance-encoding plasmid, sodium azide (NaN₃)-resistant E. coli strain J53 was used as the recipient, as described previously (18). Transconjugants were screened by using eosin-methylene blue (EMB) agar plates containing 8 μg/ml azithromycin and 100 μg/ml NaN₃. The presence of the erm(B) gene as a marker gene in the plasmid harbored by the transconjugants was determined by PCR. The MIC profiles of the transconjugants were determined to differ from those of the donor. XbaI PFGE and S1 nuclease PFGE were performed to confirm the transfer of this plasmid. Successful transconjugants of E. coli strain J53 were then treated as the donor and S. Typhimurium strain PY1 was used as the recipient to further determine the transferability of this plasmid. XLT4 agar plates containing 8 μg/ml azithromycin were used to select transconjugants. To calculate the conjugation efficiency, the culture obtained after conjugation was diluted and spread on plates containing only 100 μg/ml NaN₃, with strain J53 being used as the recipient, as well as plates without antibiotics, with strain PY1 being used as the recipient. The conjugation efficiency was calculated as the number of transconjugant cells divided by the number of recipient cells.

Antibiotic susceptibility tests. Antimicrobial susceptibility of the test strains was determined by using the microdilution method according to the guidelines provided by the Clinical and Laboratory Standards Institute (19). Antimicrobial agents including azithromycin, cefotaxime, ceftazidime, ciprofloxacin, chloramphenicol, aztreonam, ampicillin, gentamicin, amikacin, meropenem, colistin, and tigecycline were tested. All tests were performed in duplicate; each test included three biological replicates per strain.

DNA sequencing and bioinformatics. Genomic DNA from strain HK31 was extracted using the genomic purification kit for bacteria (Qiagen, Germany) according to the manufacturer’s instructions. The extracted DNA was then subjected to library preparation with the NEBNext Ultra II DNA library preparation kit for Illumina (New England Biolabs, USA) and sequenced via the 150-bp paired-end NextSeq 500 platform (Illumina, San Diego, CA). Genomic DNA was also subjected to sequencing with the long-read MinION platform, following the manufacturer’s guide (Oxford Nanopore Technologies, Oxford, United Kingdom). Both short and long reads were de novo hybrid assembled using Unicycler v0.4.8 (20). Assembled genome sequences were annotated with RAST v2.0 (21). MLST was performed by Kleborate software based on genetic variation in seven housekeeping genes (22). Capsular typing of the assembled sequences was performed using Kaptive (23). The BLAST command lines, with 80% coverage and identity cutoff values, were used to map genome sequences against antibiotic resistance genes and plasmid repilcons. The resistance gene and plasmid repilcon databases were obtained from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org). Alignment of plasmid sequences with similar structures was generated by BLAST Ring Image Generator (BRIG) v0.95.22 (24) and Easyfig_win_2.1 (25).

Data availability. All sequencing data have been deposited in GenBank under BioProject accession number PRJNA725664. GenBank accession numbers CP073906, CP073907, CP073908, and CP073909 have been assigned to sequences of the strain HK31 chromosome, plasmid pHK31_1, pHK31_2, and pHK31_p3, respectively.
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We declare no conflicts of interest.

REFERENCES

1. Mutak S. 2007. Azalides from azithromycin to new azalide derivatives. J Antimicrob Chemother 60:85–122. https://doi.org/10.1093/jac/2007.10.
2. Dinos GP. 2017. The macrolide antibiotic renaissance. Br J Pharmacol 174: 2967–2983. https://doi.org/10.1111/bph.13936.
3. Girard AE, Girard D, English AR, Gootz TD, Cimochowski CR, Faiella JA, Haskell SL, Retsema JA. 1987. Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrob Agents Chemother 31:1948–1954. https://doi.org/10.1128/AAC.31.12.1948.
4. Retsema JA, Girard A, Schelkly W, Manousos M, Anderson M, Bright G, Borovoy R, Brennan L, Mason R. 1987. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against Gram-negative organisms. Antimicrob Agents Chemother 31:1939–1947. https://doi.org/10.1128/AAC.31.12.1939.
5. Fiese EF, Steffen SH. 1990. Comparison of the acid stability of azithromycin and erythromycin A. J Antimicrob Chemother 25(Suppl A):39–47. https://doi.org/10.1093/jac/25.suppl_a.39.
6. Gomes C, Martinez-Puchol S, Palma N, Horna G, Ruiz-Roldan L, Pons MJ, Ruiz J. 2017. Macrolide resistance mechanisms in Enterobacteriaceae: focus on azithromycin. Crit Rev Microbiol 43:1–30. https://doi.org/10.1080/1040841X.2015.1136261.
7. Pons MJ, Gomes C, Martinez-Puchol S, Ruiz L, Mena L, Vila J, Gascon J, Ruiz J. 2013. Antimicrobial resistance in Shigella spp. causing traveler’s diarrhea (1995–2010): a retrospective analysis. Travel Med Infect Dis 11: 315–319. https://doi.org/10.1016/j.tmaid.2013.06.010.
8. Trivedi NA, Shah PC. 2012. A meta-analysis comparing the safety and efficacy of azithromycin over the alternate drugs used for treatment of uncomplicated enteric fever. J Postgrad Med 58:112–118. https://doi.org/10.4103/0022-3859.97172.
9. Poehlsgaard J, Douthwaite S. 2003. Macrolide antibiotic interaction and resistance on the bacterial ribosome. Curr Opin Investig Drugs 4:140–148.
10. Leclercq R. 2002. Mechanism of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 34:482–492. https://doi.org/10.1086/324626.
11. Navon-Venezia S, Kondratyeva K, Carattoli A. 2017. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 41:252–275. https://doi.org/10.1093/femsec/fux013.
12. Roe CC, Vazquez AJ, Esposito EP, Zamili R, Sahl JW. 2019. Diversity, virulence, and antimicrobial resistance in isolates from the newly emerging Klebsiella pneumoniae ST101 lineage. Front Microbiol 10:542. https://doi.org/10.3389/fmicb.2019.00542.
13. Huang Y, Lin Q, Zhou Q, Lv L, Wan M, Gao X, Wang C, Liu J-H. 2020. Identification of fsaA10, a novel plasmid-mediated fosfomycin resistance gene of Klebsiella pneumoniae origin, in Escherichia coli. Infect Drug Resist 13: 1273–1279. https://doi.org/10.2147/IDR.S251360.
14. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z, Wang Y. 2018. Emergence of a novel mobile colistin resistance gene, mcr-8, in NDM-producing Klebsiella pneumoniae. Emerg Microbes Infect 7:122. https://doi.org/10.1080/14712164.2020.1242230.
15. Lv L, Wan M, Wang C, Gao X, Yang Q, Partridge SR, Wang Y, Zong Z, Doi Y, Shen J, Jia P, Song Q, Zhang Q, Yang J, Huang X, Wang M, Liu J-H. 2020. Emergence of a plasmid-encoded resistance-nodulation-division efflux pump conferring resistance to multiple drugs, including tigecycline, in Klebsiella pneumoniae. mBio 11:e02930-19. https://doi.org/10.1128/mBio.02930-19.
16. Wang L, Liu L, Liu D, Yin Z, Feng J, Zhang D, Fang H, Qiu Y, Chen W, Yang R, Wang J, Fa Y, Zhou D. 2016. The first report of a fully sequenced resistance plasmid from Shigella boydii. Front Microbiol 7:1579. https://doi.org/10.3389/fmicb.2016.01579.
17. Svetlov MS, Syroegin EA, Aleksandrova EV, Atkinson GC, Gregory ST, Mankin AS, Polikanov YS. 2021. Structure of Ern-modified 70S ribosome reveals the mechanism of macrolide resistance. Nat Chem Biol 17: 412–420. https://doi.org/10.1038/s41589-020-00715-0.
18. Yang X, Wai-Chi Chan E, Zhang R, Chen S. 2019. A conjugative plasmid that augments virulence in Klebsiella pneumoniae. Nat Microbiol 4: 2039–2043. https://doi.org/10.1038/s41564-019-0566-7.
19. Clinical and Laboratory Standards Institute. 2021. Performance standards for antimicrobial susceptibility testing, 31th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.
20. 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.
21. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
22. Lam MMC, Wick RR, Wyres KL, Holt KE. 2020. Genomic surveillance framework and global population structure for Klebsiella pneumoniae. bioRxiv 2020.12.14.422303. https://doi.org/10.1101/2020.12.14.422303.
23. Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, Holt KE. 2016. Identification of Klebsiella capsulose synthesize loci from whole genome data. Microb Genom 2:e000102. https://doi.org/10.1099/mgen.0.000102.
24. Ali Khan NF, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.
25. Sullivan MJ, Petty NK, Beatson SA. 2011. Easyfig: a genome comparison visualizer. Bioinformatics 27:1009–1010. https://doi.org/10.1093/bioinformatics/btr039.