Multiple β-Lactam Resistance Gene-Carrying Plasmid Harbored by *Klebsiella quasipneumoniae* Isolated from Urban Sewage in Japan

© Yasunori Suzuki, Miki Ida, Hiroaki Kubota, Tsukasa Ariyoshi, Ko Murakami, Makiko Kobayashi, Rei Kato, Akihiko Hirai, Jun Suzuki, Kenji Sadamasu

*Department of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan*

**ABSTRACT** The continuous emergence of carbapenemase-producing Enterobacteriaceae (CPE) presents a great public health challenge. Mitigation of CPE spread in the environment is crucial, particularly from a One Health perspective. Here we describe the isolation of CPE strain SNI47 from influent water of a sewage treatment plant in Japan. SNI47 was identified as *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* by phylogenetic analysis and was resistant to β-lactams, including carbapenems. Of four plasmids detected from SNI47, the 185,311-bp IncA/C2 plasmid (pTMSNI47-1), which carried 10 drug resistance genes, including genes for four β-lactamases (*bla*₅₀₆₆₇₋₂, *bla*₀₆₄₁₋₁, *bla*₁₈₁₋₁, and *bla*₂₅₆₁₋₁), was transferred to *Escherichia coli* J53 via conjugation. The MICs of all tested β-lactams for the transconjugant were higher than for the recipient. We constructed recombinant plasmids, into which each β-lactamase gene was inserted, and used them to transform *E. coli* DH5α cells, demonstrating that KHM-1 enhanced carbapenem resistance. In addition, these β-lactamases were responsible for a wide-spectrum β-lactam resistance acquisition with mutual compensation. KHM-1, recognized as a rare type of metallo-β-lactamase, was detected in a transferable plasmid, from a sewage treatment plant, involved in horizontal gene transfer. The detection of such plasmids raises a health risk alarm for CPE dissemination.

**IMPORTANCE** In our investigation of urban wastewater in Japan, carbapenem-resistant *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* was isolated that carried the pTMSNI47-1 plasmid, which carries four β-lactamase genes and has transferability among Enterobacteriaceae. pTMSNI47-1 was found to encode a rarely reported carbapenemase, KHM-1. Cooperative effects of β-lactamases encoded by pTMSNI47-1 appeared to have broad-spectrum resistance to β-lactams. The detection of the KHM-1 gene in urban wastewater suggests that such a rare antimicrobial resistance (AMR) gene can be pooled in the environment, potentially emerging as an AMR determinant in a pathogen. When the number of β-lactamase resistance genes is increased in one plasmid, the transfer of this plasmid can confer broad-spectrum resistance to β-lactams, even if the individual gene confers narrow-spectrum resistance. The present study adds important information about the potential risk of sewage treatment plants as reservoirs and environmental suppliers of AMR genes, contributing to the public health from a One Health perspective.

**KEYWORDS** carbapenemase-producing Enterobacteriaceae, conjugal transfer, metallo-β-lactamase, plasmid, whole-genome sequencing, Enterobacteriaceae, β-lactamases, carbapenems, conjugation, genome analysis

Worldwide, infections caused by carbapenemase-producing Enterobacteriaceae (CPE) are of utmost interest in clinical settings because carbapenems are often antimicrobial agents of last resort (1, 2). Moreover, it is necessary to mitigate CPE spread...
in the environment, particularly from a One Health perspective. Nevertheless, CPE environmental contamination has not been investigated fully. Sewage treatment plants (STPs) are one of the most important interfaces between the human population and the aquatic environment. Several previous studies (3–5) have proposed STPs and wastewater to be the hot spots for horizontal gene transfer, facilitating the spread of antimicrobial resistance (AMR) genes, including carbapenemase genes, between different bacterial species. Additionally, sewage system diffusion has improved in recent years, leading to an increased proportion of sewage effluent in the environmental water. These facts suggest that STPs and wastewater can act as anthropogenic sources, reservoirs, and environmental suppliers of AMR genes.

A large variety of carbapenemases have been reported, including those belonging to Ambler class A (e.g., KPC and IMI), class B (e.g., IMP and NDM), and class D (e.g., OXA-48 and OXA-162) (6). In clinical fields, highly carbapenem-resistant strains harboring KPC- or NDM-producing Enterobacteriaceae have been spreading rapidly between countries (7, 8). In Japan, the most prevalent carbapenemase in Enterobacteriaceae is an IMP-type enzyme (9, 10). On the other hand, NDM-, VIM-, KPC-, or OXA-48-producing Enterobacteriaceae have been rarely isolated from sporadic cases (e.g., patients with carbapenem-resistant infections who have travelled abroad) (8, 11). Ambler class B carbapenemases are metallo-β-lactamases (MBLs) and classified into various types according to their amino acid sequences. In general, MBLs harbor hydrolytic activity against broad-spectrum β-lactams except monobactams and demonstrate reduced carbapenem susceptibility (12). Kyorin Health Science MBL-1 (KHM-1) was identified in 1997 in a multidrug-resistant Citrobacter freundii isolate from a patient with a catheter-associated urinary tract infection, in Japan. The blaKHM-1 gene was carried in a plasmid of approximately 200 kbp, designated pCF243 (13). However, reappearance of this enzyme has not been identified in clinical settings or the natural environment since its first report (14). Thus, the extent of spread and whether this MBL can contribute to CPE infections are unknown.

We reported the isolation of a novel multidrug-resistant IncA/C2 plasmid, pTMSNI47-1, containing carbapenemase gene blaKHM-1, in Klebsiella quasipneumoniae SNI47 isolated from a municipal STP in Japan. It was suggested that CPE, harboring a highly transferable and broad-spectrum resistance plasmid, had been disseminated and deposited into the sewage.

**RESULTS**

Detection of carbapenem-resistant *Klebsiella quasipneumoniae* isolate SNI47 in urban sewage. We initially used a short-read next-generation sequencer (MiSeq) to confirm the phylogenetic position of the carbapenem-resistant isolate SNI47 relative to typical strains of *Klebsiella pneumoniae*, *Klebsiella variicola*, *Klebsiella quasivaricola*, *Klebsiella quasipneumoniae subsp. quasipneumoniae*, and *Klebsiella quasipneumoniae subsp. similipneumoniae*. Average nucleotide identity (ANI) analysis showed that the nucleotide sequence of SNI47 was similar (99.18%) to that of 01A030 strain, a type strain of *Klebsiella quasipneumoniae subsp. quasipneumoniae*. In addition, the resultant tree, based on k-mer diversity, indicated that the genomic structure of SNI47 was quite similar to that of *K. quasipneumoniae* subsp. *quasipneumoniae* strains (Fig. 1). S1 pulsed-field gel electrophoresis (S1-PFGE) analysis for SNI47 revealed two bands, approximately 180 kbp and 80 kbp (Fig. 2, left). MiSeq analysis for gel-extracted chromosome or plasmid DNA fragments revealed that the AMR determinants located on the chromosome were *bla*OXA-4 (conferring reduced susceptibility to β-lactams), *qoxA* and *qoxB* (quinolones), and *fosA* (fosfomycin). An approximately 180-kbp DNA fragment carried IncA/C2 and IncFIB(K) replicons and 10 types of AMR determinants, namely, *aadA1* (aminoglycosides); *bla*CTX-M-2, *bla*DHA-1, *bla*KHM-1, and *bla*OXA-10 (β-lactams); *qnrB4* (quinolones); *cmiA5* (chloramphenicol); *arn2* (rifampin); *sul1* (sulfonamide); and *dfrA14* (trimethoprim). IncFII(K) and IncR replicons were detected in the approximately 80-kbp DNA fragment; however, AMR genes were absent.
Antibiotic susceptibility testing using BD Sensi-Discs revealed that *K. quasipneumoniae* subsp. *quasipneumoniae* SNI47 was resistant to all tested β-lactams, rifampin, streptomycin, and sulfamethoxazole-trimethoprim (SXT) and sensitive to fosfomycin, kanamycin, gentamicin, amikacin, and all tested quinolones. Intermediate susceptibility to chloramphenicol was shown (Fig. 3). MICs of the β-lactams were 24 μg/ml for aztreonam, 32 μg/ml for imipenem, meropenem, ertapenem, doripenem, benzylpenicillin, and cefotaxime, and 256 μg/ml for amoxicillin, piperacillin, ceftazidime, and ceftepime (Table 1). MICs of the aminoglycosides and fluoroquinolones were 32, 1, 2, 0.5, and 1 μg/ml for streptomycin, gentamicin, amikacin, ciprofloxacin, and levofloxacin, respectively (Table 2).

Transmission ability of pTMSNI47-1 and antimicrobial susceptibility of transconjugant. A conjugation experiment was performed with SNI47 and *Escherichia coli* J53. Transconjugants that were resistant to both sodium azide and ampicillin were obtained. According to S1-PFGE, the transconjugants contained an approximately 180-kbp plasmid (Fig. 2, right). The transconjugants exhibited a susceptibility profile similar to that of *K. quasipneumoniae* subsp. *quasipneumoniae* SNI47, although the MICs for carbapenems (imipenem, 4 μg/ml; meropenem, 2 μg/ml; ertapenem, 2 μg/ml; and doripenem, 4 μg/ml), cefepime (64 μg/ml), and aztreonam (4 μg/ml) were lower in the transconjugants than in SNI47 (Table 1).

Antimicrobial susceptibilities of five β-lactamas. To detect which β-lactamas harbored hydrolytic activity against each β-lactam, we transformed five recombinant plasmids to DH5α cells and measured the MICs of 10 β-lactams. As Table 1 shows, both CTX-M-2-producing and DHA-1-producing strains showed increased MICs of penicillin, cephem, or monobactam derivatives. In particular, the MICs of cefotaxime (>32 μg/ml), cefepime (8 μg/ml), and aztreonam (8 μg/ml) for the former were the largest among the five tested β-lactamas. The production of CTX-M-2 had a small effect on the hydrolytic activity against ceftazidime (0.5 μg/ml). On the other hand, the DHA-1-
producing strain showed a higher MIC increase for ceftazidime (4 μg/ml) and lower MIC increases for piperacillin (32 μg/ml), cefotaxime (2 μg/ml), and aztreonam (1 μg/ml) than those of the CTX-M-2-producing strain. Regarding the three cephem derivatives, clavulanic acid could inhibit CTX-M-2 activities but could not inhibit DHA-1. The KHM-1-producing strain showed generally increased MICs of carbapenems and cephems. The MICs of carbapenems were 0.5 μg/ml for imipenem, 0.5 μg/ml for meropenem, 0.25 μg/ml for ertapenem, and 0.25 μg/ml for doripenem, corresponding to 4-

FIG 2 Pulsed-field gel electrophoresis (PFGE) of S1 nuclease-digested total DNA plugs. (Left) S1-PFGE pattern observed in Klebsiella quasipneumoniae subsp. quasipneumoniae SNI47; (right) S1-PFGE pattern observed in transconjugant Escherichia coli J53. A lambda ladder (Promega, Fitchburg, WI) was used as the size marker.

FIG 3 Sizes of zones of inhibition of Klebsiella quasipneumoniae subsp. quasipneumoniae SNI47 by 21 antimicrobials in the Kirby-Bauer drug susceptibility test. The following antimicrobials were tested: 1, imipenem; 2, meropenem; 3, penicillin; 4, ampicillin; 5, cefotaxime; 6, ceftazidime; 7, cefoxitin; 8, cephalothin; 9, aztreonam; 10, fosfomycin; 11, chloramphenicol; 12, rifampin; 13, nalidixic acid; 14, norfloxacin; 15, levofloxacin; 16, ciprofloxacin; 17, sulfamethoxazole-trimethoprim; 18, streptomycin; 19, kanamycin; 20, gentamicin; and 21, amikacin. Susceptibility criteria conformed to the Clinical and Laboratory Standards Institute guidelines. S, susceptible; I, intermediate; R, resistant; U.D., susceptibility undetermined because interpretative criteria were not defined for Enterobacteriaceae.
**TABLE 1** Etest results of β-lactams on *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* SNI47, *Escherichia coli* J53, and *Escherichia coli* DH5α transconjugant and transformants

| Antimicrobial | SNI47 | JS3/ pTMSNI47-1 | DH5α | DH5α/ pHSG398 | DH5α/ pHSG-blaCTX-M-2 | DH5α/ pHSG-blaOXA-1 | DH5α/ pHSG-blaKHM1 | DH5α/ pHSG-blaDHA-1 | DH5α/ pHSG-blaOXA-10 | DH5α/ pHSG-blaOKP-A-4 | DH5α/ pHSG-blaOXA-10 |
|---------------|-------|-----------------|------|-------------|----------------------|----------------------|-------------------|---------------------|----------------------|---------------------|---------------------|
| Imipenem      | >32   | 0.125 4         | 0.125| 0.125      | 0.25                 | 0.125                | 0.5               | 0.25                | 0.125                | 4                   | 0.125 |
| Meropenem     | >32   | ≤0.06 2        | ≤0.06| ≤0.06      | ≤0.06                | ≤0.06                | ≤0.06             | ≤0.06               | ≤0.06                | 2                   | ≤0.06 |
| Ertapenem     | >32   | ≤0.06 2        | ≤0.06| ≤0.06      | ≤0.06                | ≤0.06                | 0.25             | ≤0.06               | ≤0.06                | 2                   | ≤0.06 |
| Doripenem     | >32   | 0.125 4        | ≤0.06| ≤0.06      | 0.125                | 0.125                | 0.25             | 0.125               | ≤0.06                | 4                   | ≤0.06 |
| Benzylpenicillin | >32   | >32 >32 >32 | >32  | >32 >32 >32 | >32 >32 >32 | >32 >32 >32 | >32 >32 >32 | >32 >32 >32 | >32 >32 >32 | >32 >32 >32 |
| Amoxicillin   | >256  | 8 >256 4 | 4  | 4 >256 | >256 64 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 |
| Piperacillin  | >256  | 1 >256 0.5 | 0.5 | >256 32 | 1 >256 >256 | 0.125 >256 0.125 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 | >256 >256 >256 |
| Ceftazidime   | >256  | 0.125 >256 | 0.125| 0.125 0.5 | 4 >256 0.125 >256 0.125 | >256 >256 0.125 >256 0.125 | >4 >4 >4 >4 | ≤0.125 >0.125 >0.125 >0.125 | >4 >4 >4 >4 | >0.125 >0.125 >0.125 >0.125 | >4 >4 >4 >4 |
| Ceftazidime-clavulanic acid | >4 | 0.125 >4 | ≤0.125 ≤0.125 ≤0.125 | >4 | >4 | >4 | ≤0.125 >0.125 >0.125 | >4 | >4 | >4 | >4 |
| Cefotaxime    | >32   | ≤0.06 >32 | ≤0.06| ≤0.06 ≤0.06 >32 | 2 16 0.125 ≤0.06 ≤0.06 | ≤0.06 ≤0.06 | ≤0.06 ≤0.06 | ≤0.06 ≤0.06 | ≤0.06 ≤0.06 | ≤0.06 ≤0.06 |
| Cefotaxime-clavulanic acid | >1 | ≤0.06 >1 | ≤0.06 ≤0.06 ≤0.06 | >1 | >1 | >1 | ≤0.06 ≤0.06 ≤0.06 | >1 | >1 | >1 | >1 |
| Cefepime      | >256  | ≤0.06 64 | ≤0.06| ≤0.06 ≤0.06 8 4 ≤0.06 4 | 0.125 ≤0.125 ≤0.125 | ≤0.06 0.125 ≤0.125 | ≤0.125 ≤0.125 | 64 | ≤0.125 | ≤0.125 | ≤0.125 |
| Cefepime-clavulanic acid | >4 | ≤0.125 >4 | ≤0.125 ≤0.125 ≤0.125 | ≥4 | ≥4 | ≥4 | ≤0.125 ≤0.125 ≤0.125 | ≥4 | ≥4 | ≥4 | ≥4 |
| Aztreonam     | 24    | ≤0.06 4 | ≤0.06| ≤0.06 ≤0.06 8 1 ≤0.06 | 0.25 ≤0.06 | ≤0.06 ≤0.06 | ≤0.06 ≤0.06 | 2 | ≤0.06 | ≤0.06 | 2 |
TABLE 2 Etest results of aminoglycosides and fluoroquinolones on Klebsiella quasipneumoniae subsp. quasipneumoniae SNI47, Escherichia coli J53, and transconjugant J53/pTMSNI47-1

| Antimicrobial | MIC (µg/ml) |
|--------------|-------------|
|              | SNI47 | E. coli J53 | E. coli J53/pTMSNI47-1 |
| Streptomycin | 32    | 1            | 8                |
| Gentamicin   | 1     | ≤0.06        | ≤0.06            |
| Amikacin     | 2     | 0.5          | 0.5              |
| Ciprofloxacin| 0.5   | ≤0.06        | 0.25             |
| Levofloxacin | 1     | ≤0.06        | 0.5              |

>8-, >4-, and >4-fold increases compared with those for the control DH5α strain, respectively. As expected, the production of KHM-1 had no effect on the MIC of aztreonam. The production of OXA-10 increased the MICs of amoxicillin (256 g/ml) and piperacillin (256 g/ml). The production of chromosomal β-lactamase, OKP-A-4, only increased the MICs of penicillin derivatives. DH5α transformed with pTMSNI47-1 exhibited a susceptibility profile almost identical to that of transconjugant J53/pTMSNI47-1.

Whole-genome sequencing of SNI47. The hybrid assembly of MiSeq and MinION reads gave one complete chromosome sequence (5,468,267 bp) and four complete plasmid sequences over 50 kbp, namely, 185,311 bp (pTMSNI47-1), 181,469 bp (pTMSNI47-2), 89,834 bp (pTMSNI47-3), and 85,849 bp (pTMSNI47-4). The sequence type (ST) for the chromosome was ST668. The incompatibility (Inc) type and sequence type were as follows: IncA/C2 and ST26 (pTMSNI47-1), IncFIB(K) and not available (pTMSNI47-2), IncFII(K) and allele identifier (ID) 21 (pTMSNI47-3), and IncR and not available (pTMSNI47-4). AMR genes were not found on pTMSNI47-2, pTMSNI47-3, or pTMSNI47-4 (Table 3). These results were consistent with those of S1-PFGE and MiSeq analysis using the gel-extracted DNA samples.

pTMSNI47-1 displayed overall constant levels of homology with other IncA/C2 plasmids found in K. pneumoniae (pHM881QN, GenBank accession number LC055503, 71% query cover) and in Citrobacter freundii (pCFJY-17, GenBank accession number MH763829, 71% query cover). The 223 coding DNA sequences (CDSs) were annotated in pTMSNI47-1, and the genes that contributed to conjugal transfer, such as traA, traB, and traC, were detected in two regions of the pTMSNI47-1 sequence (Fig. 4A). Ten AMR genes, which were identified using ResFinder, were detected in these CDSs, namely, aadA1 (CDS125), blaCTX-M-2 (CDS136), blaDHA-1 (CDS180), blaKHM-1 (CDS197), blaOXA-10 (CDS126), qnrB4 (CDS174), cmlA5 (CDS127), arr2 (CDS128), and dfrA14 (CDS129). sul1 was triplicated in pTMSNI47-1 (CDS123, CDS164, and CDS184). Analysis of the

TABLE 3 Whole-genome information for Klebsiella quasipneumoniae subsp. quasipneumoniae SNI47 isolated from influent water from a sewage treatment plant in Japan

| Replicon  | GC (%) | Length (bp) | Incompatibility group | Sequence type and/or allele ID | Drug resistance gene(s) | Resistance phenotype |
|-----------|--------|-------------|-----------------------|-------------------------------|-------------------------|---------------------|
| Chromosome | 57.80  | 5,468,267   | ST668                 |                               | blaOKP-A-4, aoxA, aoxB, fosa | β-Lactams, Quinolones, Fosfomycin |
| pTMSNI47-1 | 51.24  | 185,311     | IncA/C2               | ST26, cgST26.1                | aadA1, blaCTX-M-2, bladha1, blaKHM-1, blaOXA-10 | Aminoglycosides, β-Lactams, Quinolones, Chloramphenicol, Rifampin, Sulfonamides, Trimethoprim |
| pTMSNI47-2 | 50.37  | 181,469     | IncFIB(K)             | Not available                 | Not found               | Not found            |
| pTMSNI47-3 | 53.02  | 89,843      | IncFIB(K)             | Allele ID 21                  | Not found               | Not found            |
| pTMSNI47-4 | 50.13  | 85,849      | IncR                  | Not available                 | Not found               | Not found            |
FIG 4 Genome structure of pTMSNI47-1. (A) Genome representation was performed using CGview Server. The outermost two rings comprising coloured arrowheads show features extracted from the pTMSNI47-1 genome. Yellow arrowheads show drug resistance genes harbored by pTMSNI47-1. The next three (Continued on next page)
genetic context revealed insertion sequence (IS) transposase-encoding or recombinase/integrate-encoding genes in the vicinity of bla<sub>CTX-M-2</sub>, bla<sub>DHA-1</sub>, and bla<sub>KHM-1</sub> (Fig. 4B), namely, genes for an IS1380-like element ISEc9 family transposase (CDS135), IS5-like element ISEC68 family transposase (CDS196), Tn3 family transposase (CDS133, CDS134, CDS186, and CDS187), and tyrosine-type recombinase/integrate (CDS205). pTMSNI47-1 carried two distinct class 1 integrons. One was located between CDS162 (intI1) and CDS164 (sulI) with an empty gene cassette region. The other was located complementarily in the region between CDS123 (sulI) and CDS130 (intI1) and comprised the aadA1-bla<sub>OXA-10</sub>-cmI<sub>AS-arr2</sub>-dfrA14 gene cassette array. This sequence was identical to that of In633 harbored by a Providencia stuartii strain Psb/3 plasmid (GenBank accession number JN193567).

**DISCUSSION**

STPs are considered major anthropogenic sources, reservoirs, and environmental suppliers of AMR genes, including carbapenemase genes (3, 4). In fact, recent studies of Japanese STPs identified GES-5, GES-24, IMP-19, and KPC-2-type CPE isolates from wastewater (11, 15). Our study identified a CPE isolate, SNI47, from the influent water of an STP in Japan. The phylogenetic tree constructed using MiSeq data illustrated that SNI47 belongs to <i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i> (Fig. 1). In clinical settings, a higher prevalence of <i>K. pneumoniae</i> than <i>K. quasipneumoniae</i> has been reported (16, 17). Gomi et al. (15) reported that the prevalence among the carbapenemase-producing Klebsiella isolates from STPs or hospital wastewater was different from those observed in clinical isolates, with <i>K. quasipneumoniae</i> isolated more frequently than <i>K. pneumoniae</i> from wastewater. Our results support their suggestion that the difference may primarily be because <i>K. quasipneumoniae</i> is associated more frequently with carriage, whereas <i>K. pneumoniae</i> is associated with human infection.

<i>K. quasipneumoniae</i> subsp. <i>quasipneumoniae</i> SNI47 harbored four plasmids: IncA/C2 plasmid pTMSNI47-1, IncFIB(K) plasmid pTMSNI47-2, IncFII(K) plasmid pTMSNI47-3, and IncR plasmid pTMSNI47-4. Of these, only pTMSNI47-1 harbored drug resistance genes (Table 3). IncA/C plasmids have demonstrated a wide Enterobacteriaceae host range and are one of the main plasmid families that mediate AMR dissemination (18). In addition, our whole-genome sequencing results indicated that the genes contributing to conjugal transfer, such as traA, were annotated in pTMSNI47-1 (Fig. 4A). It was demonstrated experimentally that pTMSNI47-1 could transfer among Enterobacteriaceae through conjugation. The MICs of all tested β-lactams, including carbapenems, for both transconjugant J53/pTMSNI47-1 and transformants DH5α/pTMSNI47-1 were higher than those for the recipient strain, indicating that this plasmid conferred resistant properties for carbapenems. The broad host range and the high self-transferability of plasmid pTMSNI47-1 can lead to drug resistance acquisition in one horizontal gene transfer event.

Gene module transpositions are facilitated by transposons and ISs. These elements are frequently detected in plasmids, including pTMSNI47-1, that involve AMR genes, non-AMR genes, and transposable genetic elements. For example, analysis of the genetic context showed ISEC68 and recombinase/integrate-encoding genes in the vicinity of <i>bla</i>_<sub>KHM-1</sub>-<i>int</i>. The ISEC68 transposase gene was flanked by 17-bp inverted repeats IRL (5′-GGAGGTGGGATCAAGT-3′) and IRR (5′-ACTTAATCGCAGCTTCC-3′); however, the repeat regions did not encompass the KHM-1-encoding gene. On the other hand, the region from <i>bla</i>_<sub>KHM-1</sub>-<i>int</i>, encoding tyrosine-type integrase, was conserved (Fig. 4B). It is possible that both plasmids acquired this region from a common ancestor (i.e., <i>bla</i>_<sub>KHM-1</sub>-harboring mobile genetic element) via homologous recombination in the

**FIG 4 Legend (Continued)**

rings show the positions of BLAST hits detected through BLASTn comparisons of the pTMSNI47-1 genome against the two plasmid genomes (pHM881QN and pKHM-1, represented by red and green circles, respectively). Darker arcs indicate a high percent identity of the hit. The black circle displays the GC content, and inner circles display GC skew. (B) Comparison of the structures among pTMSNI47-1, pHM881QN, and pKHM-1. Comparisons were performed using the Artemis Comparison Tool with a minimum score cutoff 200 and a minimum percent identity cutoff of 80%. Forward matches are colored in red.
evolution process. Further studies are required to conclude which elements mediate a transposition mechanism involving blaKHM-1. In addition, blaOXA10 was located in a class 1 integron, which includes a site-specific recombination system capable of integrating and expressing open reading frames contained in structures called mobile gene cassettes. This integron gene cassette array, aaA1-blaOXA-10, cmiA5-arr2-dfrA14, has been identified in other plasmids harbored by several Enterobacteriaceae, such as Providencia stuartii (19), demonstrating that gene module transpositions are a vital factor in the successful spread of bla genes among various plasmids.

An increase in MICs of β-lactams was detected with transformants of E. coli DH5α with the recombinant plasmids pHSG-blaCTX-M-2, pHSG-blaDHA-1, pHSG-blaKHM-1, pHSG-blaOXA-10, and pHSG-blaOKP-A-4. Of these, blaCTX-M-2 enhanced amoxicillin, piperacillin, cefotaxime, ceftazidime, and aztreonam resistance, while the blaDHA-1 strain showed a higher MIC increase for ceftazidime than the blaCTX-M-2 strain. Clavulanic acid, a mechanism-based β-lactamase inhibitor, could inhibit CTX-M-2 activities but could not inhibit DHA-1 (Table 1). The production of OXA-10 or OKP-A-4 strongly increased the MICs of amoxicillin and piperacillin (Table 1). CTX-M-type enzymes are classified as Ambler class A extended-spectrum β-lactamases (ESBLs) and exhibit a striking substrate preference for cefotaxime and ceftriaxone over ceftazidime because of the unique geometry of the β-lactam-binding site (20). DHA-1 is an AmpC-type β-lactamase belonging to Ambler class C and generally not inhibited by clavulanic acid. AmpC-type β-lactamases harbor hydrolytic activity against cephalosporin and cefamycin and variably to aztreonam, but they remain sensitive to cefepime and carbapenems (21, 22). OXA-10-type enzyme, classified as Ambler class D, was known to have narrow-spectrum β-lactamase activity, although variants in this enzyme family (such as OXA-11 and OXA-16) exhibit expanded-spectrum activity (23). The class A β-lactamase OKP enzymes, which are similar to SHV and LEN enzymes, exist in almost all K. quasipneumoniae chromosomes and are penicillinases (24, 25). Our antimicrobial susceptibility results are completely consistent with those of these previous studies.

Only blaKHM-1 enhanced carbapenem resistance (Table 1). KHM-1 is an acquired Ambler class B MBL and harbors the hydrolytic efficiencies of carbapenems. So far, this enzyme has been recognized as a rare form of MBL because there is only one report of its isolation (14). However, our study has isolated KHM-1 in a transferable plasmid (pTMSN147-1) harbored by an efficient carrier (K. quasipneumoniae) from a hot spot for horizontal gene transfer (an STP), and this enzyme might be widely disseminated in the near future in Japan. Carbapenem resistance has been documented by several mechanisms besides carbapenemase production. One of them is the loss of outer membrane porins combined with ESBLs or AmpC β-lactamases (26, 27). We did not examine the expression of porins, CTX-M-2, or DHA-1 in this study; therefore, these may also have influenced carbapenem resistance. However, regardless of the mechanism of resistance, horizontal transfer of pTMSN147-1, harboring four different Ambler class β-lactamases, leads to a very-wide-spectrum β-lactam resistance acquisition with mutual compensation.

Except for β-lactams, SNI47 was fully resistant to SXT (Fig. 3). Previous reports have stated that sul contributes to SXT resistance, and the sul and dfrA genes could synergistically lead to high-level SXT resistance (28, 29). Both genes exist in pTMSN147-1. Additionally, it was interesting to find that the sul1 gene was triplicated in this plasmid (Fig. 4A), suggesting that these genes, especially triplicated sul1 genes, might play an important role in high-level SXT resistance. On the other hand, SNI47 was sensitive to kanamycin, gentamicin, and amikacin (Fig. 3). Aminoglycoside resistance may occur due to several mechanisms. Of them, aaA1, which is located in the class 1 integron of pTMSN147-1, encodes an aminoglycoside adenyllytransferase that influences enzymatic modification and inactivation (30). This enzyme imparts streptomycin and spectinomycin resistance by modifying the 3-hydroxyl position of streptomycin and the 9-hydroxyl position of spectinomycin, but it has marginal effects on the resistance to other aminoglycosides (30). Our results are consistent with those of these previous studies (Fig. 3 and Table 2).

The oqxA and oqxB genes, encoding the OqxA efflux pump, and plasmid-mediated
quino­lone resistance (PMQR) gene qnrB4 were found in the chromo­some of SNI47 and pTMSNI47-1, respectively. However, contrary to our expec­ta­tions, SNI47 was sensitive to all tested quinolones (Fig. 3) and did not reach the Clinical and Laboratory Standards Institute (CLSI) breakpoint (Table 2). At least three distinct mechanisms of quinolone resistance in bacteria have been identified: (i) mutations in target enzymes, (ii) alter­na­tion in mem­brane permeability, and (iii) protection of target enzymes from quinolone inhibition (31). Of them, resis­tance is mediated mainly by the accumu­lation of point mutations in the quinolone resistance-determin­ing region (QRDR) of DNA gyrase (gyrA) and DNA topo­isomerase IV (parC) (3, 31). It was reported that differ­ences in the expression level of OqxAB influ­enced reduced susceptibility to quinolones; how­ever, most fell short of suscep­tibility breakpoints (32). PMQR genes con­tribute to the low level of resistance to fluoroquinolones, and these genes exert their influence by wid­ening the mutant selec­tion window and elev­a­ting mutant pre­ven­tion (33). Our present Etest results for fluoroquinolones sup­port the abo­vemen­tioned conten­tion. The MICs for SNI47, which harbored the oqxA, oqxB, and qnrB4 genes and did not harbor mutations in QRDR, were 0.5 μg/ml for ciprofloxacin and 1 μg/ml for levo­floxacin. On the other hand, those for transconjugant J53/pTMSNI47-1 (harboring only qnrB4) were 0.25 μg/ml for ciprofloxacin and 0.5 μg/ml for levofloxacin, corre­sponding to >4- and >8-fold increases compared with those for the recip­i­ent strain, respectively (Table 2). To some extent, these genes contribute to fluoroquinolone resistance, although both SNI47 and transconjugants J53/pTMSNI47-1 did not reach the CLSI breakpoint, even for inter­mediate resistance.

The present study uncovered evidence that the horizon­tal trans­fer of pTMSNI47-1 was a causative agent of a very-wide-spectrum β-lactam resis­tance. Whole-genome sequencing illustrated that isola­te SNI47 was K. quasipneumoniae subsp. quasipe­ne­moniae and harbored four plas­mids. Of these, the IncA/C2 plasmid, pTMSNI47-1, could transfer to E. coli J53 through con­jugation and car­ried resistance genes, includ­ing those for four β-lact­amas­es (blaCTX-M-2, blaDHA-1, blaKHM-1, and blaDHAL-10). The MICs of β-lact­ams for both transconjugant J53/pTMSNI47-1 and transformant DH5α/pTMSNI47-1 were higher than those for the recip­i­ent strain. Fur­thermore, blaKHM-1 was mainly responsible for reduc­ing car­ba­penem susceptibility. Our find­ings indi­cate that this plasmid conferred prop­er­ties for wide-spectrum β-lact­am resistance, includ­ing car­ba­penems, with mu­tual com­pen­sation. The highly trans­fer­able plasmid pTMSNI47-1 was detected in effi­cient carriers isolated from hot spots for horizon­tal gene trans­fer. There­fore, it might be widely disseminated and con­cern­ing clin­i­cal issue in the near future.

MATERIALS AND METHODS

Bacterial strains used in this study. A 10-ml aliquot of influent water from an STP in Japan was cul­tured overnight in 2× brilliant green lactose bile (BGLB) broth (Eiken Chemical, Tokyo, Japan) at 37°C. A 1-ml aliquot of the gas-producing BGLB broth cul­ture was spread on a Pro-media Tricolor agar (ELMEX, Tokyo, Japan) plate to perform antimi­crobial sus­ceptibility testing with imi­penem and meropenem using BD Sensi-Discs (Becton, Dickinson and Company, Franklin Lakes, NJ). Red colonies (β-galacto­sida­se produced by coliforms hydrol­yzes the Magenta-GAL complex) formed inside the inhib­ition rings were picked, and we determined the MICs of imi­penem and meropenem using the dry-strip technique (ETest; bioMérieux, La Balme-les-Grot­tes, France) ac­cording to CLSI criteria. MICs of >32 mg/liter were iden­ti­fied for each drug. We designated this coliform isolate strain SNI47.

51 pulsed-field gel electrophoresis and gel extraction. A 10-ml aliquot of influent water from an STP in Japan was cul­tured overnight in 2× brilliant green lactose bile (BGLB) broth (Eiken Chemical, Tokyo, Japan) at 37°C. A 1-ml aliquot of the gas-producing BGLB broth culture was spread on a Pro-media Tricolor agar (ELMEX, Tokyo, Japan) plate to perform antimi­crobial sus­ceptibility testing with imi­penem and meropenem using BD Sensi-Discs (Becton, Dickinson and Company, Franklin Lakes, NJ). Red colonies (β-galacto­sida­se produced by coliforms hydrol­yzes the Magenta-GAL complex) formed inside the inhib­ition rings were picked, and we determined the MICs of imi­penem and meropenem using the dry-strip technique (ETest; bioMérieux, La Balme-les-Grot­tes, France) ac­cording to CLSI criteria. MICs of >32 mg/liter were iden­ti­fied for each drug. We designated this coliform isolate strain SNI47.

51 pulsed-field gel electrophoresis and gel extraction. S1-PFGE, for separating plas­mids from chromo­some, was per­formed ac­cording to the method of Barton et al. (34), with modifi­ca­tions. Briefly, DNA plugs digested with S1 nuclease (Takara Bio, Shiga, Japan) were elec­trophoresed on a CHEF Mapper XA PFGE system (Bio-Rad, Hercules, CA) with autoalgorithms for 5 to 250 kbp. A lambda ladder (Promega, Fitchburg, WI) was used as the size marker. Extraction of the chromo­some or plasmid DNA frag­ments from the elec­trophoresed gel for MiSeq analysis was performed using a Zymoclean large-fragment DNA recovery kit (Funakoshi Co., Ltd., Tokyo, Japan).

Preparation of genomic DNA. The genomic DNA of SNI47 was ex­tracted for MiSeq analysis using a QiAamp DNA minikit (Qiagen GmbH, Hilden, Germany). The con­cen­tra­tion of the extracted genomic DNA was de­termined using a QuantiFluor ONE double-stranded DNA (dsDNA) system (Promega), and its purity was as­sessed using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

DNA library preparation and MiSeq analysis. An index-tagged library was pre­pared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA), and 300-bp paired-end reads were se­quenced on an Illumina MiSeq instrument according to the manu­facturer’s in­struc­tions. To ensure that only high-quality data were used for assembly, reads were trimmed and filtered using the CLC Genomics
TABLE 4 Nucleotide sequences used for cloning in this study

| Gene   | Name               | Oligonucleotide sequence (5′→3′) |
|--------|--------------------|---------------------------------|
| bla<sub>CTX-M-2</sub> | CTXM2_IF_F          | CATGATTACGAATTCGAGCTAGACGACAT   |
|         | CTXM2_IF_R          | GCCCATGCGCAAAGCTCATCAGAGGTT     |
| bla<sub>NDM-1</sub> | DHA1_IF_F           | CATGATTACGAATTCGAAAAATCTGATAC  |
|         | DHA1_IF_R           | GCCATGCGCAAAGCTCATCAGAGGTT     |
| bla<sub>NDM-1</sub> | RHM1_IF_F           | CATGATTACGAATTCGAAAAATCTGATAC  |
|         | RHM1_IF_R           | GCCATGCGCAAAGCTCATCAGAGGTT     |
| bla<sub>OXA-10</sub> | OXA10_IF_F          | CATGATTACGAATTCGAAAAATCTGATAC  |
|         | OXA10_IF_R          | GCCATGCGCAAAGCTCATCAGAGGTT     |
| bla<sub>OKP-A-4</sub> | OKPA4_IF_F          | CATGATTACGAATTCGAAAAATCTGATAC  |
|         | OKPA4_IF_R          | GCCATGCGCAAAGCTCATCAGAGGTT     |

Workbench 11.0 (Qiagen) set to a minimum length of 100 bp and a quality score threshold of 30. These trimmed reads were assembled de novo using the CLC Genomics Workbench with default settings. Species identification was performed by ANI analysis (http://enve-omics.ce.gatech.edu/ani/). Phylogenetic analysis of k-mer diversity was performed according to a previously reported method (35). The k-mer length was set at 16 bases for this analysis. The MLST web server (https://cge.cbs.dtu.dk/services/MLST/) was used to determine sequence types for chromosome. The PubMLST web server (https://pubmlst.org/) was used to determine sequence types or allele ID for plasmid. The ResFinder web server (https://cge.cbs.dtu.dk/services/ResFinder/) was used to identify AMR genes. The PlasmidFinder web server (https://cge.cbs.dtu.dk/services/PlasmidFinder/) was used to identify the Inc type. The threshold for minimum coverage was set at 60% of the length of the gene sequence in the database, with a minimum sequence identity of 80%.

Antimicrobial susceptibility testing. A Kirby-Bauer disc diffusion test was performed for SNI47 using BD Sensi-Discs and Mueller-Hinton agar plates (Becton, Dickinson and Company) according to CLSI recommendations. The following antimicrobials were tested: imipenem (10 μg), meropenem (10 μg), penicillin (30 μg), ampicillin (30 μg), cephalothin (30 μg), amoxicillin (30 μg), chloramphenicol (30 μg), rifampin (5 μg), naldixic acid (30 μg), norfloxacin (10 μg), levofloxacin (5 μg), ciprofloxacin (5 μg), SXT (23.75/1.25 μg), kanamycin (30 μg), gentamicin (10 μg), and amikacin (10 μg). Then the MICs of four carbapenem derivatives (imipenem, meropenem, ertapenem, and doripenem), two penicillin derivatives (amoxicillin and piperacillin), three cepham derivatives (ceftazidime, cefotaxime, and cefepime), one monobactam derivative (aztreonam), cefazidine-clavulanic acid, cefotaxime-clavulanic acid, and ceftizime-clavulanic acid were determined using the dry-strip technique (Etest) according to the guidelines of the CLSI.

Conjugation experiment. The SNI47 strain containing a β-lactamase-encoding plasmid was used as a donor, and the spontaneous-sodium azide-resistant E. coli J53 strain was used as a recipient in our conjugation experiment. The recipient was susceptible to all antibiotics tested and did not harbor a β-lactamase-encoding plasmid. SNI47 and E. coli J53 were mixed in a 1:1 ratio, and then the mixture was cultured at 37°C for 5 h. Transconjugants were selected on Pro-media triclor agar supplemented with sodium azide (100 μg/ml) and ampicillin (50 μg/ml). The blue colonies (β-glucuronidase produced by E. coli) hydrolyzes the 5-bromo-4-chloro-3-indolyl-β-D-glucuronide [X-Gluc] complex, indicating successful plasmid transformation) were picked and subjected to S1-PFGE. Then plasmid DNA was extracted from the transconjugant colonies using a HiSpeed plasmid maxikit (Qiagen).

TABLE 5 Plasmids used in this study

| Plasmid          | Relevant characteristics                                      | Source   |
|------------------|----------------------------------------------------------------|----------|
| pHSG398          | Cm', cloning vector                                           | Takara   |
| pHSG-bla<sub>CTX-M-2</sub> | Cm'; pHSG398 with the cloned PCR product containing bla<sub>CTX-M-2</sub> full-length sequence | This study |
| pHSG-bla<sub>NDM-1</sub> | Cm'; pHSG398 with the cloned PCR product containing bla<sub>NDM-1</sub> full-length sequence | This study |
| pHSG-bla<sub>NDM-1</sub> | Cm'; pHSG398 with the cloned PCR product containing bla<sub>NDM-1</sub> full-length sequence | This study |
| pHSG-bla<sub>OXA-10</sub> | Cm'; pHSG398 with the cloned PCR product containing bla<sub>OXA-10</sub> full-length sequence | This study |
| pHSG-bla<sub>OKP-A-4</sub> | Cm'; pHSG398 with the cloned PCR product containing bla<sub>OKP-A-4</sub> full-length sequence | This study |
Whole-genome sequencing using Illumina MiSeq and Oxford Nanopore MinION. The complete genome sequence of SN47 was obtained by combining sequencing data from both Illumina MiSeq and MinION (Oxford Nanopore Technologies, Oxford, UK) sequencers. Illumina sequencing was performed as described above, and Nanopore sequencing was performed according to the manufacturer’s instructions. Briefly, a DNA library was prepared using a rapid sequencing kit (Oxford Nanopore Technologies), and the prepared library was subsequently loaded into a MinION flow cell (R9.4; Oxford Nanopore Technologies). The MinION sequencing run was performed over 48 h. Hybrid assembly of both the MiSeq and MinION reads was performed using Unicycler v0.4.2 (36) with default settings and annotated using Prokka v1.12 (37) and DFAST (https://dfast.nig.ac.jp/). Further annotation was performed manually using information from National Center for Biotechnology Information BLASTn for any unknown CDSs. The annotation of ISs was performed using ISFinder (https://isfinder.biotoul.fr). When complete, the genome of pTMSNI47-1 was viewed using CGview Server (http://stothard.afns.uaalberta.ca/cgview_server/), and a comparison of the regions of interest was facilitated using the Artemis Comparison Tool (38).

Data availability. The DDJB accession numbers for sequences obtained in this study are as follows: AP019687 (SN47 chromosome), AP019688 (pTMSNI47-1), AP019689 (pTMSNI47-2), AP019690 (pTMSNI47-3), and AP019691 (pTMSNI47-4).

REFERENCES

1. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. 2011. Carbapenemases: past, present, and future. Antimicrob Agents Chemother 55:4943–4966. https://doi.org/10.1128/AAC.00296-11.

2. McKenna M. 2013. Antibiotic resistance: the last resort. Nature 499:394–396. https://doi.org/10.1038/499394a.

3. Akiba M, Sekizuka T, Yamashita A, Kuroda M, Fujii Y, Murata M, Lee K, Joshua DI, Balakrishna K,airy J, Subramanian K, Krishnan P, Munuswamy N, Sinha RK, Iwata T, Kusumoto M, Guruge KS. 2016. Distribution and relationships of antimicrobial resistance determinants among extended-spectrum-cephalosporin-resistant or carbapenem-resistant Escherichia coli isolates from rivers and sewage treatment plants in India. Antimicrob Agents Chemother 60:2972–2980. https://doi.org/10.1128/AAC.01950-15.

4. Guo J, Li J, Chen H, Bond PL, Yuan Z. 2017. Metagenomic analysis reveals wastewater treatment plants as hotspots of antibiotic resistance genes and mobile genetic elements. Water Res 123:468–478. https://doi.org/10.1016/j.watres.2017.07.002.

5. Karkman A, Do TT, Walsh F, Virta M. 2018. Antibiotic-resistance genes in waste water. Trends Microbiol 26:220–228. https://doi.org/10.1016/j.tim.2017.09.005.

6. Queenan AM, Bush K. 2007. Carbapenemases: the versatile beta-lactamase. Clin Microbiol Rev 20:440–458. https://doi.org/10.1128/CMR.00001-07.

7. Gupta N, Limbago BM, Patel JB, Kallen AJ. 2011. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis 53:60–67. https://doi.org/10.1093/cid/cir202.

8. Logan LK, Weinstein RA. 2017. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 215(Suppl 1):S28–S36. https://doi.org/10.1093/infdis/jiw282.

9. 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, Hisashi K, Komatsu M, Nakamura F. 2017. Molecular epidemiology of multiple extended-spectrum cephalosporin resistance genes and mobile genetic elements in carbapenemase-producing Enterobacteriaceae in wastewater revealed by genomic analysis. Antimicrob Agents Chemother 62:e02301-17. https://doi.org/10.1128/AAC.02501-17.

10. Brisse S, van Hingham T, Kusters K, Verhoef J. 2004. Development of a rapid identification method for Klebsiella pneumoniae phylogenetic groups and analysis of 420 clinical isolates. Clin Microbiol Infect 10:942–945. https://doi.org/10.1111/j.1469-0691.2004.00937.x.

11. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severijn J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Huo NT, Schultz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A 112:E3574–E3581. https://doi.org/10.1073/pnas.1501491112.

12. Lang KS, Danzeisen JL, Xu W, Johnson TJ. 2012. Transcriptome mapping of pAR060302, a bla<sub>CMY-2</sub>-positive broad-host-range IncA/C plasmid. Appl Environ Microbiol 78:3379–3386. https://doi.org/10.1128/AEM.07199-11.

13. Arpin C, Thabet L, Yassin H, Messadi AA, Boukadida J, Dubois V, Coulange-Mayonnove L, Andre C, Quentin C. 2012. Evolution of an incompatibility group IncA/C plasmid harboring bla<sub>CMY-2</sub> and qnrA genes and its transfer through three clones of Providencia stuartii during a two-year outbreak in a Tunisian burn unit. Antimicrob Agents Chemother 56:1342–1349. https://doi.org/10.1128/AAC.05267-11.

14. Rosolini GM, D’Andrea MM, Muggiali C. 2008. The spread of CTX-M-type extended-spectrum-beta-lactamases. Clin Microbiol Infect 14(Suppl 1):S23–S41. https://doi.org/10.1111/j.1469-0691.2007.01867.x.

15. Gaillot O, Clement C, Simonet M, Philippon A. 1997. Novel transferable beta-lactam resistance with cephalosporinase characteristics in Salmonella enteritidis. J Antimicrob Chemother 39:85–87. https://doi.org/10.1093/jac/39.1.85.

16. Jacoby GA. 2009. AmpC beta-lactamases. Clin Microbiol Rev 22:161–182. https://doi.org/10.1128/CMR.00036-08.

17. Poirel L, Naas T, Nordmann P. 2010. Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54:24–39. https://doi.org/10.1128/AAC.00512-08.

18. Haegeman S, Lofdahl S, Pauw A, Verhoef J, Brisse S. 2004. Diversity and evolution of the class A chromosomal beta-lactamase gene in Klebsiella pneumoniae. Antimicrob Agents Chemother 48:2400–2408. https://doi.org/10.1128/AAC.48.7.2400-2408.2004.

19. Feyere P, Passet V, Weill FX, Grimont PA, Brisse S. 2005. Variants of the Klebsiella pneumoniae OPM chromosomal beta-lactamase are divided into two main groups, OKP-A and OKP-B. Antimicrob Agents Chemother 49:5149–5152. https://doi.org/10.1128/AAC.49.12.5149-5152.2005.
26. Wang XD, Cai JC, Zhou HW, Zhang R, Chen GX. 2009. Reduced susceptibility to carbapenems in Klebsiella pneumoniae clinical isolates associated with plasmid-mediated beta-lactamase production and OmpK36 porin deficiency. J Med Microbiol 58:1196–1202. https://doi.org/10.1099/jmm.0.008094-0.

27. Tsai YK, Liou CH, Fung CP, Lin JC, Siu LK. 2013. Single or in combination antimicrobial resistance mechanisms of Klebsiella pneumoniae contribute to varied susceptibility to different carbapenems. PLoS One 8:e79640. https://doi.org/10.1371/journal.pone.0079640.

28. Hu LF, Chang X, Ye Y, Wang ZX, Shao YB, Shi W, Li X, Li JB. 2011. Stenotrophomonas maltophilia resistance to trimethoprim/sulfamethoxazole mediated by acquisition of sul and dfrA genes in a plasmid-mediated class 1 integron. Int J Antimicrob Agents 37:230–234. https://doi.org/10.1016/j.ijantimicag.2010.10.025.

29. Chung HS, Kim K, Hong SS, Hong SG, Lee K, Chong Y. 2015. The sul1 gene in Stenotrophomonas maltophilia with high-level resistance to trimethoprim/sulfamethoxazole. Ann Lab Med 35:246–249. https://doi.org/10.3343/alm.2015.35.2.246.

30. 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.

31. Hopkins KL, Davies RH, Threlfall EJ. 2005. Mechanisms of quinolone resistance in Escherichia coli and Salmonella: recent developments. Int J Antimicrob Agents 25:358–373. https://doi.org/10.1016/j.ijantimicag.2005.02.006.

32. Rodriguez-Martinez JM, Diaz de Alba P, Briones A, Machuca J, Lossa M, Fernandez-Cuenca F, Rodriguez Baño J, Martinez-Martinez L, Pascual A. 2013. Contribution of OqsAB efflux pumps to quinolone resistance in extended-spectrum-beta-lactamase-producing Klebsiella pneumoniae. J Antimicrob Chemother 68:68–73. https://doi.org/10.1093/jac/dks377.

33. Jacoby GA, Strahilevitz J, Hooper DC. 2014. Plasmid-mediated quinolone resistance. Microbiol Spectr 2:PLAS-0006-2013. https://doi.org/10.1128/microbiolspec.PLAS-0006-2013.

34. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220.

35. Suzuki Y, Kubota H, Ono HK, Kobayashi M, Murauchi K, Kato R, Hirai A, Sadamasu K. 2017. Food poisoning outbreak in Tokyo, Japan caused by Staphylococcus argenteus. Int J Food Microbiol 262:31–37. https://doi.org/10.1016/j.ijfoodmicro.2017.09.005.

36. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Uncycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.

37. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.

38. Carver T, Berriman M, Tivey A, Patel C, Böhme U, Barrell BG, Parkhill J, Rajandream MA. 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. Bioinformatics 24:2672–2676. https://doi.org/10.1093/bioinformatics/btn529.